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Insect egg morphology: evolution, development, and ecology

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The insect egg can be viewed through many lenses: it is the single-celled developmental stage, a resource investment in the next generation, an unusually large and complex cell type, and the protective vessel for embryonic development. In this review, I describe the morphological diversity of insect eggs and then identify recent advances in understanding the patterns of egg evolution, the cellular mechanisms underlying egg development, and notable aspects of egg ecology. I also suggest areas for particularly promising future research on insect egg morphology; these topics touch upon diverse areas such as tissue morphogenesis, life history evolution, organismal scaling, cellular secretion, and oviposition ecology.

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Introduction

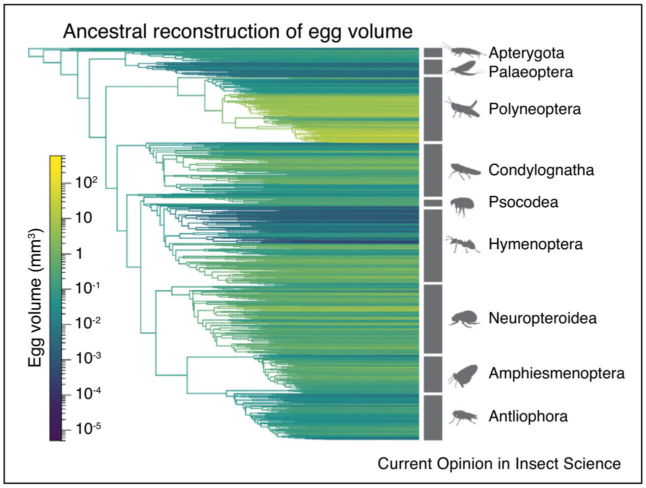
> I have a small box full of insect's eggs in my collection, so very curious, that I should not think a particular treatise ill bestowed upon them; for they all greatly differ from one another in shape and colour. Some are oblong, others oval; some again perfectly round, others angular; some pear-shaped [. . .]. Some also are soft, others hard; some are only covered with a slight membrane, whilst others have a shell or firm crust, like parchment. Again, some are sheltered by a froth that surrounds them, others are covered with hair; some are found fastened by a viscous matter to the branches of trees, so as to form a ring about them; other lie parallel to the horizon; and some are found buried in animal and vegetable substances, whilst others are only laid in a loose manner upon the surfaces of such things.[1]
>
> –17th century naturalist Jan Swammerdam

Instead of Swammerdam's box of insect eggs, we can now pore over a much larger collection: the full corpus of published morphological descriptions. This is the collective product of many naturalists and taxonomists, with particularly extensive contributions from those few who found insect eggs so enthralling that they devoted years to cataloging their multifold varieties. In the past, such biologists included Kunio Iwata [1], Takashi Kobayashi [2], Massimo Mazzini [3], and Alvah Peterson [4]; contemporary researchers in this vein include Selami Candan [5], Irina Dolinskaya [6•], Jorge Llorente-Bousquets [7•], and Jerome Rozen Jr. [8•]. Building on this work, several efforts have been made to compile traits from these published descriptions into comprehensive datasets [9–11,12••].

In this review, I draw on those assembled datasets to describe the primary axes of insect egg variation, and then highlight a selection of studies that address aspects of the evolution, development, and ecology of these diverse egg morphologies. Sections 'Life history strategy, developmental rate, and egg size', 'Ecological context of intraspecific variation in egg size', and 'Patterns of egg shape evolution' predominantly highlight findings from the most recent effort to compile and analyze insect egg traits at a broad phylogenetic scale [12••,13••]; other sections draw mainly from studies that that focus on one or a few taxa at a time. I also direct readers to sources that cover subtopics in further detail. For instance, a more thorough treatment of how insect eggs develop can be found in the recent Special Issue on Insect Oogenesis in the present journal [14]. Moreover, many single-species egg-related topics have been investigated most extensively in *Drosophila melanogaster*. This research has been particularly informative in uncovering developmental mechanisms. Because that literature rivals the scale of research on the rest of insect species combined, I have aimed to downweight the coverage of *D. melanogaster* here on those topics for which I can direct readers to other recent reviews.

[1] Quotation from the English translation of *The Book of Nature*, published posthumously in 1758 (Swammerdam, 1758).

Figure 1



Inferred ancestral states of insect egg volume.
A dataset of egg volumes was assembled from the published literature, and then used to describe the extant range and to infer the ancestral states of egg size. Volumes span more than eight orders of magnitude. Labeled groups are monophyletic clades, with the exception of Apterygota, which is polyphyletic [85]. Figure modified from Church *et al.* [13••].

Egg size

The eggs of extant insects span at least a 100 million-fold range in volume, and there have been many independent increases and decreases over the course of evolution (Figure 1) [13••]. Put differently, this is a range of eight orders of magnitude, roughly the same volume fold-change as from the moon to the sun, or from the adult body size of the smallest extant mammal to the largest. Some of the largest insect eggs are 10–15 mm in length, such as those from earth-boring beetles in the genus *Bolboleaus* [15], carpenter bees in the genus *Xylocopa* [16], and stick insects in the genus *Haaniella* [17]. The smallest eggs are 20–100 μm in length, and most of them are laid by parasitoid wasps, such as *Trichogramma cacoeciae* [18], *Aprostocetus procerae* [19], and *Platygaster vernalis* [20]; many of the parasitoid species with the smallest eggs also have polyembryonic development [13••].

Life history strategy, developmental rate, and egg size

Efforts to understand macroevolutionary patterns of egg size variation have focused on determining whether life history traits have co-evolved predictably with egg size. One long-standing hypothesis is that the energy investment in producing an egg co-varies positively with its size, which results in a generalized trade-off between egg size and fecundity [21]. A recent study tested this hypothesis by using ovariole number as a proxy for fecundity, and then quantitatively assessing the relationship between ovariole number and egg size in four clades of insects, finding that the hypothesized relationship held true in Drosophilidae, but not in three other insect clades [22]. Another life history concept extends the well-described scaling relationship between organismal size and metabolic rate to embryogenesis, hypothesizing that developmental rate should be negatively related to egg size [23–25]. A recent analysis showed that after accounting for the evolutionary relationships among taxa, the hypothesis was not supported across insects as a whole [13••].

Ecological context of intraspecific variation in egg size

The eggs of a given species vary in size, with a range in volume that is typically much less than an order of magnitude [12••]. In some cases, researchers have identified the ecological and behavioral factors that co-vary with intraspecific egg size differences. In the solitary bee *Megachile rotundata*, for instance, egg size varies seasonally [26]. In the desert locust *Schistocerca gregaria*, egg size varies with the crowdedness of the conditions experienced by the mother [27]. It is clear that in many species there exists a developmental process that enables females to adjust egg size, but it is unclear what conditions give rise to the evolutionary emergence of this trait. It is also not known how external stimuli are transduced in the body, ultimately causing changes in the process of oogenesis.

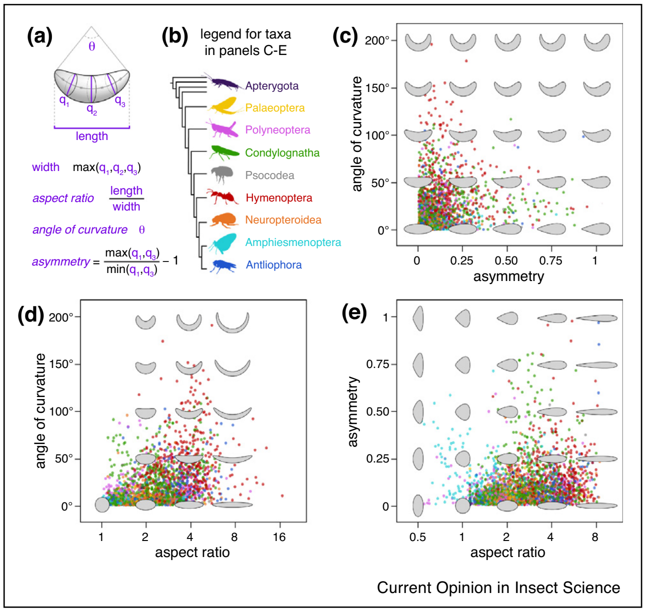
Developmental control of egg size

One approach to understanding the genetic and developmental basis of intraspecific egg size variation was conducted with *D. melanogaster*, in which researchers artificially selected independent lab populations for unusually small and large eggs. A genetically mixed pool of flies was separated into multiple populations. One group of replicate populations was selected for large eggs, another for small eggs, and some were left as control populations [28]. Over the 1.5 years of selecting (approximately 30 generations), egg volumes had increased (or decreased) in the replicate populations by roughly 10%, but it is not yet known which cellular and developmental changes were responsible for the altered egg sizes [28]. In a related experiment, researchers artificially selected for walking ability in the red flour beetle *Tribolium castaneum*, and found that the beetles with atypically high walking ability also laid eggs that were unusually small [29]. These artificially selected fruit fly and beetle lines will be a valuable resource for learning the genetic sources of intraspecific variation of egg size in a genetically tractable context.

Egg shape

For the typical insect egg, it is possible to trace an imaginary line through its middle, such that slicing orthogonal to the line at any point produces a roughly circular cross-section [12••]. Thus, an egg's shape can be approximated as a simple ellipsoid [11,30]. To quantitatively describe more of the existing shape variation, an egg can also be parameterized as an ellipsoid that has

Figure 2



Dimensions of whole-egg shape variation.
(a) A set of measurements that can be obtained from an image of an egg (see Church *et al.* [12••] for details on how each trait is defined). Traits in italics are dimensions of the morphospaces in panels **(c–e)**. **(b)** Points on panels (c–e) are colored according to these groups of insects. (c–e) Two-dimensional morphospaces illustrating the distribution of egg shapes, with each point representing an individual species. Each plot shows a pair of shape traits, along with idealized silhouettes representing the shapes of eggs throughout the morphospace. Insect eggs vary considerably in their aspect ratio, asymmetry, and angle of curvature. Note that angle of curvature is not defined for eggs with an aspect ratio at or below 1. In panels (d) and (e), aspect ratio is plotted on a log scale. Modified from Church *et al.* [13••].

been transformed to curve along an arc—and to vary asymmetrically in width—along its axis of rotational symmetry [12••]. This approximation gives rise to three predominant shape features: aspect ratio, asymmetry, and angle of curvature (Figure 2a) [12••]. Across insect species, eggs vary considerably in all three traits (Figure 2c–e).

Patterns of egg shape evolution

One issue of evolutionary allometry that has been studied in insect eggs is the question of how aspect ratio and size co-evolve. Suppose there is an evolutionary pressure for increased egg size. As evolution produces larger eggs, do they tend to become more spherical? This is what one might expect if eggshell material is particularly costly, with the evolutionary process tending to minimize surface area of the egg for a given volume. Or, alternatively, are egg sizes mostly constrained by the need to pass through an ovipositor that is developmentally costly to enlarge? In that case, we would expect that as eggs become larger, they assume a relatively higher aspect ratio, in order to maximally increase volume while minimizing the increase to cross-sectional area. Initial work on this question found a positive evolutionary allometry between egg size and aspect ratio [9], that is, eggs have tended to become relatively narrower when they have become larger over the course of evolution. A more comprehensive assessment found a similar pattern across all insects; notably, however, it also found that scaling relationships varied markedly between multiple subclades [13••].

Another way to interrogate egg shape evolution is to ask if there are shape features that have co-evolved with aspects of oviposition ecology. Both internal parasitic oviposition (laying eggs within another organism) and aquatic oviposition (laying eggs on or in water) have been evolutionarily gained and lost multiple times within insects, and it has recently been shown that both of these oviposition traits help to explain the patterns of egg shape diversification [13••]. Specifically, transitions to internal parasitic oviposition have been associated with the evolution of eggs with a higher asymmetry; transitions to aquatic oviposition have been associated with the evolution of eggs with a lower aspect ratio (and in both of these oviposition modes, eggs evolve significantly smaller sizes) [13••]. The specific evolutionary pressures underlying these trait distributions remain unknown, and it is likely that uncovering further details would require detailed work in a narrower clade, in which it is possible to parameterize egg shape traits and/or oviposition traits at a finer level of granularity. Researchers have recently done just that for stick insects [31,32,33••], establishing Phasmatodea as a promising model clade for uncovering causal relationships in the co-evolution of ecology and egg morphology.

Aspect ratio of the fruit fly egg

Even though there is a great deal of data on the diversity of whole-egg shape traits, there is only one case—the aspect ratio of the *D. melanogaster* egg—for which the trait's cellular and developmental basis has been explored in detail. Researchers have studied the various developmental processes that give rise to the particular elongated aspect ratio of the egg (i.e. an aspect ratio of ∼2.8) [30]. In doing so, they have discovered mutations in numerous genes that result in more spherical eggs (i.e. an aspect ratio closer to 1) [34]. Conspicuously, there are many more genetic manipulations that produce atypically spherical eggs, as compared to those that produce atypically elongate eggs [34]. During development, the insect egg is enclosed and supported by a sheet of cells called the *follicular epithelium*. The basal surface of these cells faces outward, and in *D. melanogaster*, it has been shown that these cells secrete an extracellular matrix called the basement membrane, which plays a crucial role in establishing the aspect ratio of the developing egg [35]. The cells are planar polarized along the circumference of the developing egg, which allows them to migrate along the interior surface of the basement membrane, causing the developing egg and the follicular epithelium to jointly rotate in place [36]. This rotation is essential for forming the proper aspect ratio of the egg because the rotational movement enables the cells to secrete oriented fibrils in the basement membrane, which in turn provides anisotropic resistance to internal forces, constraining the egg to grow preferentially along the axis of rotation [35,37]. All told, there are many morphogenetic processes required to form the correct aspect ratio of the mature egg. These include planar cell polarity [38–40], directed secretion and remodeling of extracellular matrix proteins [41,42], polarized microtubule orientation [43], cellular motility [44], and muscular contraction of the oviduct [45]. The elongation process is also regulated by hormone signaling [46]. It is an open question whether a similar developmental process establishes egg shape in taxa that are more distantly related to *D. melanogaster*, but one tantalizing datum from several decades ago is that developing eggs from the gall midge *Heteropeza pygmaea* have likewise been shown to rotate when observed in culture [78]. This would be a fruitful topic for researchers to revisit with contemporary imaging tools.

Elaborations to egg shape

The traits shown in Figure 2a—aspect ratio, asymmetry, and angle of curvature—are attributes of the egg as a whole. There are, however, many other structures on insect eggs that have been described at finer levels of detail in subsets of the insect phylogenetic tree, typically because they have proven useful for distinguishing eggs for taxonomic purposes. An *operculum* is a region of the eggshell that is specialized as a relatively weak point, often with the appearance of a "lid". When embryogenesis has completed, the hatchling ruptures the eggshell along the margin of the operculum to exit the egg. Opercula are particularly prominent in some Hemiptera [47] and Phasmatodea [32]. A *micropyle* is an entry point for sperm at the time of egg fertilization. There can be one or multiple micropyles on an egg, they can be pits or protrusions, and their morphologies have been described for many species (e.g. Refs. [48–50]). Because of the established functional genetic toolkit and live-imaging protocols, development of the micropyle of *D. melanogaster* is a promising model process for understanding how cells collectively produce a complex three-dimensional shape [51••]. There are also several types of egg *appendages*. These have been best described in *D. melanogaster* and its congeners, where the *dorsal appendages* elongate in three dimensions, ultimately forming multicellular tubular protrusions that are thought to facilitate gas exchange for the embryo [52]. Appendages feature prominently on the eggs of some other clades as well, such as in the stalked eggs of lacewings [53] and in the tubular respiratory structures of some parasitic wasps [54].

Eggshell morphology

The eggshell is an extracellular matrix that is secreted by the follicular epithelium, whereupon it assembles into a structure that protects the embryo and mediates the flux of gases and water between the contents of the egg and its environment. There are several detailed reviews on the structure, development, and function of the insect eggshells [10,55,56], with the most recent by Rezende *et al.* [57]. The eggshell is composed of a series of secreted layers that collectively contain the egg's contents, protect it from predators and parasitoids, and mediate the passage of gases and water between the developing embryo and its environment.

The evolution and development of diverse surface textures

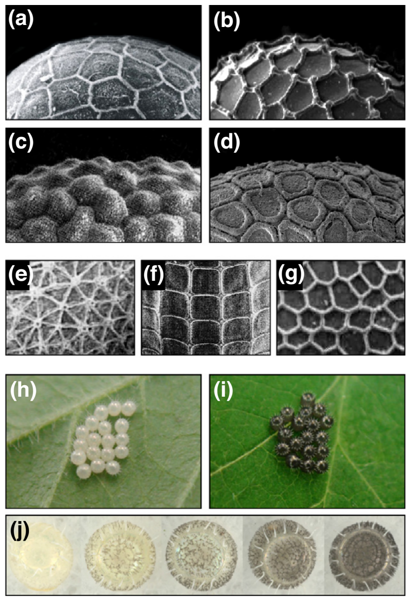
The outermost layer of the eggshell is called the chorion [57]. Since the advent of the scanning electron micrograph microscope (SEM), researchers have been capturing SEM images of egg chorions, revealing an enormous diversity of textural forms. They have found that some are largely smooth [58,59], some have ridges that span the full length of the egg [60,61], some have features that are at the scale of follicular epithelium cells [62], and others have fine-scale structures that are much smaller than the width of such cells [63,64]. Stick insects have evolved some of the most diverse and elaborate external eggshell structures [31]. Recent work has assessed evidence that the external appearance of stick insect eggs serves, at least in part, to mimic seeds—a potential case of evolutionary mimicry between animal eggs and plant 'eggs' [33••].

One conspicuous textural feature, found in many taxa, is a *closed polygonal pattern*, with a typical polygon diameter that is consistent with them being developmental 'footprints' of the interfaces of the follicular epithelium cells at the time of chorion secretion. These polygonal textures have been observed in many insect species (see Figure 3a–d for examples). They are also present in *D. melanogaster*, where it has been shown that these polygonal patterns correspond directly to the apical cell interfaces of the follicular epithelium at the time when the final layer of the egg shell is secreted [62]. Similarly, in two other species where the arrangement of cells in the follicular epithelium has been documented, their geometry also matches the closed polygonal pattern on surface of their chorions [65,66]. Thus, the polygonal patterning of eggshells is a particularly promising trait for uncovering the cellular basis of morphological diversity in the future.

Eggshell structure and egg physiology

The exchange of gas and water across the eggshell at the proper rate is crucial for embryo development within the egg [10,67]. An eggshell's morphology can include a complex internal structure and pores such as *hydropyles* and *aeropyles*; collectively these determine the respiratory traits of the egg [59,67,68]. In some insects, there is a

Figure 3



Insect eggshells vary in their surface textures and colors.
(a–d), SEM images of insect eggshells with diverse surface height patterns. Each panel image was cropped and rotated from its referenced source as follows: (a) the lowland mayfly *Ametropus fragilis* [84]; (b) the myrmecophile butterfly *Aricoris propitia* [82]; (c) the threadwing antlion *Lertha sofiae* [86]; (d) the spiny crawler mayfly *Ephemerella maculate* [81]. **(e–g)** SEM images of eggshells with diverse polygonal geometries. (e) the regal hairstreak butterfly *Evenus regalis* [80], (f) the red postman butterfly *Heliconius erato* [83], (g) the cucumber beetle *Diabrotica virgifera* [87]. **(h)** and **(i)** Females of the predatory stinkbug *Podisus maculiventris* can alter the amount of pigmentation in the eggshells of the eggs they lay. Images reproduced from Abram *et al.* [75]. (h) On the undersides of leaves they tend to lay lighter-colored eggs. (i) On the sun-facing sides of leaves they tend to lay darker-colored eggs. **(j)** The extent of egg pigmentation falls along a continuum.

period of embryogenesis during which the egg absorbs water from its environment, increasing in overall size [69,70]. The movement of water across the eggshell can be further modified by the developing embryo itself, when it secretes an additional inner layer to the eggshell called the serosal cuticle [57,79]. It has recently been shown, for instance, that in several disease vector mosquitos, the chemical composition of the serosal cuticle can have a dramatic effect on resistance to dessication in developing embryos [71]. It has also been hypothesized that the evolutionary advent of this embryo-secreted cuticle enabled hexapods to lay eggs in drier conditions, facilitating the terrestrialization of hexapods [72•].

Egg and eggshell color

Most insect eggs are white or light yellow in color, but there are some captivating exceptions, such as the turquoise eggs of *Plodia interpunctella* [73], or the jewel toned eggs of Heliconiini [74]. The color of insect eggs—and eggshell pigmentation in particular—is poorly understood from a developmental perspective. There has been more work done on the ecological and evolutionary aspects of the topic, as was described in the recent review by Guerra-Grenier [88]. One of the more remarkable recent observations in this theme concerns the predatory stinkbug *Podisus maculiventris*. It was recently discovered that females of this species can alter their egg color in response to the perceived brightness of the leaf where the eggs are laid (Figure 3e–g) [75]. Moreover, the addition of pigmentation improves survival in those offspring exposed to higher levels of UV [76•]. This simple observation—intraspecific variation in egg color—revealed a previously unknown developmental mechanism (control of pigmentation quantity) with ecological relevance to the species.

Concluding remarks

A theme of the research on insect egg morphology is that meticulous descriptive work at the single-species scale is an essential component of the larger project to reveal general patterns of evolution and previously undiscovered mechanisms of development. Doubtless there will be more such gems in the coming years, and discoveries like this will be most valuable if the data underpinning them are maximally usable by the community. Therefore, those of us engaged in the collective project to document insect egg diversity would do well to adopt the norms of *phenomics* [77]. Specifically, I urge taxonomists and entomologists to publish data in the form of high-quality images, and where possible, measure multiple quantitative traits with estimates of variability. Ultimately this will maximize the extent to which dataset can be analyzed and augmented by other scientists. Finally, there is a particularly pressing need for more preserved insect eggs to be included in museum collections. This will be a potent way to empower the researchers in the future.

Conflict of interest statement

Nothing declared.

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