Global diversity and adaptations of avian eggshell thickness indices

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The amniote eggshell is a fundamental aspect of the embryo life-support system, protecting it from UV light, microbes and mechanical damage, while regulating gas exchange and providing calcium for growth. The thickness of eggshells is highly diverse across modern birds and influences multiple eggshell functions, yet the selective pressures driving eggshell thickness have not been clearly identified. Here, we use a global dataset of avian eggshell thickness indices for 4260 (> 41%) avian species to assess trends in eggshell thickness across the phylogeny, as these indices are strongly correlated with direct measures of eggshell thickness and are non-destructive to the sample. We analysed the dataset within a phylogenetic framework to assess the relative importance of climatic and ecological explanations for variation in eggshell thickness indices. The distribution of avian eggshell thickness indices across species was found to be primarily driven by phylogenetic relatedness, in addition to evolutionary processes that do not match a Brownian model of evolution. Across modern birds, thicker eggshells were more prevalent in species (1) with precocial young, (2) which exhibit a scavenger-based diet, (3) which primarily feed on vertebrates or plants (excluding nectivores, seed and fruit specialists) and (4) which breed in open habitats. Thicker eggshells found in species with precocial young probably enable higher rates of calcium removal for the more advanced embryo development. Excessive light transmission through the shell damages developing embryos, while too little light transmission can impede development. Eggs in shaded habitats experience low light exposure, and thus thinner shells are more prevalent in these environments potentially to increase light transmission through the shell. Overall, variation in eggshell thickness indices appears to be driven largely by phylogeny, with certain life-history traits linked to embryo growth rate, calcium content of their food, and the need to mitigate excessive light transmission through the shell.

Keywords: avian, dietary calcium, eggs, eggshell thickness indices, habitat, life-history, mode of development.

Birds (class Aves) are highly diverse, with more than 10 000 extant species distributed globally (Jetz *et al.* 2012). Birds are unique as the only vertebrate class to be exclusively oviparous (Blackburn & Evans 1986). Laying eggs has multiple evolutionary benefits, particularly allowing for a significantly higher fecundity compared with most mammals (Holliday 1994, Etterson *et al.* 2011). Bird eggshells are a compelling model system to investigate macroevolutionary hypotheses relating to adaptive functions and environmental effects on biological diversity; they are critical to embryo survival, are morphologically diverse, and must withstand the physiological and mechanical challenges of being laid in a variety of environments (Maurer

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et al. 2015). These environments can include wetlands, extreme temperatures, Arctic regions and deserts (Attard & Portugal 2021). Embryo survival in these diverse conditions is reliant on specific physical characteristics – i.e. adaptations – of the eggshells.

The global diversity in eggshell traits for colour and patterning (Kilner 2006, Wisocki *et al.* 2019), stiffness (Hung *et al.* 2022), shape (Stoddard *et al.* 2017), size and structural components (D'Alba *et al.* 2016) have been attributed to abiotic (humidity, temperature and altitude) and biotic (parent incubation strategies, nest structure and microbes) selective pressures that influence the survival and metabolic requirements of the developing embryo. For instance, egg shapes with a low surface area are more commonly found in hot, dry and sparsely vegetated environments to reduce water loss and exposure to UV radiation (Englert Duursma *et al.* 2018). In contrast, eggs in humid infection-prone environments are more likely to have pore-plugging nanospheres to minimize the risk of microbial infection and flooding of the embryo (D'Alba *et al.* 2016). Eggs in low-oxygen environments, such as high altitudes, have small pores to reduce water loss, while eggs of most burrowing birds have high shell porosity to cope with high humidity and carbon dioxide concentrations (Vieco-Galvez *et al.* 2021). These studies provide strong evidence for factors underlying structural diversification of bird eggshells. Although large-scale macroecological studies have examined egg shape (Stoddard *et al.* 2017), maculation (Kilner 2006, Wisocki *et al.* 2019) and cuticle structures (D'Alba *et al.* 2016), other important eggshell factors are less well understood. This is particularly important given that eggshell traits are not mutually exclusive, and thus each needs to be integrated with other eggshell characteristics successfully to fulfil multiple purposes.

Eggshell thickness has been demonstrated to be a particularly useful indicator of environmental change, hatching success and population health in birds (Cooke 2009). Eggshell thickness varies within species, yet variation is far more extensive between species (Peterson *et al.* 2020), ranging from 29 μm in Blue-tailed Emeralds *Chlorostilbon mellisugus* to 1920 μm in Ostriches *Struthio camelus* (Schönwetter 1960). The thickness of an eggshell is a key determinant of how well the egg contents are protected from mechanical impacts, dehydration, ultraviolet light and contamination by microorganisms, which otherwise would be detrimental to the embryo. For example, eggs with thicker shells tend to be stronger (Ketta & Tůmová 2018) and are less prone to contamination, as debris and microorganisms have a further distance to travel across shell pores (Chen *et al.* 2019). Eggshell thickness also influences the delicate balance of water vapour loss and diffusion of respiratory gases across the shell (Paganelli 1980). Greater light transmission through the shell is required to assist embryonic development in nests with low light exposure, which may be accomplished by producing thinner shells (Maurer *et al.* 2015). Previous studies have focused on a limited number of ecological traits and/or species that could influence eggshell thickness variation. For example, Birchard and Deeming (2009) tested the influence of clutch size and parental care strategies across 968 species of non-Passeriformes, after accounting for egg mass. They concluded that birds with single-egg clutches invest more in the shell, particularly in thickness, to improve its strength due to the higher fitness consequence if the egg breaks. Whether a similar association between clutch size and shell thickness is widespread across the avian kingdom is yet to be fully tested.

Here, we undertake the first phylogenetically informed analysis of shell thickness variation across the full spectrum of life-histories and geographical ranges for nearly half of the world's 10 000 bird species. In doing so, we establish a strong foundation for future research to evaluate to what degree eggshells of wild birds have acclimatized to (or been affected by) different environmental stressors. Most previous attempts to assess eggshell thickness variability across species were restricted to non-Passeriformes (Rahn & Paganelli 1989, Birchard & Deeming 2009, Peterson *et al.* 2020) and did not apply phylogenetically informed analyses (Peterson *et al.* 2020). Since these earlier studies, avian-wide complementary datasets on ecological, behaviour and other life-history variables have become available (Sheard *et al.* 2020), allowing broad-scale analyses to be conducted. Shell thickness, like many eggshell traits, scales with adult body mass and egg mass (Birchard & Deeming 2009, Peterson *et al.* 2020); our study accounts for these allometric effects. We include two climatic and eight ecological variables (see Table 1 for hypotheses) to assess key drivers of interspecific variation in eggshell thickness, both across all bird species, and separately within the two major groups:

Passeriformes and non-Passeriformes. The reproductive strategies and eggshell characteristics of these two taxonomically distinct groups vary considerably, which is likely to result in different eggshell thickness patterns within each group (Attard & Portugal 2021). For example, Passeriformes (comprising 60% of the world's birds) are land birds with altricial young, whereas non-Passeriformes include water and land birds and vary in their mode of development. Passerines are generally (but not exclusively) arboreal and build a diversity of nests to incubate their eggs, from cups and domes to cavities (McEntee *et al.* 2018), whereas dome nests are much less common among non-Passeriformes (Collias 1997). Furthermore, most Passeriformes lay coloured eggs, whereas most non-Passeriformes lay white eggs, except certain ground-nesting species that lay coloured eggs for camouflage (Gosler *et al.* 2005). Many of these traits are intrinsically linked to the structure of eggs, including their thickness (Martín-Vivaldi *et al.* 1999, Maurer *et al.* 2015). Here, we use shell morphometric values from all extant bird species listed in Max Schönwetter's (1960) *Handbuch der Oologie*, to identify broad-scale trends in eggshell thickness across different life-histories while accounting for phylogenetic relatedness. Many previous comparative analyses of eggshell thickness have not corrected for allometric effects of body mass, which can hide potentially important adaptive information relating to the environment and nesting behaviour of the species. We predicted eggshell thickness would be primarily explained by body mass, as adult body mass is strongly correlated with egg mass (Rahn *et al.* 1985, Mehlum *et al.* 1987, Dyke & Kaiser 2010). By contrast, we predict that mass-independent eggshell thickness would be primarily associated with embryo development and clutch size as well as climate (Table 1).

METHODS

Eggshell thickness indices

We compared eggshell thickness indices across 4260 extant bird species. Shell thicknesses were taken from Schönwetter's *Handbuch der Oologie* (1960–1992) and are based on several indices of shell thickness (eggshell thickness indices, ETIs) that approximate the true thickness of the shell as a function of egg shape and shell density (see section 'Equations for eggshell thickness indices' for calculations of each ETI). In *Handbuch der Oologie*, a single mean shell thickness value (*d*, in mm) is provided for 7146 avian species and subspecies (Rahn & Paganelli 1989), based on one of the ETIs. The specific calculation used for each species and subspecies is unclear; however, Maurer *et al.* (2012) conclusively demonstrated the high accuracy of each ETI compared with direct measures of shell thickness taken from 230 species across 44 families; both of Schönwetter's ETIs were strongly related to thickness measured directly from the shell (Pearson's correlation coefficient, $r > 0.97$). ETIs from *Handbuch der Oologie* have been used in previous comparative studies (Ar *et al.* 1979, Rahn & Paganelli 1989, Birchard & Deeming 2009) and we used here to compare mean shell thickness across modern birds. Where multiple subspecies were present, we used the *d* value for the subspecies with the greatest sample size to represent the species overall.

Equations for eggshell thickness indices

All values for shell thickness *d* presented in Schönwetter's tables (Schönwetter 1960) are based on the average of measured values for egg length (*A*, in mm), breadth (*B*, in mm) and shell mass (*g*, in grams). Shells with defects, drill holes or signs of thinning were included in Schönwetter's measurements without applying any correction factor, as their effect on shell mass was negligible (vol. IV, pp. 37–39, tables 4 & 5).

Two equations were used by Schönwetter (1960) to calculate *d*, called explicit ETI and simple ETI (Maurer *et al.* 2012). Simple ETI is the simplest of the two indices and was used to approximate the shell thickness of most species with typical ovoid equations as follows:

$$d = \left(\frac{0.175g}{AB}\right) \qquad (1)$$

Schönwetter calculated most species values of *d* using explicit ETI as follows:

$$d = \left(\frac{k}{Kcy}\right)\left(\frac{g}{AB}\right) = m\left(\frac{g}{AB}\right) \qquad (2)$$

To account for deviations from the standard egg shape, explicit ETI includes a shape factor (*c*, no units), reduction factor (*K*, no units) and specific

Table 1. Putative predictions for a series of possible explanations for variation in eggshell thickness in birds.

Predictor	Hypothesis	Definition	Source
Body mass	The eggs of larger birds are expected to have thicker shells as egg mass scales positively with adult body mass, and larger eggs tend to have thicker shells (Rahn *et al.* 1985, Mehlum *et al.* 1987, Dyke & Kaiser 2010)	Mean body mass (g) of adult birds	Dunning (2008), Portugal *et al.* (2014), Sheard *et al.* (2020)
Clutch size	In some species, eggs from smaller clutches have thicker shells (Rothstein 1972, Hunt & Hunt 1973, Olsen & Olsen 1979, Falk & Møller 1990), whereas others produce thicker shells when they lay larger clutches (Falk & Møller 1990). In the latter case, females with higher predicted fitness are expected to produce large clutches as well as thick-shelled eggs when breeding under good conditions	Number of eggs per brood, measured as geometric mean of the typical minimum and maximum clutch size	Jetz *et al.* (2008)
Egg maculation	Eggs in low-calcium areas are more heavily spotted than eggs in high-calcium areas to compensate for eggshell thinning (Gosler *et al.* 2005, Higham *et al.* 2006, Jubb *et al.* 2006). As such, maculated eggs are expected to have thinner eggshells than immaculate eggs	(1) Immaculate: No spotting or markings on eggshell surface (2) Maculate: Maculation present on eggshell surface	This paper
Habitat	Thinner eggshells permit greater light transmission through the shell to assist embryonic development (Shafey 2004) in habitats with low light exposure. Therefore, species occupying semi-open and dense habitats are expected to have relatively thin shells compared to species breeding in open habitats	(1) Open: Breeds in desert, grassland, open water, open moorland, low shrubs, rocky habitats, seashores and cities (2) Semi-open: Breeds in open shrubland and bushland, scattered bushes, parkland, forest edge (3) Dense: Breeds in forest with a closed canopy, or in the lower vegetation strata of dense thickets, shrubland, mangroves or marshland	Tobias *et al.* (2016)
Mode of development	Precocial species are expected to have thicker shells than altricial species of the same egg mass because they are more susceptible to breaking due to their longer incubation period (Portmann 1955) and require more calcium to produce a more developed chick (Bond *et al.* 1988)	(1) Altricial: Newly born young are relatively immobile, naked, and usually require care and feeding by the parents (2) Precocial: Newly born young are relatively mobile, covered in feathers, and independent	Starck & Ricklefs (1998)
Migration	Migration promotes the evolution of thicker eggshells relative to adult body mass, as migratory birds have more opportunities to access calcium-rich resources (Drent & Woldendorp 1989)	(1) Nomadic: Moves in response to resources that are sporadic in time and distribution (2) Altitudinal Migrant: Regularly/seasonally makes cyclical movements to higher/lower elevations with predictable timing and destinations (3) Full Migrant: A substantial proportion of the global or regional population makes regular or seasonal cyclical movements beyond the breeding range, with predictable timing and destinations	Sheard *et al.* (2020)

(continued)

Table 1. (continued)

Predictor	Hypothesis	Definition	Source
Hand-wing index (HWI)	Species with high HWI tend to have more elliptical or asymmetric eggs, which may be related to the demands of having streamlined bodies (Stoddard *et al.* 2017). More asymmetric eggs are mechanically weaker than rounder eggs (Richards & Swanson 1965, Tung *et al.* 1968), unless compensated for by having a thicker shell. Therefore, species with a higher HWI are expected to have thicker eggshells	The ratio of Kipp's distance (the distance between the tip of the longest primary and the tip of the first secondary feather) to total wing chord (distance between the carpal joint and wingtip)	Sheard *et al.* (2020)
Island	Thinner eggshells may be more common in island environments as there are fewer predators to defend against, and these eggs are exposed to less-seasonal climates (Losos & Ricklefs 2009). Inversely, the longer developmental period and biparental care shown by many island colonizers (Covas 2012) may promote the evolution of thicker eggshells to prevent accidental egg breakage	Proportion of species range intersecting islands with landmass below 2000 km^2	Sheard *et al.* (2020)
Temperature seasonality	Thicker eggshells are thought to protect the embryo better from sudden temperature fluctuations and cooler climates (De Frenne *et al.* 2013), and therefore thicker eggshells are expected to be more common in highly seasonal environments	Average temperature seasonality (BIO4) of breeding/resident range, based on WorldClim v1 data. Defined as the amount of temperature variation over a given year (or averaged years) based on the standard deviation of monthly temperature averages	Hijmans *et al.* (2005), Sheard *et al.* (2020)
Precipitation seasonality	Eggs incubated in environments with highly variable precipitation are expected to have thicker eggshells to combat temporary periods of excessive rain (Veldsman *et al.* 2020), which could otherwise cause the embryo to suffocate or drown	Average precipitation seasonality (BIO15) of breeding/resident range, based on WorldClim v1 data. This is a measure of the variation in monthly precipitation totals over the course of the year and is expressed as a percentage	Hijmans *et al.* (2005), Sheard *et al.* (2020)

Hypotheses and definitions of the predictors, the categories contained within, and their sources are provided. Predictors excluded from analysis due to collinearity are provided in Table S1.

mass of the egg (γ, in grams). Schönwetter calculated γ for 50 bird families (see Schönwetter 1960, vol. IV, p. 118, table 20), which includes the mass of the shell and shell membrane. The thickness factor m is defined as $k/Kc\gamma$, and $k = A/B$. The shape factor was determined using table 3 in Schönwetter (1960) and the specific mass of the shell was determined at the family level using table 2 in Schönwetter (1960). The reduction factor was defined as:

$$K = 0.914 + 2.228k \qquad (3)$$

For our study, we had considered calculating eggshell thickness for a single ETI. Explicit ETI, one of the indices used, was the preferred choice, as it has the best fit when compared with direct measures of equator thickness ($r^2 = 0.995$, $y = 1.017x + 0.002$) and overall shell thickness ($r^2 = 0.996$, $y = 0.998x + 0.004$) (Maurer *et al.* 2012). This formula requires the specific mass of the shell c, determined at the family level, following table 20 in Schönwetter (1960). The specific mass of the shell is only available for a select number of families, so unfortunately explicit ETI cannot be used for the full dataset. Instead, we used species-specific d values taken directly from Schönwetter (1960), which include a mixture of explicit ETI and simple ETI values.

Limitations of egg thickness indices

A limitation of ETI is that it produces a single value for shell thickness (Maurer *et al.* 2012), and thus it does not account for variation in shell thickness along the long-axis of the egg (i.e. from the sharp pole to the blunt pole) (Orłowski *et al.* 2019). Eggshells are generally thickest at the equator, with thickness declining towards the poles (Peterson *et al.* 2020). Latitudinal variation in thickness along the shell is potentially biologically relevant, as it could determine areas of greatest gas conductance (de Araújo *et al.* 2017), calcium mobilization (Orłowski *et al.* 2019) and shell breaking strength (Sun *et al.* 2012).

In museum collections, clutch sizes may be biased by the collector having sampled larger clutches because they were more valuable (Falk & Møller 1990). Eggshell thickness index data compiled by Schönwetter were primarily based on the 19 206 egg clutches across 3849 taxa in his own collection. As such, it is likely that there is some degree of bias in this dataset. Schönwetter collected the remaining data from private and public collections worldwide as well as data from published literature and private correspondence (Maurer *et al.* 2010). The locations where clutches were collected encompass a wide geographical range, as specified in the *Handbuch der Oologie* tables. Overall, Schönwetter remains one of the largest egg morphometric datasets. Very few extensive datasets exist for direct measures of eggshell thickness, the largest of which encompasses a few hundred species (Maurer *et al.* 2012). Future research would benefit from direct measures of shell thickness from different regions of the egg across numerous species to assess different functional advantages based on thickness distribution.

A further limitation of our approach is the assumption that the previously determined relationship (Maurer *et al.* 2012) between direct eggshell thickness measurements and ETI (230 species across 44 families) is applicable to all species. Although the relationship was strong in those species measured ($r^2 = 0.94$), the dataset was Western Palaearctic-focused, possibly not encompassing all egg types, shapes and thicknesses. Moreover, the relationship between measured eggshell thickness and Schönwetter's ETI was influenced by egg shape (Maurer *et al.* 2012). The relationship, for example, was slightly weaker for spherical eggs (e.g. 'Owls', $r^2 = 0.975$), compared witho more pyriform egg shapes (e.g. 'Waders', $r^2 = 0.995$). However, all regression relationships and associated Pearson's correlations remained significant for all egg shapes.

Study species

We included ETI measures for all modern species, except brood parasites because (i) their clutch size is difficult to define, as females usually spread eggs over many host species (Jetz *et al.* 2008) and (ii) the evolution of thick-shelled brood parasite eggs are driven by very different selective pressures to other birds, such as resisting bacterial infection caused by parasitic females visiting numerous host nests (Hahn & Reisen 2011) and resisting damage when parasitic eggs are laid from an elevated position (López *et al.* 2018).

Life-history and climate traits

We collated data on factors hypothesized to influence the evolution of eggshell thickness (Table 1). Previous research supporting our rational for inclusion of each hypothesis and sources for predictor definitions are included in Table 1. Following previous studies (Tobias *et al.* 2016, Sheard *et al.* 2020), we classified species according to their mode of development (altricial, precocial); movement (not a migrant, altitudinal migrant, full migrant, nomadic); habitat (open, semi-open and dense), and diet (nectar, seeds, plants, invertebrates, omnivores, scavengers and vertebrates; see Table 1 for full definitions and Supporting Information Table S1 for sample sizes). For analyses within Passeriformes, vertebrate consumers and scavengers were dropped from the diet category due to insufficient sample sizes (Table S1). Similarly, mode of development was excluded as a predictor for Passeriformes, as they all have altricial young. Mean clutch size and scores based on their association with islands (Sheard *et al.* 2020) were also included. Other traits previously linked to eggshell thickness were considered (Supporting Information Table S2) but were removed from analysis due to multicollinearity (Supporting Information Fig. S1).

Statistical analysis

In all, 1000 phylogenetic trees were constructed online (http://www.birdtree.org) using the primary backbone tree of Hackett *et al.* (2008) and were summarized into a single consensus tree. All statistical analyses were performed using the software R v3.6.1 (R Core Team 2020).

Phylogenetic generalized least square (PGLS) regressions were performed following protocols by Attard and Portugal (2021) to test for relationships between ecological traits and ETI. First, we tested for collinearity using variance inflation factor (VIF) and pairwise correlations to determine whether any predictor variables need to be removed from the PGLS model. VIF of each predictor in a linear model (using the *lm* and *vif* functions in 'stats' and 'reclass' packages) was used to identify multicollinearity (Fox & Monette 1992). As our linear models included predictors with more than 1 degree of freedom, generalized variation-inflation factors (GVIF) with degrees of freedom (df) were used, where df is the number of coefficients in the subset. Predictors in the linear model with a squared $GVIF^{1/(2*df)}$ above 10 were considered to be highly correlated. The predictor with the highest value was removed and the model was rerun until all predictors in the linear model had a squared $GVIF^{1/(2*df)}$ below 10 (Supporting Information Tables S2 and S3). Correlations were then inspected between each numerical variable included in the original linear model to confirm independence. Numerical variables that resulted in pairwise correlations greater than 0.75 were considered highly correlated (Paz *et al.* 2015) and were removed from the PGLS analysis.

Body mass was highly correlated (> 0.75) with multiple eggshell traits (egg mass, egg length, egg breadth and incubation days) and two of these (egg mass, egg length) had a $GVIF^{1/(2*df)}$ above 10, and thus only body mass was retained for the PGLS. Annual temperature, temperature range and absolute latitude were highly correlated with each other (Fig. S1) and annual temperature had a squared value of $GVIF^{1/(2*df)}$ above 10 (Tables S2 and S3), and thus both absolute latitude and temperature range were removed from the PGLS analysis. The final predictors included in the PGLS for ETI were body mass, clutch size, HWI, diet, habitat, island, migration, annual precipitation and latitude. The association with islands

was arcsine-transformed, and all other numerical variables were log_{10}-transformed (except annual temperature, temperature seasonality, HWI and absolute median latitude) to reduce skewness.

We ran the full PGLS model containing all predictors, then used the 'pdredge' function from the package MuMIn (Burnham & Anderson 2002) to fit all model combinations with a maximum of five predictors, including a null model. As eggshell thickness correlates with body mass, we ran two full models. The first full model used adult body mass as a predictor and ETI as the response variable. The second full model used response variable ETI_R, computed as residuals from a PGLS regression of ETI on log(body mass) (slope = 0.26 ± 0.004 se; intercept = −1.28 ± −45.45 se; $\lambda = 0.66$; Supporting Information Fig. S2). We identified the best subset of models using the Akaike Information Criterion, adjusted for small sample sizes (AIC_c). We report all models with $\Delta AIC_c \leq 2$ of the lowest ranked model, and applied model averaging to quantify the importance for each trait present in this subset (Angert *et al.* 2011). Pagel's lambda (λ) was calculated in the package 'phytools' (Revell 2012) to measure phylogenetic dependence of ETI across all species investigated. Lambda values vary continuously from 0 to 1, where $\lambda = 0$ indicates no phylogenetic signal in the trait (i.e. close relatives are not more similar on average than distant relatives) and $\lambda = 1$ indicates strong phylogenetic signal in the trait (i.e. close relatives are more similar on average than distant relatives).

RESULTS

ETI and ETI_R are phylogenetically conserved

Phylogenetic signal was high and significantly different from 0 for ETI ($\lambda = 0.99$, $P < 0.001$ for all birds, and $\lambda = 0.99$, $P < 0.001$ for non-Passeriformes and Passeriformes) and ETI_R ($\lambda = 0.97$, $P < 0.001$ for all birds, $\lambda = 0.87$, $P < 0.001$ for Passeriformes, and $\lambda = 0.96$, $P < 0.001$ for non-Passeriformes; Table 2). This demonstrates that, on average, closely related species are more similar in ETI than more distantly related species, irrespective of allometric effects. Pagel's λ was also significantly different from 1 for all birds and both taxonomic groups, for ETI and

Table 2. Estimates of phylogenetic signal in eggshell thickness (ETI) in all birds.

Taxonomic group	Response variable	Pagel's λ	Log likelihood	Log likelihood for λ = 0	Log likelihood for λ = 1	P for λ = 0	P for λ = 1
All birds	ETI	0.99	4372.78	−673.49	3557.31	<0.001	<0.001
	ETI$_R$	0.97	5605.08	3080.02	4183.16	<0.001	<0.001
Passeriformes	ETI	0.99	2780.13	1554.60	2284.93	<0.001	<0.001
	ETI$_R$	0.87	3304.37	2977.58	2460.87	<0.001	<0.001
Non-Passeriformes	ETI	0.99	1728.60	3742.91	1368.85	<0.001	<0.001
	ETI$_R$	0.96	2390.02	1813.99	1774.62	<0.001	<0.001

Phylogenetic signal was analysed separately for log$_{10}$-transformed eggshell thickness (ETI) calculated by Schönwetter (1960) and their residuals (ETI$_R$). The *P*-value tests the null hypothesis of no phylogenetic signal (λ = 0) and Brownian motion model (λ = 1) of evolution.

ETI$_R$ (*P* < 0.001). The evolution of ETI is therefore influenced largely by relatedness, with some evolutionary processes (i.e. life-history traits).

Distribution of ETI$_R$ across birds

ETI$_R$ varied markedly across species, ranging from −0.38 (Abyssinian Longclaws *Macronyx flavicollis*) to 0.76 (Davison's Leaf-warblers *Phylloscopus davisoni*; Fig. 1 and see Exc file in Figshare repository (doi: 10.6084/m9.figshare.14763147) for all ETI$_R$ values. At the order level, eggshells were thicker than predicted for a given body mass for flightless birds (Struthioniformes, Rheiformes, Casuriiformes, Apterygiformes and Sphenisciformes), tropicbirds (Phaethontiformes), flamingos (Phoenicopteriformes) and stork-like birds (Ciconiiformes). Avian orders with thinner eggshells than expected for a given body mass include most swifts and treeswifts (Apodiformes), mousebirds (Coliiformes), turacos (Musophagiformes), sandgrouses (Pteroclidiformes), woodpeckers (Piciformes) and tinamous (Tinamiformes).

Life-history influences ETI across all birds

Against this backdrop of evolutionary conservatism, we tested the relationships between ETI and different life-history traits while accounting for phylogenetic and allometric effects. We found strong support for the hypotheses that variation in ETI in birds is influenced by body mass, mode of development, diet and habitat (Figs 2 and 3, Supporting Information Tables S5 and S6). Similarly,

differences in ETI$_R$ across birds were influenced by mode of development, diet and habitat (Supporting Information Tables S7 and S8). Body mass was significantly and positively associated with ETI (Fig. 2a, Table S6). Altricial species had significantly lower ETI and ETI$_R$ than precocial species (Fig. 2b, Tables S5–S8). Species occupying open habitats had higher ETI and ETI$_R$ than those in dense habitats (Fig. 2c, Tables S5–S8). Species that primarily feed on nectar, seeds or fruit had significantly thinner shells than all non-plant consumers (Fig. 2d, Tables S5–S8). Conversely, vertebrate consumers had significantly higher ETI and ETI$_R$ compared with omnivores, invertivores and plant-based consumers (Fig. 2d, Tables S5–S8).

Life-history influences ETI among Passeriformes

Among Passeriformes, body mass and diet were the main predictors of ETI, and diet, migration and HWI were the most important predictors of ETI$_R$ (Fig. 3). ETI among Passeriformes was positively associated with body mass (Supporting Information Tables S9 and S10), whereas ETI$_R$ was negatively associated with HWI (Supporting Information Tables S11 and S12). Unexpectedly, across Passeriformes, nectar and fruit specialists had lower ETI than seed specialists, invertivores and omnivores, and seed specialists had significantly higher ETI than omnivores (Table S10). ETI$_R$ was also significantly higher in invertivores than in plant, fruit and nectar specialists. Passeriformes in open habitats had significantly higher ETI than those in semi-open and dense habitats.
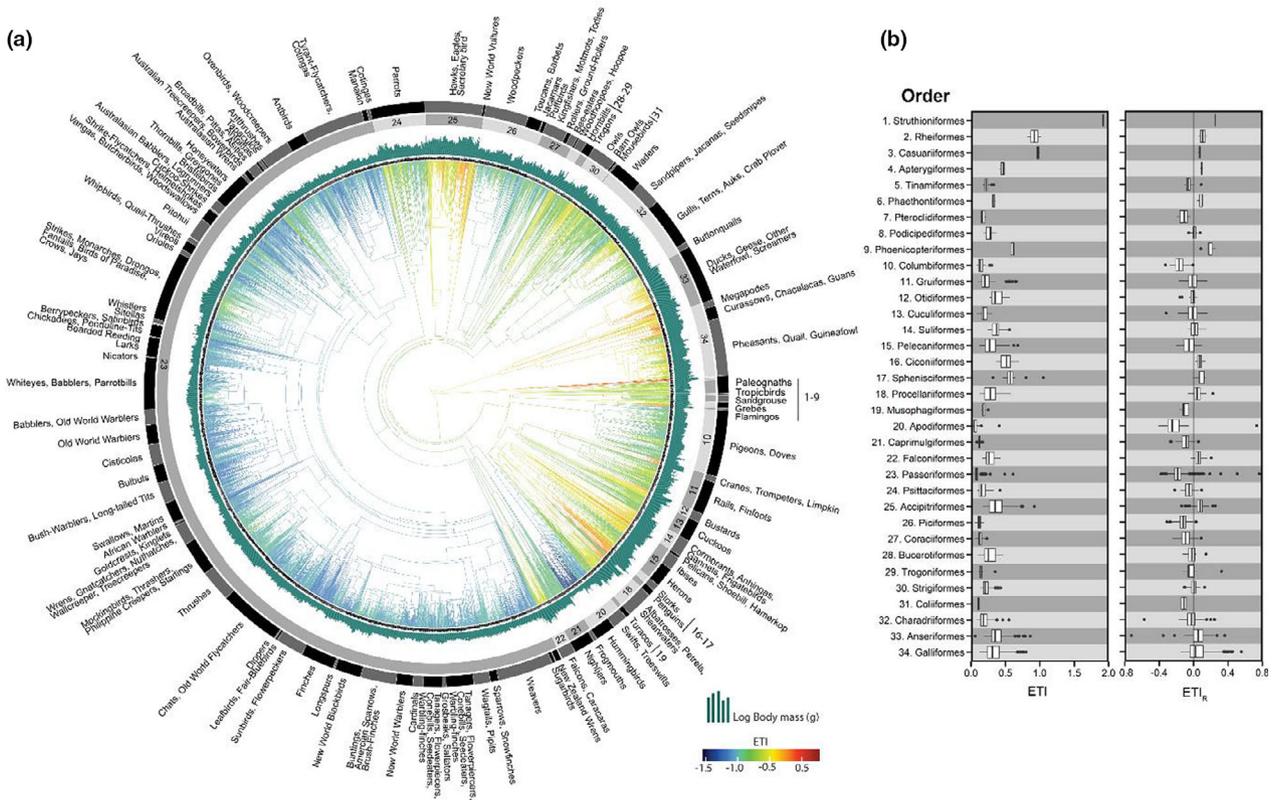
Figure 1. Species-level relationship between $\log_{10}$-transformed eggshell thickness (ETI) and adult body mass across birds. (a) Phylogeny of 4260 extant species showing branches coloured with tip-level diversification in ETI based on ancestral trait estimates. Zoom in to the branch tips to see species labels (online version). Bar plots around the phylogeny represent $\log_{10}$-transformed adult body mass. Numbered clade labels on the inner ring correspond to avian orders listed in (b). The outer ring corresponds to families listed in the tree periphery. (b) Box plots of ETI and their residuals (ETI_R) for each avian order. Outlier species values are shown as filled circles, vertical lines indicate the median, box shows the interquartile range (IQR) and the whiskers are 1.5× IQR. A vertical line is drawn at 0 in the right box plot to denote shell thickness indices that are higher ($ETI_R > 0$) or lower ($ETI_R < 0$) than expected for the species body mass.

Life-history influences ETI among non-Passeriformes

Body mass, mode of development, habitat and temperature seasonality were significant predictors of ETI in non-Passeriformes, and similar patterns were found for ETI_R (Fig. 3). ETI and ETI_R were negatively associated with temperature seasonality among non-Passeriformes (Supporting Information Tables S12–S16). Non-Passeriformes occupying open habitats had significantly higher ETI and ETI_R compared with species occupying semi-open and dense habitats. Altricial species also had significantly lower ETI and ETI_R than precocial species.

DISCUSSION

Our results show that mass-independent eggshell thickness, referred to here as ETI_R, is predominantly

influenced by phylogeny, mode of development, habitat and diet. Furthermore, variation in ETI_R among Passeriformes was influenced by diet, migration and HWI, whereas in non-Passeriformes, ETI_R was influenced by mode of development, habitat and temperature seasonality.

Effect of precocity on ETI across modern birds

The observed increase in ETI with development mode (precocity) across all birds combined and among non-passerines as a separate group may be related to differences in their respective rate of embryo development and stage of development upon hatching. Thicker eggshells probably enable higher rates of calcium removal for embryo growth (Karlsson & Lilja 2008), contributing to the development of large brains, mature muscles and highly
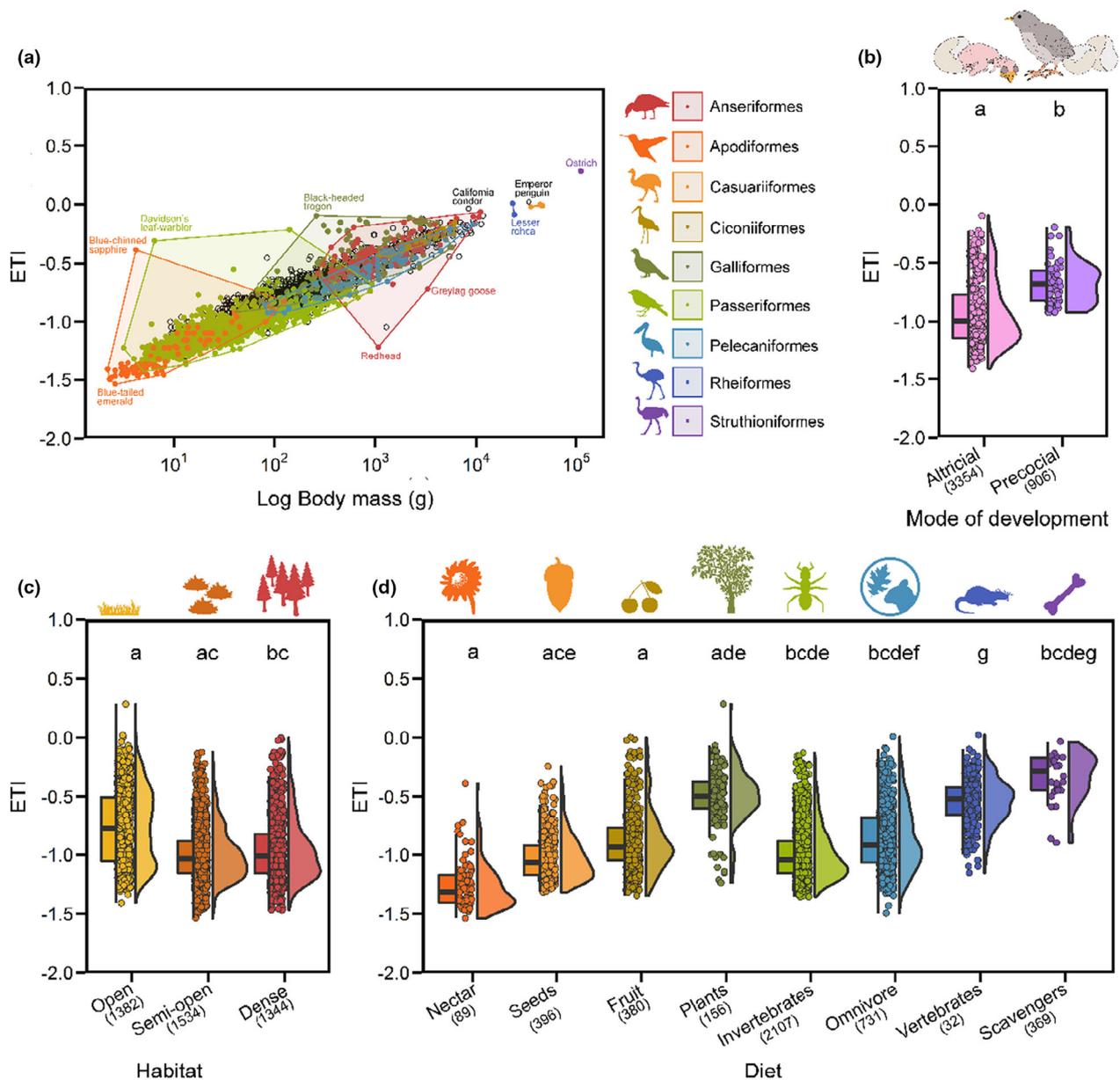
Figure 2. Significant predictors of $\log_{10}$-transformed eggshell thickness (ETI) across all birds. ETI plotted as a function of (a) body mass, (b) mode of development, (c) habitat type and (d) diet. In each graph, dots (one per species) ($n = 2277$) show the distribution of ETI. (a) Partitioning of variation of ETI among key avian orders using different colours and convex polygons. (b–d) In the hybrid box plots, species mean ETIs ($n = 4260$) are shown as filled circles, vertical line indicates the median, box shows the interquartile range (IQR) and the whiskers are $1.5\times$ IQR, and their distribution is shown as histograms. Sample sizes for each category are in parentheses. Different letters indicate a significant difference among levels of precipitation ($P < 0.05$). These visualizations do not take phylogeny into account. Artwork from phylopic and other sources under Public domain licences (see Supporting Information Appendix S1 for detailed credits).

ossified skeletons in precocial hatchlings, whereas altricial hatchlings have small brains, undeveloped muscles and poorly ossified skeletons (Blom & Lilja 2004, 2005). This is supported by Bond

et al. (1988), who found that precocial species experience greater eggshell thinning than altricial species, suggesting a higher calcium requirement for precocial embryos (but see Williams *et al.* 1982,
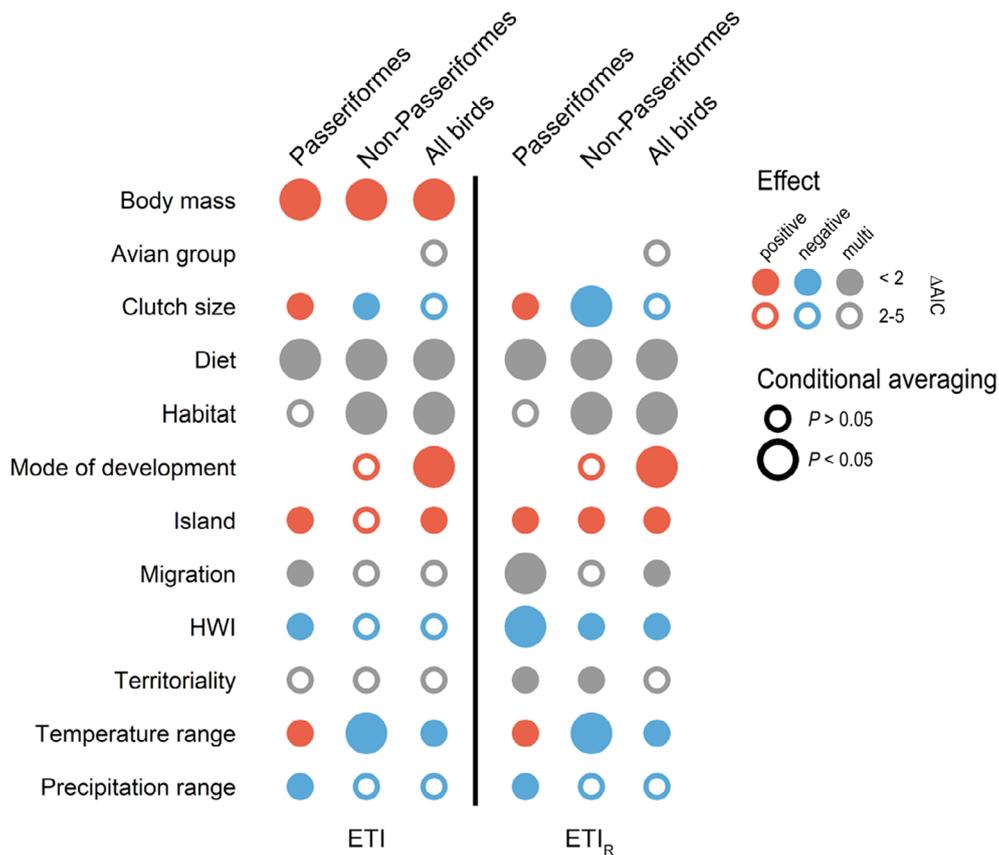
Figure 3. Predictors of $\log_{10}$-transformed eggshell thickness (ETI) and their residuals (ETI$_R$) for phylogenetically controlled comparative analyses. Passeriformes and non-Passeriformes were analysed together (labelled as 'all birds') and separately. Unfilled circles indicate factors that were not present in the top ranked model set. Red and blue points indicate predictors with positive and negative effects, respectively. Predictors with grey points (e.g. diet) represent categorical variables with >2 ('multi') levels or continuous variables with positive and negative effects in models. ΔAIC$_c$ values indicate the change in model support when the focal predictor was dropped from conditional averaging, with ΔAIC$_c$ < 2 values indicating greater support for the importance of a predictor. Gaps indicate factors where there were too few samples or that were non-applicable, and were therefore not included in the multi-predictor model. HWI = hand wing index.

Orłowski & Hałupka 2015). An additional benefit of a thicker eggshell, at least for the initial portion of incubation and development, would be water conservation. Egg water loss, through the conductance of water through the shell, is largely determined by eggshell thickness (Ar *et al.* 1974). A greater ETI for precocial species at the onset of incubation would work to ensure sufficient water stores are available at the latter stages of development, with conductance potentially increasing in conjunction with the eggshell thinning, as the embryo gets larger. In the eggs of Common Quails *Coturnix coturnix* and Greylag Geese *Anser anser*, both precocial species, metabolic rate increased during the first 75% of incubation but then remained relatively constant until shortly before hatching (Vleck

et al. 1979), possibly coinciding with a stabilization of the eggshell thinning.

A closer examination of changes in the eggshell membrane, which is attached to the inner layer of the shell, may help verify distinctions in shell thinning across developmental modes. We categorized species as either altricial or precocial but, in reality, differences in developmental modes occur across the 'altricial–precocial' spectrum (Scheiber *et al.* 2017). Our interpretation, therefore, possibly oversimplifies the effects of developmental mode on ETI. Species may increase membrane thickness during development to provide more protection towards the end of incubation (Castilla *et al.* 2010). This has been reported in Falcons and Partridges and is accomplished via separation of

the membrane from the shell once the mammillary layer (inner shell) is eroded. Hatched eggs of altricial species show little disruption of their mammillae, so much of the shell membrane remains in place (Schmidt 1965). This would suggest that the thickness of the membrane and shell is less prone to change in altricial species than in precocial species. If more of the shell is eroded in precocial species, there could be adaptive differences in the amount of calcium in the shell and hence differences in ETI_R. Thicker shells may also increase mechanical restriction of the embryo (Walsberg & Schmidt 1992), atherefore precocial young may benefit from greater shell thinning to better manoeuvre inside the egg as they grow.

Effect of diet on ETI across modern birds

We demonstrate that diet is a strong predictor of ETI and ETI_R across birds, and in Passeriformes. This form–function link can probably be attributed to distinctions in calcium availability between different food resources. Birds require at least 10–15 times more calcium for eggshell formation compared with egg-laying monotremes and reptiles (Simkiss 1967). This calcium is primarily derived from food consumed by the female during egg formation and laying (Graveland 2008), as the amount of calcium in a typical clutch can exceed that present in the female's whole skeleton (Perrins & Birkhead 1983). Females with high calcium content diets usually produce thicker shells and lay larger eggs and clutches (Tilgar *et al.* 1999, Patten 2007). Although the typical diet of most small birds (insects, seeds and fruit) contains insufficient calcium for eggshell formation or skeletal growth (Simkiss 1967), many birds will compensate for this by supplementing their diet with calcium-rich sources prior to laying (Graveland 2008). When considering their typical diet, we find that nectar, seed and fruit specialists have exceptionally low ETI_R compared with scavengers and vertebrate and plant specialists. Most adult female birds are osteoporotic due to the depletion of medullary bone reserves for eggshell formation, and this is most extreme among individuals with calcium-deficient diets (Reynolds & Perrins 2010). Overall, this suggests that eggshells of typically calcium-poor species (except plant consumers) have thinner eggshells than calcium-rich species. Plant consumers in our study had, on average, 6.7 eggs per clutch, whereas scavengers and vertebrate-consumers typically have smaller clutches (average 1.7 and 2.7 eggs per clutch, respectively). We propose that plant-consumers, particularly those that lay large clutches, will rely more heavily on calcium stores in their skeleton to build eggshells (Simkiss 1967), thereby reducing the need for calcium-rich foods during the laying period. This could be tested by assessing differences in calcium storage, surface area, quantity and activity level of osteoclasts in the mendullary bone (Prondvai & Stein 2014) of herbivorous and carnivorous birds during the egg-laying cycle.

Effect of habitat and temperature seasonality among non-Passeriformes

Non-Passeriformes occupying semi-open and dense habitats had lower ETI_R compared with species in open habitats, permitting greater light transmission through the shell to assist embryonic development in habitats with low light exposure. As light improves embryonic growth and hatchability of avian eggs (Shafey 2004), species nesting in enclosed spaces more commonly have immaculate, white or pale-coloured eggshells to improve light transmission through the shell (Maurer *et al.* 2015). Furthermore, we propose that the heat capacity of thicker eggs is less than that of thinner eggs of the same size; therefore, thinner eggs will warm and cool more quickly (Yang *et al.* 2018). We found that species that nest in open habits have a higher ETI_R than those species nesting in semi-open and/or dense habitats. Previously, it has been demonstrated that the eggs of open habitat nesting species have a lower conductance (rate of water loss) under standard conditions, thought to aid in conserving water and reducing water loss, in an environment which is exposed to the wind and sun (Board & Scott 1980, Portugal *et al.* 2014). As eggshell thickness is a major determinant of conductance and associated water loss (Ar *et al.* 1974), a thicker ETI_R will probably aid in water conservation for those species nesting in open habitats.

In contrast to our expectations, we found that non-Passeriformes had lower ETI_R in regions of greater temperature seasonality. Initially, we had predicted that species in more seasonal climates would have higher ETI_R to reduce heat loss at lower temperatures (De Frenne *et al.* 2013). Functionally, eggs are more prone to heat loss at low

ambient temperatures unless regulated by the incubating bird (Turner 2002), whereas eggs laid in warmer and more humid environments are more prone to microbial infection (Cook *et al.* 2005). Thinner eggshells have been consistently reported in bird populations breeding at high temperatures (Warren & Schnepel 1940, Heywang 1946), potentially in response to thermally stressed environments (Oguntunji & Alabi 2010). Some birds cannot regulate their own body temperature if it gets too hot (Heywang 1938), causing disruption of physiological processes involved in egg production (Huston *et al.* 1957). For example, the amount of calcium circulating in the bloodstream of birds decreases at higher temperatures, reducing calcium availability for egg production (Conrad 1939), but recovers slowly when ambient temperature is lowered (Warren & Schnepel 1940). Hens exposed to a 24-h linear temperature cycle ranging from 26.7 °C to 35 °C had thinner and mechanically weaker eggshells than hens exposed to 21.1–35 °C and 15.6–35 °C linear cycles; therefore, lowering the temperature during the cool portion of a 24-h cycle positively influenced shell quality (Deaton *et al.* 1981). Warran *et al.* (1950) found that birds housed at 18 °C produced thinner shells in winter than birds in uncontrolled conditions. Species evolving in areas with substantial temperature variation have broader thermal tolerances than species living in areas with small temperature variation (Sheldon & Tewksbury 2014). Multiple aspects of animal physiology, ecology and behaviour are influenced by temperature seasonality, and it seems likely that parents assume a greater role in moderating egg temperature in highly seasonal environments, given their thinner eggshells.

Effects of hand-wing index, diet and migration among Passeriformes

Passeriformes with a higher HWI had low ETI_R, whereas migratory Passeriformes had high ETI_R. High HWI is common among highly dispersive species and those that specialize in foraging during flight (Sheard *et al.* 2020). The effects of flight capability on ETI_R appear to vary with taxonomic group. Legendre and Clarke (2021) identified flight as a limiting factor on ETI_R in modern birds and dinosaurs; yet, unlike our findings, flying birds included in their study had a higher ETI_R. The earliest theropod dinosaurs to have acquired powered flight laid smaller eggs with absolutely thinner, but relatively thicker eggshells. In our study, we propose that Passeriformes that forage in flight are more likely to consume high-calcium resources, so would be expected to have a high HWI and ETI_R. Species with a high HWI also tend to have more elliptical or asymmetric eggs, which may reflect the demands of having streamlined bodies (Stoddard *et al.* 2017). Shells of elongated and asymmetric eggs are usually thicker at the equator and thinner at the poles (Peterson *et al.* 2020), which might compensate for having a higher surface-to-volume ratio compared with rounder eggs (Hoyt 1976).

Drent and Woldendorp (1989) found that resident birds breeding in regions with calcium-limited sandy soils laid eggs with no shells or such thin shells that embryos died from desiccation, whereas eggshells of migratory birds breeding in the same area were unaffected. This suggests that migratory birds have more opportunity to find calcium-rich foods and thus have high ETI_R compared with non-migratory Passeriformes. Therefore, it appears that avian dispersal promotes the evolution of ETI_R in Passeriformes.

Within a rising need to understand how avian hatchability may be impacted by environmental change, we can improve predictions by identifying selective pressures driving vital eggshell traits. Previous studies have shown temperature-related effects on eggshell thickness (Warren *et al.* 1950, Deaton *et al.* 1981), and the incorporation of life-history evolution is urgently needed to understand egg resilience under changing climate.

AUTHOR CONTRIBUTIONS

Marie Attard: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); software (lead); validation (lead); visualization (lead); writing –

original draft (lead); writing – review and editing (equal). **Steven Portugal:** Conceptualization (equal); data curation (supporting); funding acquisition (lead); investigation (supporting); methodology (equal); project administration (equal); resources (lead); supervision (lead); validation (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal).

FUNDING

ETHICAL NOTE

None.

CONFLICT OF INTEREST

The authors declare no competing or financial interests.

Data Availability Statement

The data, code and data sources are available from the Figshare Digital Repository (doi: 10.6084/m9.figshare.14763147).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1 Silhoutte credits.

Figure S1. Correlation matrix heat map for numerical predictors considered for PGLS and MCMCglmm analytical models.

Figure S2. Phylogenetic regression between $\log_{10}$-transformed body mass and eggshell thickness (ETI). The phylogenetic regression was used (a) to take into account allometric effects on ETI and (b) to obtain a relative measure based on the residuals (ETI_R) across all birds.

Table S1. Sample size of species in each categorical predictor included in phylogenetic comparative analysis.

Table S2. List of putative predictions for a series of possible explanations for variation in eggshell thickness in birds that were excluded from phylogenetic comparative analysis due to high collinearity between predictors.

Table S3. Variance inflation factor of final predictors used in PGLS across all birds ($n = 4260$) for ETI. All numerical predictors except annual temperature were $\log_{10}$-transformed to reduce skewed distributions.

Table S4. Variance inflation factor of final predictors used in PGLS across all birds ($n = 4260$) for residual shell thickness (ETI_R). All numerical predictors except annual temperature were $\log_{10}$-transformed to reduce skewed distributions (see main text for details).

Tables S5–S16. Model outputs from PGLS conditional averaging.