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Independent life history evolution between generations of bivoltine species: a case study of cyclical parthenogenesis

Glen R. Hood[1] **· James R. Ott**[2]

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Abstract Successive generations of bi- and multivoltine species encounter differing biotic and abiotic environments intra-annually. The question of whether selection can independently adjust the relationship between body size and components of reproductive effort within successive generations in response to generation-specific environmental variation is applicable to a diversity of taxa. Herein, we develop a conceptual framework that illustrates increasingly independent life history adjustments between successive generations of taxa exhibiting complex life cycles. We apply this framework to the reproductive biology of the gall-forming insect, *Belonocnema treatae* (Hymenoptera: Cynipidae). This bivoltine species expresses cyclical parthenogenesis in which alternating sexual and asexual generations develop in different seasons and different environments. We tested the hypotheses that ecological divergence between the alternate generations is accompanied by generational differences in body size, egg size, and egg number and by changes in the relationships between body size and these components of reproductive effort. Increased potential reproductive effort of sexual generation *B. treatae* is attained by increased body size and egg number (with no trade-off between egg number and egg size) and by a significant increase in the slope of the relationship between body size and potential fecundity. These generation-specific relationships, interpreted in the context of the model framework, suggest that within each generation selection has independently molded the relationships relating body size to potential fecundity and potential reproductive effort in *B. treatae.* The conceptual framework is broadly applicable to comparisons involving the alternating generations of bi- and multivoltine species.

Keywords Body size · Complex life cycles · Cynipidae · Fecundity · Heterogony

Introduction

Complex life cycles are defined as “the passage through two or more ecologically distinct phases” (Istock 1967). Understanding the factors that promote, and the processes that underlie, the origin, diversification, and maintenance of life cycle complexity represents a long-standing challenge to evolutionary biologists (Slade and Wassersug 1975; Moran 1994; Normark 2003; Minelli and Fusco 2010). Most organisms with complex life cycles undergo abrupt morphological, physiological, and behavioral changes associated with a change in habitat (e.g., metamorphosis in anurans and holometabolous insects; Wilbur 1980). Thus, there are both ecological and evolutionary implications of the ontogenetic change in a species’ niche during development (Wilbur 1980; Ebenman 1992). For species with complex life cycles that undergo a single generation per year (univoltine species), each individual experiences the suite of environmental challenges encountered during development. In contrast, when life cycle complexity involves the production of two or more generations annually (bi- or multivoltinism), each generation independently experiences temporal variation in biotic and abiotic environments (Wolda

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James R. Ott
jimott@txstate.edu

[1] Department of BioSciences, Rice University, Houston, TX 77251, USA

[2] Population and Conservation Biology Program, Department of Biology, Texas State University, San Marcos, TX 78666, USA

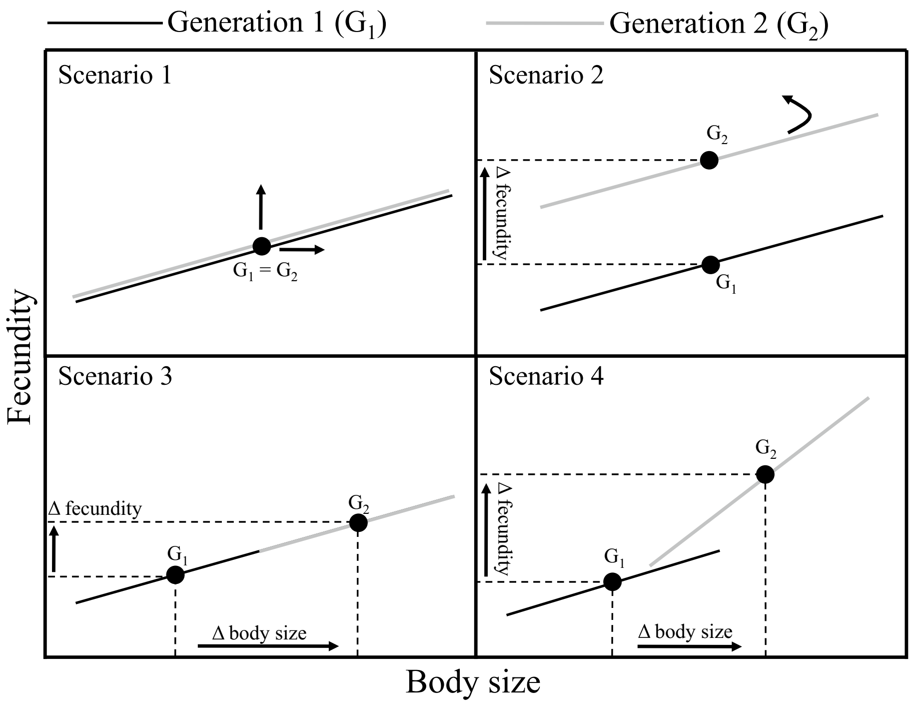

Fig. 1 Four pathways to increased fecundity for taxa that exhibit bivoltinism and (or) heterogony. *Scenario 1*: Body size and slope and intercept of the body size × fecundity relationship are coupled between generations. Selection for increased fecundity in one of the alternate generations, for example, G_2 (with or without a change in body size), leads to a coupled increase in G_1. *Scenario 2*: Body size coupled, slope, and intercept uncoupled. Selection for increased fecundity in G_2 is achieved without a change in body size (with or without a change in the intercept and/or slope of the body size × fecundity relationship). *Scenario 3*: Body size uncoupled, intercept, and slope coupled. Selection for increased fecundity in G_2 is achieved via increased body size without a change in slope of the body size × fecundity relationship. *Scenario 4*: Body size and slope and intercept uncoupled. Selection for increased fecundity in G_2 is achieved via an increase in body size and a change in the body size × fecundity relationship

1988; Moran 1992; Kivela et al. 2013). Thus, species characterized by bi- or multivoltinism have been considered to be composed of distinct populations within a species, with successive generations evolving semi-independently in response to generation-specific selection (Istock 1967; Wilbur 1980; Askew 1984; Werner 1988; Ebenman 1992; Moran 1994).

Heterogony, or cyclical parthenogenesis, is a specific type of bivoltinism in which asexual and sexual generations obligately alternate to complete the life cycle. Heterogony is present in the class Trematoda, the phylum Rotifera, the subphylum Crustacea, and four insect orders: Coleoptera, Diptera, Homoptera, and Hymenoptera (Bell 1982; Hebert 1987; Stone et al. 2002). Species expressing heterogony in these diverse taxa share two patterns. First, the alternate generations are exposed to differences (often dramatic) in biotic and abiotic environmental conditions. Second, the alternate generations are characterized by striking divergence in ecology, morphology, physiology, behavior, and life history (Simon et al. 2002; Stone et al. 2002; Galaktionov and Dobrovolskij 2004; Schroder and Gilbert 2004). Research across heterogonous taxa and more broadly across bi- and multivoltine taxa is united by the following questions: (1) how are evolutionary adjustments to biotic and abiotic factors achieved within each generation, and (2) to what extent are adjustments in each generation independent of adjustments in the alternate generation (Werner 1988; Wolda 1988; Ebenman 1992; Moran 1994; Minelli and Fusco 2010)?

Specific to the present study of life history evolution of heterogonous species, we are interested in how adjustments in body size, egg size, egg number, and reproductive effort (the product of egg number and egg size) are achieved within alternate generations, and whether adjustments of these components of reproductive strategy are independent between generations. Change in fecundity necessarily involves the joint consideration of potential changes in body size, egg number, and egg size, as well as potential changes in the intercept and (or) slope of the regression relating variation in fecundity to variation in body size in each generation. To frame the analysis of the independence of life history adjustments within alternate generations of heterogonous species (and more broadly across bi- and multivoltine species), four hypothetical pathways to increase fecundity of one generation of a bivoltine species and concomitant changes in the alternate generation are illustrated in Fig. 1. Here, we consider scenarios in which selection favors an increase in fecundity in one of the alternating generations (referred to in our model as G_2, but the designation is arbitrary) with two potential outcomes: (1) an equivalent shift in the fecundity of the alternate generation (G_1) or (2) an increase in fecundity of G_2 independent of G_1. In scenario 1, adjustments are not independent and thus increased fecundity in G_2 attained with or without a change in body size results in an equivalent shift in the alternate generation. In scenario 2, selection for increased fecundity in G_2 proceeds independently of G_1 without a concomitant increase in body size, perhaps at the expense of a trade-off with egg size. Scenario 3 illustrates increased fecundity in G_2 achieved via increased body size. Here the generations, which differ in both body size and fecundity, are simply proportionately scaled versions of one another. Finally, scenario 4 depicts an increase in fecundity in G_2 achieved through both an increase in body size and a change in the body size—fecundity relationship. In the context of this model, we test the hypothesis that ecological divergence between the sexual and asexual generations of the gall-forming wasp; *Belonocnema treatae* (Hymenoptera: Cynipidae) is accompanied by independent adjustment of the relationships among four important life history components: body size, egg size, egg number, and reproductive effort.

The biology of gall-forming wasps makes them appropriate and tractable systems for the study of life cycle complexity. Heterogony is commonly observed among the ~1400 species in the tribe Cynipini (Pujade-Villar et al. 2001; Stone et al. 2002). Both generations are typically host specific and feed and develop obligately within nutrient-rich compartments of plant tissue (galls) induced by adult oviposition or larval feeding (Schonrogge et al. 2000). The alternate generations (sexual and asexual—otherwise known as gamic and agamic) are exposed to generation-specific biotic and abiotic conditions that likely drive the evolution of alternative morphologies, ecologies, and reproductive tactics (Askew 1984; Stone et al. 2002; Egan et al. 2012, 2013). The generations develop within galls initiated at different times and on different plant organs and (or) different plant species (Stone et al. 2002). Furthermore, each generation is exposed to natural enemy communities that typically differ in abundance and composition and induce variable rates of mortality (Askew 1984; Eliason and Potter 2000; Hood and Ott 2010; Forbes et al. 2015). Similarly, host plant defenses are variable and can inflict levels of mortality that differ between generations (Egan and Ott 2007; Hood and Ott 2011). Alternate generation adults typically differ in body size (Rokas et al. 2003), and since body size and fecundity are positively correlated in hymenopterans (Honek 1993), differences in body size are expected to translate into fecundity differences. Adult cynipids are short lived and rarely feed, thus intrinsic reproductive characteristics linked to resource acquisition during the larval feeding stage can be evaluated by examining newly emerged adults. Finally, females of many species are pro-ovigenic (born with the lifetime production of eggs fully matured), facilitating measurements of egg number and egg size (Hood and Ott 2011).

Herein, we compare body size, egg number, egg size, and reproductive effort of the sexual and asexual generations of *B. treatae*. We then compare slopes of the regressions relating variation in body size to components of potential reproductive effort (e.g., egg number and egg size) and reproductive effort itself to test the hypothesis that selection has independently altered the body size–reproductive potential relationship between generations of this heterogonic species. Our study provides a framework for, and an example of, testing alternative hypotheses for observed asymmetries in body size and fecundity in heterogonic species and more broadly among the diversity of taxa characterized by bivoltine and multivoltine life cycles. As well, in conjunction with our earlier analysis of generation-specific alteration of egg shape and size in *B. treatae* (Hood and Ott 2011), this study represents an entryway to inspection of generation-specific adjustments of components of reproductive effort in the species-rich Cynipidae.

Materials and methods

Study system

Belonocnema treatae Mayr exhibits a heterogonous life cycle typical of cynipids in which alternating sexual and asexual generations are spatially and temporally segregated (Lund et al. 1998). Both generations are host specific and induce galls on plateau live oak, *Quercus fusiformis* Small, section Virentes (Muller 1961), in the Edwards Plateau region of central Texas, USA (Lund et al. 1998). The asexual generation develops throughout spring, summer, and fall within spherical, single-chambered leaf galls, whereas the sexual generation develops during winter and spring within multichambered root galls (Lund et al. 1998). Adults of both generations are short lived, do not feed, oviposit upon eclosion, and are pro-ovigenic (Lund et al. 1998).

Sample processing and variable definitions

In total, 1155 asexual *B. treatae* were reared from leaf galls randomly collected from live oak trees at Texas State University's Freeman Center, Hays County, TX, USA (29°55′N, 98°00′W). Additionally, 676 sexual female *B. treatae* were reared from 144 root galls collected from these same trees. Galls were housed in collection traps outdoors at Texas State University. Emergent females were collected daily and stored in 95% ethanol. Because female *B. treatae* only oviposit into actively growing plant tissue, measures of fecundity represent maximum potential fecundity prior to any egg loss due to oviposition.

Female body size was indexed by measuring right hind tibia length (TL), a reliable indicator of body size and body mass (Rogers et al. 1976; Honek 1993), to ±1 ocular unit (0.0257 mm) with a stereo dissecting microscope fitted with an ocular micrometer (Hood and Ott 2011). A preliminary analysis showed TL measurements were repeatable ($r = 0.99$; $n = 60$). Our large samples allowed us to compare the observed distribution of body sizes that characterized natural populations of each generation at our study site and estimate the predicted distribution of fecundities (see below).

We next dissected females to count eggs. Owing to the detailed nature and time required to count and measure the size of eggs produced by individual females, we subsampled each generation. To ensure equivalent representation of females across the range of body sizes in each generation, we first binned all individuals per generation into six TL size classes following Hood and Ott (2011). We then randomly selected a subset for measurement from within each size class for a total of 63 asexual and 70 sexual females. This pseudo-random or purposive selection ensured sampling across the full spectrum of body sizes and approximately equal representation of females of each body size class in subsequent analyses; it is superior to random selection of an equivalent number of individuals for regressions involving a set number of individuals (Kenkel et al. 1989). Thus, our sampling scheme matched our goal, which was to describe the functional form of the relationship between body size and elements of reproductive effort more so than capturing the regressions describing these relationships in the natural populations. To enumerate eggs, the abdomen of each female was removed 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 (Hood and Ott 2011). Eggs were then removed, stained with methylene blue, and counted using a stereo dissecting scope. Counts of egg number were repeatable ($r = 0.98$; $n = 10$ females per generation). The number of eggs dissected from each female represents the maximum number of eggs potentially deposited (defined as potential fecundity).

The size (volume) of individual eggs produced by *B. treatae* is most parsimoniously estimated by modeling egg shape as a prolate spheroid (Hood and Ott 2011). For 30 of the 70 sexual and 30 of the 63 asexual females, again selected to represent the range of body sizes in each generation, we determined the average egg size based on length and width measurements of five eggs per female. Measures of egg width and length are repeatable ($r > 0.98$), and egg shape is not influenced by egg size, body size, or potential fecundity (Hood and Ott 2011). We used these estimates of average egg size per female in combination with counts of the number of eggs dissected from each female to predict potential reproductive effort measured as mm^3. Volumetric estimates of individual egg size and total reproductive effort were then used in analyses of the relationships between variation in female body size and egg size, body size, and reproductive effort and the potential trade-off between egg size and egg number within and between generations.

Alternate generation comparisons

One-way ANOVA was used to test the null hypothesis of no difference in the body size of sexual ($N = 676$) and asexual ($N = 1155$) females. The null hypothesis of no difference in potential fecundity and reproductive effort between alternate generations was tested using the size-structured subsamples of sexual ($N = 70$) and asexual ($N = 63$) females, again using one-way ANOVA. We then used generation-specific linear regressions as justified by results of ANCOVA (see below) to predict fecundity and reproductive effort on the basis of body size (TL) for the 676 sexual and 1155 asexual females for which we initially measured body size. This procedure allowed us to approximate the distributional properties of potential fecundity and reproductive effort that characterized the natural populations of the alternate generations at our study site in central Texas, USA.

Relationship of body size and reproduction within and between generations

We used simple linear regressions to assess the relationship between variation in (1) body size and potential fecundity, (2) body size and egg size, and (3) body size and reproductive effort within each generation ($N = 70$ sexual and $N = 63$ asexual females). We also tested for a trade-off between egg number and individual egg volume within each generation by examining the sign and significance of the slope between relationships for each generation. Specifically, each regression provided a test of the hypothesis that $\beta = 0$ and an estimate of the strength of each relationship (r^2) within each generation. Where a significant regression was present in either or both generations, we conducted an ANCOVA with generation as a covariate to test the null hypothesis of homogeneity of slopes between generations. In these analyses, a significant covariate term indicates differences between generations (Fig. 1, scenarios 2 and 4), whereas a significant generation × body size interaction term indicates heterogeneity of slopes (i.e., independent adjustment of slopes between generations; Fig. 1, scenario 4). Following ANCOVA, a mixed-effect variance component analysis was conducted for the body size × potential fecundity comparison to determine the proportion of the overall variation attributable to within and between generation factors.

Allometry of reproduction

To determine if potential fecundity, egg size, and reproductive effort scale iso- or allometrically with body size within generations and to test if the scaling coefficients differ between generations, we first natural log (ln) transformed TL and each response variable (Wickman and Karlsson 1989; Preziosi et al. 1996). While reduced major axis regression (RMA) is a popular method to analyze allometric relationships of reproductive characteristics (e.g., Berger et al. 2012), RMA is more appropriate than standard ordinary regression only when the independent variable is measured with error (Sokal and Rohlf 2012). Measurement error in body size was only 1.7 and 2.5% of average body size in the sexual and asexual generations, respectively; therefore, we used linear regression to estimate the allometric slope (β) that describes the change in each ln-transformed response variable in relation to ln body size (isometry, $\beta = 3$; negative allometry, $\beta < 3$; positive allometry, $\beta > 3$). We then conducted an ANCOVA with generation as a covariate to test the hypothesis of homogeneity of scaling coefficients between asexual and sexual generations for each relationship. All statistical analyses were performed in R version 3.1.1 (R Core Team 2014). Means ± standard errors (SE) are presented throughout.

Results

Body size, potential fecundity, and reproductive effort within and between generations

Body size indexed by tibia length varied considerably within both sexual [coefficient of variation (CV) = 0.13] and asexual generations (CV = 0.14) (Fig. 2a). While the distribution of body sizes overlapped between generations, the average body size of sexual females ($\overline{\mathrm{TL}} = 1.46 \pm 0.007$ mm, range = 0.67–1.80 mm, $N = 676$) exceeded that of asexual females ($\overline{\mathrm{TL}} = 1.05 \mp 0.004$ mm, range = 0.51–1.39 mm, $N = 1155$; $F_{1,1829} = 2578.5$, $P < 0.00001$). Based on inspection of the size-structured samples, we found potential fecundity varied broadly within both the sexual (CV = 0.46) and asexual (CV = 0.39) generations. On average, sexual females produced significantly more eggs ($\bar{x} = 696 \pm 38$, range = 128–1138, $n = 70$) than asexual females ($\bar{x} = 186 \pm 9$, range = 46–427, $n = 63$; $F_{1,131} = 154.5$, $P < 0.0001$). Prior analysis established that the average size (volume) of individual eggs produced by sexuals ($\bar{x} = 0.00282 \pm 0.00016$ mm^3) exceeds that of asexuals ($\bar{x} = 0.00197 \pm 0.00008$ mm^3) (Hood and Ott 2011). Based on these average volumes and numbers of eggs produced per female, the average potential reproductive effort of sexual generation females ($\bar{x} = 2114.4 \pm 223.9 \times 0.001$ mm^3, range = 603–5756 × 0.001 mm^3, $n = 30$) greatly exceeded that of the asexual generation females

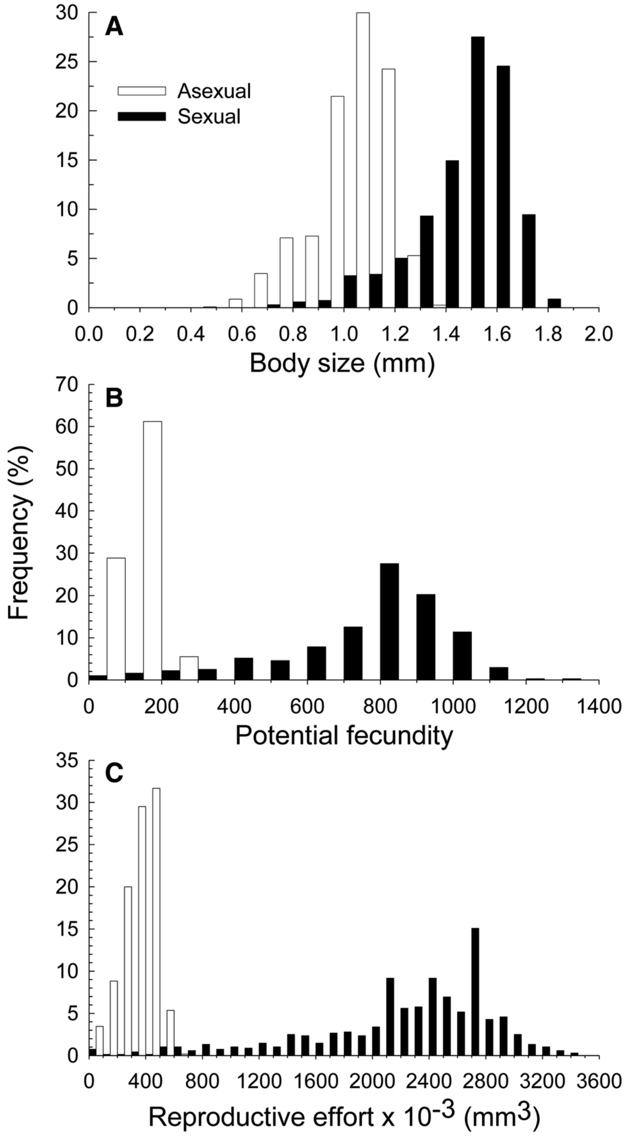

Fig. 2 Distribution of **a** body size, **b** potential fecundity, and **c** potential reproductive effort for 1155 asexual (*open bars*) and 676 sexual (*filled bars*) generation *B. treatae*. Body size (tibia length) was binned into 0.1 mm size classes, potential fecundity into classes of 50 eggs, and reproductive effort into volume classes of 1 × 10^{-3} mm^3. Body size distributions formed from randomly sampled females. Fecundity and reproductive effort distributions were predicted using generation-specific linear regression models developed from size-structured samples as described in Fig. 3

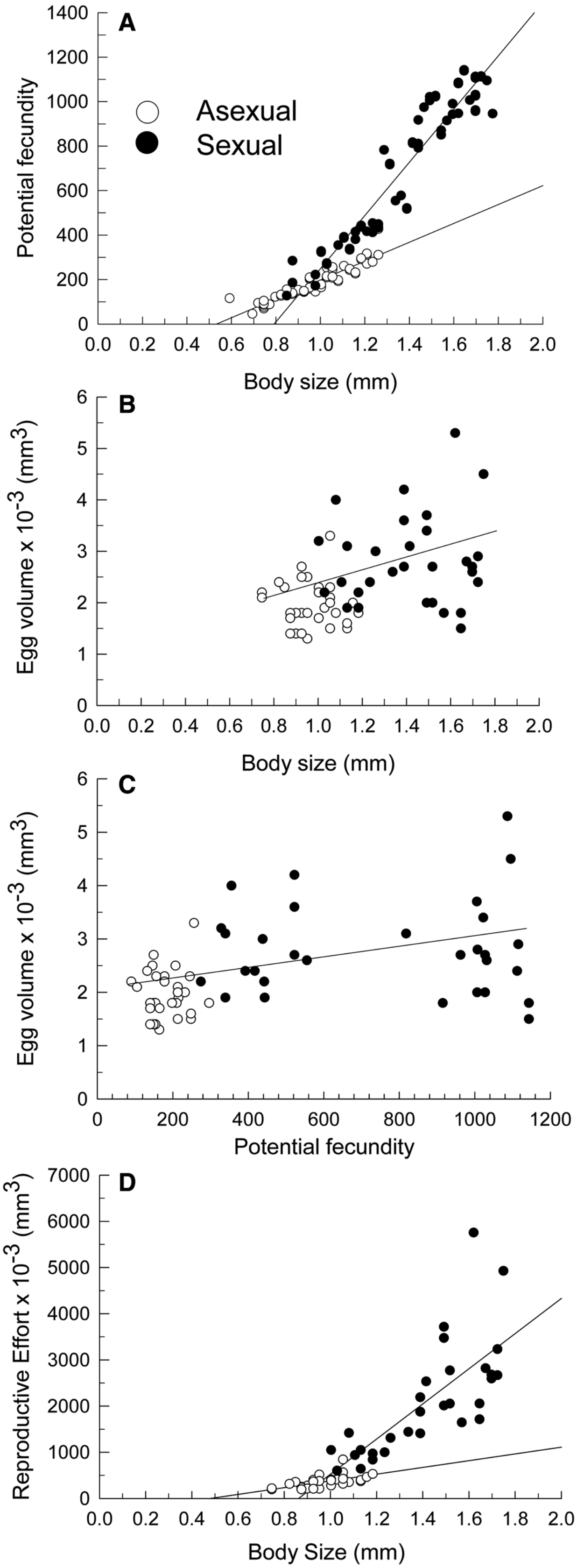

Fig. 3 Relationships between **a** body size and potential fecundity, ▶ **b** body size and egg size, **c** potential fecundity and egg size, and **d** body size and potential reproductive effort for asexual (*open circles*) and sexual (*closed circles*) generation *B. treatae*. Within-generation regressions were justified by ANCOVA analysis for the relationships depicted in Fig. 3a, d. In Fig. 3a, $n = 63$ asexual and 70 sexual generation individuals. In Fig. 3b–d, $n = 30$ sexual and 30 asexual generation individuals. *Error bars* are omitted from egg volume measurements in Fig. 3b, c for clarity

($\bar{x} = 361.3 \pm 25.2 \times 0.001$ mm^3, range = 196–845 × 0.001 mm^3, $n = 30$; $F_{1,58} = 60.56$, $P < 0.0001$).

Relationship between body size and reproductive characteristics

Potential fecundity increased with body size in both the asexual ($-225.4 + 423.9 \times$ TL; $P < 0.0001$) and sexual ($-946.6 + 1196.3 \times$ TL; $P < 0.0001$; Fig. 3a) generations. Variability in body size explained most of the variation in potential fecundity within both generations ($r^2 = 0.85$ and 0.90 for the asexual and sexual generations, respectively). Importantly, the slopes of the regressions relating variation in body size to variation in potential fecundity differed significantly between generations ($\beta_{\text{sex}} > \beta_{\text{asex}}$), as indicated by the significant generation × body size interaction term ($F_{1,129} = 106.35$, $P < 0.0001$) in the ANCOVA (Fig. 3a). The intrinsic difference in fecundity between generations (indicated by the significant generation term in the ANCOVA; $F_{1,129} = 85.39$, $P < 0.0001$) is apparent by inspecting that region of Fig. 3a where body size of each generation overlaps: equivalent-size females of each generation produced different numbers of eggs. If the analysis is restricted to just these asexual ($n = 50$) and sexual ($n = 27$) individuals that overlapped in body size (TL range = 0.85–1.26 mm), equivalent-size sexual females produced ~68% more eggs, on average, than asexual females (sexual: 351 ± 18; range = 128–438; asexual: 209 ± 9; range = 151–427). A variance component analysis revealed that the intrinsic differences between generations accounted for 15% of the variation in egg number while body size variation accounted for 85%.

The size of individual eggs varied considerably within both the asexual (CV = 0.23) and sexual (CV = 0.31) generations and overlapped marginally between generations (Fig. 3b). However, variation in egg size was not related to variation in female body size within either generation (asexual: $r^2 = 0.01$, $P = 0.67$; sexual: $r^2 = 0.01$, $P = 0.67$). Additionally, variation in egg size was not related to variation in potential fecundity within each generation (asexual: $r^2 = 0.003$, $P = 0.76$; sexual: $r^2 = 0.03$, $P = 0.87$; Fig. 3c). Therefore, the increase in egg number that

Table 1 Slopes (± SE) and ANCOVA results of natural log (ln) transformed body size (BS), potential fecundity (FX), egg volume (EV), and reproductive effort (RE) relationships for asexual and sexual generation (Gen) *B. treatae*

Relationship	Slope (±SE)			ANCOVA results			
	Asexual	Sexual	Factors	*df*	Mean Sq	*F*	*P*
BS × FX	2.28 (0.13)	2.73 (0.10)	BS	1	26.25	939.2	**<0.0001**
			Gen	1	2.87	102.78	**<0.0001**
			BS × Gen	1	0.21	7.51	**0.007**
			Residual	129	0.03		
BS × EV	−0.17 (0.35)	0.07 (0.33)	BS	1	0.003	0.04	0.84
			Gen	1	0.67	9.3	**<0.01**
			BS × Gen	1	0.02	0.21	0.65
			Residual	56	0.07		
BS × RE	2.00 (0.42)	2.78 (0.35)	BS	1	6.30	71.25	**<0.0001**
			Gen	1	3.26	36.93	**<0.0001**
			BS × Gen	1	0.17	1.90	0.174
			Residual	56	0.09		

Bold values indicate the significant *P* values

characterizes sexual generation *B. treatae* is not achieved at the expense of a reduction in egg volume. When asexual and sexual females were pooled for regression analysis, as justified above, egg size increased with both body size (0.93 + 1.22 × TL, $P < 0.0005$, $r^2 = 0.19$; Fig. 3b) and potential fecundity (1.95 + 0.001 × potential fecundity, $P < 0.001$, $r^2 = 0.18$; Fig. 3c). In both generations, potential reproductive effort increased with body size (asexual: −347.6 + 729.9 × TL, $r^2 = 0.36$, $P < 0.0005$; sexual: −3278.5 + 3806.3 × TL, $r^2 = 0.53$, $P < 0.0001$; Fig. 3d). ANCOVA confirmed the difference in average reproductive effort between generations (generation term: $F_{1,56} = 6.38$, $P = 0.01$) and demonstrated that the slope of the body size × reproductive effort relationship differed between generations (generation × body size interaction term: $F_{1,56} = 7.64$, $P = 0.01$).

Predicted potential fecundity and reproductive effort

Using the above generation-specific linear regressions, in conjunction with the observed distribution of body sizes for females randomly sampled at our study sites (Fig. 2a), we estimated and then compared potential fecundity and potential reproductive effort for the 676 sexual and 1155 asexual generation *B. treatae* females (Fig. 2b, c). Potential fecundity was predicted from measurements of body size. Because our analyses revealed that egg size was not related to body size or potential fecundity within either generation (Fig. 3b, c), we used the average egg size for each generation estimated in Hood and Ott (2011) with the appropriate generation-specific prediction of fecundity as a function of body size to predict reproductive effort (Fig. 3d). Based on the observed distributions of female body sizes (Fig. 2a), the sexual generation was predicted to produce an average of ~ 3.6 times more eggs than the asexual generation (asexual: $\bar{x} = 221 \pm 1.8$, range = 0–363; sexual: $\bar{x} = 799 \pm 8.9$, range = 0–1206; Fig. 2b), which translated into an ~5.2 times increase in reproductive effort (asexual: $\bar{x} = 436.8 \pm 3.6$, range = 0–715 × 0.001 mm^3; sexual: $\bar{x} = 2255.3 \pm 25.0$, range = 0–3402.88 × 0.001 mm^3; Fig. 2c).

Allometry of reproduction

Within each generation, potential fecundity exhibited negative allometry ($\beta < 3$), and the relationship differed between generations (Table 1). ANCOVA results for the test of the body size × generation interaction term for the ln (fecundity) × ln (body size) regression confirm the heterogeneity of slopes between generations demonstrated earlier by the analysis of the untransformed data. Egg volume did not exhibit allometry within either generation. A significant generation term indicated that egg volume differed as a function of body size between generations (Table 1) and also confirmed results of the untransformed data. Last, potential reproductive effort exhibited negative allometry with body size within each generation (Table 1); however, the interaction term in the ANCOVA for this relationship was not significant when the data were ln transformed.

Discussion

Despite the promotion of bivoltine taxa exhibiting heterogony as model systems in evolutionary ecology (Stone et al. 2002; Brisson and Stern 2006; Stollewerk 2010), we are not aware of comparative studies of the relationships between body size and reproduction for alternate generations of heterogonic species. In our study of the asexual and sexual generations of the heterogonic gall former *B. treatae*, we (1) examined variation within and between alternate generations in body size, egg size, and potential fecundity and reproductive effort, (2) assessed whether components of reproductive effort are coupled to body size, and (3) tested the hypothesis that the relationships between body size × fecundity and body size × reproductive effort show evidence of independent adjustments between generations. We found that sexual *B. treatae* are 39% larger and produce ~3.6 times more eggs and ~5.2 times the potential reproductive effort of asexuals. Increased fecundity of the sexual generation was achieved primarily through an increase in body size but, importantly, also through increases in the slopes relating body size to fecundity (and reproductive effort).

We presented a conceptual model to explore alternative pathways to asymmetrical allocation of resources to reproductive effort between generations for taxa that exhibit bivoltine and (or) heterogonic life cycles. This model provides a framework for comparative analysis for these taxa. We use this model to interpret our results as supporting the view that the evolution of observed asymmetries in body size and fecundity between the asexual and sexual generations of *B. treatae* has been accompanied by selection that has independently molded the body size × fecundity relationship within each generation (Fig. 1d, scenario 4). Below we further explore the relationships between body size and aspects of reproductive effort for *B. treatae* and examine alternative mechanistic bases for, and sources of selection driving, asymmetrical allocation of resources to reproductive effort between generations of bivoltine taxa.

As expected for Insecta (Wickman and Karlsson 1989; Honek 1993), both potential fecundity and reproductive effort increased with negative allometry in relation to body size for *B. treatae* in each generation. However, the slopes of the relationship between fecundity and body size and reproductive effort and body size were both greater in the sexual generation. Importantly, the significant body size × generation term for the ANCOVA of the ln-transformed data rules out the possibility that the cubic increase in body volume that accompanies increased body size (and hence cubic increase in fecundity) explained the heterogeneity of slopes. Notably, the generational difference in potential fecundity was achieved without a reduction in egg size. Reproductive effort also showed evidence of independent adjustment between generations but only for the analysis of untransformed data. The use of a single value (mean) for egg volume applied to females across all body sizes within each generation may underlie this discrepancy by under or over estimating the expected size of eggs produced per body size class. We emphasize that we estimated potential fecundity via dissection, thus whether generational differences in potential fecundity translate into differences in realized fecundity in nature are unknown. A 1:1 relationship is unlikely (Leather 1988; Berger et al. 2008). While knowledge of the factors that link potential and realized fecundity are well developed for other members of the parasitic Hymenoptera (Jervis et al. 2008), relevant studies are largely lacking for the Cynipidae (Graziosi and Rieske 2014). Studies of the relationships among variation in body size, longevity, age-specific fecundity, and realized lifetime fecundity in the alternate generations of cynipid gall formers are needed.

Models of change in body size × fecundity relationships between alternate generations

To identify alternative trait configurations and combinations among traits that underlie generational differences in body size and fecundity relationships for bivoltine and heterogonic species, we envisioned scenarios to describe how selection could result in generational asymmetries in fecundity (Fig. 1). In scenario 1 (the null model), the slope (and intercept) of the body size × fecundity relationship is equivalent in alternate generations. Thus, the evolutionary response to selection appears coupled between generations and change in fecundity and (or) body size in either generation would result in concomitant changes in the alternate generation. In this case, it would not be clear whether selection favored increased fecundity in either or both generations. However, if the response to selection in the two generations were partially to completely uncoupled, as divergence in the ecology and life history of alternative generations of heterogonic species suggests (Askew 1984; Moran 1994; Stone et al. 2002), the evolution of increased fecundity in either generation can be described by at least three scenarios that depict increasingly independent life history adjustments. In scenario 2, selection favors increased fecundity in one generation (G_2) but the response proceeds without an increase in body size (i.e., body size evolution is constrained). In the case of *B. treatae* as in other gall-forming cynipids (Visser et al. 2013; Graziosi and Rieske 2014), resources for growth and reproduction are accrued only during the active larval feeding stage, thus a trade-off between more but smaller eggs might be expected (Smith and Fretwell 1974; Fox and Czesak 2000). For *B. treatae*, however, we found that even when the body size of sexual generation individuals overlapped that of asexuals (Fig. 3a), the increase evident in potential fecundity of the sexual generation females was not accompanied by a decrease in egg size (Fig. 3b). Increasing independence between the generations in evolutionary adjustments could result in increasingly unequal slopes for the relationship between body size and fecundity (indicated by arrow in Fig. 1, scenario 2). In scenario 3, selection could result in increased fecundity within one generation (G_2) via an increase in body size with no change in the relationship between body size and fecundity. In this scenario, individuals in each generation simply represent isometric or allometric versions of the alternative generation. This change again may or may not be accompanied by a trade-off between egg number and egg size. For *B. treatae,* the negative correlation indicative of a trade-off is absent. In fact, we observed that when asexual and sexual females are pooled, a weak positive relationship between egg number and egg size is present in analyses of untransformed data (Fig. 3c). In scenario 4, an increase in fecundity is achieved via an increase in body size and a change in how fecundity is related to body size. Increasing differences in the slopes between generations indicates increasing independence of the response to selection between the generations. Again, no trade-off in egg number and egg size is necessarily predicted.

The observed relationships between body size and potential fecundity (Fig. 3a) suggest that the divergence between the asexual and sexual generation *B. treatae* in average body size (Fig. 2a) and potential fecundity (Fig. 2b) follow scenario 4. The patterns revealed by our analysis beg three questions: (1) What is the mechanistic basis of independent life history adjustments between generations? (2) What selective forces promote divergence between generations in body size and reproduction and their inter-relationship? and (3) How common are asymmetrical relationships between body size and components of reproductive effort between generations of heterogonic cynipids or bivoltine species in general?

Mechanisms of independent life history adjustments between generations

The reproductive asymmetries we documented and have interpreted as independent responses to environmental-specific selection encountered by each generation can suggest specific loci that underlie the body size × fecundity relationship and that can respond separately to selection within each generation (Mank 2017). Alternatively, the contrasting responses to selection evident between the generations can represent differential gene expression similar to patterns of differential expression observed between intraspecific sexual dimorphisms (Mank 2017) and ecological morphs (Manousaki et al. 2013). In this latter case the differential slopes, we observed for the body size × fecundity relationships between generations represent phenotypic plasticity with differing but constrained optima between generations. Phenotypic plasticity in the presence of environmental variation (West-Eberhard 1989; Pigliucci 2005) is a common response among populations of multivoltine organisms (Ishihara 1999; Whitham and Agrawal 2009; Minelli and Fusco 2010; Ayabe et al. 2015). Regardless of underlying mechanisms, consideration of the selective forces that potentially drive reproductive asymmetries between generations represents an additional fruitful line of inquiry.

Natural enemies as potential drivers of body size × fecundity patterns of alternate generations

Body size is at the center of life history adjustments and reflects the outcome of myriad forces acting over variable time scales and across all stages of the life history (Roff 2002; Stearns 1992). For the subset of cynipid species for which both generations are described, (Pujade-Villar et al. 2001), differences in adult body size of sexuals and asexuals are common. (Rokas et al. 2003). Typically, asexuals exhibit larger body size (Wachi and Abe 2010), but the direction of the difference varies, as illustrated by *B. treatae* and other species (Ide et al. 2010). Establishing the relationship between body size and components of reproductive effort for the alternate generations for multiple cynipid species represents a necessary first step toward understanding (1) the observed asymmetry in body size between generations, (2) the variation among species as to which generation is largest and (or) more fecund, and, ultimately, (3) the patterns of diversification in reproductive strategies in the group. Given the diversity and complexity of forces driving body size evolution, single-factor analyses are necessarily limited. Nevertheless, we next explore a non-mutually exclusive driver that could generate selection for increased fecundity in either generation of bivoltine species.

Ecologist have long evoked “the ghost of parasitism past” to explain current patterns of host use by herbivorous insects (Brown et al. 1995; Feder 1995). The hypothesis is that historically natural selection has favored the shift of species exposed to high levels of natural enemy morality to novel niches where mortality was (is) lower. We postulate an analogous role of natural enemies in driving the reproductive asymmetries observed in alternate generations of *B. treatae* and propose two tests to evaluate this hypothesis more broadly across heterogonous or otherwise bivoltine species. This analysis of taxonomic patterns carries with it the assumption that current drivers equate to historical drivers. First, we predict that generational differences in natural enemy mortality drives the asymmetry in fecundity and body size between generations by generating selection for increased fecundity in the generation preceding (e.g., producing) the generation with the higher mortality. Second, we predict that the greater the differences in mortality due to natural enemies between generations, the greater the expected asymmetries in predicted fecundities of the alternate generations. These two predictions mirror Darwin's fecundity advantage hypothesis (Darwin 1874) and are irrespective of the sexual system of the generation experiencing higher mortality. That is, if the sexual generation experiences higher mortality, the asexual generation would be predicted to have the highest per capita fecundity and vice versa. We are aware of two examples in the Cynipidae with the requisite data to illustrate these alternate outcomes. In *B. treatae,* the asexual generation consistently experiences dramatically higher mortality (Hood and Ott 2010; Forbes et al. 2015) and the sexual generation as shown herein is as expected, larger and more fecund. In contrast, in the gall wasp *Callirhytis cornigera*, the sexual generation experiences higher mortality, and the asexual generation as predicted is characterized by higher fecundity and larger body size (Eliason and Potter 2000). These examples illustrate only that the differing outcomes are possible and come with the caveat that multiple hypotheses operating simultaneously, and (or) synergistically may explain variation in body size and life history adjustments between generations of species with alternating generation life cycles. A robust test of this hypothesis would require data from multiple species and comparative phylogenetic analysis (Hernandez et al. 2013).

Yet another alternative hypothesis for the asymmetrical fecundities of sexual and asexual generations is that the "cost of sex" (Maynard Smith 1978) should, *ceteris paribus*, result in sexual generations of cynipids being characterized by fecundity twice that of asexuals to compensate for the 2 × advantage of asexual reproduction. From this perspective, we note that the fecundity of the sexual generation of *B. treatae* is ~3.6 times that of the asexual generation, and in *C. cornigera* fecundity of the asexual generation would be expected to be half that of the sexual generation. These observed deviations from expectations of equalizing the cost of sex further suggest a potent driver(s) of asymmetries in reproduction between generations. A systematic review of body size, fecundity (egg number and egg size), body size × fecundity relationships, and generational mortality for Cynipidae would allow examination of the generality of the hypothesis that differences in natural enemy mediated mortality can drive asymmetries in reproduction between generations of species that exhibit alternation of generations.

The presence of heterogony in Cynipidae and other taxa has provided a long-standing challenge to biologists interested in understanding the factors that promote and constrain the evolution of life cycle complexity (Folliot 1964; Moran 1994; Stone et al. 2002). Assessing the reproductive potential and the relationship between body size and reproductive potential within and between generations of heterogonic species represent critical first steps in understanding the diversification and maintenance of this complex life cycle. However, a broader understanding of the evolution of life cycle diversity in the Cynipidae and other taxa exhibiting heterogony will remain elusive until more species have complete life cycle and life history information available. The empirical framework and testable hypotheses presented herein are intended to motivate additional studies of, and provide a framework for, better understanding asymmetries in fecundity, body size, and the scaling of fecundity with body size between generations for the diversity of taxa that exhibit complex life cycles.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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