ARTICLE

https://doi.org/10.1038/s41586-019-1302-4

Insect egg size and shape evolve with ecology but not developmental rate

Samuel H. Church[1,4]*, Seth Donoughe[1,3,4], Bruno A. S. de Medeiros[1] & Cassandra G. Extavour[1,2]*

Over the course of evolution, organism size has diversified markedly. Changes in size are thought to have occurred because of developmental, morphological and/or ecological pressures. To perform phylogenetic tests of the potential effects of these pressures, here we generated a dataset of more than ten thousand descriptions of insect eggs, and combined these with genetic and life-history datasets. We show that, across eight orders of magnitude of variation in egg volume, the relationship between size and shape itself evolves, such that previously predicted global patterns of scaling do not adequately explain the diversity in egg shapes. We show that egg size is not correlated with developmental rate and that, for many insects, egg size is not correlated with adult body size. Instead, we find that the evolution of parasitoidism and aquatic oviposition help to explain the diversification in the size and shape of insect eggs. Our study suggests that where eggs are laid, rather than universal allometric constants, underlies the evolution of insect egg size and shape.

Size is a fundamental factor in many biological processes. The size of an organism may affect interactions both with other organisms and with the environment[1,2], it scales with features of morphology and physiology[3], and larger animals often have higher fitness[4]. Previous studies have aimed to identify the macroevolutionary forces that explain the observed distributions in animal size[1,5,6]. However, the limited availability of data on the phylogenetic distribution of size has precluded robust tests of the predicted forces[4,7]. Here we address this problem by assembling a dataset of insect egg phenotypes with sufficient taxon sampling to rigorously test hypotheses about the causes and consequences of size evolution in a phylogenetic framework.

Insect eggs are a compelling system with which to test macroevolutionary hypotheses. Egg morphologies are extraordinarily diverse[8], yet they can be readily compared across distant lineages using quantitative traits. Changes in egg size have been studied in relation to changes in other aspects of organismal biology[9], including adult body size[10–12], features of adult anatomy[13] and offspring fitness through maternal investment[14]. Eggs must also withstand the physiological challenges of being laid in diverse microenvironments, including in water, air, or inside plants or animals[15]. Furthermore, because the fertilized egg is the homologous, single-cell stage in the lifecycle of multicellular organisms, egg size diversity is relevant to the evolution of both cell size and organism size[8,14].

Three classes of hypotheses have been proposed to explain the evolution of egg size and shape. The first suggests that geometric constraints due to the physical scaling of size and shape explain the diversity of egg morphology[13,16–19]. The second suggests that there is an interaction between egg size and the rate of development[20–22]. Finally, the third suggests that the diversification of size and shape is a response to ecological or life-history changes[10,13,15,23]. We use a phylogenetic approach to test all three of these hypotheses, and show that many presumed universal patterns in the size, shape and embryonic development of eggs are not supported across insects. Instead, we find that models that account for ecological changes best explain the morphological diversity in eggs of extant insects..

Using custom bioinformatics tools, we assembled a dataset of 10,449 published descriptions of eggs, comprising 6,706 species, 526 families and every currently described extant hexapod order[24] (Fig. 1a and Supplementary Fig. 1). We combined this dataset with backbone hexapod phylogenies[25,26] that we enriched to include taxa within the egg morphology dataset (Supplementary Fig. 2) and used it to describe the distribution of egg shape and size (Fig. 1b). Our results showed that insect eggs span more than eight orders of magnitude in volume (Fig. 1a, c and Supplementary Fig. 3) and revealed new candidates for the smallest and largest described insect eggs: respectively, these are the parasitoid wasp *Platygaster vernalis*[27] (volume $= 7 \times 10^{-7}$ mm^3; Fig. 1c) and the earth-boring beetle *Bolboleaus hiaticollis*[28] (volume $= 5 \times 10^{2}$ mm^3; Fig. 1c).

Plotting eggs by morphology revealed that some shapes evolved only in certain clades (Fig. 1a and Supplementary Figs. 4–7). For example, oblate ellipsoid eggs (aspect ratio < 1) are found only in stoneflies, moths and butterflies (Plecoptera and Lepidoptera; Fig. 1c, Supplementary Figs. 4, 5). Egg cases (oothecae) have evolved in multiple insect lineages[29]. To test whether oothecae constrain shape or size, we measured individual eggs within cases, and found that these eggs are morphologically similar to those of freely laid relatives (Supplementary Fig. 8). The most prominent pattern was that distantly related insects have converged on similar morphologies many times independently (Fig. 1a and Supplementary Fig. 7). This high degree of morphological convergence allowed us to robustly test trait associations across independent evolutionary events.

Evolutionary allometry of insect eggs

Two opposing hypotheses based on predicted geometric constraints have been proposed to explain the evolutionary relationship between egg shape and size. One hypothesis posits that when eggs evolve to be larger, they become wider (increases in egg size are associated with decreases in aspect ratio)[17,18]. This hypothesis predicts a reduction in relative surface area as size increases, which has been proposed as a solution to the presumed cost of making eggshell material[18]. The alternative hypothesis proposes that when eggs evolve to be larger, they become longer (increases in egg size are associated with increases in aspect ratio)[13,18,19]. This hypothesis predicts a reduction in relative cross-sectional area as eggs become larger, which has been proposed

[1]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA. [2]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA. [3]Present address: Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, USA. [4]These authors contributed equally: Samuel H. Church, Seth Donoughe. *e-mail: church@g.harvard.edu; extavour@oeb.harvard.edu

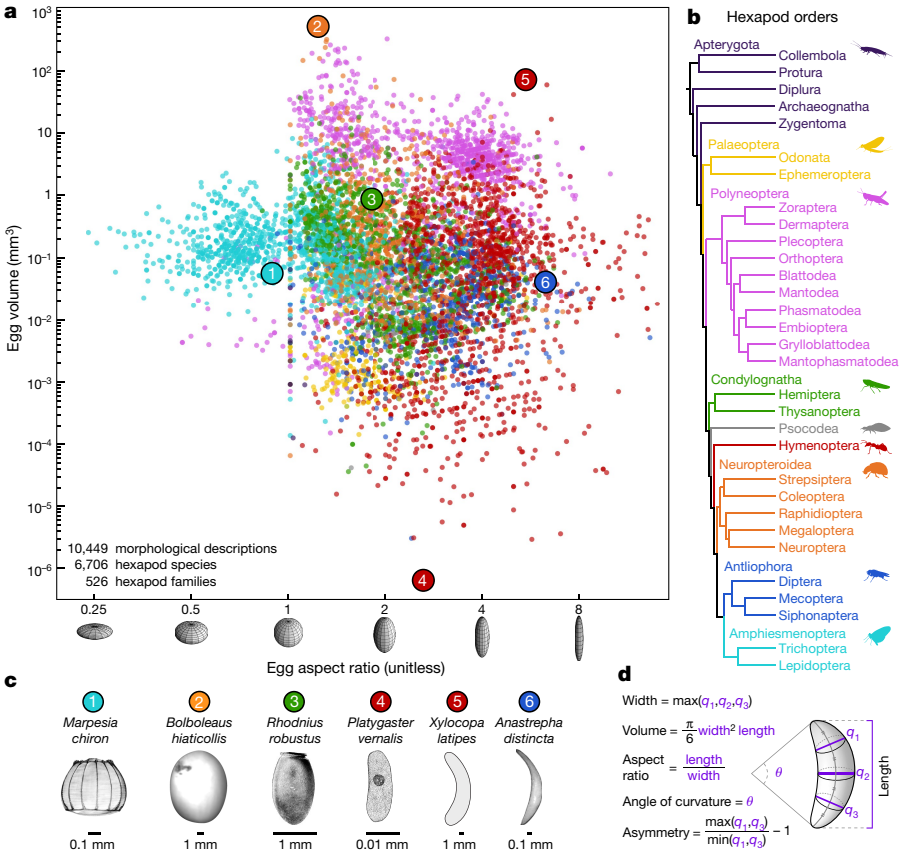

Fig. 1 | The shapes and sizes of hexapod eggs. a, Eggs are plotted in a morphospace defined by volume (mm^3) and aspect ratio (unitless) on a log scale. Points are coloured by clades as shown in **b**. **b**, Relationships are shown according to a previous study[25], one of the backbone phylogenies used in this study. Numbered points correspond to six eggs shown in **c**. **c**, Eggs selected to show a range of sizes and shapes, arranged by aspect ratio[27,28,48–51]. **d**, Size and shape are described using six features, calculated as shown.

as a solution to the need for eggs to pass through a narrow opening during oviposition[13,19].

To test these hypotheses about the physical scaling of size and shape, we began by modelling the evolutionary history of each morphological trait. This allowed us to determine whether distributions of extant shape and size have been shaped by phylogenetic relationships. For egg volume, aspect ratio, asymmetry and angle of curvature (Fig. 1d), we compared four models of evolution: Brownian motion, Brownian motion with evolutionary friction (Ornstein–Uhlenbeck), Brownian motion with a decreasing rate of evolution (early burst) and a non-phylogenetic model of stochastic motion (white noise). We found that models that accounted for phylogenetic covariance fit our data better than a non-phylogenetic model (white noise); in other words, the morphology of insect eggs tends to be similar in closely related insects (Supplementary Table 5). For egg size and aspect ratio, an early burst model in which evolutionary rate decreases over time, best describes the data (Supplementary Figs. 9–11). In previous studies, early burst models were rarely detected[30]. However, our findings are consistent with recent studies evaluating datasets that—similar to our data—comprise many taxa and orders of magnitude in morphological variation[31,32]. Having established appropriate phylogenetic models, we used these results to test hypotheses about the relationship between egg shape and size.

To test which aforementioned scaling relationship best describes insect egg evolution, we compared support for each of the two opposing hypotheses described above using a phylogenetic generalized least-squares approach to determine the scaling exponent of length and width (the slope of the regression of log-transformed length and log-transformed width). A slope less than one would support the first hypothesis (Fig. 2a), whereas a slope greater than one would support the second hypothesis[33] (Fig. 2b). An alternative third hypothesis is that egg shape remains the same as size changes; this would result in a slope near one (an isometric relationship; Fig. 2c). The relationships describing these hypotheses are shown in Fig. 2a–d. We found that across all insects, the second hypothesis is best supported: larger eggs have higher aspect ratios than smaller eggs ($0 < P < 0.005$, slope $= 0.78$; Fig. 2e and Supplementary Table 6), even when controlling for adult body size (Supplementary Fig. 14 and Supplementary Table 8). We found no support for the first hypothesis, which suggests that future hypotheses of egg shell evolution may need to account for additional factors such as chorion composition and thickness when considering potential fitness cost. However, the allometric relationship between size and shape evolves dynamically across the phylogeny, which has also been shown for metabolic scaling in mammals[34]. The third hypothesis, isometry, could not be rejected for beetles and their relatives, nor for butterflies, moths and caddisflies (respectively, Neuropteroidea $P = 0.04$ and Amphiesmenoptera $P = 0.01$; Fig. 2f, Supplementary Fig. 12 and Supplementary Table 7). Calculating the scaling relationship on lineage subgroups revealed that additional clades, including mayflies, crickets and shield bugs, also show an isometric relationship (Supplementary Fig. 13). The marked differences in scaling exponents are evidence that egg evolution was not governed by a universal allometric constant. Instead, evolutionary forces beyond the constraints of physical scaling (for example, development or ecology) are required to explain the morphological diversification of insect eggs.

Developmental traits and egg evolution

The egg is the starting material for embryogenesis, and the size of the hatchling is directly related to the size of the egg at fertilization[35]. It has been reported that embryogenesis takes longer in species with larger eggs[22] and that this relationship could influence size evolution[20,21]. This would be consistent with the observation that larger adult species

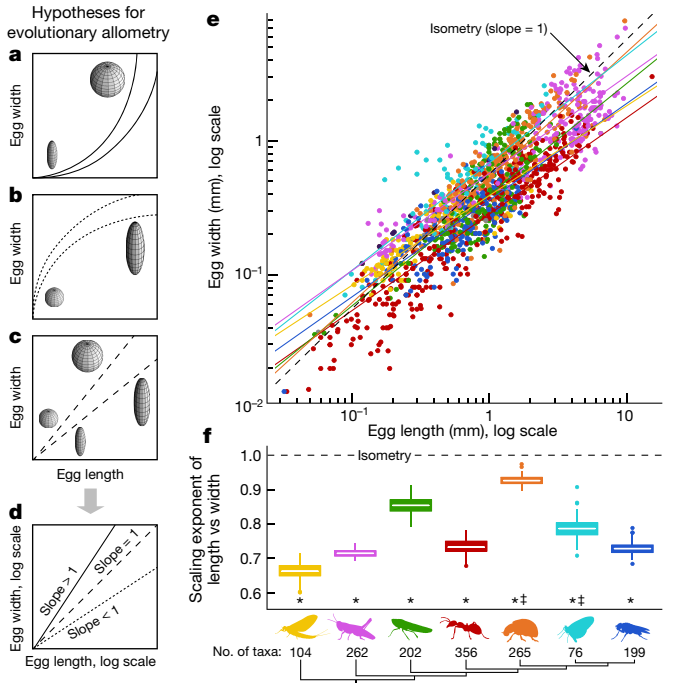

Fig. 2 | The allometric relationship of egg shape and size evolves across insects. a–c, Hypothesized relationships between size and shape: larger eggs are proportionally wider (**a**, solid line); larger eggs are proportionally longer (**b**, dotted line); shape and size scale isometrically (**c**, dashed line). **d**, Each hypothesis predicts a different scaling exponent—the slope of the regression between the log-transformed length and log-transformed width. Lines are as in **a–c**. **e**, Egg length and width plotted in log–log space. The dashed line represents a hypothetical 1:1 relationship (**c**). Solid lines are clade-specific phylogenetic generalized least-squares regressions; points are randomly selected representatives per genus. *n* numbers (genera): Palaeoptera, $n = 104$; Polyneoptera, $n = 262$; Condylognatha, $n = 202$; Hymenoptera, $n = 356$; Neuropteroidea, $n = 265$; Amphiesmenoptera, $n = 76$; Antliophora, $n = 199$. **f**, The distribution of scaling exponents from phylogenetic generalized least-squares regressions, calculated over the posterior distribution. White lines, boxes, bars and dots represent median, 25–75th percentiles, 5–95th percentiles and outliers, respectively. Asterisks indicate a significant relationship ($P < 0.01$, exact values are shown in Supplementary Table 6) and double daggers indicate that the relationship is not distinguishable from isometry ($P > 0.01$, exact values are shown in Supplementary Table 7). $n = 100$ phylogenetic generalized least-squares regressions. Colours correspond to Fig. 1b.

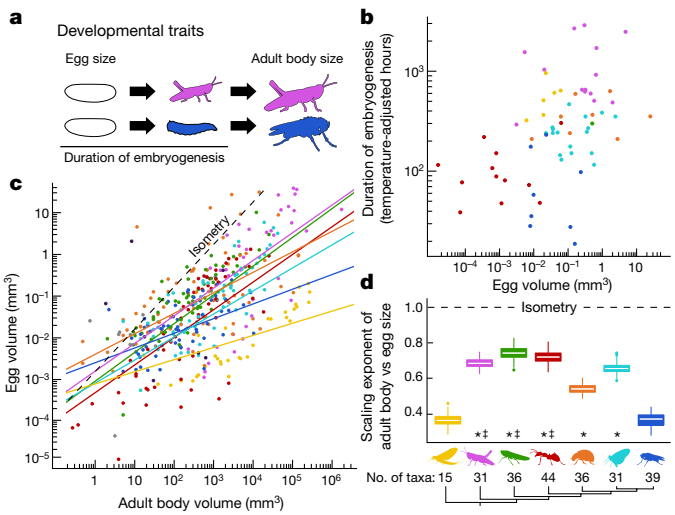

Fig. 3 | Developmental features do not co-vary with egg size. a, Mature eggs undergo embryonic development, hatch and grow into adults. **b**, Egg volume (mm^3) compared to duration of embryogenesis, defined as time from egg laying to hatching (hours), adjusted for incubation temperature. When phylogeny is accounted for, there is no significant relationship. **c**, Egg volume (mm^3) compared to adult body volume, calculated as body length cubed (mm^3). Dashed line represents a hypothetical 1:1 relationship (isometry). Solid lines are clade-specific phylogenetic generalized least-squares regressions; points are family- or order-level average egg size and median adult size. *n* numbers (family- or order-level averages): Palaeoptera, $n = 15$; Polyneoptera, $n = 31$; Condylognatha, $n = 36$; Hymenoptera, $n = 44$; Neuropteroidea, $n = 36$; Amphiesmenoptera, $n = 31$; Antliophora, $n = 39$. **d**, The distribution of scaling exponents from phylogenetic generalized least-squares regressions. White lines, boxes, bars and dots represent median, 25–75th percentiles, 5–95th percentiles and outliers, respectively. Asterisks indicate a significant relationship ($P < 0.01$, exact values are shown in Supplementary Table 12) and double daggers indicate that the relationship is not distinguishable from isometry ($P > 0.01$, exact values are shown in Supplementary Table 13). $n = 100$ phylogenetic generalized least-squares regressions. Colours correspond to Fig. 1b.

have lower metabolic rates than smaller species[36]. To test this prediction across our egg dataset, we assembled published embryological records, and found that simply comparing egg volume and duration of embryogenesis yields the previously reported positive relationship[22] (Supplementary Fig. 17). However, a linear regression that does not account for phylogenetic relationships is inappropriate for this analysis owing to the covariance of traits on an evolutionary tree[37]. When we accounted for phylogenetic covariance, we found that there was no significant relationship between egg size and duration of embryogenesis across insects, such that eggs of very different sizes develop at a similar rate and vice versa ($0.02 < P < 0.10$; Fig. 3b and Supplementary Table 11). These results suggest that the often-invoked trade-off between size and development[20–22] does not hold across insects.

We also tested the hypothesis that the size of the egg has a positive relationship with adult body size. Previous studies have reported this relationship in subsets of insects and have suggested that smaller insects lay proportionally larger eggs for their bodies[11,35,38]. Such a relationship between egg size and body size would result in an allometric scaling exponent that is less than one. We combined our dataset of egg size with published adult body length data for insect families[39], and found that this relationship was not generalizable across all insect lineages. For example, in flies and their relatives (Antliophora), as well as in mayflies and odonates (Palaeoptera), egg size is not predicted by body size, meaning that insects of similar body size lay eggs of different sizes (Antliophora $P = 0.02$, Palaeoptera $P = 0.19$; Fig. 3c, d and Supplementary Table 13). In Polyneoptera, thrips and true bugs (Condylognatha), and bees, ants and wasps (Hymenoptera), an isometric relationship between egg size and body size cannot be rejected (Polyneoptera $P = 0.02$, Hymenoptera $P = 0.01$, Condylognatha $P = 0.01$; Supplementary Fig. 18 and Supplementary Table 13). In general, the predictive power of the relationship between body size and egg size is low: average egg volume can vary by up to four orders of magnitude among species with a similar body size (Fig. 3c).

At the time of fertilization an egg is a single cell. We therefore tested whether the size of this cell evolved with the size of the genome, as has been observed for other cell types[40], using a database of genome size for hexapods[41]. Although the data appeared to show a positive relationship between egg size and genome size (Supplementary Table 14), we found that this relationship was driven entirely by the lineage Polyneoptera (specifically grasshoppers, Acrididae). This lineage has evolved genome sizes that are an order of magnitude larger than other insects and has relatively large eggs (Supplementary Fig. 19). Across other insect lineages, egg volume and genome size are not significantly related ($0 < P < 0.08$; Supplementary Table 14), and egg volume can range over six orders of magnitude for species with a similar genome size (Supplementary Fig. 19c). This indicates that genome size is not a general driver of egg size. The decoupling of genome size, body size and developmental rate from the evolution of egg sizes suggests that the diversification of insect eggs has not been universally constrained by development.

Oviposition ecology explains egg morphology

Egg size and shape have been predicted to evolve in response to changes in life history and ecology. Recent studies in birds have highlighted one such relationship, suggesting that birds with increased flight capability have more elliptical and asymmetrical eggs[13]. We investigated whether an analogous relationship exists between insect flight capability and egg shape. Unlike birds, insects have undergone hundreds of evolutionary shifts to flightless and even wingless forms[42]. We focused on two clades in which flight evolution has been extensively studied. Stick insects (Phasmatodea) have flightless and wingless species[43,44] (Supplementary Fig. 22), and many butterflies (Lepidoptera) show migratory behaviour[45], which we used as a proxy for increased flight capability relative to non-migratory taxa (Supplementary Fig. 22). We found that, in contrast to birds, evolutionary changes in flight ability in these two insect clades were not associated with changes in egg shape (Ornstein–Uhlenbeck model with multiple optima per regime; ΔAICc (Akaike information criterion) < 2, exact values are included in Supplementary Tables 18, 19).

Similar to flight capacity, the microenvironment that insect eggs experience varies widely, including being exposed to air, submerged or floating in water, or contained within a host animal[8]. Each microenvironment places different demands on the egg, such as access to oxygen and water during development[15]. Preliminary studies in small groups of insects have suggested that evolutionary changes in oviposition ecology and life history may drive the evolution of egg size and shape[10,23]. To test this prediction across all insects, we compiled records on two modes of oviposition ecology that have been extensively studied: oviposition within an animal host (internal parasitic oviposition) and oviposition in or on water. For each mode, we reconstructed ancestral changes along the insect phylogeny, and found that both aquatic and internal parasitic oviposition modes have been gained and lost multiple times independently (Fig. 4a, b and Supplementary Figs. 20, 21). This extensive convergent evolution allowed us to perform a strong test of whether egg size and shape evolution are explained by the evolution of oviposition ecology.

We found that the evolution of new oviposition environments is linked to changes in egg size and shape. Models that accounted for shifts to either aquatic or internal parasitic oviposition better explained size and shape distributions than models that did not (Ornstein–Uhlenbeck model, ΔAICc > 2, exact values are shown in Supplementary Tables 15–17). In this analysis, we compared model fit for each ecology–trait pair separately, and found that these two ecological states were correlated with different egg morphologies. Specifically, shifts to aquatic oviposition were significantly associated with the evolution of smaller eggs with a lower aspect ratio (Fig. 4c, d and Supplementary Table 17), whereas shifts to internal parasitic oviposition were significantly associated with smaller, more asymmetric eggs (Fig. 4c, e and Supplementary Table 15). Moreover, we note that the smallest eggs are from parasitoid wasps that develop polyembryonically (that is, multiple embryos form from a single egg[46]; Supplementary Fig. 23). Neither oviposition mode is associated with consistent changes in the allometric relationship between size and shape (Supplementary Fig. 24).

Given that Ornstein–Uhlenbeck models can be favoured when dataset size and measurement error are large[47], we repeated these analyses 100 times using simulated ecological states independent of egg morphological traits. The results of this bootstrap analysis showed that our observed result, which favoured ecological models of morphological evolution, is unlikely to be caused by dataset size alone ($P = 0.01$; Supplementary Table 20). Moreover, these results were robust to uncertainty in phylogenetic relationships, and to uncertainty in how taxa were classified for oviposition ecology (Supplementary Table 16). These findings provide evidence that the microenvironment that is experienced by the egg has had an important role in morphological evolution.

Insect eggs present an ideal case for testing the predictability of macroevolutionary patterns in size and shape. By comparing insect egg size and shape, we found that previous hypotheses about evolutionary trade-offs with developmental time, body size or the presumed cost of egg shells do not hold. Although we showed that developmental time is not linked to egg size, we suggest that other features of development (for example, cell number and distribution) may scale in predictable ways across eight orders of magnitude in egg size. Finally, we provide evidence that the ecology of oviposition drives the evolution of egg size and shape.

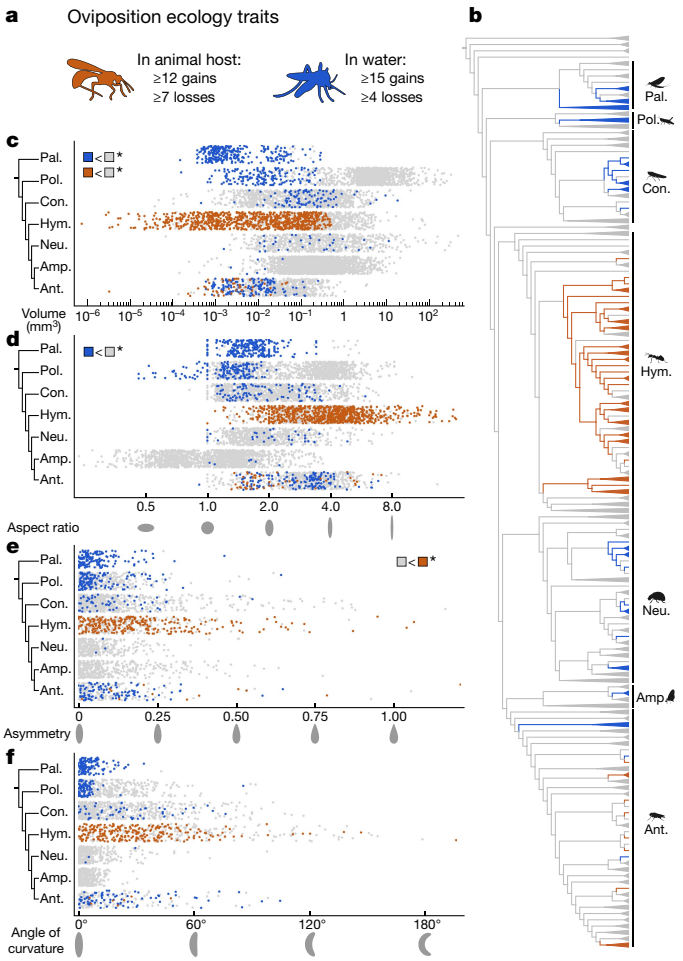

Fig. 4 | Shifts in oviposition ecology are associated with changes in egg morphology. a, Two modes of oviposition ecology: laying eggs within an animal host (orange; for example, parasitoid wasps), and in water (blue; for example, mosquitoes). Other oviposition substrates (for example, terrestrial or within plants) are shown in grey. **b**, Ancestral state reconstruction of oviposition mode reveals both evolved multiple times (see Supplementary Figs. 17, 18). **c**–**f**, The distribution of egg features, coloured by ecology. **c**, Volume (mm^3; shown on a log scale). **d**, Aspect ratio (unitless; shown on a log scale). **e**, Asymmetry (unitless). **f**, Angle of curvature (degrees). Asterisks indicate that the model that accounts for ecology fits the data better than a non-ecological model (Ornstein–Uhlenbeck model with multiple optima, ΔAICc > 2, exact values are shown in Supplementary Tables 14–19).

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1302-4.

Received: 28 November 2018; Accepted: 14 May 2019;
Published online 3 July 2019.

1. Peters, R. H. *The Ecological Implications of Body Size* (Cambridge Univ. Press, 1983).
2. Allen, R. M., Buckley, Y. M. & Marshall, D. J. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *Am. Nat.* **171**, 225–237 (2008).
3. Blanckenhorn, W. U. The evolution of body size: what keeps organisms small? *Q. Rev. Biol.* **75**, 385–407 (2000).

4. Kingsolver, J. G. & Pfennig, D. W. Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution* **58**, 1608–1612 (2004).
5. Stanley, S. M. An explanation for Cope's rule. *Evolution* **27**, 1–26 (1973).
6. LaBarbera, M. Analyzing body size as a factor in ecology and evolution. *Annu. Rev. Ecol. Syst.* **20**, 97–117 (1989).
7. Chown, S. L. & Gaston, K. J. Body size variation in insects: a macroecological perspective. *Biol. Rev. Camb. Philos. Soc.* **85**, 139–169 (2010).
8. Hinton, H. E. *Biology of Insect Eggs* vols I–III (Pergammon, 1981).
9. Thompson, D. W. *On Growth and Form* (Cambridge Univ. Press, 1917).
10. Fox, C. W. & Czesak, M. E. Evolutionary ecology of progeny size in arthropods. *Annu. Rev. Entomol.* **45**, 341–369 (2000).
11. Berrigan, D. The allometry of egg size and number in insects. *Oikos* **60**, 313–321 (1991).
12. García-Barros, E. Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biol. J. Linn. Soc.* **70**, 251–284 (2000).
13. Stoddard, M. C. et al. Avian egg shape: form, function, and evolution. *Science* **356**, 1249–1254 (2017).
14. Bernardo, J. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *Am. Zool.* **36**, 216–236 (1996).
15. Hinton, H. E. Respiratory systems of insect egg shells. *Annu. Rev. Entomol.* **14**, 343–368 (1969).
16. Legay, J. M. Allometry and systematics of insect egg form. *J. Nat. Hist.* **11**, 493–499 (1977).
17. Blackburn, T. Evidence for a 'fast-slow' continuum of life-history traits among parasitoid Hymenoptera. *Funct. Ecol.* **5**, 65–74 (1991).
18. Kratochvíl, L. & Frynta, D. Egg shape and size allometry in geckos (Squamata: Gekkota), lizards with contrasting eggshell structure: why lay spherical eggs? *J. Zoological Syst. Evol. Res.* **44**, 217–222 (2006).
19. Bilder, D. & Haigo, S. L. Expanding the morphogenetic repertoire: perspectives from the *Drosophila* egg. *Dev. Cell* **22**, 12–23 (2012).
20. Steele, D. & Steele, V. Egg size and duration of embryonic development in Crustacea. *Int. Rev. Gesamten Hydrobiol. Hydrograph.* **60**, 711–715 (1975).
21. Sargent, R. C., Taylor, P. D. & Gross, M. R. Parental care and the evolution of egg size in fishes. *Am. Nat.* **129**, 32–46 (1987).
22. Maino, J. L. & Kearney, M. R. Ontogenetic and interspecific metabolic scaling in insects. *Am. Nat.* **184**, 695–701 (2014).
23. Iwata, K. & Sakagami, S. F. Gigantism and dwarfism in bee eggs in relation to the modes of life, with notes on the number of ovarioles. *Jap. J. Ecol.* **16**, 4–16 (1966).
24. Church, S. H., Donoughe, S. D., de Medeiros, B. A. S. & Extavour, C. G. A dataset of egg size and shape from more than 6,700 insect species. *Sci. Data* https://doi.org/10.1038/s41597019–0049-y (2019).
25. Misof, B. et al. Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763–767 (2014).
26. Rainford, J. L., Hofreiter, M., Nicholson, D. B. & Mayhew, P. J. Phylogenetic distribution of extant richness suggests metamorphosis is a key innovation driving diversification in insects. *PLoS ONE* **9**, e109085 (2014).
27. Leiby, R. & Hill, C. The polyembryonic development of *Platygaster vernalis*. *J. Agric. Res.* **28**, 829–839 (1924).
28. Houston, T. F. Brood cells, life-cycle stages and development of some earth-borer beetles in the genera *Bolborhachium, Blackburnium* and *Bolboleaus* (Coleoptera: Geotrupidae), with notes on captive rearing and a discussion of larval diet. *Aust. Entomol.* **55**, 49–62 (2016).
29. Goldberg, J. et al. Extreme convergence in egg-laying strategy across insect orders. *Sci. Rep.* **5**, 7825 (2015).
30. Harmon, L. J. et al. Early bursts of body size and shape evolution are rare in comparative data. *Evolution* **64**, 2385–2396 (2010).
31. Uyeda, J. C., Hansen, T. F., Arnold, S. J. & Pienaar, J. The million-year wait for macroevolutionary bursts. *Proc. Natl Acad. Sci. USA* **108**, 15908–15913 (2011).
32. Cooper, N. & Purvis, A. Body size evolution in mammals: complexity in tempo and mode. *Am. Nat.* **175**, 727–738 (2010).
33. Peters, R. H. & Wassenberg, K. The effect of body size on animal abundance. *Oecologia* **60**, 89–96 (1983).
34. Sieg, A. E. et al. Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models matter? *Am. Nat.* **174**, 720–733 (2009).
35. Polilov, A. A. Small is beautiful: features of the smallest insects and limits to miniaturization. *Annu. Rev. Entomol.* **60**, 103–121 (2015).
36. Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. & Charnov, E. L. Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251 (2001).
37. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).
38. Rensch, B. Histological changes correlated with evolutionary changes of body size. *Evolution* **2**, 218–230 (1948).
39. Rainford, J. L., Hofreiter, M. & Mayhew, P. J. Phylogenetic analyses suggest that diversification and body size evolution are independent in insects. *BMC Evol. Biol.* **16**, 8 (2016).
40. Gregory, T. R. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev. Camb. Philos. Soc.* **76**, 65–101 (2001).
41. Gregory, T. R. Animal Genome Size Database. Release 2.0 http://www.genomesize.com (2019).
42. Roff, D. A. The evolution of flightlessness in insects. *Ecol. Monogr.* **60**, 389–421 (1990).
43. Whiting, M. F., Bradler, S. & Maxwell, T. Loss and recovery of wings in stick insects. *Nature* **421**, 264–267 (2003).
44. Trueman, J., Pfeil, B., Kelchner, S. & Yeates, D. Did stick insects really regain their wings? *Syst. Entomol.* **29**, 138–139 (2004).
45. Stancă-Moise, C. et al. Migratory species of butterflies in the surroundings of Sibiu (Romania). *Sci. Pap. Ser. Manage. Econ. Eng. Agric. Rural Dev.* **16**, 319–324 (2016).
46. Ivanova-Kasas, O. M. in *Developmental Systems: Insects* vol. 1 (eds Counce, S. J. & Waddington, C. H.) Ch. 5, 243–271 (Academic, 1972).
47. Cooper, N., Thomas, G. H., Venditti, C., Meade, A. & Freckleton, R. P. A cautionary note on the use of Ornstein Uhlenbeck models in macroevolutionary studies. *Biol. J. Linn. Soc.* **118**, 64–77 (2016).
48. Nieves-Uribe, S., Flores-Gallardo, A., Hernández-Mejía, B. C. & Llorente-Bousquets, J. Exploración morfológica del corion en Biblidinae (Lepidoptera: Nymphalidae): aspectos filogenéticos y clasificatorios. *Southwest. Entomol.* **40**, 589–648 (2015).
49. Barata, J. M. S. Morphological aspects of Triatominae eggs. II. Macroscopic and exochorial characteristics of ten species of the genus Rhodnius Stal, 1859 (Hemiptera - Reduviidae) (in Portuguese). *Rev. Saude Publica* **15**, 490–542 (1981).
50. Iwata, K. The comparative anatomy of the ovary in Hymenoptera (records on 64 species of Aculeata in Thailand, with descriptions of ovarian eggs). *Mushi* **38**, 101–109 (1965).
51. Dutra, V. S., Ronchi-Teles, B., Steck, G. J. & Silva, J. G. Egg morphology of *Anastrepha* spp. (Diptera: Tephritidae) in the fraterculus group using scanning electron microscopy. *Ann. Entomol. Soc. Am.* **104**, 16–24 (2011).

Acknowledgements This work was supported by the National Science Foundation (NSF) under grant no. IOS-1257217 to C.G.E., NSF GRFP DGE1745303 to S.H.C. and by a Jorge Paulo Lemann Fellowship to B.A.S.d.M. from Harvard University. We thank members of the Extavour laboratory and B. Farrell, C. Dunn, D. McCoy, D. Rice, A. Kao, E. Kramer, J. Boyle, L. Bittleston, M. Srivastava, M. Johnson, P. Wilton, R. Childers and S. Prado-Irwin for discussion, and the Ernst Mayr Library at the Museum of Comparative Zoology at Harvard, and specifically M. Sears, for assistance in gathering references.

Reviewer information *Nature* thanks Clay Cressler and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions S.H.C. and S.D. conceived the project and generated the dataset. S.H.C. performed statistical analyses. B.A.S.d.M. performed phylogenetic analyses. All authors contributed to experimental design, interpretation and writing.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1302-4.

Correspondence and requests for materials should be addressed to S.H.C. or C.G.E.

METHODS

Creating the insect egg dataset. A list of the 1,756 literature sources used to generate the egg dataset is provided in the Supplementary Information. A full description of the methods used to assemble the insect egg dataset has been published elsewhere[24]. Egg descriptions were collected from published accounts of insect eggs using custom software to parse text from PDFs and measure published images (Fig. 1d), followed by manual verification. Each entry in the egg dataset includes a reference to an insect genus and, when reported, species name. Scientific names were validated using TaxReformer[24], which relies on online taxonomic databases[52–56]. The final sample size of the dataset (over 10,000 egg descriptions) was determined to be sufficient because it included thousands of instances of repeated evolution of similar egg size and shape.

Measuring egg features. Full trait definitions are described in the Supplementary Information and summarized in brief 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 millimetres (mm) from one end to the other 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 (that is, the egg is a flattened ellipsoid[57]), 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 (mm^3) was calculated using the equation for the volume of an ellipsoid: $(1/6)\pi lw^2$, following previous studies[12,58].

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, minus one. The first quartile was always defined as the larger of the two diameters.

Angle of egg curvature. The angle of curvature was measured as the angle (degrees) of the arc created by the end points and mid-point of the length axis.

Phylogenetic methods. A genus-level phylogeny was built by combining mitochondrial 18S and 28S sequencing data from the SILVA database[59–62] with phylogenetic constraints from published higher-level insect phylogenies[25,26]. To account for phylogenetic uncertainty in comparative analyses, trees were estimated using a hierarchical approach[63,64]. Separate phylogenies for each insect order were inferred in a Bayesian framework using MrBayes v.3.2.6[65] and 100 post-burn-in trees were randomly chosen for each order using the order-level backbone trees of two previous studies[25,26]. See Supplementary Information for further details.

Annotating the egg dataset with developmental trait data. For developmental traits, a set of references was assembled from the embryological and ecological literature, and then used to compile data on interval between syncytial mitoses, time to cellularization and duration of embryogenesis. Developmental rate observations were rescaled to approximate rates at a standardized temperature of 20 °C following previous studies[66]. For a full list of sources, methods used in this calculation, and further discussion of developmental trait definitions, see Supplementary Information.

Annotating the egg dataset with life-history trait data. For each of the ecological features of interest (internal parasitic oviposition, aquatic oviposition, flightlessness and migratory behaviour), taxonomic descriptions from the literature were matched to taxa in the insect egg dataset. For some taxonomic groups, it was not possible to classify all members unambiguously. In these cases, the ecological state was coded 'uncertain' and the potential effect of this uncertainty on results was tested. For each trait the ancestral state reconstruction was estimated using an equal-rates model (R package corHMM[67], function rayDISC, node.states = marginal). For a full list of sources and methods used see Supplementary Information.

Data analysis and evolutionary model comparison. Egg length, width, volume and aspect ratio were $\log_{10}$-transformed. Angle of curvature and asymmetry were square-root-transformed.

Models of evolution were compared using the R package geiger[68]. For each trait (egg length, width, volume, aspect ratio, asymmetry and angle of curvature), the model fits of Brownian motion, Ornstein–Uhlenbeck and early-burst models were compared against a null hypothesis of a white noise model that assumes no evolutionary correlation (see Supplementary Information for details). The performance of the best-fitting model was further analysed by comparing expected values of parameters from simulations under the model to observed parameters using the R package arbutus[69].

The ancestral state of volume, aspect ratio and angle of curvature were mapped on the summary phylogeny using the R package phytools[70] (v.0.6-44, function contMap). Evolutionary rate regimes of volume, aspect ratio and the angle of curvature were fitted on the summary phylogeny using the program BAMM[71,72] (v.2.5.0, R package BAMMtools v.2.1.6, setBAMMpriors, prior for expected number of shifts set to 10, for 10,000,000 generations).

All evolutionary regression analyses were performed using a phylogenetic generalized least-squares approach in the R packages ape[73] (v.5.0, correlation structure = corBrownian) and nlme[74] (v.3.1-131.1). Given that the early-burst models best fit the data, we also tested a corBlomberg correlation structure, which invokes an accelerating–decelerating model of evolution, with the decelerating rate of trait change fixed at 1.3.

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

For phylogenetic regressions controlling for a third variable, we calculated the phylogenetic residuals of each variable against the dependent variable, and then calculated the phylogenetic regression of the residuals[75]. To test alternative hypotheses, new data were simulated using a fixed scaling exponent and the parameters of the best-fitting model with the R package phylolm[76] (v.2.5, function 'rTrait').

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

Following ancestral state reconstruction of ecological regimes, for each ecology–trait pair (internal parasitic or aquatic oviposition combined with volume, aspect ratio, asymmetry or curvature) the fit of a Brownian motion model, an Ornstein–Uhlenbeck model with a single optimum and an Ornstein–Uhlenbeck model with an independent optimum for each ecological state were compared using the R package OUwie[77] (version 1.50). These analyses were repeated over 100 trees randomly drawn from the posterior distribution, and randomly selecting a representative egg for each genus.

Plots were generated in R. Figures were assembled with Adobe Illustrator. Egg images that were reproduced from other publications were converted to greyscale, contrast adjusted, rotated, and then masked from their backgrounds using Adobe Photoshop.

Statistical information. For evolutionary regressions and parametric bootstraps, a significance threshold of 0.01 was used. All *P* values were rounded to the nearest hundredth. Exact values for all statistical comparisons are available in the figure legends and Supplementary Information. For evolutionary model comparisons, weighted AICc values were compared at a significance threshold of 2. Evolutionary regressions were performed 100 times each, taking into account phylogenetic and phenotypic uncertainty. For more details see Supplementary Information.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The dataset of insect eggs is publicly available at Dryad (https://datadryad.org) with doi:10.5061/dryad.pv40d2r and has been described elsewhere[24]. The phylogenetic posterior distributions are provided as Supplementary Information (phylogeny_posterior distribution_misof_backbone.nxs and phylogeny_posterior_distribution_rainford_backbone.nxs).

Code availability

All code required to reproduce the analyses and figures shown here is available at https://github.com/shchurch/Insect_Egg_Evolution.

52. Patterson, D., Mozzherin, D., Shorthouse, D. P. & Thessen, A. Challenges with using names to link digital biodiversity information. *Biodivers. Data J.* **4**, e8080 (2016).
53. Pyle, R. L. Towards a global names architecture: the future of indexing scientific names. *ZooKeys* **550**, 261–281 (2016).
54. Rees, J. A. & Cranston, K. Automated assembly of a reference taxonomy for phylogenetic data synthesis. *Biodivers. Data J.* **5**, e12581 (2017).
55. Hinchliff, C. E. et al. Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proc. Natl Acad. Sci. USA* **112**, 12764–12769 (2015).
56. GBIF. GBIF: The Global Biodiversity Information Facility https://www.gbif.org/en/ (2018).
57. Clark, J. The capitulum of phasmid eggs (Insecta: Phasmida). *Zool. J. Linn. Soc.* **59**, 365–375 (1976).
58. Markow, T. A., Beall, S. & Matzkin, L. M. Egg size, embryonic development time and ovoviviparity in *Drosophila* species. *J. Evol. Biol.* **22**, 430–434 (2009).
59. Glöckner, F. O. et al. 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J. Biotechnol.* **261**, 169–176 (2017).
60. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2013).
61. Yilmaz, P. et al. The SILVA and "all-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res.* **42**, D643–D648 (2014).
62. Pruesse, E., Peplies, J. & Glöckner, F. O. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829 (2012).

63. Smith, S. A. & Brown, J. W. Constructing a broadly inclusive seed plant phylogeny. *Am. J. Bot.* **105**, 302–314 (2018).
64. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. *Nature* **491**, 444–448 (2012).
65. Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
66. Maino, J. L., Pirtle, E. I. & Kearney, M. R. The effect of egg size on hatch time and metabolic rate: theoretical and empirical insights on developing insect embryos. *Funct. Ecol.* **31**, 227–234 (2017).
67. Beaulieu, J. M., O'Meara, B. C. & Donoghue, M. J. Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Syst. Biol.* **62**, 725–737 (2013).
68. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131 (2008).
69. Pennell, M. W., FitzJohn, R. G., Cornwell, W. K. & Harmon, L. J. Model adequacy and the macroevolution of angiosperm functional traits. *Am. Nat.* **186**, E33–E50 (2015).
70. Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).
71. Rabosky, D. L. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* **9**, e89543 (2014).
72. Rabosky, D. L. et al. Bamm tools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods Ecol. Evol.* **5**, 701–707 (2014).
73. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
74. Pinheiro, J. et al. nlme: linear and nonlinear mixed effects models. R package version 3.1-117 https://cran.r-project.org/web/packages/nlme/index.html (2014).
75. Revell, L. J. Phylogenetic signal and linear regression on species data. *Methods Ecol. Evol.* **1**, 319–329 (2010).
76. Tung Ho, L. s. & Ané, C. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. *Syst. Biol.* **63**, 397–408 (2014).
77. Beaulieu, J. M., Jhwueng, D.-C., Boettiger, C. & O'Meara, B. C. Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. *Evolution* **66**, 2369–2383 (2012).

nature research

Corresponding author(s): Samuel H. Church and Cassandra G. Extavour

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Data in this study were collected from descriptions of insect eggs in the primary literature. We used custom software to extract text descriptions and measure published images. All code used to generate the insect egg dataset is made freely available. Python code used to compile the dataset and extract text information from sources, as well as the R code used to convert the raw dataset to the final dataset is available at https://github.com/shchurch/Insect_Egg_Evolution. Python code used to measure published images of eggs is available at https://github.com/sdonoughe/Insect_Egg_Image_Parser. Python code to cross-reference the egg dataset with taxonomic tools is available at https://github.com/brunoasm/TaxReformer.
Data analysis	All code required to reproduce the analyses in this study is made freely and publicly available at https://github.com/shchurch/Insect_Egg_Evolution., directory 'analyze_data'. The software R, version 3.4.2, was used for all statistical analyses. Additional versions for R packages are listed in the methods and on the github repository.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data were made available with no restrictions. Egg data files have been uploaded to Dryad https://datadryad.org/review?doi=doi:10.5061/dryad.pv40d2r. The final data files include the raw dataset in tab delimited format, which includes all values extracted from the text and images, as well as the final dataset in tab delimited format. The code to convert the raw dataset to the final dataset, as well the code to generate all figures is located in https://github.com/shchurch/Insect_Egg_Evolution, directory analyze_data. This code can be executed directly from that directory, with the versions specified therein, and no additional information required.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	The study describes the evolutionary analyses of egg size and shape from thousands of insect species. The dataset was assembled from the primary literature following an explicit and reproducible workflow. Phylogenies were assembled that were enriched for taxa in the egg dataset, and incorporated published relationships between insects. Using the dataset and phylogenies, regression analyses and ecological model comparisons were performed to test trait relationships across taxonomic groups. Regression analyses were performed 100 times to assess the sensitivity of results to both phylogenetic and phenotypic variation. Ecological model comparisons were performed over a series of classification methods to assess sensitivity to bias in ecological definitions. Significance thresholds were set for p-values < 0.01, and for model comparisons, deltaAICc > 2. In all comparisons, the maximum number of descriptions that had both phylogenetic and phenotypic data were used. All results were robust to measures of sensitivity - no results were excluded from the publication based on conflicting or negative outcomes.
Research sample	The research sample used to generate these results is a dataset of hexapod egg measurements collected from the primary literature. Hexapods were chosen as the appropriate scale because existing hypotheses about egg size and shape were made based on preliminary hexapod data. The dataset was collected from the literature following the methods described in Church et al. "A dataset of egg size and shape from more than 6,700 insect species", Scientific Data, (2019). The sample was collected using methods to maximize the number of descriptions as well as the representation across the phylogeny. The sample includes representatives from every major lineage, and our results assessing sampling bias indicated that our sampling scales with the diversity of described insects per lineage, such that most lineages have 1 representative per 100 species (see Church et al. 2018). The final sample size of the dataset (>10,000 egg descriptions) was determined to be sufficient because it included thousands of instances of repeated evolution of similar egg size and shape. This allowed for robust tests of evolutionary patterns and hypotheses.
Sampling strategy	Evolutionary analyses were performed in such a way as to maximize the number of samples that could be compared using an evolutionary tree. Regression analyses were repeated 100 times to include both the effects of phylogenetic uncertainty, as well as the sampling uncertainty within an insect clade. This was accomplished by choosing a random tree from the posterior distribution, and by choosing a random representative description for each taxon, for each of the 100 repeated analyses. The sample size of each lineage specific regression was determined by the maximum number of egg descriptions which were available and could be placed on an enriched phylogenetic tree. Clades with too few taxa that met these criteria (threshold < 20 taxa, e.g. Psocodea) were excluded from the analyses.
Data collection	The data was originally recorded by many thousands of entomologists, in separate publications, over 250 years. The data was aggregated following an explicit and reproducible workflow, which included using a number of predetermined search terms to query online databases and gather relevant publications. We used custom software (made freely available) to then extract egg descriptions from the literature, maximizing both the number of descriptions and the consistency across publications.
Timing and spatial scale	Online literature databases were queried for relevant publications between October 2015 to August 2017, after which all predetermined terms had been searched and data collection was stopped. Publications were not excluded based on geography or language.
Data exclusions	No text descriptions of eggs were excluded from the study, but a select number of re-measurements of published images of eggs were excluded based on sensitivity tests of the image measuring software using simulated egg shapes. Our analysis of this software indicated that in particular extreme combinations of traits, the software was less accurate in measuring features of egg shape (see Church et al. 2018). Therefore, using a pre-determined exclusion criterion based these results, the top 0.01% of entries for aspect ratio and asymmetry were excluded (~10 entries each), and curvature data was excluded for eggs with an aspect ratio <1. No further

data was excluded from any evolutionary analysis (e.g. regressions, model comparisons).

Reproducibility	All experiments performed here are fully reproducible using the R code available at https://githu,b.com/shchurch/Insect_Egg_Evolution. All the data required to generate the figures is included in that repository, and a description of each code file is provided. In no case was an analysis repeated which provided a different result or a failed result, compared to what is reported here.
Randomization	For evolutionary analyses, a random tree from the posterior distribution and a representative egg description for each taxon was randomly chosen for each iteration of the regression experiments. Randomness was determined by shuffling the datasets in R.
Blinding	The data collection was not fully blinded, as the custom software cannot currently fully automate the process of data extraction from the literature. Therefore all data collection was assisted automatically based on explicit rules, and then manually verified. Evolutionary analyses were blinded, given that analyses for each lineage, model, or trait comparison were performed exactly equivalently using objective criteria (e.g. predetermined significance thresholds) and results were reported exactly as generated by R.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging