

1 Insect egg size and shape evolve with ecology, not develop- 2 mental rate

3 Samuel H. Church^{*,1,†}, Seth Donoughe^{*,1,2}, Bruno A. S. de Medeiros¹, Cassandra G. Extavour^{1,3,†}

4 Contents

5	1 Abstract	2
6	2 Body text	4
7	2.1 Evolutionary patterns of egg shape and size	5
8	2.2 Developmental traits and egg evolution	6
9	2.3 Changes in size and shape are explained by oviposition ecology	8
10	3 Acknowledgements	10
11	4 Author contributions	11
12	5 Competing interests	11
13	Main References	11
14	6 Methods	14
15	6.1 Data availability	14
16	6.2 Creating the insect egg database	14
17	6.3 Measuring egg features	14
18	6.4 Phylogenetic methods	15
19	6.5 Annotating the egg database with developmental trait data	15
20	6.6 Annotating the egg database with life-history trait data	15
21	6.7 Data analysis and evolutionary model comparison	15
22	6.8 Statistical Information	16
23	Methods References	17

* Samuel H. Church and Seth Donoughe contributed equally to this work.

1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, United States

2 *Current address:* Department of Cell and Molecular Biology, University of Chicago, Chicago, IL 60637, United States

3 Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, United States

† Correspondence to church@oeb.harvard.edu and extavour@oeb.harvard.edu

24 **1 Abstract**

25 The evolution of organism size is hypothesized to be predicted by a combination of development, morphological
26 constraints, and ecological pressures. However, tests of these predictions using phylogenetic methods have been
27 limited by taxon sampling. To overcome this limitation, we generated a database of more than ten thousand
28 observations of insect egg size and shape from the entomological literature and combined them with published
29 genetic and novel life-history datasets. This enabled us to perform phylogenetic tests of long-standing predictions
30 in size evolution across hexapods. Here we show that across eight orders of magnitude in egg volume variation,
31 the relationship between egg shape and size itself evolves, such that predicted universal patterns of scaling do not
32 adequately explain egg shape diversity. We test the hypothesized relationship between size and development, and
33 show that egg size is not correlated with developmental rate across insects, and that for many insects egg size is not
34 correlated with adult body size either. Finally, we show that the evolution of parasitism and aquatic oviposition both
35 help to explain the diversification of egg size and shape across the insect evolutionary tree. Our study challenges
36 assumptions about the evolutionary constraints on egg morphology, suggesting that where eggs are laid, rather than
37 universal mathematical allometric constants, underlies egg size and shape evolution.

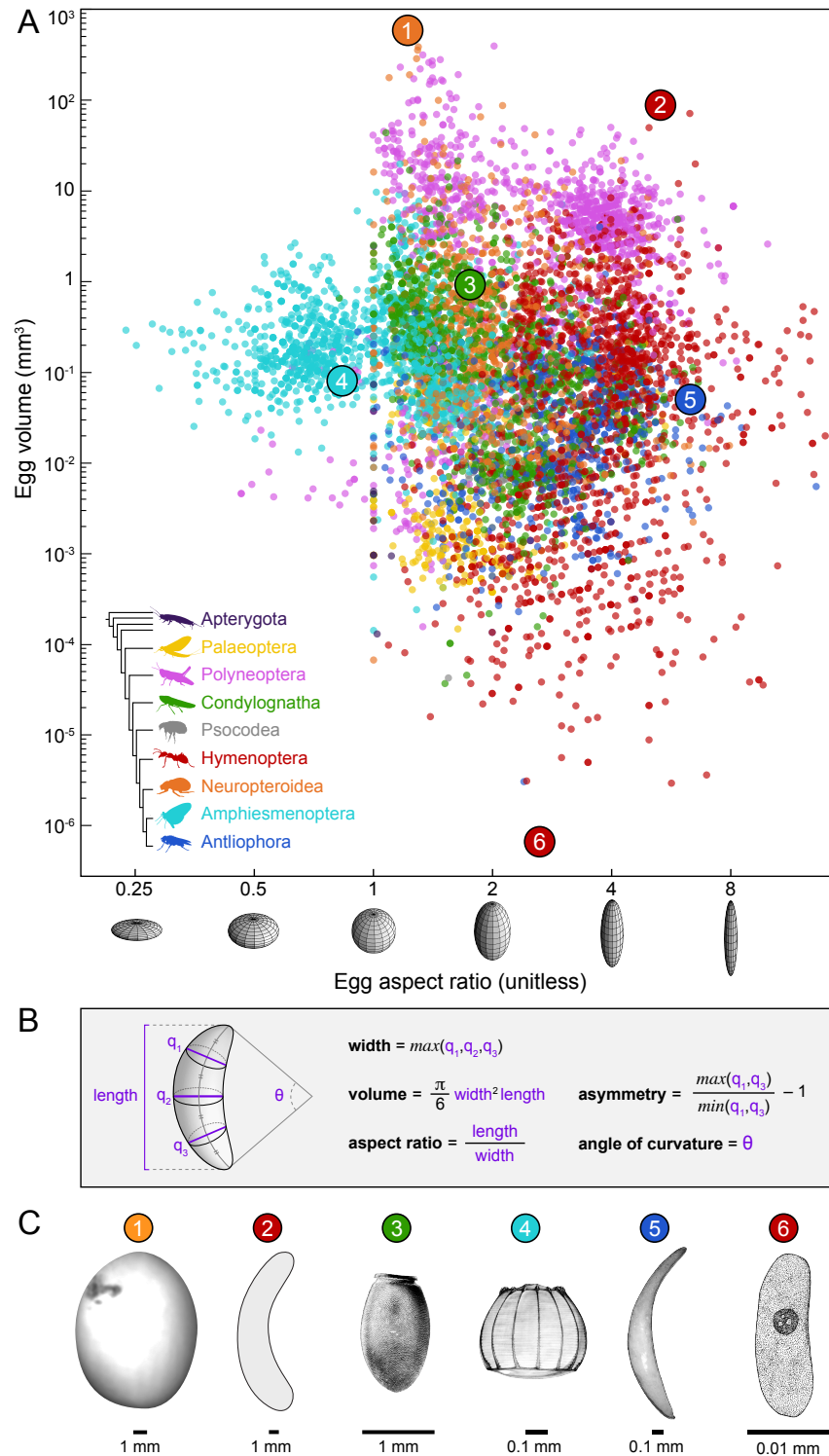


Figure 1: **The shapes and sizes of hexapod eggs.** **A**, The distribution of egg descriptions in a morphospace defined by egg volume (mm^3) and aspect ratio (unitless). Both traits are plotted on a log scale. Points are colored according to the groups on the in the lower left, which illustrates relationships according to Misof et al. 2014¹, one of the backbone phylogenies used in this study. Numbered points correspond to the six eggs shown in C, chosen as examples of specific or extreme sizes or shapes. There are 10,449 published egg descriptions in the database; the plot shows the 7,935 descriptions with both volume and aspect ratio data. **B**, Egg shape is described in the database with three parameters (aspect ratio, asymmetry, and angle of curvature) calculated from the measurements shown in purple (see text for details). **C**, Images of six example eggs, arranged from largest to smallest and oriented with the axis of rotational symmetry vertical: 1. Earth-borer beetle *Bolboleaus hiaticollis*², 2. Tropical carpenter bee *Xylocopa latipes*³, 3. Kissing bug *Rhodnius robustus*⁴, 4. Many-banded daggerwing butterfly *Marpesia chiron*⁵, 5. Tephritid fruit fly *Anastrepha distincta*⁶, 6. Parasitoid wasp *Platygaster vernalis*⁷.

38 2 Body text

39 Size is a fundamental factor in many biological processes. Size may impact ecological interactions^{8,9}, it scales with
40 features of morphology and physiology¹⁰, and in many cases larger animals have higher fitness¹¹. Previous studies
41 have aimed to identify the macroevolutionary forces that explain observed size distributions^{8,12,13}, but limited
42 data on the phylogenetic distribution of size has precluded robust tests of these predicted forces^{11,14}. Here we
43 address this problem by assembling a dataset of insect egg phenotypes with sufficient taxon sampling to rigorously
44 test evolutionary hypotheses in a phylogenetic framework, and use these data to elucidate the roles of allometric
45 constraints, development, and ecology in size and shape evolution.

46 We have chosen insect eggs as a compelling system in which to test macroevolutionary hypotheses about the causes
47 and consequences of size evolution¹⁴. Insect eggs are extraordinarily diverse¹⁵, yet their morphologies can be readily
48 compared across distant lineages using quantitative traits. Changes in egg size are often assumed to be linked to
49 changes in other aspects of organismal biology¹⁶, including adult body size^{17,18}, features of adult anatomy^{19,20},
50 and offspring fitness via maternal investment²¹. The egg is an independent ecological vessel that must withstand
51 the physiological challenges of being laid in diverse microenvironments, including water, air, or internal to plants
52 or animal hosts²². Furthermore, because the fertilized egg is the homologous, single-cell stage in the life-cycle of
53 multicellular organisms, egg size diversity is relevant to both cell size and organism size evolution^{15,21}.

54 Three classes of hypotheses have historically been proposed to explain the evolution of egg size and shape: [1]
55 geometric constraints due to the physical scaling of size and shape^{19,20,23–25}, [2] the interaction between size and
56 the rate of development^{26–29}, and [3] the diversification of size and shape in response to ecological or life-history
57 changes^{17,19,22,30}. We use a large-scale phylogenetic approach to test all three of these hypotheses, and show that many
58 presumed universal patterns in egg size, shape, and embryonic development are not supported across insects. Instead,
59 our results show that models accounting for ecological change best explain extant egg morphological diversity.

60 Using custom bioinformatic tools, we assembled a database of 10,449 morphological descriptions of eggs from
61 the published scientific literature, comprising 6,706 species, 526 families, and every extant insect and non-insect
62 hexapod order (Figs. 1A, S1, S2)³¹. We combined this database with backbone hexapod phylogenies^{1,32} that we
63 enriched to include taxa in the egg morphology database (Fig. S3), and used it to describe the distribution of egg
64 shape and size (Fig. 1B). Our results showed that insect eggs span more than eight orders of magnitude in volume
65 (Figs. 1C, S4), and revealed new candidates for the smallest and largest described insect eggs: respectively, a parasitoid
66 wasp *Platygaster vernalis*⁷ (volume = 7×10^{-7} mm³), and an earth-boring beetle, *Bolboleaus hiaticollis*² (volume = 5
67 $\times 10^2$ mm³) (Fig. 1C).

68 Plotting eggs by morphological traits revealed that some egg shapes have evolved only in certain clades (Fig. 1C, S5,
69 S6). For example, oblate ellipsoid eggs (with an aspect ratio smaller than one) are found only in moths, butterflies,
70 and stoneflies (Amphismenoptera: Lepidoptera and Polyneoptera: Plecoptera; image 4 in Fig. 1C). However, the
71 most prominent pattern was that distantly related insects have converged on similar egg morphologies many times
72 independently (Fig. 1C, S6). This high degree of morphological convergence allowed us to perform robust tests of
73 phenotypic associations across independent evolutionary events.

74 **2.1 Evolutionary patterns of egg shape and size**

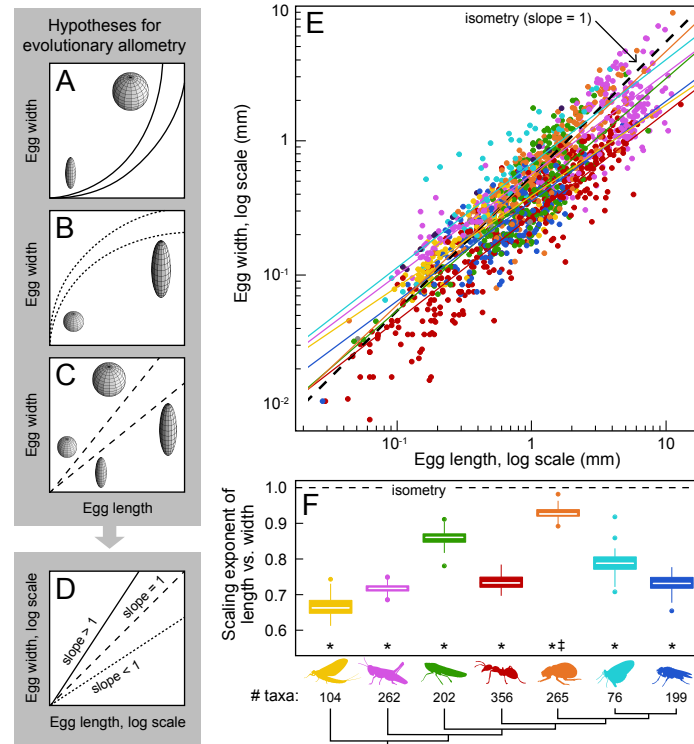


Figure 2: **The allometric relationship of egg shape and size evolves across insect lineages.** Historically hypothesized allometric relationships between egg size and shape: **A**, larger eggs are proportionally wider than smaller counterparts, (solid line); **B**, larger eggs are proportionally longer (dotted line); and **C**, insect egg shape and size scale isometrically (dashed line). Hypothetical eggs for each hypothesis are shown as schematics with length axis vertical. **D**, Each hypothesis (solid line = A, dotted line = B, dashed line = C) predicts a different value of the scaling exponent—that is, the slope of the regression between egg length and width in log-log space. **E**, The distribution of egg length and width in log-log space. The dashed black line represents a hypothetical 1:1 relationship (isometry, hypothesis C). Solid colored lines are the phylogenetic regressions for seven clades, and each colored point is a randomly selected representative egg for a genus. **F**, Distribution of the scaling exponents for the seven monophyletic insect clades included in this analysis, calculated over the posterior distribution of trees. We resampled tree topology and genus-level representatives from the egg morphology dataset 100 times, calculating a scaling exponent each time. White lines, boxes, bars, and dots represent median, 25-to-75th percentiles, 5-to-95th percentiles, and outliers, respectively. Asterisks indicate that the relationship between length and width is significant (p -value threshold <0.01 , exact values in Table S6), and double-dagger indicates the relationship is not statistically distinguishable from isometry (p -value threshold >0.01 , exact values in Table S7). In **E** and **F** the colors correspond to the clades shown in Fig. 1A.

75 Two opposing hypotheses based on predicted geometric constraints have been proposed to explain the evolutionary
 76 relationship between propagule shape and size. *Hypothesis A*: When eggs evolve to be larger, they get wider (increases
 77 in egg size are associated with decreases in aspect ratio)^{24,25}. This hypothesis predicts a reduction in relative surface
 78 area as size increases, which has been proposed as a solution to the presumed cost of making eggshell material²⁵.
 79 *Hypothesis B*: When eggs evolve to be larger, they get longer (increases in egg size are associated with increases in
 80 aspect ratio)^{19,20,25}. This hypothesis predicts a reduction in relative cross sectional area as eggs get larger, which has
 81 been proposed as a solution to the need for eggs to pass through a narrow opening during oviposition^{19,20}.

82 To test these hypotheses about the physical scaling of size and shape, we first modeled the individual evolutionary

83 history of each morphological trait across insects. This allows us to first determine whether distributions of extant
84 shape and size have been shaped by phylogenetic relationships, or if these traits are instead stochastically distributed
85 across insects. For egg volume, aspect ratio, asymmetry, and angle of curvature (Fig. 1B), we considered four
86 models of potential evolutionary change in egg size and shape, and asked which of these best fit our data: Brownian
87 motion (BM), Brownian motion with evolutionary friction (Ornstein-Uhlenbeck, OU), Brownian motion with a
88 decreasing rate of evolution (Early Burst, EB), and a non-phylogenetic model of stochastic motion (white noise,
89 WN). We found strong evidence that models that account for covariance based on phylogeny fit our data better than
90 a non-phylogenetic model (WN); in other words, insect egg morphology tends to be similar in closely related insects.
91 For egg size and aspect ratio, an ‘Early-Burst’ (EB) model in which the rate of evolution decreases over time, best
92 describes the observed distributions of these two traits. This change in rate is distributed non-uniformly over the
93 insect phylogeny, with some clades evolving more rapidly than others (Figs. S8-10). In earlier studies EB models were
94 rarely detected³³; however our findings are consistent with recent work evaluating datasets that, like ours, comprise
95 many taxa and orders of magnitude in morphological variation^{34,35}. Having established the phylogenetic models
96 that best describe egg trait evolution, we used these results to test hypotheses about the relationship between egg
97 shape and size.

98 To test which aforementioned scaling relationship best describes insect egg evolution, we compared support for
99 Hypotheses A and B using a phylogenetic generalized least-squares approach (PGLS) to determine the scaling
100 exponent of egg length and width (the slope of the regression of log-length and log-width). A slope less than
101 one would support Hypothesis A, while a slope greater than one would support Hypothesis B³⁶. An alternative,
102 Hypothesis C, is that egg shape remains the same as size changes, which would result in a slope near one (an isometric
103 relationship). We found that Hypothesis B is best supported across all insects: larger eggs have higher aspect ratios
104 than smaller eggs ($0 < p\text{-value} < 0.005$, slope = 0.76, Fig. 2B, Table S6), even when controlling for adult body size
105 (Fig. S13, Table 8). However, the allometric relationship between size and shape evolves dynamically across the
106 phylogeny, as has also been shown for metabolic scaling in mammals³⁷. Among the large clades we tested, Hypothesis
107 C could not be rejected for Neuropteroidea ($p\text{-value of alternative hypothesis test} = 0.02$; Fig. 2C, 2F, S11, S12,
108 Table S7). Calculating the scaling relationship on subgroups of each of these major lineages revealed that many
109 additional insect clades, including mayflies, crickets and grasshoppers, and shield bugs, also had eggs with isometric
110 scaling of shape and size (Fig. S12). The marked differences in scaling exponents are evidence that throughout the
111 course of insect diversification, egg evolution was not governed by a universal allometric scaling constant. Instead,
112 evolutionary forces beyond the constraints of physical scaling (e.g. development or ecology) are required to explain
113 egg morphological diversification.

114 2.2 Developmental traits and egg evolution

115 The egg is the starting material for embryogenesis, and the size of the hatchling is directly related to the size of the
116 egg at fertilization³⁸. It was previously reported that embryogenesis takes longer in species with larger eggs²⁹, and
117 that this relationship could influence size evolution²⁶⁻²⁹. This prediction is consistent with the observation that
118 larger adult species have a lower metabolic rate than smaller species³⁹. To test this prediction across our expanded egg
119 dataset, we assembled embryological records from published studies, and found that indeed, simply comparing egg

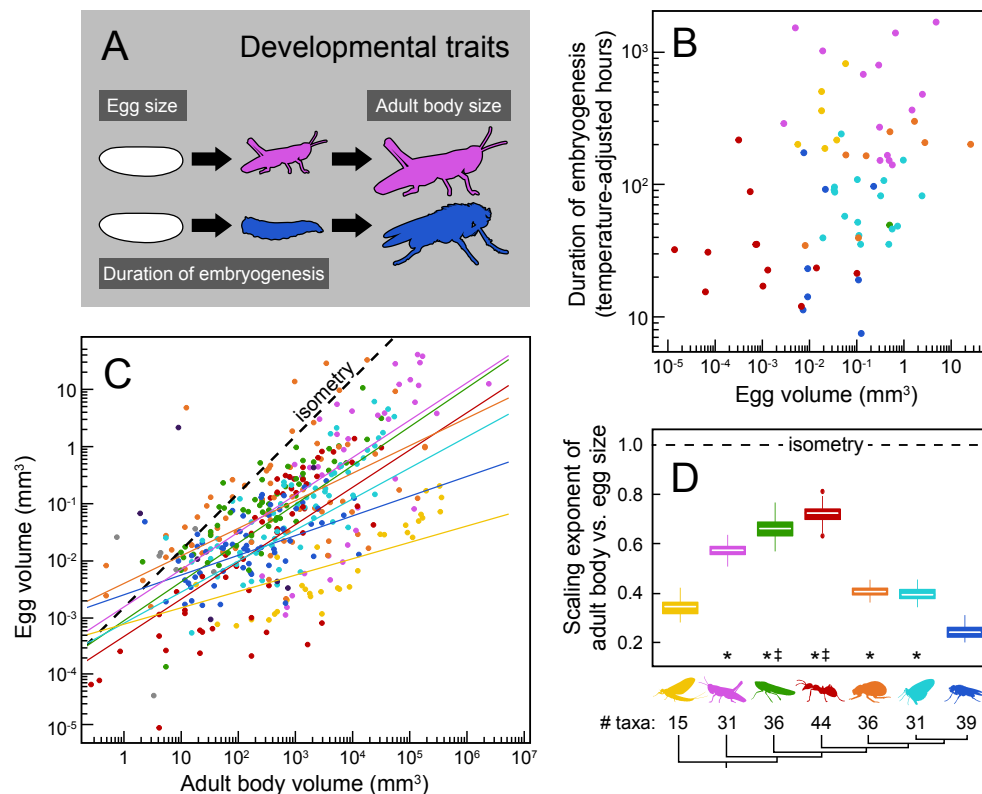


Figure 3: Rate of embryogenesis does not co-vary predictably with egg size. **A**, Mature eggs undergo embryonic development, hatch, and grow into adult insects. We define duration of embryogenesis as the time elapsed from egg-laying to the point at which the nymph (in Hemimetabola) or larva (in Holometabola) emerges from the egg. **B**, Duration of embryogenesis, adjusted for incubation temperature (hours, plotted on a log scale), compared to egg volume (mm^3 , plotted on a log scale). Each colored point represents an insect species for which duration of time-to-hatching has been reported (see Supplemental Information for sources), and egg morphological data are contained in our database³¹. When phylogenetic relationships are taken into account, there is no significant relationship between egg volume and duration of embryogenesis. **C**, Egg volume (mm^3 , plotted on a log scale) compared to adult body volume, calculated as body length cubed (mm^3 , plotted on a log scale). The dashed black line shows an example 1:1 relationship (isometry), solid colored lines are the phylogenetic regression for the seven clades included in this analysis, and colored points are family- and order-level averages for egg size and median adult insect size. **D**, The distributions of the allometric exponents for the seven monophyletic insect clades included in this analysis. Asterisks indicate that the relationship between length and width is significant (p -value threshold <0.01 , exact values in Table S12), and double-dagger indicates the relationship is not statistically distinguishable from isometry (p -value threshold >0.01 , exact values in Table S13). Colors and labels in B–E correspond to the clades shown in Fig. 1A.

120 volume and duration of embryogenesis yields the previously reported positive relationship²⁹ (Fig. S22). However, a
121 linear regression that does not account for phylogenetic relationships is inappropriate for this analysis due to the
122 covariance of traits on an evolutionary tree⁴⁰. When we accounted for such potential phylogenetic covariance of
123 data, we found that there is no significant relationship between egg size and duration of embryogenesis across insects,
124 such that eggs of very different sizes can develop at a similar rate and vice versa ($0.08 < p\text{-value} < 0.26$; Fig. 3B, S21,
125 Table S21). These results suggest that the often-invoked trade-off between size and development^{26–29} does not hold
126 across insect species.

127 We also tested the hypothesis that the size of the egg has a positive relationship with adult body size. Previous work
128 reported this relationship in subsets of insects, and moreover suggested that smaller insects lay proportionally larger
129 eggs for their bodies^{18,38,41}. Such a relationship between egg size and body size would result in an allometric scaling
130 exponent less than one. We combined our dataset of egg size with published adult body length data for insect
131 families⁴², and found that this relationship was not generalizable across all insect lineages. For example, in flies and
132 their relatives (Antliophora) and mayflies and odonates (Palaeoptera), egg size is not predicted by body size, meaning
133 that insects of similar body size lay differently sized eggs ($p\text{-value}$, Antliophora = 0.04, Palaeoptera = 0.03; Fig. 3C,
134 3D, Tables S22). In thrips and true bugs (Condylognatha) and in bees, ants, and wasps (Hymenoptera), an isometric
135 relationship between egg size and body size cannot be rejected ($p\text{-value}$ of alternative hypothesis test, Hymenoptera
136 = 0.02, Condylognatha = 0.02, Fig. S23, Table S23). In general, the predictive power of the relationship between
137 body size and egg size is low: average egg volume can vary by up to four orders of magnitude among species with
138 similar body size (Fig. 3C). This decoupling of both body size and duration of embryogenesis from egg size evolution
139 suggests that egg diversification has not been universally constrained by development across insects.

140 **2.3 Changes in size and shape are explained by oviposition ecology**

141 Egg size and shape have been predicted to evolve in response to changes in life-history and ecology. Recent work in
142 birds has highlighted one such relationship, suggesting that birds with increased flight capability have more elliptical
143 and asymmetrical eggs¹⁹. We asked whether an analogous relationship exists between insect flight capability and egg
144 shape. Unlike birds, insects have undergone many hundreds of evolutionary shifts to flightless and even wingless
145 forms⁴³. We focused on two clades in which the patterns of flight evolution have been extensively studied. Stick
146 insects (Phasmatodea) have flightless and wingless species^{44,45} (Fig. S17), and many butterflies (Lepidoptera) show
147 migratory behavior⁴⁶, which could be considered a proxy for increased flight capability relative to non-migratory
148 taxa (Fig. S17). We found that, in contrast to birds, evolutionary changes in flight ability in these two insect clades
149 were not associated with changes in egg shape (OU model with multiple optima per regime; $\Delta\text{AICc} < 2$, exact
150 values in Tables S17, S18).

151 Like flight capacity, the microenvironment that eggs experience varies widely across insects, including being exposed
152 to air, submerged or floating in water, or contained within a host animal¹⁵ (Fig. 4A). Each of these microenvi-
153 ronments places different demands on the egg, such as variable access to oxygen and water during development²².
154 Preliminary studies in small groups of insects suggested that evolutionary changes in oviposition ecology and life
155 history may drive the evolution of egg size and shape^{17,30}. To test this prediction robustly across all insects, we

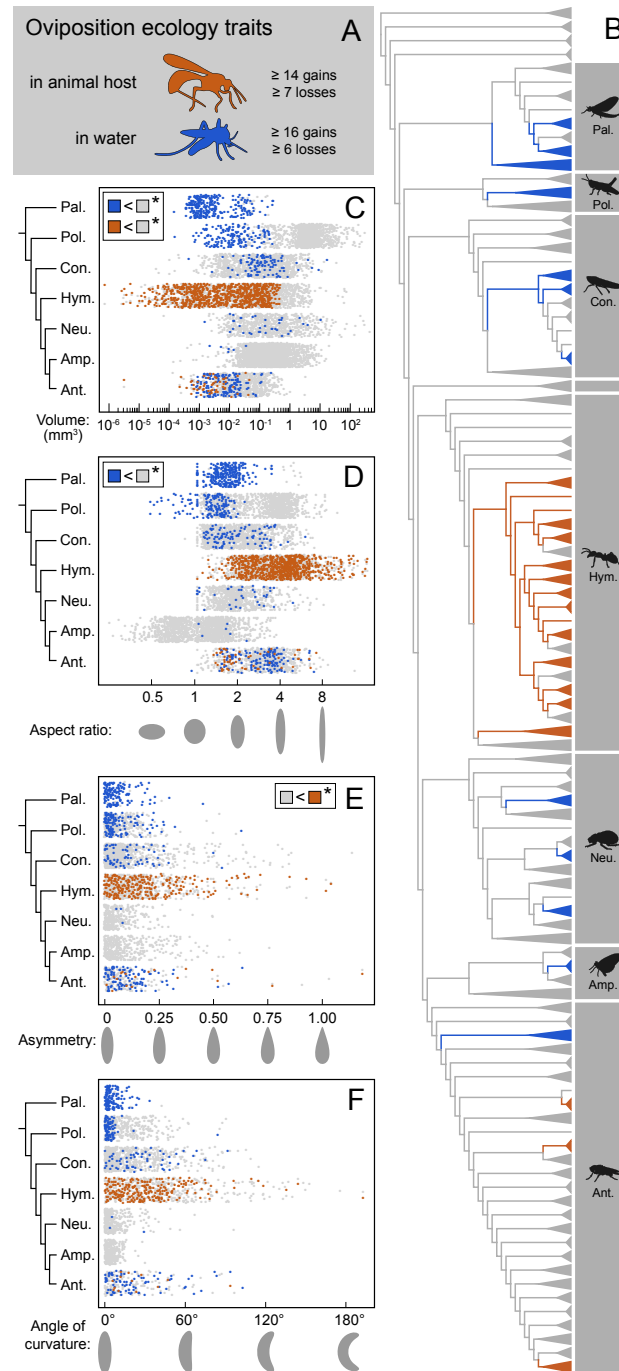


Figure 4: Shifts in oviposition ecology are associated with morphological changes in insect eggs. **A**, Laying eggs within an animal host (orange; e.g. parasitoid wasps) and in water (blue; e.g. mosquitoes) are two modes of insect oviposition ecology. Other oviposition substrates (e.g. terrestrial) are shown in gray (see SI for further details on oviposition substrate classification). **B**, Ancestral state reconstruction of each oviposition ecology trait reveals that they have arisen multiple times in distantly related insect lineages (see Fig. S17 and S18 for full phylogeny). **C-F**, The distribution of four egg morphology parameters across insect clades, colored by oviposition ecology and plotted by phylogenetic group: **C**, volume (mm^3 , plotted on log scale); **D**, aspect ratio (unitless, plotted on log scale); **E**, asymmetry (unitless); and **F**, angle of curvature (degrees). The x-axes of **D-F** include theoretical egg silhouettes shown with the length axis vertical. Asterisks indicate that the model accounting for oviposition ecology (Ornstein-Uhlenbeck process with multiple regimes) fits the data better ($\Delta\text{AICc} > 2$, exact values in Table S14-S19) than a non-ecological model. Clade labels in **B-F** are abbreviations of clades shown in Fig. 1A.

156 compiled records on two specific oviposition ecology modes that have been extensively studied: oviposition within
157 an animal host (which can be in the host adult body or in the host egg, called internal parasitic oviposition) and
158 oviposition in or on water. For each mode we reconstructed ancestral changes along the insect phylogeny, and found
159 that both aquatic and internal parasitic oviposition have been gained and lost multiple times independently (Fig.
160 4A, B, S15, S16). This extensive convergent evolution allowed us to perform a strong test of whether egg size and
161 shape evolution is predicted by the evolution of oviposition ecology.

162 We found that the evolution of new oviposition environments was linked to changes in egg size and shape. Models
163 that accounted for shifts to new oviposition environments better explained egg size and shape distributions than
164 models that did not (OU multistate model, $\Delta AICc > 2$, exact values in Tables S14-S19). Specifically, shifts to
165 aquatic oviposition were significantly associated with the evolution of smaller, wider eggs (Fig. 4C-D, Tables S11,
166 S14), and shifts to internal parasitic oviposition were significantly associated with smaller, more asymmetric eggs
167 (Figs. 4C, 4E, Table S11). Moreover, we note the smallest eggs in the dataset are from wasps with internal parasitic
168 oviposition that develop polyembryonically (i.e. multiple embryos form from a single egg⁴⁷; Fig. S7). Neither
169 ecological change was associated significantly with consistent changes in the scaling relationship between size and
170 shape (Fig. S18). These results were robust to uncertainty in how taxa had been classified for oviposition ecology;
171 using broader ecological definitions, such as combining endo- and ectoparasites or semi-aquatic and riparian insects,
172 did not change these results (Tables S12, S15, S13, S16). Taken together, these convergent evolutionary events are
173 evidence that the microenvironment experienced by the egg following oviposition plays an important role in the
174 evolution of egg size and shape.

175 We have shown that insect eggs present an ideal example case for testing the predictability of macroevolutionary
176 patterns in size and shape. By comparing egg size and shape across taxa, we find that prevalent assumptions about
177 evolutionary trade-offs with developmental time, body size, or the cost of making egg shells do not hold across
178 insects. Although we showed that time of development is not linked to egg size, we speculate that other features of
179 development, such as cell number and distribution, may scale in predictable ways across eight orders of magnitude
180 in egg size variation. We leveraged the power of the vast descriptive literature to overcome barriers common to
181 macroevolutionary studies, establishing computational tools and methods that can be followed in future work.
182 Finally, we provide evidence that the ecology of oviposition drives the evolution of egg size and shape.

183 **3 Acknowledgements**

184 This work was supported by the National Science Foundation (NSF) under Grant No. IOS-1257217 to CGE, and
185 NSF Graduate Research Training Fellowship No. DGE1745303 to SHC, and by a Jorge Paulo Lemann Fellowship to
186 BdM from Harvard University. We thank the Extavour lab and Brian Farrell for discussion, and Casey Dunn, Dakota
187 McCoy, Dan Rice, Elena Kramer, Jack Boyle, Leonora Bittleston, Mansi Srivastava, Milo Johnson, Peter Wilton,
188 Richard Childers, and Sofia Prado-Irwin for suggestions on initial versions of this manuscript. We acknowledge
189 the Ernst Mayr Library at the Museum of Comparative Zoology at Harvard, and specifically thank Mary Sears, for
190 countless hours of support in gathering the references used in this study.

191 4 Author contributions

192 SHC and SD contributed equally to database generation, experimental design, data analysis, writing, and figure
193 preparation. SHC wrote all code to perform statistical analyses. BdM performed the phylogenetic analyses. BdM
194 and CGE contributed to experimental design, interpretation, and writing.

195 5 Competing interests

196 The authors declare no competing interests.

197 Main References

- 198 1. Misof, B. *et al.* Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763–767
199 (2014).
- 200 2. Houston, T. F. Brood cells, life-cycle stages and development of some earth-borer beetles in the genera *Bol-*
201 *borhachium*, *Blackburnium* and *Bolboleaus* (Coleoptera: Geotrupidae), with notes on captive rearing and a
202 discussion of larval diet. *Austral Entomology* **55**, 49–62 (2015).
- 203 3. Iwata, K. The comparative anatomy of the ovary in Hymenoptera. (Records on 64 species of Aculeata in
204 Thailand, with descriptions of ovarian eggs). *Mushi* **38**, 101–109 (1965).
- 205 4. Barata, J. M. S. Morphological aspects of Triatominae eggs: II. Macroscopic and exochorial characteristics of
206 ten species of the genus *Rhodnius* Stal, 1859 (Hemiptera-Reduviidae). *Revista de Saúde Pública* **15**, 490–542
207 (1981).
- 208 5. Nieves-Urbe, S., Flores-Gallardo, A., Hernández-Mejía, B. C. & Llorente-Bousquets, J. Exploración morfológ-
209 ica del corion en Biblidinae (Lepidoptera: Nymphalidae): aspectos filogenéticos y clasificatorios. *Southwestern*
210 *Entomologist* **40**, 589–648 (2015).
- 211 6. Dutra, V. S., Ronchi-Teles, B., Steck, G. J. & Silva, J. G. Egg morphology of *Anastrepha* spp. (Diptera: Tephri-
212 tidae) in the fraterculus group using scanning electron microscopy. *Annals of the Entomological Society of*
213 *America* **104**, 16–24 (2011).
- 214 7. Leiby, R. & Hill, C. The polyembryonic development of *Platygaster vernalis*. *Journal of Agricultural Research*
215 **28**, 829–839 (1924).
- 216 8. Peters, R. H. & Peters, R. H. *The ecological implications of body size* (Cambridge University Press, London,
217 1986).
- 218 9. Allen, R. M., Buckley, Y. M. & Marshall, D. J. Offspring size plasticity in response to intraspecific competition:
219 an adaptive maternal effect across life-history stages. *The American Naturalist* **171**, 225–237 (2007).
- 220 10. Blanckenhorn, W. U. The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology*
221 **75**, 385–407 (2000).
- 222 11. Kingsolver, J. G. & Pfennig, D. W. Individual-level selection as a cause of Cope’s rule of phyletic size increase.
223 *Evolution* **58**, 1608–1612 (2004).

- 224 12. Stanley, S. M. An explanation for Cope's rule. *Evolution* **27**, 1–26 (1973).
- 225 13. LaBarbera, M. Analyzing body size as a factor in ecology and evolution. *Annual Review of Ecology and*
226 *Systematics* **20**, 97–117 (1989).
- 227 14. Chown, S. L. & Gaston, K. J. Body size variation in insects: a macroecological perspective. *Biological Reviews*
228 **85**, 139–169 (2010).
- 229 15. Hinton, H. E. *Biology of insect eggs* (Pergamon Press, Oxford, 1981).
- 230 16. Thompson, D. W. *On growth and form* (The University Press; The Macmillan Company, Cambridge, 1942).
- 231 17. Fox, C. W. & Czesak, M. E. Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*
232 **45**, 341–369 (2000).
- 233 18. Berrigan, D. The allometry of egg size and number in insects. *Oikos* **60**, 313–321 (1991).
- 234 19. Stoddard, M. C. *et al.* Avian egg shape: Form, function, and evolution. *Science* **356**, 1249–1254 (2017).
- 235 20. Bilder, D. & Haigo, S. L. Expanding the morphogenetic repertoire: perspectives from the *Drosophila* egg.
236 *Developmental Cell* **22**, 12–23 (2012).
- 237 21. Bernardo, J. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of
238 evidence and interpretations. *American Zoologist* **36**, 216–236 (1996).
- 239 22. Hinton, H. Respiratory systems of insect egg shells. *Annual Review of Entomology* **14**, 343–368 (1969).
- 240 23. Legay, J. M. Allometry and systematics of insect egg form. *Journal of Natural History* **11**, 493–499 (1977).
- 241 24. Blackburn, T. Evidence for a 'fast-slow' continuum of life-history traits among parasitoid Hymenoptera.
242 *Functional Ecology*, 65–74 (1991).
- 243 25. Kratochvíl, L. & Frynta, D. Egg shape and size allometry in geckos (Squamata: Gekkota), lizards with contrast-
244 ing eggshell structure: why lay spherical eggs? *Journal of Zoological Systematics and Evolutionary Research* **44**,
245 217–222 (2006).
- 246 26. Steele, D. & Steele, V. Egg size and duration of embryonic development in Crustacea. *Internationale Revue*
247 *der Gesamten Hydrobiologie und Hydrographie* **60**, 711–715 (1975).
- 248 27. Steele, D. Correlation between egg size and developmental period. *The American Naturalist* **111**, 371–372
249 (1977).
- 250 28. Sargent, R. C., Taylor, P. D. & Gross, M. R. Parental care and the evolution of egg size in fishes. *The American*
251 *Naturalist* **129**, 32–46 (1987).
- 252 29. Maino, J. L. & Kearney, M. R. Ontogenetic and interspecific metabolic scaling in insects. *The American*
253 *Naturalist* **184**, 695–701 (2014).
- 254 30. Iwata, K. & Sakagami, S. F. Gigantism and dwarfism in bee eggs in relation to the modes of life, with notes on
255 the number of ovarioles. *Japanese Journal of Ecology* **16**, 4–16 (1966).
- 256 31. Church, S. H., Donoughe, S. D., De Medeiros, B. A. S. & Extavour, C. G. A database of egg size and shape
257 from more than 6,700 insect species. *bioRxiv*. doi:[10.1101/471953](https://doi.org/10.1101/471953) (2018).
- 258 32. Rainford, J. L., Hofreiter, M., Nicholson, D. B. & Mayhew, P. J. Phylogenetic distribution of extant richness
259 suggests metamorphosis is a key innovation driving diversification in insects. *PLoS One* **9**, 1–7 (2014).
- 260 33. Harmon, L. J. *et al.* Early bursts of body size and shape evolution are rare in comparative data. *Evolution* **64**,
261 2385–2396 (2010).

- 262 34. Uyeda, J. C., Hansen, T. F., Arnold, S. J. & Pienaar, J. The million-year wait for macroevolutionary bursts.
263 *Proceedings of the National Academy of Sciences of the United States of America* **108**, 15908–15913 (2011).
- 264 35. Cooper, N. & Purvis, A. Body size evolution in mammals: complexity in tempo and mode. *The American*
265 *Naturalist* **175**, 727–738 (2010).
- 266 36. Peters, R. H. & Wassenberg, K. The effect of body size on animal abundance. *Oecologia* **60**, 89–96 (1983).
- 267 37. Sieg, A. E. *et al.* Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models
268 matter? *The American Naturalist* **174**, 720–733 (2009).
- 269 38. Polilov, A. A. Small is beautiful: features of the smallest insects and limits to miniaturization. *Annual Review*
270 *of Entomology* **60**, 103–121 (2015).
- 271 39. Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. & Charnov, E. L. Effects of size and temperature on
272 metabolic rate. *Science* **293**, 2248–2251 (2001).
- 273 40. Felsenstein, J. Phylogenies and the comparative method. *The American Naturalist* **125**, 1–15 (1985).
- 274 41. Rensch, B. Histological changes correlated with evolutionary changes of body size. *Evolution* **2**, 218–230
275 (1948).
- 276 42. Rainford, J. L., Hofreiter, M. & Mayhew, P. J. Phylogenetic analyses suggest that diversification and body size
277 evolution are independent in insects. *BMC Evolutionary Biology* **16**, 8 (2016).
- 278 43. Roff, D. A. The evolution of flightlessness in insects. *Ecological Monographs* **60**, 389–421 (1990).
- 279 44. Whiting, M. F., Bradler, S. & Maxwell, T. Loss and recovery of wings in stick insects. *Nature* **421**, 264 (2003).
- 280 45. Trueman, J., Pfeil, B., Kelchner, S. & Yeates, D. Did stick insects really regain their wings? *Systematic Entomology*
281 **29**, 138–139 (2004).
- 282 46. Stancă-Moise, C. *et al.* Migratory species of butterflies in the surroundings of Sibiu (Romania). *Scientific*
283 *Papers Series Management, Economic Engineering in Agriculture and Rural Development* **16**, 319–324 (2016).
- 284 47. Ivanova-Kasas, O. M. in *Developmental Systems: Insects* 243–271 (Academic Press, London, 1972).

6 Methods

6.1 Data availability

The database of insect eggs is publicly available at Dryad <https://datadryad.org/review?doi=doi:10.5061/dryad.pv40d2r> and described in Church *et al.* 2018¹. All code required to reproduce the analyses and figures shown here is available at https://github.com/shchurch/Insect_Egg_Evolution. The phylogenetic posterior distributions are provided as supplemental files ‘phylogeny_posterior_distribution_misof_backbone.nxs’ and ‘phylogeny_posterior_distribution_rainford_backbone.nxs’.

6.2 Creating the insect egg database

A list of the 1,756 literature sources used to generate the egg database is provided as a supplemental file, ‘bibliography_egg_database.pdf’. A full description of the methods used to assemble the insect egg database have been published separately¹. Egg descriptions were collected from published accounts of insect eggs using custom software to parse text from PDFs and measure published images (Fig. 1B), followed by manual verification. Each entry in the egg database includes a reference to an insect genus and, when reported, species name. Scientific names were validated using TaxReformer¹, which relies on online taxonomic databases^{2–6}.

6.3 Measuring egg features

Full trait definitions are described in the Supplementary Information and summarized briefly below. To resolve ambiguous cases and to measure published images, we used the definitions below.

Egg length: We defined egg length as the distance in millimeters (mm) of the axis of rotational symmetry.

Egg width: We defined egg width as the widest diameter (mm), measured perpendicular to the axis of rotational symmetry of the egg. For eggs described in published records as having both a width and breadth or depth (i.e. the egg is a flattened ellipsoid⁷), we defined width as the wider of the two diameters, and breadth as the diameter perpendicular to both the width and length.

Egg volume: Volume (mm³) was calculated using the equation for the volume of an ellipsoid: $\frac{1}{6}lw^2$, following previous workers^{8,9}.

Egg aspect ratio: Aspect ratio was calculated as the ratio of length to width.

Egg asymmetry: Asymmetry was calculated as the ratio between the two egg diameters at the first and third quartile of the length axis. The first quartile was always defined as the larger of the two diameters, such that asymmetry is always between zero and one.

Angle of egg curvature: The angle of curvature was measured as the angle (degrees) of the arc created by the endpoints and midpoint of the length axis.

315 **6.4 Phylogenetic methods**

316 A genus-level phylogeny was built by combining mitochondrial 18S and 28S sequence data from the SILVA
317 database^{10–13} with phylogenetic constraints from published higher-level insect phylogenies^{14,15}. To account for
318 phylogenetic uncertainty in comparative analyses, trees were estimated using a hierarchical approach^{16,17}. Separate
319 phylogenies for each insect order were inferred in a Bayesian framework using MrBayes v. 3.2.6¹⁸ and 100 randomly
320 chosen post-burn-in trees for each order using the order-level backbone trees of Rainford et al.¹⁵ and Misof et al.¹⁴.
321 See Supplemental Information, section “Sequence alignment and phylogenetic methods” for more details.

322 **6.5 Annotating the egg database with developmental trait data**

323 For developmental traits, a set of references were assembled from the embryological and ecological literature, and then
324 used to compile data on interval between syncytial mitoses, time to cellularization, and duration of embryogenesis.
325 Developmental rate observations were rescaled to approximate rates at a standardized temperature of 20°C following
326 previous work¹⁹. For a full list of sources, methods used in this calculation, and further discussion of developmental
327 trait definitions, see the Supplemental Information, section “Collecting developmental time data”.

328 **6.6 Annotating the egg database with life-history trait data**

329 For each of the ecological features of interest (internal parasitic oviposition, aquatic oviposition, flightlessness, and
330 migratory behavior) taxonomic descriptions from the literature were matched to taxa in the insect egg database. For
331 some taxonomic groups it was not possible to classify all members unambiguously. In these cases, the ecological
332 state was coded “uncertain”, and the potential impact of this uncertainty on results was tested. For each trait the
333 ancestral state reconstruction was estimated using an equal-rates model (R package corHMM²⁰, function rayDISC,
334 node.states=‘marginal’). For a full list of sources and methods used see the Supplemental Information, section
335 “Evolutionary history of ecological traits”.

336 **6.7 Data analysis and evolutionary model comparison**

337 Egg length, width, volume, and aspect ratio were log₁₀ transformed. Angle of curvature and asymmetry were square
338 root transformed.

339 Models of evolution were compared using the R package geiger²¹. For each trait (egg length, width, volume, aspect
340 ratio, asymmetry, and angle of curvature), the model fit of a Brownian Motion (BM), Ornstein-Uhlenbeck (OU),
341 and Early-Burst (EB) model was compared against a null hypothesis of a white noise (WN) model that assumes
342 no evolutionary correlation. See Supplemental Information, section ‘Evolutionary model fitting’ for details. The
343 performance of the best fitting model was further analyzed by comparing expected values of parameters from
344 simulations under the model to observed parameters, using the R package arbutus²².

345 The ancestral state of volume, aspect ratio, and angle of curvature were mapped on the summary phylogeny using
346 the R package phytools²³ (version 0.6-44, function contMap). Evolutionary rate regimes of volume, aspect ratio,
347 and the angle of curvature were fit on the summary phylogeny using the program BAMM^{24,25} (version 2.5.0, R
348 package BAMMtools version 2.1.6, setBAMMpriors, prior for expected number of shifts set to 10, for 10,000,000
349 generations).

350 Following ancestral state reconstruction for ecological regimes (see above), for each trait (volume, aspect ratio,
351 asymmetry, curvature) the fit of a Brownian-Motion model (BM), an Ornstein-Uhlenbeck model with a single
352 optimum (OU1), and an Ornstein-Uhlenbeck model with an independent optimum for each ecological state (OUM)
353 were compared using the R package OUwie²⁶ (version 1.50).

354 All evolutionary regression analyses were performed using a phylogenetic generalized least squares (PGLS) approach
355 in the R packages ape²⁷ (version 5.0, correlation structure = corBrownian) and nlme²⁸ (version 3.1-131.1). Given
356 that the EB models best fit the data, we also tested a corBlomberg correlation structure, which invokes an accelerating-
357 decelerating model of evolution, with the decelerating rate of trait change fixed at 1.3.

358 For comparisons performed at the genus level, each regression was repeated over 100 trees randomly drawn from the
359 posterior distribution randomly selecting a representative egg database entry per genus. For comparisons performed
360 at the family level, each regression was repeated 100 times calculating the family level average egg data from 50% of
361 entries per family.

362 For phylogenetic regressions controlling for a third variable, we calculated the phylogenetic residuals of each variable
363 against the dependent variable, and then calculated the phylogenetic regression of the residuals²⁹. To test alternative
364 hypotheses, new data were simulated using a fixed scaling exponent and the parameters of the best fitting model
365 with the R package phylolm³⁰ (version 2.5, function 'rTrait').

366 Allometric regressions were performed over all insect taxa as well as for seven monophyletic groups of insects
367 individually (Palaeoptera, Polyneoptera, Condylgnatha, Hymenoptera, Neuropteroidea, Amphiesmenoptera,
368 Antliophora). In addition, the scaling exponent between egg length and width was calculated for each monophyletic
369 group of taxa with more than 20 tips but fewer than 50.

370 **6.8 Statistical Information**

371 For evolutionary regressions and parametric bootstraps, a significance threshold of 0.01 was used. All p-values
372 were rounded to the nearest hundredth. Exact values for all statistical comparisons are available in the figure
373 legends and Supplemental Information. For evolutionary model comparisons, weighted AICc values were compared
374 at a significance threshold of 2. Evolutionary regressions were performed 100 times each, taking into account
375 phylogenetic and phenotypic uncertainty. For more details see Supplemental Information, section “Calculating
376 allometric exponents using phylogenetic generalized least squares (PGLS)”.

377 **Methods References**

- 378 1. Church, S. H., Donoughe, S. D., De Medeiros, B. A. S. & Extavour, C. G. A database of egg size and shape
379 from more than 6,700 insect species. *bioRxiv*. doi:[10.1101/471953](https://doi.org/10.1101/471953) (2018).
- 380 2. Patterson, D., Mozzherin, D., Shorthouse, D. P. & Thessen, A. Challenges with using names to link digital
381 biodiversity information. *Biodiversity Data Journal* (2016).
- 382 3. Pyle, R. L. Towards a global names architecture: The future of indexing scientific names. *ZooKeys* **550**, 261–
383 281 (2016).
- 384 4. Rees, J. & Cranston, K. Automated assembly of a reference taxonomy for phylogenetic data synthesis. *Biodi-
385 versity Data Journal* **5**, e12581 (2017).
- 386 5. Hinchliff, C. E. *et al.* Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceedings of
387 the National Academy of Sciences of the United States of America* **112**, 12764–12769 (2015).
- 388 6. GBIF. *GBIF: The Global Biodiversity Information Facility* 2018.
- 389 7. Clark, J. The capitulum of phasmid eggs (Insecta: Phasmida). *Zoological Journal of the Linnean Society* **59**,
390 365–375 (1976).
- 391 8. Markow, T., Beall, S. & Matzkin, L. Egg size, embryonic development time and ovoviviparity in *Drosophila*
392 species. *Journal of Evolutionary Biology* **22**, 430–434 (2009).
- 393 9. García-Barros, E. Body size, egg size, and their interspecific relationships with ecological and life history traits in
394 butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biological Journal of the Linnean Society* **70**, 251–284
395 (2000).
- 396 10. Glöckner, F. O. *et al.* 25 years of serving the community with ribosomal RNA gene reference databases and
397 tools. *Journal of Biotechnology* **261**, 169–176 (2017).
- 398 11. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based
399 tools. *Nucleic Acids Research* **41**, 590–596 (2013).
- 400 12. Yilmaz, P. *et al.* The SILVA and “all-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids
401 Research* **42**, 643–648 (2014).
- 402 13. Pruesse, E., Peplies, J. & Glöckner, F. O. SINA: Accurate high-throughput multiple sequence alignment of
403 ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829 (2012).
- 404 14. Misof, B. *et al.* Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763–767
405 (2014).
- 406 15. Rainford, J. L., Hofreiter, M., Nicholson, D. B. & Mayhew, P. J. Phylogenetic distribution of extant richness
407 suggests metamorphosis is a key innovation driving diversification in insects. *PLoS One* **9**, 1–7 (2014).
- 408 16. Smith, S. A. & Brown, J. W. Constructing a broadly inclusive seed plant phylogeny. *American Journal of
409 Botany* **105**, 302–314 (2017).
- 410 17. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and
411 time. *Nature* **491**, 444–448 (2012).
- 412 18. Ronquist, F. *et al.* MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large
413 model space. *Systematic Biology* **61**, 539–542 (2012).

- 414 19. Maino, J. L., Pirtle, E. I. & Kearney, M. R. The effect of egg size on hatch time and metabolic rate: theoretical
415 and empirical insights on developing insect embryos. *Functional Ecology* **31**, 227–234 (2017).
- 416 20. Beaulieu, J. M., O’Meara, B. C. & Donoghue, M. J. Identifying hidden rate changes in the evolution of a
417 binary morphological character: the evolution of plant habit in campanulid angiosperms. *Systematic Biology*
418 **62**, 725–737 (2013).
- 419 21. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. GEIGER: investigating evolutionary
420 radiations. *Bioinformatics* **24**, 129–131 (2007).
- 421 22. Pennell, M. W., FitzJohn, R. G., Cornwell, W. K. & Harmon, L. J. Model adequacy and the macroevolution
422 of angiosperm functional traits. *The American Naturalist* **186**, E33–E50 (2015).
- 423 23. Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in*
424 *Ecology and Evolution* **3**, 217–223 (2012).
- 425 24. Rabosky, D. L. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic
426 trees. *PLoS One* **9**, e89543 (2014).
- 427 25. Rabosky, D. L. *et al.* BAMM tools: an R package for the analysis of evolutionary dynamics on phylogenetic
428 trees. *Methods in Ecology and Evolution* **5**, 701–707 (2014).
- 429 26. Beaulieu, J. M., Jhwueng, D.-C., Boettiger, C. & O’Meara, B. C. Modeling stabilizing selection: expanding the
430 Ornstein–Uhlenbeck model of adaptive evolution. *Evolution* **66**, 2369–2383 (2012).
- 431 27. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinfor-*
432 *matics* **20**, 289–290 (2004).
- 433 28. Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. R Core Team (2014) nlme: linear and nonlinear mixed effects
434 models. R package version 3.1-117. Available at <http://CRAN.R-project.org/package=nlme> (2014).
- 435 29. Revell, L. J. Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution* **1**,
436 319–329 (2010).
- 437 30. Tung Ho, L. S. & Ané, C. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models.
438 *Systematic Biology* **63**, 397–408 (2014).