

Within-individual consistency and among-individual
variation in shapes of tree swallow (*Tachycineta*
bicolor) eggs

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Bird eggs come in a wide variety of shapes and recent large-scale studies across species have renewed interest in understanding the factors that generate and maintain this diversity. However, these advances focus on species average egg shapes and do not take into account that shape varies dramatically within species: among populations, among individuals, and between eggs produced by the same individual. We measured the shape and size of 1382 eggs produced by 205 tree swallows (*Tachycineta bicolor*) in 259 nests with 45 females having eggs measured in two or three years. We found that intra-specific variation in the asymmetry and ellipticity of tree swallow eggs spanned the range of typical egg shapes from 69% of species reported in a recent study of 1400 species. Variation in shape and size was largely explained by individual identity, as the repeatability of egg shape and size was remarkably high both for eggs produced within a clutch and for clutches produced in successive years by the same female. All characteristics of mother and daughter eggs were positively correlated, although with a sample size of only 15 pairs these relationships were not significant. Female mass and structural size were correlated with the size of eggs produced, but not with their shape. Older females produced eggs that were larger, more asymmetrical, and less elliptical; this pattern was driven by a combination of both longitudinal changes in egg characteristics as females aged and by differential overwinter survival of females with particular egg characteristics. We found no evidence that any aspect of shape or size that we measured was related to nestling measurements or fate. Finally, we found that the patterns of covariation in aspects of egg size and shape among individuals differed from that observed within individuals, suggesting that the mechanisms driving egg shape variation might differ across these levels. A complete understanding of avian egg shapes will need to incorporate variation at multiple scales and we discuss the interpretation of our results in light of recent large-scale comparative studies that focus only on mean species egg shapes.

Keywords: egg shape; egg size, among individual variation, scale-dependence

LAY SUMMARY

- Birds' eggs vary enormously in their shape and size and ornithologists have long been interested in understanding this variation.
- Recent studies have renewed interest in this field and shed light on why egg shape varies among species, but they generally overlook the fact that eggs also vary enormously within species or even within individuals over the course of their lifetime.
- We found that the asymmetry and ellipticity of eggs produced by different individual tree swallows resulted in eggs that spanned most of the shapes seen across 1400 species in a recent comparative study.
- This variation mostly resulted from differences among individual tree swallows, because shape and size of eggs produced by the same female were repeatable.
- We discuss the ways that egg shape might vary across different scales and what our findings mean for interpreting recent studies that compare different species.

INTRODUCTION

Birds vary enormously in the shape and size of the eggs they produce and explaining this remarkable variation has been a longstanding goal of ornithologists (e.g., Thompson 1908, Preston 1969). Recently, advances in the ease of making accurate measurements of egg shape and the ability to conduct large-scale comparative analyses have led to renewed efforts to understand what factors drive variation in egg shape between species (Stoddard et al. 2017, Biggins et al. 2018, Shatkovska et al. 2018, Montgomerie et al. 2021). To date, few studies have used these tools to examine variation in shape among populations, among individuals, or within individuals of the same species (but see Adamou et al. 2018, Bañbura et al. 2018).

Among species, there is general agreement that differences in life history demands and morphology contribute to egg shape variation, though the details of these patterns and their generality across different taxonomic scales is debated (Stoddard et al. 2017, 2019; Birkhead et al. 2019, Montgomerie et al. 2021). At the broadest level, much of the variation in egg shape can be explained by phylogenetic history and relative egg size (Stoddard et al. 2017, Montgomerie et al. 2021). After accounting for these differences, wing morphology explains additional variation in egg shape (Stoddard et al. 2017), possibly due to its association with selection on body shape and mode of locomotion (Montgomerie et al. 2021). Montgomerie et al. (2021) argue that different aspects of shape are driven by different selection pressures, with elongation being largely associated with anatomical constraints while asymmetry is an adaptation to conditions during the incubation period. More targeted studies on narrower taxonomic scales have also identified other correlates of egg shape, including pelvis shape (Shatkovska et al. 2018), climate conditions (Duursma et al. 2018), incubation behavior and chick developmental mode (Birkhead et al. 2019), nest characteristics (Nagy et al. 2019), and egg composition (Deeming 2018), among others, but the underlying mechanisms generating these correlations are not known.

Whereas among species variation has received considerable recent research attention, there is also widespread recognition that egg shape varies within species (Adamou et al. 2018, Bañbura et al. 2018). The causes of this variation are less clear, both because few studies measure the shape of many eggs from known individuals over multiple breeding attempts and because it is often unclear how or if explanations for egg shape variation based on among-species life history differences should apply within species or among populations. In many cases, the interspecific correlates of egg shape variation may not be relevant for understanding intraspecific variation. For example, Stoddard et al. (2017) found that aspects of wing morphology are associated with inter-specific variation, yet this variable is unlikely to differ much among individuals within a population (though it might among populations of a species). Similarly, Birkhead et al. (2018) argue that common murre (*Uria aalge*) eggs are shaped to provide stability on sloping ledges, but this mechanism cannot explain the maintenance of variation among individuals in a species that are all selected for nesting on a similar type of substrate.

If there is an optimal egg shape for different species (Andersson 1978, Barta and Székely 1997) based on differences in life history traits (Stoddard et al. 2017, e.g., Montgomerie et al. 2021), then what mechanism maintains the substantial variation in egg shape within species

where life history and ecology are relatively similar across individuals? Reconciling explanations for variation in egg shape among species with the apparently large variation in egg shape that exists within at least some species requires a careful consideration of the scale over which mechanisms operate. A recent debate has focused on the general usefulness of comparing egg shape across all species of birds versus within smaller taxonomic groups that have more similar life histories (Birkhead et al. 2019, Stoddard et al. 2019). Montgomerie et al. (2021) show that relationships at the family level can differ in both strength and direction, such that broad comparisons may be subject to Simpson’s paradox. This phenomenon occurs when relationships within sub groups disappear or reverse after groups are combined (Simpson 1951). Despite this recognition that scale is an important consideration for understanding inferences about egg shape, heterogeneity in egg shape among populations or individuals is not considered in recent comparative studies.

Because phenotypic variation is hierarchically organized (Westneat et al. 2015), the same principle of scale-specific inference can be applied to understanding variation in egg shape among populations of a species, individuals within a population, or repeated production of eggs within an individual female. Inferences derived from higher levels can always be subject to Simpson’s paradox or ecological fallacies. Recent comparative studies largely ignore the possibility of different drivers of egg shape variation at these levels by measuring only a small number of eggs per species and by conducting analyses on only a single average value for each species (e.g., Stoddard et al. 2017 and Montgomerie et al. 2021 measured a median of 8 and 3 eggs per species, respectively). There is no guarantee that relationships observed at broader levels (e.g., family level or across all birds) will explain variation among populations or individuals of the same species. A complete understanding of egg shape variation will require a scale-dependent framework (Agrawal 2020) that makes predictions that explicitly consider the scale, based on an understanding of the mechanism(s) involved. Analyses at these different levels are complementary, rather than contradictory (Stoddard et al. 2019), but at present, very few studies provide even descriptive data on correlates of variation in egg shape within species or individuals (but see Adamou et al. 2018, Bañbura et al. 2018).

In contrast to egg shape, many studies have explored among and within individual variation in egg size, mass, or composition as it relates to maternal effects and nestling outcomes (reviewed in Christians 2002, Krist 2011, Groothuis et al. 2019). Christians (2002) comprehensively reviewed studies of individual variation in egg mass and found that the largest egg in a population is typically 1.5 to 2 times larger than the smallest egg. This enormous variation in egg size tends to be both highly repeatable (generally repeatability above $r = 0.60$) and heritable (generally $h^2 > 0.50$, Christians 2002). Some individual studies demonstrate that egg size changes with dietary supplementation (Hogstedt 1981, Ramsay and Houston 1997), ambient temperature (Nager and Van Noordwijk 1992, Whittingham et al. 2007), and female age (Croxall et al. 1992, Hipfner et al. 1997), but the results of these studies are decidedly mixed and the amount of variation in egg size explained is typically small (no more than 10-15%, Christians 2002). Even female mass and size are inconsistently related to egg size variation in these studies (Christians 2002). While egg mass often predicts early life mass and growth of nestlings, it is less clear whether there are long term fitness consequences of initial egg mass (Potti 1999, Christians 2002). Given these patterns, it is possible that much of the variation in intra-specific egg shape might be explained by

consistent differences in egg mass, but few studies present the data to address this question and the causes of differences in egg mass themselves remain unclear (Christians 2002).

To begin to understand variation in egg shape at the among and within individual level, we studied variation in the shape and size of tree swallow (*Tachycineta bicolor*) eggs over three years. Tree swallows are a migratory cavity nesting species with a distribution spanning most of North America. They typically produce clutches containing 4-7 eggs (Winkler et al. 2020a). While we are not aware of prior studies directly measuring egg shape, a number of previous studies in different populations of tree swallows have documented substantial variation in egg mass (Wiggins 1990, Ardia et al. 2006, Bitton et al. 2006) or yolk characteristics (Whittingham and Schwabl 2002). These differences are sometimes attributed to variation in temperature, food availability, or body condition (Ardia et al. 2006, Whittingham et al. 2007, Pellerin et al. 2016), but also appear to be predominantly attributable to female identity (Wiggins 1990).

We were initially interested in comparing the degree of variation in egg shape within tree swallows to the degree of variation described among species in comparative studies. If there is generally strong selection for an optimal egg shape matching life history traits, then we expected there would be relatively little variation in shape within tree swallows (i.e., ellipticity or asymmetry), even if there was variation in egg size associated with investment (Wiggins 1990, Ardia et al. 2006). Next, our multi-year study allowed us to assess individual repeatability and correlates of egg shape and size both within a clutch and across multiple years. We also took advantage of the fact that some female nestlings returned to breed as adults, which allowed us to compare the shape and size of eggs produced by mothers and their daughters. Finally, we asked whether there was any evidence that variation in egg shape or size was related to nestling morphology or fate. We interpret the correlations that we observed within tree swallows in light of the results of recent comparative studies.

METHODS

We studied tree swallows breeding near Ithaca, New York from April to July of 2019 to 2021. Tree swallows at this site have been studied continuously since 1984 and we followed well established protocols for general monitoring of breeding activity (Vitousek et al. 2018, Winkler et al. 2020b). Briefly, we checked all nest boxes every other day during the breeding season to record the timing of the onset of nest building activity, the initiation and completion of egg laying, the timing of hatching and fledging, and the fate of nestlings. This schedule also allowed us to compile accurate information on clutch size and the number of eggs that hatched at each nest, but we did not have information on the laying order of eggs within a nest.

For this study, we visited each nest during the first week of incubation and photographed eggs to measure size and shape (example photographs in Figure 1). Eggs were placed on a flat black surface and photographed from a height of approximately 14 inches. For these photographs, we placed eggs in a relaxed position rather than attempting to set them perfectly horizontal by propping up one end. Biggins et al. (Biggins et al. 2018) showed that

large and highly pyriform egg measurements differ systematically when photographed in relaxed versus perfectly horizontal posture. However, it is unclear how much this positioning matters for smaller eggs and we planned to compare our measurements to Stoddard et al. (2017), which used relaxed photographs. The potential error in measurements even for large eggs when relaxed is very small relative to between egg shape variation and tree swallow eggs are small enough that the correction would have been nearly impossible to achieve in the field (i.e., we would have needed to adjust one end by $< 1\text{mm}$ in height).

During the years of study, many nests at these sites were included in a variety of experiments focused on manipulating environmental stressors (e.g., Injaian et al. 2021, Taff et al. 2022). However, all of these experimental manipulations began during mid-incubation, after eggs had been laid and pictures had been taken. We focus primarily on pre-treatment female and egg characteristics in this study, but we also include an analysis of nestling growth and fate from a subset of 55 nests that were not subject to any experimental manipulations (see below).

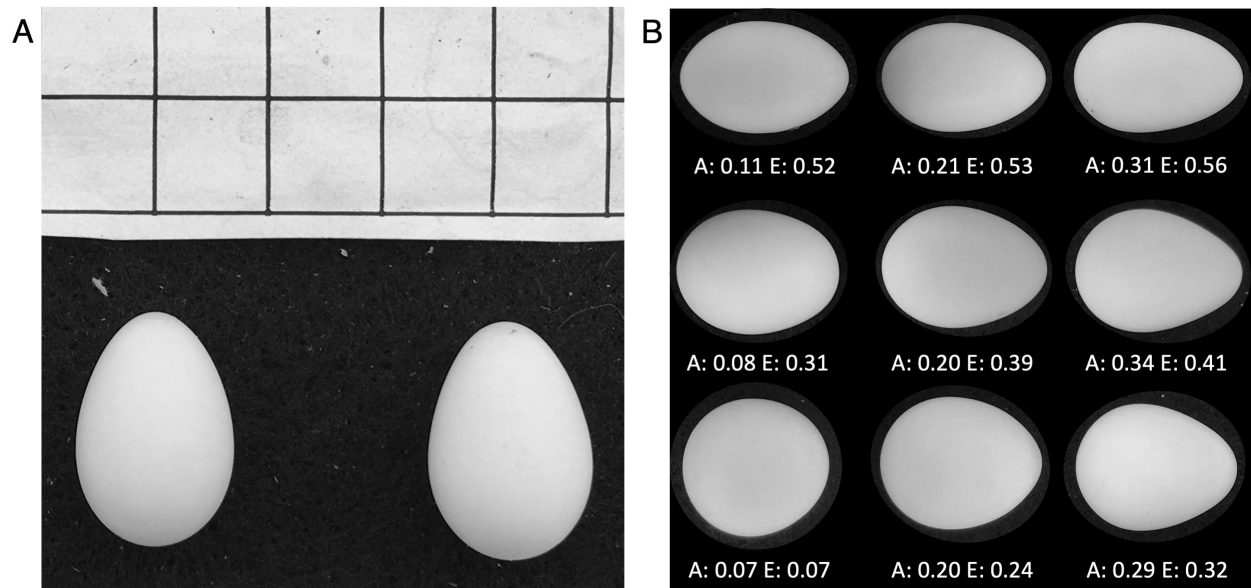


Figure 1: Panel A shows an example of a photograph of two eggs from the same clutch with an included scale grid for measurement. Panel B shows measurements of asymmetry and ellipticity produced from EggxTractor software with a selection of tree swallow eggs from this study. Numbers below each egg indicate the asymmetry and ellipticity. Note that eggs are not shown at scale but are rather shown at a similar size with shape maintained for illustration only.

Adult females were captured on day 6-8 of incubation between 6 and 10 am Eastern Standard Time. At the time of capture, we took a series of three blood samples and a set of standard morphological measurements that included mass, flattened wing length, and the length of the head plus bill (Vitousek et al. 2018). Any females captured for the first time were banded with a USGS aluminum leg band. Depending on the experiment, females were captured again later in incubation or provisioning and males were captured during provisioning, but those captures occurred after experimental manipulations and the data are not included in this paper.

Nestlings were banded and measured on day 12 after hatching. At this time, we measured mass, wing length, head plus bill length, and took a small blood sample. Following a return visit to experimental nests on day 15, we avoided checking nests to prevent forced fledging until day 24. We did a final nest check to determine fledging fate for each nestling. Individuals not in the nest at this point were considered fledged and we recorded the band numbers from any dead nestlings recovered. Given our sampling strategy, we cannot link individual egg characteristics to individual nestling morphology or fates, but we can explore correlations between average egg and nestling characteristics at the nest level. While nestling characteristics and fates were recorded at every nest, we only included analyses of nests that were not subject to any experimental manipulations.

Egg measurements

Using the photographs described above, we characterized the size and shape of eggs from each nest. To measure shape, we followed the approach developed by Baker (2002), which results in measures of the degree of ellipticity (deviation from circularity) and asymmetry (difference in shape of the two egg poles) as described in Stoddard et al. (2017). The measurements were performed using the **EggxTractor** software in MatLab, provided by Stoddard et al. (2017). While there are a variety of other methods to characterize egg shape that differ slightly, our approach performs well for the range of shapes covered by tree swallow eggs (Stoddard et al. 2017) and allowed for a direct comparison with Stoddard et al. (2017).

Biggins et al. (2018) demonstrates that Preston’s method, which describes shape based on elongation, polar asymmetry, and pointedness, is generally slightly more accurate than Baker’s, especially for pyriform eggs. We remeasured a subset of our eggs ($n = 900$) using the script provided in Biggins et al. (Biggins et al. 2018). Our measure of ellipticity was nearly identical to ‘elongation’ ($R^2 = 0.98$). Asymmetry was positively correlated with both ‘polar asymmetry’ ($r = 0.67$) and ‘pointedness’ ($r = 0.72$) and a simple model with both variables explained most of the variation in asymmetry ($R^2 = 0.79$). Given the high similarity between measures and the fact that we were interested in comparing directly with Stoddard et al. (2017), we focus only on the measures derived from **EggxTractor**, but the dataset archived with the paper includes both measurement approaches.

To characterize the size of eggs, we used ImageJ (Schneider et al. 2012). We loaded photographs and set a scale using the scale grid that was included in every image. We then used the straight line segment tool to measure the maximum length from pole to pole (egg length) and the maximum egg width for each egg. For our photographs in the field, the scale bar was located on the same surface that the egg was placed on rather than at the mid-line of the egg. This discrepancy created a small parallax issue when scaling eggs, such that linear distance measurements of the egg width and length were 2.5% longer than true values. We corrected for this scale issue in all of our analyzed measurements. The scale does not have any impact on shape measurements, which are scale independent.

It is important to note that we could not link individual eggs to individual nestlings with our

methods. Thus, our egg measurements include those of eggs that hatched along with those that did not hatch for any reason. Many species occasionally lay ‘runt’ eggs or double-yolked eggs that can differ substantially in size and shape. We did not identify any eggs that clearly fell into these categories, but it is possible that a small number of these eggs or otherwise infertile eggs were included in our measurements.

Data analysis

We initially examined the overall amount of intra-specific variation in egg shape from our study in comparison with the amount of inter-specific variation presented in Stoddard et al. (2017). For this qualitative comparison, we estimated a 90% ellipse for tree swallow egg shapes based on asymmetry and ellipticity values and determined how many of the 1400 species included in Stoddard et al. (2017) had average egg shapes that fell within this ellipse. We used a 90% ellipse because it described typical tree swallow egg shape without being overly influenced by unusual eggs or including areas of morphospace with very few egg observations. We note that Stoddard et al. (2017), specifically excluded pyriform eggs and represents a non-random sampling of all possible species, so this comparison is meant only as a qualitative description of tree swallow egg shape variation relative to intraspecific variation.

Given the substantial variation in egg shape among individual tree swallows, we next asked whether aspects of egg shape or size were repeatable within a female. We used linear mixed models implemented in the **rptr** package in R (Stoffel et al. 2017) to assess repeatability, measured as the intra-class correlation. For each egg characteristic, we fit a model with random effects for nest identity nested within female identity. This allowed us to directly assess the amount of variation in shape explained by a single shared nesting attempt and by female identity. Because eggs laid in a nest occur over a few day period we expect that any immediate environmental conditions that directly impact egg size or shape would be captured by that predictor (e.g., temperature or food availability). Since we only had one nest measurement per female in any given year, the intra-class correlation represents between year repeatability for females. Finally, after calculating repeatability, we used a subset of 15 mother-daughter pairs that both had eggs measured to ask whether a female’s egg characteristics predicted her daughter’s egg characteristics one or more years later. Most tree swallows at our site disperse after their natal year, so the sample size for recruiting nestlings with known mothers was small (Winkler et al. 2005).

We next asked whether individual characteristics of females explained variation in egg shape or size. For these questions, we fit a series of four linear mixed models with egg shape (asymmetry, ellipticity) or size (width, length) as the response variable and with female mass, wing length, head plus bill length, and age as predictors. We included age as a categorical predictor of ‘first-time breeders’ or ‘returning breeders.’ Female tree swallows have delayed plumage maturation and first time breeders can be identified by their brown plumage regardless of prior capture history (Winkler et al. 2020a). Each of these models also included a random effect for nest identity nested within female identity (to account for multiple eggs measured from the same nest). The exact age of returning breeders was not always known, but see below for a subsequent analysis comparing only known age birds. We standardized

all continuous predictors to a mean of 0 and standard deviation of 1 so that effect sizes are directly comparable. Models were fit with the `lme4` package and model diagnostics were examined with the `DHARMA` package in R to ensure appropriate fits (Bates et al. 2015, Hartig 2021).

After finding that female age was related to some egg characteristics, we asked whether this pattern might be best explained by a longitudinal change in egg characteristics as females age or by the selective return of females with particular egg characteristics. We used a subset of 29 females that were measured in multiple years and that were initially observed as first time breeders to ask whether egg characteristics changed longitudinally within females as they aged using paired *t*-tests for each egg characteristic. We used the full set of observations from 2019 and 2020 to ask whether any egg characteristics predicted the likelihood of returning to breed in the following year (we could not include 2021 females in this analysis because survival to the next year was not known). We compared the average egg characteristics in year 1 for birds that did or did not return in year 2 using *t*-tests. Returning the following year is not a perfect proxy for survival because adults may occasionally disperse or go undetected, but in this population previous work using the full historical database has shown that dispersal distance in adulthood is generally very small and detection of returning birds is high (Winkler et al. 2020b).

We analyzed within individual covariation in egg characteristics using a subset of 185 females that had at least 5 eggs with complete measurements. Using this subset, we calculated the pairwise correlation and R^2 value for each pair of egg characteristics for each individual female and examined the distribution and average value of these correlations across all females. We qualitatively compared the pattern of covariation within females to that observed when using average egg characteristics among females. We examined clutch size in a set of linear mixed models including only nests that had at least 4 eggs to test whether egg size or shape were associated with clutch size.

To ask whether any aspects of egg characteristics were associated with nestling characteristics, we used a subset of 55 nests that were not part of any experiment. For these nests, we calculated the average egg shape and size and fit simple linear models with average nestling morphology (mass, wing, head plus bill) as response variables and with egg shape and size as predictors. For nestling fate, we fit a single model with the number of nestlings that fledged and the number that did not as the binomial response and with the four egg characteristics as predictors. We used average values at a nest level in these analyses because we were not able to link individual egg shape or size measurements with individual nestling characteristics.

All analyses and figures were produced in R version 4.0.2 (R Core Team 2020). The complete set of code and data required to reproduce all analyses and figures is available at https://github.com/cct663/tres_egg_shape and will be permanently archived on Zenodo upon acceptance.

RESULTS

In total, we measured the shape and size of 1382 eggs produced in 259 nests by 205 unique females. A total of 38 females had eggs measured in two years and 7 females had eggs measured in 3 years. Overall, there was enormous variation in the shape and size of tree swallow eggs. A 90% ellipse drawn based on tree swallow eggs measured in this study included the species average egg shapes for 965 out of 1400 (69%) species reported in Stoddard et al. (2017) (Figure 2). When comparing average egg characteristics among females, the four measures of egg size and shape that we examined were moderately correlated with each other (Table 1).

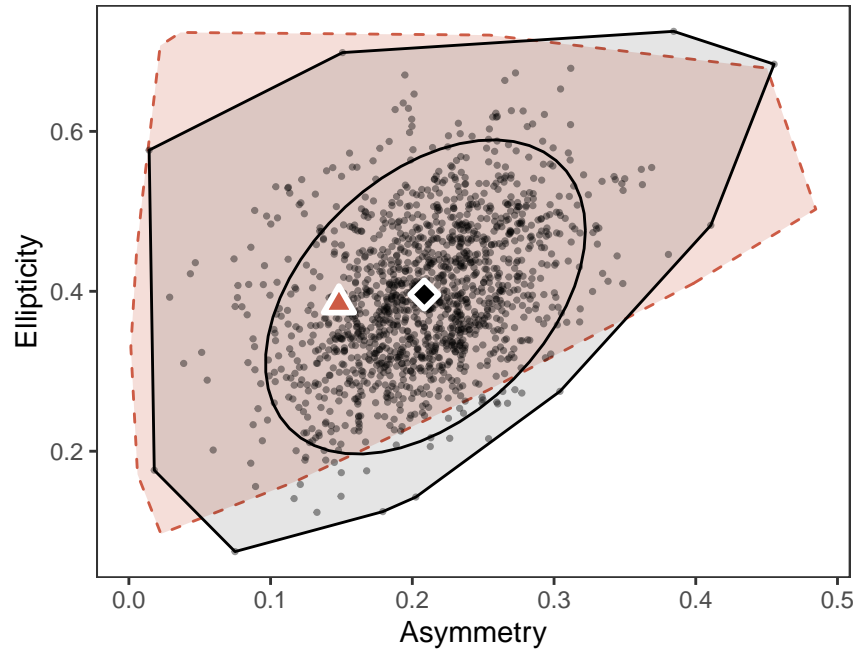


Figure 2: Morphospace of tree swallow egg shapes from 1382 measured eggs. Black polygon is the full minimum convex polygon for all tree swallow eggs and black ellipse indicates the region containing 90% of eggs. The red dashed polygon shows the minimum convex polygon for the 1400 species included in Stoddard et al. 2017. Large symbols show the overall average tree swallow egg shape (black diamond) and overall average egg shape across all species (red triangle).

Table 1: Among-individual correlation for each pair of egg characteristics based on the average of each characteristic for each female. Pearson correlation is shown below the diagonal and *P*-value above the diagonal. Correlations that are significant after Bonferroni correction for multiple comparisons are shown in bold.

	Asymmetry	Ellipticity	Width	Length
Asymmetry		<0.001	0.74	<0.001
Ellipticity	0.46		<0.001	<0.001
Width	0.02	-0.45		0.004
Length	0.46	0.77	0.2	

Individual repeatability in egg shape and size

Despite the enormous variation overall, the shape and size of eggs produced by individual females were repeatable. Female identity explained 34.7% of the variation in egg asymmetry and nest identity explained an additional 11.8% (female identity 95% CI = 24.2 to 43.3%, $P < 0.001$; nest identity CI = 4.4 to 20.7%, $P < 0.001$). For ellipticity, female identity explained 41.4% of the variation and nest identity explained an additional 14.2% (female identity 95% CI = 30.2 to 50.9%, $P < 0.001$; nest identity CI = 7 to 23.2%, $P < 0.001$).

The size of eggs was also repeatable within females. Female identity explained 48.2% of the variation in egg length and nest identity explained an additional 15.1% (female identity 95% CI = 36.8 to 57.8%, $P < 0.001$; nest identity CI = 7.4 to 24.8%, $P < 0.001$). Egg width had the lowest repeatability, with female identity explaining 20.2% of the variation and nest identity explained an additional 10.6% (female identity 95% CI = 9.7 to 30%, $P < 0.001$; nest identity CI = 2.1 to 20.8%, $P < 0.001$).

When comparing the eggs of mothers and their daughters, there was a positive association between all egg characteristics (Pearson's correlation between mother and daughter for asymmetry $r = 0.31$, $P = 0.26$; ellipticity $r = 0.28$, $P = 0.31$; width $r = 0.25$, $P = 0.36$; length $r = 0.24$, $P = 0.39$). However, with a sample size of only 15 mother and daughter pairs, none of these relationships were significant.

Predictors of egg shape and size

In linear mixed models, no aspect of female morphology or age was significantly related to egg asymmetry or ellipticity (Table 2). Female head plus bill length was positively correlated with egg width, while female mass was negatively correlated with egg width (Table 2; head plus bill length $\beta = 0.08$, $P = 0.01$; mass $\beta = -0.07$, $P = 0.04$). No aspect of female morphology significantly predicted egg length, but older females produced eggs that were both wider and longer (Table 2; female age for egg width $\beta = 0.24$, $P < 0.001$; female age for egg length $\beta = 0.24$, $P < 0.02$). Despite the fact that age was significantly related to egg width and length and that morphology was significantly related to egg width, the overall amount of variation explained by the fixed effects in these models was low (Table 2; egg width full model marginal $R^2 = 0.04$; egg length full model marginal $R^2 = 0.02$).

Clutch size was unrelated to egg shape (asymmetry $\beta = -0.0$, CI = -0.01 to 0.0, $P = 0.88$; ellipticity $\beta = -0.0$, CI = -0.01 to 0.01, $P = 0.59$) or size (width $\beta = 0.01$, CI = -0.06 to 0.06, $P = 0.92$; length $\beta = 0.07$, CI = -0.03 to 0.18, $P = 0.17$).

Table 2: Linear mixed models with female band and nest included as random effects ($n = 1353$ eggs, 254 nests, 202 females). All continuous predictors are standardized to a mean of 0 and standard deviation of 1.

Response	Predictor	Estimate	CI	P
Asymmetry ^a	Intercept	0.21	0.20 - 0.21	<0.001
	Headbill	0.00	-0.01 - 0.00	0.129

Response	Predictor	Estimate	CI	P
Ellipticity ^b	Wing	0.00	-0.00 - 0.01	0.709
	Mass	0.00	-0.00 - 0.01	0.670
	Age (ASY)	0.00	-0.01 - 0.01	0.785
	Intercept	0.40	0.38 - 0.41	<0.001
	Headbill	0.00	-0.01 - 0.01	0.460
Width ^c	Wing	-0.01	-0.02 - 0.00	0.097
	Mass	0.01	-0.00 - 0.02	0.141
	Age (ASY)	-0.01	-0.03 - 0.01	0.394
	Intercept	14.07	13.98 - 14.16	<0.001
	Headbill	0.08	0.02 - 0.14	0.012
Length ^d	Wing	0.02	-0.05 - 0.08	0.566
	Mass	-0.07	-0.13 - -0.00	0.037
	Age (ASY)	0.24	0.12 - 0.36	<0.001
	Intercept	19.23	19.07 - 19.39	<0.001
	Headbill	0.09	-0.02 - 0.19	0.112
	Wing	-0.10	-0.21 - 0.01	0.088
	Mass	0.02	-0.09 - 0.13	0.776
	Age (ASY)	0.24	0.04 - 0.44	0.021

Note: ^a asymmetry: marginal $R^2 = 0.01$, conditional $R^2 = 0.47$ ^b ellipticity: marginal $R^2 = 0.01$, conditional $R^2 = 0.56$ ^c width: marginal $R^2 = 0.02$, conditional $R^2 = 0.64$ ^d length: marginal $R^2 = 0.04$, conditional $R^2 = 0.32$

Age related changes

The difference in egg size with female age could arise from longitudinal increases in egg size as females age or from selective survival of females that lay larger eggs. Among females that were measured as both first time breeders and as returning breeders, asymmetry did not change longitudinally, but ellipticity decreased slightly (Figure 3 A-B; paired t -test for asymmetry: $t = -0.34$, $df = 28$, $P = 0.73$; ellipticity: $t = -2.3$, $df = 28$, $P = 0.03$). Both the width and length of eggs increased significantly from a female's first to second breeding year (Figure 3 C-D; paired t -test for width: $t = 5.31$, $df = 28$, $P < 0.001$; length: $t = 2.25$, $df = 28$, $P = 0.03$).

Nestling characteristics and fate

There was no evidence that nestling mass or size on day 12 was related to any characteristics of egg shape or size at a nest level (linear model for nestling mass: asymmetry $\beta = 0.74$, $P = 0.13$, ellipticity $\beta = 1.99$, $P = 0.34$, width $\beta = 1.55$, $P = 0.23$, length $\beta = -2.41$, $P = 0.20$; model for head plus bill length: asymmetry $\beta = 0.18$, $P = 0.31$, ellipticity $\beta = 0.55$, $P =$

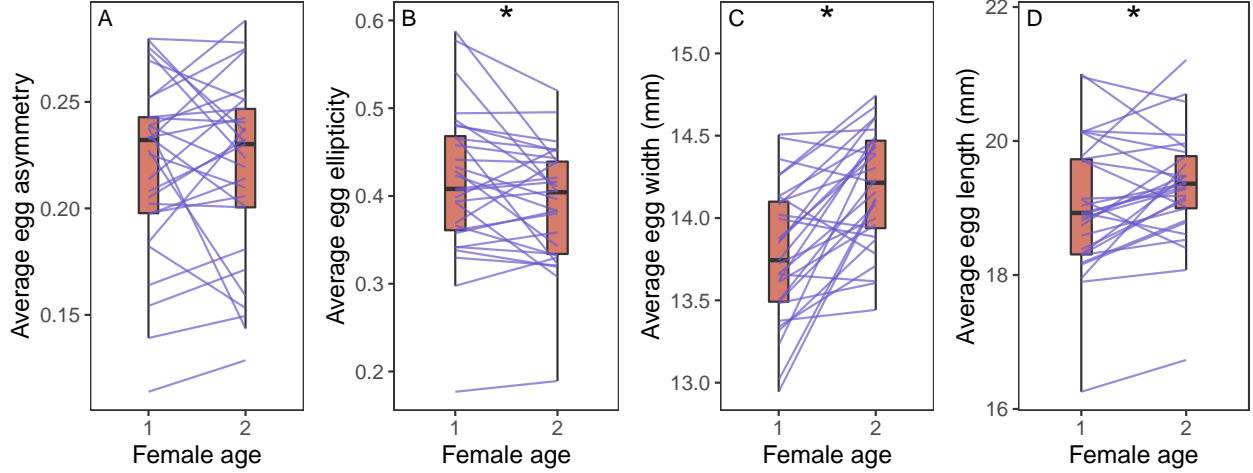


Figure 3: Change in average egg shape (A, B) and size (C, D) for females that were observed as one year old first time breeders and again in the subsequent year. Boxes show the mean and interquartile range for each age group and whiskers extend to 1.5 times the interquartile range. Lines connect observations from the same individual. Asterisks denote significant difference between ages.

0.47, width $\beta = 0.39$, $P = 0.41$, length $\beta = -0.58$, $P = 0.40$; model for wing length: asymmetry $\beta = 1.19$, $P = 0.29$, ellipticity $\beta = -0.93$, $P = 0.85$, width $\beta = -0.91$, $P = 0.76$, length $\beta = 1.13$, $P = 0.79$). There was also no evidence that any average egg characteristic predicted the likelihood of survival to fledging among these nests (generalized linear model with binomial response: asymmetry $\beta = -0.22$, $P = 0.60$, ellipticity $\beta = 1.92$, $P = 0.14$, width $\beta = -1.55$, $P = 0.20$, length $\beta = 2.17$, $P = 0.21$).

DISCUSSION

We found that variation in the shape of eggs from a single population of tree swallows encompassed most of the variation observed in average egg shape across 1400 species (Stoddard et al. 2017). Consistent with prior studies of egg size in many species (Christians 2002), most of this variation was attributable to among-individual differences, as we found that egg shape was repeatable within females and stable across multiple years. Although some aspects of egg shape and size increased as females aged and were associated with overwinter survival, we found no conclusive evidence that either egg shape or size was directly related to any aspect of reproductive performance or nestling survival. Because global patterns of egg shape diversity are, ultimately, the consequence of selection operating on variation at the within-species level, our results have implications for understanding the mechanisms and processes that contribute to egg shape variation among species.

#Despite finding substantial variation in egg shape among females, we found little evidence that these differences were driven by overall morphology or directly related to any important fitness proxies, other than adult female survival. While a great deal is known about egg shape variation among species (Stoddard et al. 2017, Montgomerie et al. 2021), understanding what generates and maintains variation in egg shape within species is largely

an open question. Egg size is highly heritable in many species (Christians 2002) and the correlations that we found between egg shape and size among-individuals, it is likely that variation in egg shape is partially genetically determined. It is worth noting that additive genetics could contribute to egg shape directly or to morphological features, such as pelvis shape (Shatkovska et al. 2018) that subsequently influence egg shape. It also seems likely that early life developmental conditions might play a strong role in determining egg size and shape throughout the lifetime. Potti (1999) found that pied flycatcher (*Ficedula hypoleuca*) egg volume as an adult was related to volume of the egg they had hatched from, but after controlling for maternal effects, early life body condition also had a strong effect on the volume of subsequent eggs and this effect persisted throughout the lifetime. This mechanism also suggests that, at least in some cases, associations between egg characteristics and female condition or performance might arise from a shared cause (early life conditions) rather than from a direct consequence of variation in initial egg shape or size.

In a meta-analysis of 283 studies that examined variation in egg size within species, Krist (2011) found that egg size is consistently related to hatching success, chick survival, growth rate, condition, and morphology. These effects are strongest early in life, but sometimes persist long after chicks have hatched (Krist 2011). Therefore, even though we found no clear link between egg size and nestlings in our study, it is plausible that the variation in egg size that we detected could have important consequences for fitness under some conditions (e.g., when food is more limited). Prior studies of tree swallows have suggested that egg size has consequences for nestlings (Ardia et al. 2006, Bitton et al. 2006) and studies in a variety of species demonstrate that female quality or environmental conditions can generate variation in egg size (Kouwenberg et al. 2013, Kvalnes et al. 2013). This pattern might also explain the associations that we saw between egg size, survival, and age among females. Variation in individual quality is an important driver of reproductive success in female tree swallows and older females are typically in better body condition and produce more offspring (Winkler et al. 2020b). In this case, the association between overwinter survival and egg size might arise because females that are higher quality or in better current condition are able to invest more in large eggs while also maintaining high survival prospects. Because of the within-individual correlations between egg shape and size, changes in overall investment (egg size) between years might have caused the smaller, but still significant, changes in shape that we detected between years. Alternatively, these correlations may be unrelated to reproductive investment *per se* and instead arise as the product of some other constraint that differs as females age, such as changes in abdominal musculature.

One question that our study cannot address is whether tree swallows have an unusually high or a typical amount of intra-specific variation in egg shape. It seems likely that species that experience strong selection for optimal egg shapes would have less between and within-individual variation in the shape of eggs produced as a consequence of consistent selection. For example, if the egg shape of the common murre is optimized to promote stability on a rocky ledge, we might expect very strong selection for the optimal shape, resulting in little variation among individuals (Birkhead et al. 2018). In contrast, tree swallows nest in cavities, produce variable clutch sizes, and have a widespread geographic distribution that results in highly variable climate conditions and resource availability during breeding (Winkler et al. 2020b, Zimmer et al. 2020). Given this life history, there may be

relaxed selection on egg shape and the variation we observed may result as a by-product of selection on more consequential traits, such as morphology or yolk deposition (Ardia et al. 2006). Our study focused on only a single population, but because clutch size and environment vary predictably across the breeding range (Winkler et al. 2014, Zimmer et al. 2020), subsequent work comparing egg characteristics across different populations would be illuminating.

Large-scale comparative studies that directly address the amount of within species variation in egg shape—in addition to species mean values—also have the potential to demonstrate how selection for optimal egg shape differs across clades. Understanding which species have more or less variable egg shapes would help to clarify why the factors that explain variation in egg shape differ in their explanatory power at different scales (Stoddard et al. 2017, 2019; Montgomerie et al. 2021) and why different factors are sometimes correlated with egg shape in particular groups of birds (Birkhead et al. 2019). We suggest that comparative studies should report the values measured from individual eggs rather than only species mean values in data archives (as in Montgomerie et al. 2021) along with information on location of the original nest and female identity. For example, if multiple eggs are measured from the same clutch or from different regions of a species range this information should be indicated even when the main analyses presented focus on overall species level differences.

In current databases, some common species have many egg measurements; however, most species are represented by only a few egg measurements that might come from even fewer females from a single population (Stoddard et al. 2017, Montgomerie et al. 2021). Given the huge variation in intra-specific egg shape that we identified here, the choice of individuals in these databases could have major impacts on the results. At best, relying only on species averages might add noise to comparative studies, but in some cases failing to account for different patterns of within species variation might also obscure biologically meaningful patterns (e.g., among species differences in the amount of variation in egg shape, Garamszegi and Møller 2010, Garamszegi 2014). Available egg shape databases are not well designed to fully analyze the hierarchical nature of variation in egg shape, but museum collections house an estimated five million bird eggs around the world (Marini et al. 2020) and adding photographs of clutches to measure egg shape to ongoing field studies is a low cost addition, though care must be taken to standardize photography for proper measurement (Biggins et al. 2018). Field studies will be particularly valuable when information about the parents is also collected and when the same individuals are measured repeatedly under different conditions or after experimental manipulations.

CONCLUSION

The past few years have seen huge advances in our understanding of how and why eggs vary in shape across all birds (Stoddard et al. 2017, Montgomerie et al. 2021). However, an understanding of how and why egg shape varies within species and within individuals has not been well integrated with interspecific results. We demonstrate enormous variation in egg shape in tree swallows and suggest that more work is needed to understand whether this pattern is typical and the extent to which intra-specific egg shape variation itself differs

among species. These studies are important for two reasons. First, there are interesting biological questions to address in their own right within species about the consequences and causes of egg shape variation. Second, understanding the mechanisms that generate variation in egg shape among individuals and how these mechanisms translate across scales of organization has the potential to clarify when large-scale comparative studies can or cannot adequately explain egg shape variation. A complete understanding of the diversity of eggs will include multi-scale explanations from individuals to all birds.

ETHICAL NOTE

All of the procedures described were approved by the Cornell University Institutional Animal Care & Use Board (IACUC protocol 2019-0023). Captures and sampling were approved by state and federal permits to MNV.

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AUTHOR CONTRIBUTION STATEMENT

CCT and TAR conceived the study. CCT, TAR, JJU, and ASI collected the field data. CCT measured the photographs, analyzed data, and drafted the manuscript. MNV contributed to funding for the project. All authors provided feedback and editing on the manuscript.

DATA AVAILABILITY STATEMENT

The complete set of code and data required to reproduce all analyses and figures is available at https://github.com/cct663/tres_egg_shape and will be permanently archived on Zenodo upon acceptance.

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