Generational shape shifting: changes in egg shape and size between sexual and asexual generations of a cyclically parthenogenic gall former

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Abstract

Successive generations of multivoltine species experience selection specific to the spatiotemporal environments encountered that may lead to adaptive divergence in reproductive traits among generations. To compare reproductive effort within and between generations, appropriate volumetric models, selected on the basis of the analysis of egg shape, are required to estimate the sizes (volumes) of individual eggs. We assessed the shape and estimated the volume of individual eggs produced by the temporally and spatially segregated sexual and asexual generations of the gall former, *Belonocnema treatae* Mayr (Hymenoptera: Cynipini: Cynipidae). Egg shape, indexed as the difference between the polar and equatorial axes of the ellipsoidal eggs, was independent of egg size, but differed between generations. The relationship of egg shape and female body size within and between generations confirmed that egg shape is an intrinsic property of each generation. Generational differences in egg shape then informed the selection of volumetric models to estimate egg size. We modeled asexual generation eggs as both spheres and prolate spheroids, and sexual generation eggs as both cylinders and prolate spheroids. Choice of volumetric model changed estimates of egg size within the asexual generation by 23% and within the sexual generation by 50%. Comparisons between generations based on the above models produced estimated differences in egg volume that ranged from 16 to 114%. In both generations, a prolate spheroid was the most parsimonious model of egg volume. Based on this model, sexual generation eggs averaged 43% larger than asexual generation eggs. The increased size of sexual eggs was achieved via conservation of the egg's equatorial axis and elongation of the polar axis. The shift in egg shape between sexual and asexual *B. treatae* is the first documented dimorphism in an egg characteristic expressed between generations of a cyclically parthenogenic organism.

Introduction

Facultative or obligate multivoltine lifecycles are present in all nine orders of plant-feeding insects (Danks, 1987; Wolda, 1988). Thus, multivoltinism characterizes a great diversity of phytophagous insects. In many phytophagous insects, successive generations oviposit and/or develop using different organs on the same or different host plant species (Moran, 1992; Stone et al., 2002). This spatiotemporal variation in feeding niche gives rise to generation-specific exposure to biotic and abiotic factors that can result in selection leading to adaptation of each generation to a unique set of environmental conditions (Zera, 1984; Denno & Roderick, 1990; Stone et al., 1995). Adaptation of alternative phenotypes to specific environments extends to the reproductive biology of multivoltine insects and culminates in notable variation in reproductive traits between generations (Denno & McCloud, 1985; Blankenhorn & Fairbairn, 1995). Primary reproductive traits of insects include egg size (volume) and shape. Egg size is important because the egg phenotype represents an extended maternal phenotype that can influence initial offspring growth and survival (Fox & Czesak, 2000). Also, variation in egg size can reflect environmentally driven tradeoffs between egg size and number (Berrigan, 1991; Braby, 1994; Azeve-

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do et al., 1996; Fox & Czesak, 2000; Olofsson et al., 2009). Although a great diversity of egg shapes exists among insect taxa, including closely related taxa (Kambysellis, 1993; Garcia-Barros, 2000a; Vardal et al., 2003), the literature on the determinants and consequences of variation in egg shape is considerably less advanced than is the corresponding literature for egg size. As a consequence, we lack incisive demonstrations of the adaptive significance of variation in egg shape (Noriyuki et al., 2010). Characterizing shape is the first step in such investigations, and observations of interspecific, and as reported here, intraspecific variation in egg shape invite inquiry.

Studies that examine variation in egg size, whether at the population, generation, or species level, must ensure that appropriate volumetric models are selected to estimate egg size in the groups being compared. Assessing egg shape is a necessary pre-requisite to estimating egg volume as the determination of shape guides selection of appropriate volumetric models. This task is more difficult with complex egg shapes, but methods are well established in vertebrate taxa (Peterson, 1992; Kratochvil & Frynta, 2006). Thus, the first steps in exploring causes and consequences of variation in egg volume are reliably and accurately estimating egg shape coupled with understanding the relationships (including allometric relationships) among egg shape, egg size, and female body size within and across study groups. The specific null hypothesis being tested within each study group is that egg shape is invariant to changes in egg size and female body size. In the case of multivoltine and cyclically parthenogenic species, these relationships must be understood for each generation.

Many species of gall-forming cynipids (Hymenoptera) exhibit cyclic parthenogenesis (heterogony), whereby obligate sexual and asexual generations alternate to complete the life cycle (Pujade-Villar et al., 2001). The sexual and asexual generations of cynipids typically differ in the tissue exploited for gall formation (Stone et al., 2002), the morphology and size of galls (Stone & Schönrogge, 2003), the number of siblings contained within galls (Stone et al., 2002), and adult body size (Rokas et al., 2003). The alternate generations may also be attacked by dissimilar suites of natural enemies (Hall, 2001; Stone et al., 2002) and may differ markedly in natural enemy-related mortality (Stone et al., 1995; Hayward & Stone, 2005). Given the generational differences in ecology and life history, heterogonic cynipids provide an opportunity to examine intraspecific variation in reproductive traits in relation to the environments in which successive generations develop.

Herein, we estimated and compared the shape of eggs produced by the sexual and asexual generations of the cyclically parthenogenic gall former, *Belonocnema treatae* Mayr (Hymenoptera: Cynipidae). We then tested the competing hypotheses that the observed difference in egg shape between generations is (1) related to differences in body size of the generations, or (2) represents an intrinsic characteristic of each generation. Finally, on the basis of above analyses, we applied volumetric models to estimate and then compared the size (volume), surface area, and surface area–volume ratio of individual eggs produced by asexual and sexual females. The generational shift in egg shape described by our results represents a dimorphism expressed between generations. The difference in egg size produced by each generation highlights the importance of including both egg number and egg size, following selection of appropriate volumetric models, in comparative life history studies of multivoltine species.

Materials and methods

Study system

Belonocnema treatae is host-specific to plateau live oak, *Quercus fusiformis* Muller (Fagaceae) in the Edwards Plateau region of central Texas, USA (Lund et al., 1998). In central Texas, the asexual and sexual generations alternate to complete the yearly life cycle. Asexuals and sexuals, respectively, develop in spherical, unilocular leaf galls and multilocular root galls (Lund et al., 1998). Upon emergence in the fall, asexuals oviposit into oak rootlets. Coinciding with spring bud break sexual generation adults emerge and mate, and females then oviposit into developing leaves (Hood & Ott, 2010). Adults of both generations do not feed (Lund, 1998) and, based on detailed inspection of oviducts, females of both generations are pro-ovigenic (i.e., emerge with the entire potential lifetime complement of eggs fully matured).

Characterization of egg shape

To sample eggs produced by females of each generation, we first collected leaf and root galls from plateau live oak trees exhibiting high gall densities at Texas State University's Freeman Ranch, Hays County, TX, USA (29°55′N, 98°00′W). For each generation, galls were returned to the laboratory and husbanded until gall formers emerged. Upon emergence, *B. treatae* were cold-stored in 95% ethanol. Body size, indexed as hind tibia length (Rogers et al., 1976), was then measured using a stereo dissecting microscope fitted with an ocular micrometer for the 676 sexual and 1 155 asexual generation females that emerged. Hind tibia length is correlated with potential fecundity and potential reproductive effort (i.e., the product of potential fecundity and egg size) in both generations of *B. treatae* (JR Ott & GR Hood, unpubl.). However, tibia length is not correlated with egg size in either generation (JR Ott & GR Hood, unpubl.) and, as shown herein, is only weakly cor-

related with egg shape within either generation. To ensure equal representation of all size classes in the subsequent regressions of egg characteristics on body size (see below), we first binned females of each generation into six size classes based on tibial lengths (0.13 ± 0.03 mm width classes). From the resultant frequency distributions, we then selected five females at random from each size class in each generation. In total, 30 females were sampled for each generation. Abdomens from the 60 females were removed, placed in depression slides, and treated with a 1:1:13 acetic acid, glycerol, and water solution for 2 h to loosen the ovarian follicular tissue binding eggs to the abdomen wall. Eggs were then removed and stained with methylene blue while in the depression slide. To minimize error in counting and to preserve egg shape, eggs were counted in the depression slide.

Five randomly selected eggs from each of the 60 females were then slide-mounted within a 70% glycerol solution and measured using a Nikon SMZ1000 dissecting microscope (Nikon, Tokyo, Japan). Initial inspections showed that eggs produced by both generations consisted of an egg body (posterior end) characterized by a major and a minor axis, and a projection (peduncle) at the anterior end (Figure 1A). The peduncle in this species represents a small percent of total egg volume and was not included in estimates of egg volume. Thus, minimum estimates of egg volume are presented herein. A repeatability study showed that re-measurements of the minor axes of individual eggs varied little (r = 0.987 and 0.979, asexual and sexual generation, respectively; both n = 30). Thus, egg shape could be characterized by just two measurements: length and width. Egg length (L) was measured along the midline of the longest (major = polar) axis and egg width (W, minor = equatorial axis) was measured as the maximum width of the egg perpendicular to the major axis. Egg dimensions were measured to the nearest ±0.01 mm. The shape of each egg was defined as (L − W), a measure of two-dimensional symmetry. Values of L − W ≈ 0 indicate symmetrical dimensions, that is, perfectly spheroidal eggs, whereas increasing departure from symmetry is indicated by L − W>0. All statistical analyses were performed using the mean lengths and widths of the five eggs measured for each of the 30 females for each generation.

To test the hypothesis that egg shape differs between asexual and sexual *B. treatae*, we first compared ($\overline{L} − \overline{W}$) for the females sampled in each generation by means of a two-tailed t-test. To test the null hypothesis that egg shape was independent of a linear dimension of egg size in each generation, while controlling for the possible effect of variation in egg number per female on egg shape, we conducted an ANOVA. We used ($\overline{L} − \overline{W}$) per female as the response variable and $\overline{W}$ per female, egg number per

female, and generation coded as continuous, continuous, and categorical independent variables, respectively. To visualize the difference in egg shape between generations, $\overline{L}$ vs. $\overline{W}$ was plotted using the data from each female in each generation. A $\overline{L} : \overline{W}$ ratio of 1:1 indicates that a spherical model is appropriate to estimate egg volume, whereas a ratio >1:1 indicates that the volume of the ellipsoid shape is best estimated by a prolate spheroid. The cylinder, also indicated by a ratio >1:1, is a candidate volumetric model for the sexual generation based on the shape of eggs depicted in Figure 1A.

To determine whether observed differences in the shape of eggs produced by each generation represented an intrinsic characteristic of each generation or was related to differences in the body size of sexual and asexual females or possibly the number of eggs produced per female, we used ANOVA to test the hypothesis that ($\overline{L} − \overline{W}$) increased equivalently with body size in each generation independent of egg number per female. Following ANOVA, a variance component analysis was used to determine the proportion of overall variation in egg shape due to within and between-generation factors.

Estimation of egg volume

Following the analysis of shape, we estimated the average volume (V) of individual eggs produced by females of each generation based on a set of volumetric models that included sphere [$V = 4/3\pi r^3$, where r = ¼(L + W)], prolate spheroid [$V = 4/3\pi(½L × ½W^2)$], or cylinder [$V = \pi(½W^2 × L)$]. Paired t-tests were used to compare estimates of volume produced by the different models within generations, and unpaired two-tailed t-tests were used to compare estimates between generations. The surface areas of eggs produced by both generations were estimated using a prolate spheroid model and calculated as $2\pi(a^2 + [ab\alpha/\sin(\alpha)])$, where a is the horizontal, transverse (equatorial) radius (½W), b is the vertical, conjugate (polar) radius (½L), and α = arccos (a/b). Subsequently, the surface area–volume ratios for each generation were calculated, and both surface areas and surface area–volume ratios of the generations were compared using two-tailed t-tests. All statistical analyses were performed in JMP Version 7 (SAS Institute, 2007).

Results

Determination of egg shapes

The symmetry of eggs produced by the sexual and asexual generations differed significantly ($\overline{L} − \overline{W}_{asex}$ = 5.5 ± 0.002 and $\overline{L} − \overline{W}_{sex}$ = 17.2 ± 0.002 mm; t = 42.06, d.f. = 58, P<0.0001; Figure 1A and B). Thus, for a given egg width, sexual generation females produced corre-
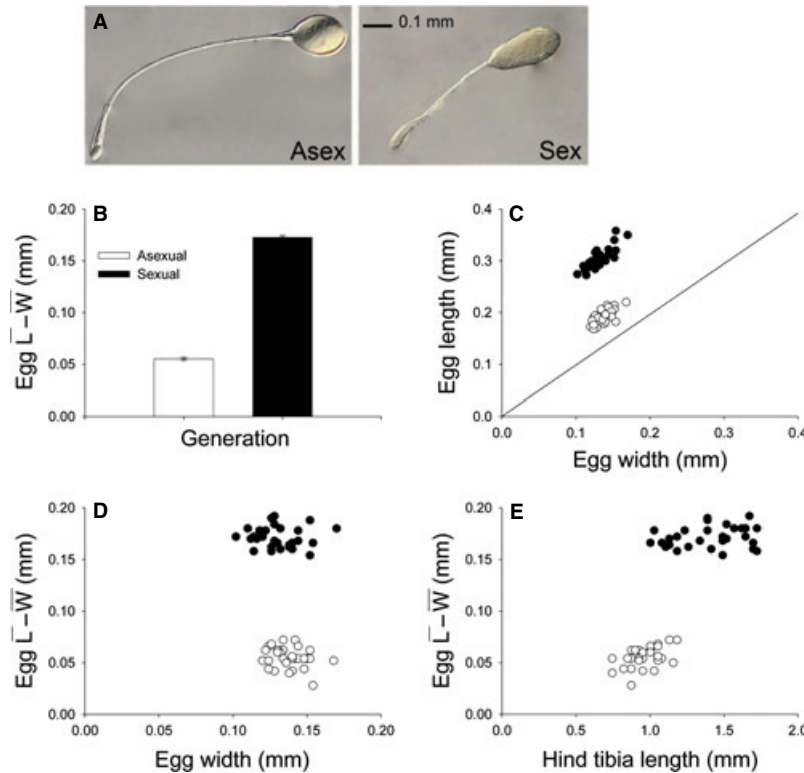
Figure 1 (A) Eggs dissected from asexual and sexual generation females of the cynipid gall former, *Belonocnema treatae*, showing the egg body and the peduncle. (B) Average shape ($\overline{L} - \overline{W} \pm SE$) of asexual and sexual generation eggs (n = 150 eggs per generation). (C) Average length of eggs produced by each of 30 asexual (open circles) and 30 sexual (closed circles) females plotted against average width. The diagonal indicates L = W, a perfect sphere. (D) Egg shape in relation to egg width, and (E) egg shape in relation to female body size (hind tibia length) in each generation. SEs are omitted for clarity in panels C, D, and E.

spondingly longer eggs than did asexual females (Figure 1C). The multifactor ANOVA confirmed that egg shape differed between generations ($F_{1,55} = 613.89$, P<0.0001) and showed that egg shape was independent of egg size in both generations as illustrated by the test of the main effect of egg size ($F_{1,55} = 0.55$, P>0.46; Figure 1D) and by the absence of an interaction of egg size and generation ($F_{1,55} = 1.54$, P>0.22). Egg shape was not influenced by the number of eggs produced per female ($F_{1,55} = 2.04$, P>0.16).

A separate multifactor ANOVA revealed that variation in female body size influenced egg shape equivalently within each generation as shown by tests of the main effect of body size ($F_{1,55} = 9.08$, P<0.0039) and the absence of an interaction of body size and generation ($F_{1,55} = 0.001$, P>0.97). Although both body size and generation influenced egg shape, a variance component analysis revealed that only 1.7% of the variation in egg shape was accounted for by within-generation variation in body size, whereas 98.3% of variation in egg shape was explained by the between-generation factor. Inspection of Figure 1E shows

that equivalent-size females in each generation produce markedly differently shaped eggs.

Egg volume and surface area

Estimates of the volume of individual eggs produced by females of both generations depended on the choice of volumetric model (Table 1). In the asexual generation, characterizing egg shape as a sphere increased the mean estimated volume by 23% compared with a prolate spheroid, and use of a cylindrical model in the sexual generation increased mean estimated egg volume by 50% compared with a prolate spheroid (Table 2). Regardless of the choice of volumetric model used, the estimated volume of individual eggs produced by sexual generation females exceeded that of asexual generation females (Table 2). However, the magnitude of the difference in egg volume (range = 16–114%) depended on how egg shape was classified in each generation. The most conservative estimates of the between-generation difference in egg volumes arose when sexual generation eggs were considered to be prolate spheroidal in shape and asexual generation eggs were con-

Table 1 Estimated average volume (± SE) of individual eggs produced by the asexual and sexual generation of *Belonocnema treatae* based on modeling egg volume as spheres, cylinders, or prolate spheroids

Generation	Volumetric model		
	Sphere	Cylinder	Prolate spheroid
Asexual	2.43 ± 0.09		1.97 ± 0.08
Sexual		4.22 ± 0.24	2.82 ± 0.16

Volume estimates were based on measurements of egg length and egg width for five eggs per female for each of 30 females per generation. Units are ×0.001 mm^3.

Table 2 Comparison of model-specific estimates of egg volume within (rows 1 and 2) and between (rows 3–6) generations of *Belonocnema treatae*

| Model comparison	t	d.f.	P	% difference
Asexual (sphere) vs. asexual (prolate)	22.38	29	<0.0001	23
Sexual (cylinder) vs. sexual (prolate)	17.14	29	<0.0001	50
Sexual (cylinder) vs. asexual (prolate)	8.78	58	<0.0001	114
Sexual (cylinder) vs. asexual (sphere)	6.89	58	<0.0001	74
Sexual (prolate) vs. asexual (prolate)	4.70	58	<0.0001	43
Sexual (prolate) vs. asexual (sphere)	2.09	58	0.041	16

Values of t represent the results of paired t-tests within generations and unpaired t-tests between generations.

sidered to be either prolate spheroid (43%) or spherical (16%). Given the minor but consistent deviation of asexual generation eggs from a perfect sphere (Figure 1C) and the consistent elongation (Figure 1C) and tapering of the distal ends of sexual generation eggs (Figure 1A), which indicate deviation from a perfect cylinder, we chose to classify eggs produced by both generations as a prolate spheroid. Using this model, we then estimated the distribution of volumes and compared the surface areas and surface area–volume ratios of eggs produced by asexual and sexual generation females. Although overlap in egg volumes exists, the 30 sexual females produced eggs that typically exceeded the average volume of eggs produced by the 30 asexual females (Figure 2). Surface area differed between generations as eggs of sexuals had 37% greater area than eggs of asexuals ($\bar{x}_{asex} = 7.67 \pm 0.002$ mm^2;

$\bar{x}_{sex} = 10.54 \pm 0.0037$ mm^2; t = 6.81, d.f. = 58, P<0.001). However, the surface area–volume ratios did not differ between the generations ($\bar{x}_{asex} = 39.51 \pm 0.54$ mm^{-1}; $\bar{x}_{sex} = 38.76 \pm 0.81$ mm^{-1}; t = 0.76, d.f. = 58, P>0.1).

Discussion

Understanding relationships among life history traits and alternative reproductive strategies demands accurate assessment of the size (volume) of individual eggs. We documented differences in both the shape and size of eggs produced by the alternate generations of the cyclically parthenogenic gall former, *B. treatae*. Our results thus provide the foundation to explore the partitioning of reproductive effort between egg number and egg size and the scaling of these reproductive traits with female body size both within and between generations of this host-specific gall former (JR Ott & GR Hood, unpubl.). More generally, our results highlight the importance of selecting appropriate models to estimate egg volume in life history studies as our results indicated that estimates of egg volume within both generations and the magnitude of between-generation differences in egg volume depended on which volumetric models were chosen. Finally, our approach to the problem of determining egg volume provides a method for selecting appropriate models to estimate volume, and our application of the method shows the value of basing selection of volumetric model on a quantitative assessment of shape rather than a simple visual inspection of eggs.
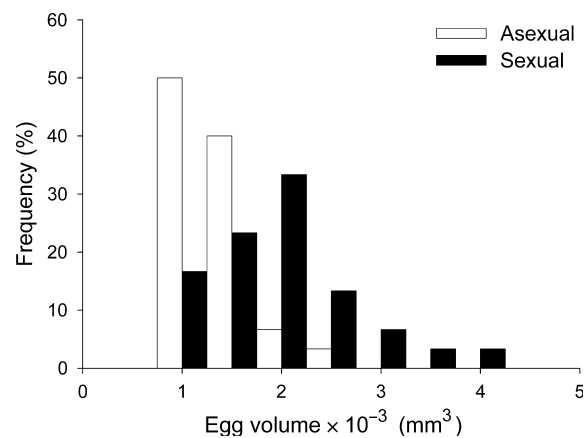
Figure 2 Distribution of egg volumes of *Belonocnema treatae*, modeled as a prolate spheroid, for both the asexual and sexual generations. Volumes were binned into 0.0005-mm^3 size classes.

Importance of shape in volumetric model selection

Assessing egg shape is a pre-requisite for selecting appropriate volumetric models to estimate egg size. However, egg shape can be highly variable, even between closely related insect species (see figure 1 in Vardal et al., 2003). Vardal et al. (2003) documented extreme variation in the length and width of eggs produced by 21 species of cynipoids. Using measurements reported by Vardal et al. (2003), we calculated that the ratio of egg width to egg length among six closely related species of cynipids varied from 0.18 to 0.57.

The sizes of approximately spherical insect eggs are typically estimated by modeling egg volume as an ellipsoid (Berrigan, 1991; Garcia-Barros, 2000b; Vardal et al., 2003). Ellipsoids range from perfect spheres to prolate spheroids to oblate spheroids. The volume of ellipsoids can be estimated based on three or even two orthogonal measurements dependent on shape. For insect eggs, typically only length and width are measured (Berrigan, 1991). For more complex egg shapes such as those depicted by Vardal et al. (2003), which include asymmetric, pedunculate, or banana-shaped eggs or eggs tapered at both ends, more complex measurements and models will be required [see Kratochvil & Frynta (2006), for estimates of complex egg shapes in geckos]. In the case of the cynipid *B. treatae* with its relatively simple egg geometry, by defining egg shape as L − W we captured two linear dimensions of shape that allowed us to visualize changes in shape as a function of egg size and female body size and compare the pre-oviposition shapes of eggs produced by sexual and asexual *B. treatae*. Inspection of shapes both within and between generations, in conjunction with comparisons of the magnitude of estimated differences in egg volumes based on alternative volumetric models, allowed us to justify our selection of the model deemed most appropriate to estimate volume in each generation.

We considered (and examined the consequences of modeling) the volume of eggs produced by asexual generation females as both spheres and prolate spheroids – two versions of an ellipsoid – and the eggs produced by sexual generation females as both cylinders and prolate spheroids. Upon first inspection, it was tempting to model the volume of asexual eggs as spheres. The sphere-like egg form suggested by Figure 1A and C was evaluated by inspection of the degree to which asexual eggs deviated from a perfect sphere, that is the percent by which the long axis exceeded the average diameter of the egg $[(\overline{L} - \{\overline{L} + \overline{W}\}/2)/(\{\overline{L} + \overline{W}\}/2) \times 100]$. On average, the long axis of asexual generation eggs exceeded the average diameter of eggs by 16.6 ± 0.6%, thus a spherical model appeared to be a close match to the three-dimensional form of asexual eggs. This result raises the question: by how much does a volume

have to deviate from a perfect spheroid before it is better considered to be a prolate spheroid? For a sphere, a = b = c, where a and b represent equatorial radii (shortest axes) and c represents the polar radius (longest axis), with each dimension measured from the origin in an x, y, z Cartesian coordinate system. In contrast, for a prolate spheroid a = b<c. As our initial repeated measurements of the width (diameter) of individual eggs showed little variability (i.e., no evidence of unequal equatorial radii; r>0.98 in both generations), we assumed a = b and measured only two dimensions of egg shape, width and length. Dimensions a and b thus equate to ½ of the measured egg width. Similarly, c, the polar radius, is equivalent to ½ of egg length. We then asked: by how much does the polar radius exceed the equatorial radius? As egg length and width represent 2c and 2a, respectively, we ascertained the extent to which the egg's polar axis is elongated by simply examining $(\overline{L} - \overline{W})/\overline{W}$. We found that the extension of the polar axis in the asexual generation egg represented a 40.4 ± 1.6% increase over the equatorial axis. This result suggested a far more dramatic deviation from the dimensions that indicate perfect sphericity than the assessment of the degree to which asexual eggs deviate from a perfect sphere and justified modeling the volume of asexual eggs based on a prolate spheroid.

In contrast, eggs produced by sexual generation females, as shown in Figure 1A and C, are decidedly not a perfect sphere. Examination of $(\overline{L} - \overline{W})/\overline{W}$ showed that extension of the polar radius of the sexual generation egg represented a 134 ± 3.3% increase over the equatorial radius. Thus, the volumes of individual eggs produced by sexual generation females were estimated by modeling egg shape as a cylinder and a prolate spheroid. We distinguished between these two models and selected the prolate spheroid as the appropriate model to estimate the volume of sexual generation eggs based on the pronounced tapering of the diameter at the distal ends of the eggs, which indicated that the cylindrical model would overestimate volume.

By comparing estimates of egg size based on choice of volumetric models, we documented the consequences of erroneous model selection on estimates of volume within generations and on the outcome of egg volume comparisons between generations. The choice of model yielded estimated differences between the generations in egg size that varied from as little as 16% to as much as 114%.

Generational differences in egg morphology

Cyclically parthenogenic insects encounter variable environments that can drive divergence in life history characteristics (Moran, 1992; Stone et al., 1995, 2002), and characteristics of insect eggs are well known to reflect environmental conditions and constraints (Braby, 1994;

Azevedo et al., 1997; Fox, 1997; Fox & Czesak, 2000; Noriyuki et al., 2010). Given that body size is continuously distributed within, and overlaps between, generations of *B. treatae* (Cryer, 2003; Hood, 2009), the hypothesis that egg size and/or shape varies within and between generations is a natural one. Underlying this hypothesis is the precept that variation in body size reflects differences in maternal environments that potentially affect the allocation of resources available for provisioning eggs and hence influences egg size, egg quality (Fox, 1997; but see McIntyre & Gooding, 2000), and/or shape. Thus, for *B. treatae*, egg character states are predicted to be expressed as norms of reaction across the maternal environments. Specifically, egg size and shape are predicted to exhibit continuous variation with eggs produced by the smallest of asexual females and the largest of sexual females representing the most divergent expression of egg characters. We found that egg size (volume) met this expectation: egg size was continuously distributed in each generation and overlapped between generations. In contrast, egg shape was highly conserved within generations, and intraspecific variation in this trait was largely explained by whether eggs were produced within the oviducts of sexual or asexual females. Although many examples of dimorphisms and polymorphisms exist within generations of sexually reproducing insects in response to spatial and/or temporal variation in environmental conditions (Blankenhorn & Fairbairn, 1995; Fox & Czesak, 2000; Nice & Fordyce, 2006; Noor et al., 2008), to the best of our knowledge the intrinsic difference in egg shape between generations of *B. treatae* is the first example of a dimorphism for egg shape expressed between generations of a cyclically parthenogenic organism. Such a dimorphism may reflect divergent evolution among generations (Nijhout, 2003; Normark, 2003; West-Eberhard, 2003).

Adaptation and constraints: egg shape and size

Mechanical forces exerted as eggs move through the ovipositor can permanently alter egg shape following oviposition (Quicke et al., 1994). Thus, estimating egg volume and investigating the basis of intraspecific variation in egg shape may be further complicated if eggs assume complex shapes during or following oviposition. Thus arises a conundrum: assessment of shape, needed to guide the selection of appropriate models to estimate volume, is perhaps best conducted using eggs examined prior to oviposition, whereas investigations of the adaptive significance of variation in egg shape, size, and/or surface area–volume ratios may necessitate studying eggs following oviposition. To understand the consequences of variation in egg size and/or shape fully, both pre- and post-oviposition studies may be needed.

Comparison of pre-oviposition egg dimensions for *B. treatae* shows that the larger sexual generation eggs are produced by elongation of the polar axis. The equivalent width of eggs across generations suggests the possibility of a constraint on egg width. Thus, shape differences of asexual and sexual generation eggs may simply represent a constrained solution to increasing the volume of sexual generation eggs (i.e., the difference in egg shape between generations may have no adaptive explanation). In *B. treatae* and in other heterogonic cynipids, the alternate generations exhibit marked variation in body size (Rokas et al., 2003), emergence and oviposition phenology (Stone et al., 2002), and host plant tissues selected for oviposition (Weis et al., 1988) – all factors possibly important in influencing allocation of resources to, and hence the shape and size of, individual eggs. Although we did not investigate the adaptive significance of the generational differences in egg shape and size, we can envision hypotheses to guide research into the possible mechanistic basis of the observed dimorphism in egg shape and the observed between-generation variation in egg size. For example, egg shape of the sexual generation may reflect adaptation for entry into, or placement within, the relatively thin developing leaves used for oviposition, whereas asexual generation egg shape may reflect an adaptation facilitating oviposition into underground rootlets. Alternatively, the basis of differences for egg size (and hence shape) may reflect asymmetries in the initial provisioning of first instars required in each oviposition substrate. Furthermore, Vardal et al. (2003) proposed that variation in cynipid eggs could be related to desiccation risk. However, we found no difference in surface area to volume ratios between generations. A manipulative experiment involving oviposition by each generation into both leaves and roots would be required to examine the adaptive basis of variation in egg characters.

Conclusion

We have shown how assessment of egg shape informs selection of volumetric models used to estimate egg size and we have illustrated the consequences of erroneous volumetric model selection for estimating egg size within and comparing egg size between generations of a cyclically parthenogenic gall former. Our analyses have revealed a dimorphism in egg shape between generations of *B. treatae*. Cyclic parthenogenesis is a common life cycle adaptation within Cynipidae and is also known from a number of insect orders (Diptera, Hemiptera, and Coleoptera) and a diverse range of other taxa. We hope that the issues we have raised here will promote fuller consideration of egg shape when estimating egg size and stimulate interests in comparative studies of the reproductive biology and life

history of multivoltine and cyclically parthenogenic species.

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