Viewpoint

Evolution of avian egg shape: underlying mechanisms and the importance of taxonomic scale

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Diversity in the shapes of avian eggs has intrigued biologists for centuries, and recent studies at a range of taxonomic scales suggest that egg shape can be a powerful lens through which to view morphological adaptation. At a broad taxonomic level, we previously examined egg shape in 1400 species (Stoddard *et al.* 2017). Our study, which contained a detailed analysis of the egg shape morphospace and a new biophysical model of egg shape formation, included a broad-scale phylogenetic comparative analysis of egg shape across more than 1200 species representing 34 orders and 143 families. We found that, at this global scale, most variation in egg shape is correlated with phylogenetic history, an adult bird's body mass and egg size. We also found a significant correlation between egg shape and hand-wing index, a measure of wing shape that provides a general estimate of flight ability, prompting us to consider the

possibility that adaptations for flight might be important drivers of egg shape variation.

In our earlier paper (Stoddard *et al.* 2017), we encouraged additional work on egg shape variation within specific avian lineages, stating that the global patterns we observed across more than 1200 species do not apply equally to all smaller clades. It is exciting to see recent studies in this area, which have explored egg shape with respect to incubation behaviour (Deeming & Mayr 2018, Birkhead *et al.* 2019), composition (eggshell, yolk and albumen; Deeming 2018), strength and stability (Birkhead *et al.* 2017a, Hays & Hauber 2018, Birkhead *et al.* 2018), protection from contamination by soil and feces (Birkhead *et al.* 2017b), morphology and locomotion (Anten-Houston *et al.* 2017, Shatkovska *et al.* 2018), diet (Bańbura *et al.* 2018) and climate conditions (Duursma *et al.* 2018) in a number of avian families. In a new study in *Ibis*, Birkhead *et al.* (2019) investigated egg shape in 30 species belonging to two avian families – the alcids (Alcidae) and penguins (Spheniscidae). They found that egg size and factors related to incubation, including chick developmental mode, clutch size and incubation site, are correlated with egg shape in these two groups. They present these findings as an alternative to some of the results we reported in Stoddard *et al.* (2017), suggesting that 'selection acting during incubation may influence egg-shape variation across birds as a whole'.

Contrary to Birkhead *et al.* (2019), who argue that their new findings regarding incubation provide an opposing explanation for egg shape variation in birds, we see no conflict between the results of Birkhead *et al.* (2019) and Stoddard *et al.* (2017), which were performed at different taxonomic scales. Here, we expand on this point and emphasize the complementary – rather than contradictory – nature of our joint findings. First, we wish to clarify that we did include factors related to incubation in our global analysis (Stoddard *et al.* 2017). We tested hypotheses related to clutch size, nest location, nest type and chick developmental mode. We did not find any of these factors to be a significant predictor of egg shape variation at a global scale across the more than 1200 species for which data were available. To conduct our analysis, we searched the literature for hypotheses related to the function of egg shape and used this to guide our collection of extensive biometric, life history and environmental data for species in our sample, including adult body mass, egg length, diet, clutch size, nest type, nest location, chick developmental mode, latitude, temperature, precipitation and hand-wing index. All of these factors were included in our comparative models, which used backbone phylogenies based on Jetz *et al.* (2012) and Prum *et al.* (2015). Controlling for differences in phylogenetic relatedness, only adult body mass, egg size and hand-wing index were significantly correlated with egg shape at the global scale in

both of the examined tree topologies. Therefore, at the global scale, we concluded that hand-wing index was a predictor of egg shape variation, whereas factors related to diet, incubation and the environment were not.

Birkhead *et al.* (2019) point out that 'as powerful as comparative studies can be, broad-based analyses like that of Stoddard *et al.* (2017) can mask effects that differ from taxon to taxon'. We certainly agree with this, which is why in our study we also investigated the extent to which global patterns of egg shape variation applied to smaller taxonomic groups: seabirds, shorebirds (order: Charadriiformes) and passerines (order: Passeriformes). We found that hand-wing index was not a significant predictor of egg shape variation in seabirds and shorebirds, and we highlighted the fact that 'life history traits may have a substantial secondary influence on egg-shape evolution on smaller taxonomic scales' (Stoddard *et al.* 2017). From this perspective, we fully expect – and indeed have demonstrated in some groups – that the factors correlated with egg shape variation across a large sample of families do not always translate to individual families. This is the beauty and challenge of comparative biology: it can reveal generalizations across a taxonomically diverse group, but it can also blur the details of smaller clades. Consequently, we might reject the hypothesis that clutch size and nest type are significant drivers of egg shape variation at the global scale, but this does not mean that these factors are unimportant for cliff-nesting alcids or penguins. Therefore, our results (Stoddard *et al.* 2017) at the global scale do not necessarily contradict the more taxonomically focused analyses of Birkhead *et al.* (2019) but rather suggest that different rules emerge at different taxonomic scales.

Just as we cannot generalize trends at the global scale to all subgroups, we should not assume that the factors explaining variation in particular subgroups will scale up. Birkhead *et al.* (2019) have focused on egg shape in two extreme seabird families. Alcids, which include cliff-nesting guillemots (*Uria* spp.) and razorbills (*Alca torda*), lay famously pyriform eggs – among the most asymmetric of all bird eggs (see Stoddard *et al.* 2017 fig. 1) – particularly in species nesting on rocky cliff-ledges. Penguins are flightless birds that incubate their eggs in shallow cup-nests, in crevices or burrows, or directly on their feet. Birkhead *et al.* (2019) emphasize the importance of incubation site and the role of the incubating parent's posture as selection pressures acting on egg shape. Certainly, these factors may be influential for birds that must position the egg in a way that reduces the chance of it rolling away, as is the case for some penguins and alcids. However, for the vast majority of bird species, eggs are not at great risk of rolling away because they are contained in deep burrows, cavities or cup-shaped nests. We acknowledge that there are many advantages to exploring egg shape variation in clades with extreme eggs, and the alcids are among the best

studied in this respect. However, alcids as a group are unlikely to reflect broader patterns of egg shape variation across all birds, particularly because unprotected cliff-ledge and bare-rock nests are extremely rare or absent in most avian families.

Birkhead *et al.* (2019) note that hand-wing index only explained about 4% of the total variance in egg shape in our comparative models across ~1200 species. This is true, because most of the variance in egg shape was explained by phylogenetic relatedness, adult body size and egg size. As we described above, however, hand-wing index was the only significant predictor of egg shape variation – across two different phylogenetic topologies – after controlling for phylogeny, body size and egg size. Birkhead *et al.* (2019) report a large proportion of variance explained by a single factor, showing that more than 60% of the variation in egg shape across alcids and penguins is explained by incubation site. However, this result is not especially surprising because it is based on an analysis that included only incubation site as a predictor, whereas a separate analysis revealed that egg volume and 'taxon' (alcids vs. penguins) also explained a large proportion (41–76%) of variation in egg shape parameters. Even in these two clades, in which experimental evidence suggests that incubation site might exert selection pressure on egg morphology in some species (e.g. Birkhead *et al.* 2018), there appears to be no significant relationship between egg shape and incubation site after controlling for egg volume and shared phylogenetic history (Birkhead *et al.* 2019, supporting information).

Birkhead *et al.* (2019) suggest that the correlation we (Stoddard *et al.* 2017) found between hand-wing index and egg shape is difficult to interpret because the effect size of hand-wing index is small. However, our P-values for the correlations between hand-wing index and egg shape fall below the widely accepted threshold of $\alpha = 0.05$ and below the stricter threshold of $\alpha = 0.005$ (Benjamin 2018). Additionally, significant predictors in broad-scale comparative analyses routinely explain a relatively small amount of overall variation in dependent variables. For example, the major findings of recent comparative studies of avian cooperative breeding (Cornwallis *et al.* 2010) and plumage evolution (Dale *et al.* 2015) are based on significant predictors with similarly limited explanatory power. The increased power of a particular explanatory variable in models conducted at smaller taxonomic scales does not refute the findings of broadly sampled comparative analyses. On the contrary, increased power with reduced sampling can be expected because of the simplified set of selective mechanisms playing out across a narrower subset of evolutionary history (Graham *et al.* 2018).

An additional point of clarification concerns the mechanism by which hand-wing index may be linked to the shape of eggs. Birkhead *et al.* (2019) state that we

'did not offer any convincingly plausible mechanism' for a possible association between flight and egg shape. From this, readers might conclude that we included hand-wing index in our analyses without a clear *a priori* hypothesis, which was not the case. When we searched the literature, we found that most hypotheses about egg shape were related to clutch size, diet, nest characteristics and chick developmental mode. However, an often overlooked hypothesis suggested that 'reduced abdominal space typical of birds (presumably an adaptation for flight, as is the habit of carrying only a single shelled egg at a time), may therefore be the most important determinant of egg-shape in birds' (Iverson & Ewert 1991).

To test this idea, we included hand-wing index, a standard proxy for several aspects of avian flight performance (Claramunt *et al.* 2012, Pigot & Tobias 2015, Kennedy *et al.* 2016), in our comparative models. When hand-wing index emerged as a significant predictor of egg shape, we proposed several ways in which adaptations for flight might influence a bird's body morphology, which could in turn affect egg shape. We did not suggest that a female's flight behaviour during egg formation alters egg shape directly. Rather, we hypothesized that general adaptations for strong flight selected for a constrained, streamlined body plan, which could influence egg shape. We fully acknowledged that 'the precise physiological mechanisms by which morphological adaptations for flight might affect egg shape are unknown', and we highlighted the need for further research exploring whether hand-wing index is correlated with other anatomical features, such as pelvic width. Pelvic shape is correlated with egg shape (Rensch 1947, Warham 1990), and recent work indicates that pelvic shape is also related to some locomotion styles in birds (Anten-Houston *et al.* 2017). Thus, while we agree with Birkhead *et al.* (2019) that more work is needed to determine whether hand-wing index is correlated with body shape, reproductive organ size and additional aspects of flight behaviour, we believe the underlying hypothesis that streamlined bodies adapted for flight are associated with asymmetric or elongated eggs is clearly plausible.

To understand the drivers of egg shape variation, it is also important to consider how best to quantify egg shape from 2D photographs. In a recent study, Biggins *et al.* (2018) presented a comprehensive analysis and comparison of egg shape metrics. They showed that a four-parameter model proposed by Preston (1953) provides a better fit to egg shapes than other methods, particularly for highly pyriform eggs. They also showed that, for eggs that are not highly pyriform, two broad sets of indices (corresponding to pointedness/polar asymmetry and elongation) can be sufficient to provide a general description of egg shape (in addition to a third measure, 'bicone', that is less directly related to the principal features of egg shape). How should researchers

proceed? The Preston (1953) method offers clear advantages over alternative metrics because its four parameters can be used to describe all egg shapes well. However, other methods – including the simpler two-parameter model we used (Stoddard *et al.* 2017) – probably capture much of the variation in egg shape expressed by two broad sets of indices (mentioned above) discussed by Biggins *et al.* (2018). For example, for the 49 175 eggs in our dataset (Stoddard *et al.* 2017), our measures of asymmetry and ellipticity are highly correlated with two measures proposed by Biggins *et al.* (2018), pointedness ($R^2 = 0.87$) and elongation ($R^2 = 0.97$), respectively. Finally, Biggins *et al.* (2018) highlighted the importance of photographing eggs in a horizontal position, to reduce errors in estimating egg shape that arise when eggs are in a resting position (with the pointed end of the egg facing downward). In the future, museums interested in digitizing their egg collections should heed this advice, but an analysis by Biggins *et al.* (2018) suggests that in most cases the errors introduced by estimating shape from resting eggs are likely to be small. For 193 eggs (of various species) photographed in the horizontal and resting positions (Biggins *et al.* 2018, supporting information), the average percentage errors in estimating pointedness and elongation from resting images appear to be relatively minor ($< 1.5\%$ for both measures, based on our calculations derived from their supporting information).

In summary, egg shape is a complex phenotype that is tugged in multiple directions by various selective forces. Which selective forces are the most salient is likely to differ at different phylogenetic scales (Graham *et al.* 2018). Investigations into the drivers of egg shape variation must therefore address a range of scales, from smaller clades (order, family, genus, species) to the broad (class) level. The results presented by Birkhead *et al.* (2019) in penguins and alcids need not challenge those of our broad-scale study (Stoddard *et al.* 2017). Instead, our findings are generally compatible. To obtain a richer picture of the adaptive function of egg shape, it will be vital to continue similarly detailed investigations in diverse avian (and non-avian) lineages, at a range of taxonomic scales. Understanding the function of egg shape will also require a deeper mechanistic appreciation of egg shape formation in the oviduct. To this end, a critical part of our study (Stoddard *et al.* 2017) involved a detailed description of the egg shape morphospace, based on a quantitative analysis of nearly 50 000 eggs, and the development of a new biophysical model of egg shape. We showed that by adjusting two parameters – variation in the material properties of the eggshell stretchy membrane, and variation in pressure across the membrane – we could simulate shapes that span the egg shape morphospace. Uncovering the details of egg formation is likely to lead to new insights regarding the

function and evolution of egg shape, and we encourage future integrative work in this area.

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