Like mother, like daughter? Phenotypic plasticity, environmental covariation, and heritability of size in a parthenogenetic wasp

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Abstract

Dinocampus coccinellae (Hymenoptera:Braconidae, Euphorinae) is a solitary, generalist Braconid parasitoid wasp of over fifty diverse species of coccinellid ladybeetles worldwide that reproduces through thelytokous parthenogenesis, an asexual process in which diploid daughters emerge from unfertilized eggs. Here we utilized a common garden and reciprocal transplant experiment using parthenogenetic lines of *D. coccinellae* presented with three different host ladybeetle species of varying sizes, across multiple generations to investigate heritability, plasticity, and environmental covariation of body size. Since unilineal (reared on same host species) lines restrict environmental variation on clones, we expected positively correlated parent-offspring parasitoid regressions, indicative of heritable size variation. Whereas multilineal (reared on different host species) lines would quantify phenotypic plasticity of clones reared in varying environments, we expected negatively correlated parent-offspring parasitoid regressions. Contrary to expectations, our results indicate (1) little heritable variation in body size, (2) strong dependence of offspring size on the host environment, (3) a consistent signal of size-host tradeoff wherein small mothers always produced larger offspring, and vice versa, independent of host environment. Our study offers support for a constrained fecundity advantage model of Cope's Law, wherein *D. coccinellae* maintains phenotypic plasticity in body size despite parthenogenetic reproduction.

Introduction

Size of an organism is a complex and often plastic trait that is correlated with key adaptive traits such as reproductive success (Bosch and Vicens 2005, Berger et al., 2012), fecundity (Honek 1993), response to varying environments and hosts (Chown and Gaston 2010), developmental rates (Davidowitz et al., 2003), survival (Callier and Nijhout 2013), and greater depredation success (Oliveira et al., 2019). At the same time, larger bodied organisms face challenges such as increased resource need, and strong evolutionary constraints on reproductive tradeoffs (Blanckenhorn 2000, Shine 1988), which set "thresholds'' on size. Theory therefore predicts that a fecundity advantage for body size only occurs in the presence of energy availability (Shine 1988). The evolution of organismal size has been studied extensively over speciation timescales (reviewed in Hone and Benton 2005), often pointing to multiple independent transitions to larger body size (termed as Cope's Rule) across diverse animal taxa, indicating that there is no one definitive "pathway" or evolutionary strategy for size among species. Several lines of evidence instead support that plasticity of body size evolves at microevolutionary scales (Maurer et al., 1992), with standing genetic variation providing the basis for adaptability of body size plasticity (Gotanda et al., 2015). Parthenogenetic wasps provide an ideal natural experimental system to test hypotheses of plasticity of body size, considering their clonal mode of reproduction that maintains genetic variation, specifically utilizing a combination of common-garden and reciprocal transplant experiments to control for genetic and environmental variation.

The parasitoid wasp, *Dinocampus coccinellae* (Hymenoptera: Braconidae), is a generalist that is capable of successfully parasitizing over fifty species of predatory ladybeetles (Coleoptera: Coccinellidae, subfamily Coccinellinae) across a global distribution (Balduf, 1926; Ceryngier et al., 2018, Fei et al 2023). *D. coccinellae* primarily displays solitary behavior, and is only known to asexually reproduce through thelytoky, a mode of parthenogenesis in which females emerge from unfertilized eggs; with males rarely observed in this species (Slobodchikoff and Daly, 1971; Wright, 1979; Heimpel and De Boer, 2008; Ceryngier et al., 2018). Briefly, thelytoky is a parthenogenetic mode of reproduction in which diploid female adults develop from unfertilized egg clones (Beukeboom et al., 2007; Heimpel and Jetske, 2008; Slobodchikoff and Daly, 1971). There are genetic forms of thelytoky in which no crossing over occurs (apomictic thelytoky or premeiotic doubling) or where the fusion of sister or non-sister recombinant chromosomes form diploid eggs (automictic thelytoky) (Heimpel and Jetske, 2008), regardless restricting genomic variation from parent to offspring. Characteristic to the Euphorinae subfamily of Hymenoptera, a parasitoid larva of *D. coccinellae* consumes the adipose tissue of a parasitized adult ladybeetle as a koinobiont endoparasitoid, (Balduf, 1926; Orr et al., 1992; Ceryngier et al., 2018), although it has been documented to oviposit within host ladybeetle larvae and pupae (Obrycki et al., 1985). Across the diverse range of host ladybeetles, *D. coccinellae* has been reported to preferentially oviposit in coccinellids which are more mobile, larger, adult, female hosts (Davis *et al.*, 2006; Obrycki, 1989). Once an adult *D. coccinellae* locates a sufficient adult ladybeetle, they arch their stinger under the beetle and thrust into the abdomen of the host, injecting clonal daughter egg(s) along with accompanying venom enzymes and the RNA-virus, the *Dinocampus coccinellae* Paralysis Virus (DcPV) (Balduf, 1926; Orr *et al.,* 1992; Dheilly *et al.*, 2015). This is yet another unique facet of the *D. Coccinellae*, as their life cycle involves an endosymbiotic relationship established with DcPV, an RNA virus in the Iflaviridae family (Dheilly et al., 2015). In concert with the host behavior modifications mediated by this virus, *D. coccinellae* then use their captive adult host (as a bodyguard) to the advantage of the next generation. After approximately a week following oviposition within a host beetle, the larva emerges from its egg into the fat body of the host's abdomen, where it undergoes four larval instar stages of development (Balduf, 1926). Multiple eggs may be deposited within the same host, which is referred to as superparasitism, which has been documented in several field studies (summarized by Ceryngier et al. 2012). When this occurs, the 'most fit' larval first instar, in terms of rapid emergence from egg and stronger mandibles, survives while crushing others (Balduf, 1926). In these cases of superparasitism, the survivor then cannibalizes its host-mate(s) as its first meal; otherwise, the larva feeds on adipose tissue and ovaries of coccinellid host throughout development (15-20 days) (Balduf, 1926). Tetratocytes, which originate from the parasitoid's egg, aid in providing an initial food source, in addition to the host itself (Okuda *et al*., 1995). Following pupation in an external cocoon, the daughter wasp emerges as an adult with fully developed eggs, with some of these females leaving a varying percentage of their hosts alive (Orr et al., 1992). The intricate behavioral relationship between an adult *D. coccinellae* wasp and its host ladybeetle have been described, with successful parasitization, measured as the percentage of emerged daughter wasps as a proxy for fecundity, varying between different host species (Orr et al., 1992). However, little is known about fitness consequences of the emergent parthenogenetic daughter wasps.

In a previous study by Vansant et al., 2019, a positive relationship was determined by regressing a variety of size morphological traits (e.g. dry mass, wing length, abdominal length) between the host beetle and the emergent daughter *D. coccinellae*; indicating that larger wasps emerged from larger hosts and vice versa. This conclusion resonates with the highly dependent the size morphology relationship is between parasitoid wasps and their hosts (Brandl and Vidal, 1987; Mackauer and Chau, 2001; Harvey et al., 2006; Henry et al., 2006; Symonds and Elgar, 2013). The developmental environmental conditions, including resources that a developing parasitoid can uptake from its host substantially determines the body size phenotype of the emerging parasitoid. However, as *D. coccinellae* reproduces via thelytokous parthenogenesis (thelytoky) with little to no additive genetic variation, this brings into question the balance between heritability or phenotypic plasticity of body size as a proxy for individual fitness.

To test and quantify the heritability and phenotypic plasticity of body size, we established multigenerational experiments with unilineal and multilineal reciprocal crosses of three host ladybeetle species exposed to parthenogenetic lines of *D. coccinellae*. Given that total phenotypic variation of a trait is composed of genetic and environmental variation, a unilineal line consisted of rearing wasps and resultant clonal daughters on a single host beetle species background with little variation in size. Restricting the environmental variability via a single host species, would therefore indicate if the size of the wasp is a plastic response. We therefore expect that the phenotypic variation from mother to clonal daughter are independent of each other and would positively correlate with host size morphological traits. Alternately, to test if body size is an adaptive heritable trait, multilineal lines were set up by raising clonal mother and daughter wasps on reciprocally alternating host beetles of varying size. A positive correlation between mother and daughter pairs would therefore indicate that phenotypic variation is strongly dependent on the parthenogenetic heritability of the wasp, and independent of the host size.

Materials and Methods

Experimental setup

D. *coccinellae* wasps used to start the lineages for reciprocal host-transplants were obtained from field collections in Kentucky of parasitized adult *Coccinella septempunctata* (*C. septempunctata* or C7 - JJO personal comm.) and from *Hippodamia convergens* (*H. convergens* or H. con) from an insectary in San Marcos, CA in . Parthenogenetic lines of *D. coccinellae* were then maintained for at least 4 generations on laboratory populations of three species of lady beetles – C7, H. con, and *Coleomegilla maculata* (*C. maculata* or C. mac) which were obtained from field sites in Kentucky (JJO personal comm.). These beetle populations were maintained on an *ad libitum* diet of *Acyrthosiphon pisum* (pea aphids), which in turn were maintained on fava bean plants (*Vicia faba*) in insect tents in the California State University San Marcos (CSUSM) greenhouse in San Marcos, CA until March 2020. Following the COVID-19 outbreak, all insect tents, subsequent crosses, and experimentation were performed (socially distanced and masked) in AT’s garage in Oceanside, CA. Despite temporary relocation of the experimental setup, all experimental conditions were maintained constant to minimize random effects, including daily variations in temperature and diurnal cycles.

In each experimental setup, one adult *D. coccinellae* wasp (‘mother’) was placed into a paper soup cup along with four individual ladybeetle hosts, moth (*Ephestia*) eggs for hosts to feed on and a honey-water soaked cotton ball for both the wasp and beetles to drink from; only one wasp was introduced per each experiential cup setup and was sealed using a mesh sheet and an open-face lid. After the ‘mother’ wasp oviposited into her hosts and died, the adult wasp was collected along with her original host ladybeetle. The remaining four host ladybeetles were fed and tended to until initial appearance of the cocoon spun by the larval D. coccinellae parasitoid. It was always the case that the adult D. coccinellae ‘mother’ died before the larval D. coccinellae ‘daughter’ egressed from her host and spun a cocoon. Once finished developing in her cocoon, an adult D. coccinellae ‘daughter’ egressed from her cocoon. This ‘daughter’ D. coccinellae is then placed in another experimental cup setup as the next ‘mother’ D. coccinellae with another four individual ladybeetles of the next type of host coccinellid species, *Ephestia* (moth) eggs for hosts to feed on and honey-water for both host and wasp to drink. In every introduction cup, the life history data recorded were: wasp introduction date, parent removal and collection date, cocoon date (if noticed), daughter eclosion date, and host mortality rate.

Parasitized beetles were reared until the egression of the *D. coccinellae* larva from the infected host as a cocoon woven between the host legs (Vansant et al., 2019). Of the morphological crosses, 92 wasp-host pairs and 40 mother-daughter pairs were collected for morphological observations. An expanded polystyrene foam stage and ruler (mm) was assembled to standardize and scale the photographed parasitoid-host pairs. Using a Nikon dissection microscope, adult *D. coccinellae* wasps were photographed in the lateral position, and the corresponding ladybeetle host was photographed from the dorsal, lateral, and ventral positions. These images were uploaded into ImageJ (NIH) to obtain the following morphometric measurements in mm for the wasp: head length, head depth, thorax length, thorax depth, abdomen length and wing length (Figure 1); and for the host beetle: dorsal body length and depth; lateral body depth, elytron chord length and pronotum length; and ventral pronotum width, and abdominal length and width (Figure 2); based on body segments measured in Vansant et al., 2019. Morphometric measurements were repeated independently by four individuals and standardized to control for observational bias. Each parent and daughter wasps were then paired for regression analysis, in addition to pairing host beetle and emergent wasp measurements.

Statistical analysis

Size distributions of all wasp and host ladybeetle morphological variables were visualized as box plots, grouped by the host species from which the wasp eclosed. To determine differences in body size morphology between all three lady beetle species, box plots, one-way ANOVAs and three paired T-tests (two samples, assuming unequal variances) were performed across each body segment morpho-measurement (Figure 3a-h, Table 1, Supplementary Data File). Additionally, owing to non-significant size differences among the *C. maculata*, *H. convergens* hosts when compared to *C. septempunctata* hosts, we grouped *C. maculata* and *H. convergens* under one ‘Small’ identifier, and repeated the same tests described above to compare them to the ‘Large’ *C. septempunctata* ladybeetles (Figure 4a-h, Table 2, Supplementary Data File). Similarly, box plots, one-way ANOVA and three paired T-tests (two samples assuming unequal variances) were performed across *D. coccinellae* morphological measurements (with their respective host species as a factor) (Figure 5a-h). All tests were p-value corrected for multiple testing using a conservative Bonferroni correction at an FPR of 0.05.

Linear regression models for each offspring variable were made using R to test how each variable was affected by (1) the size of both the parent and offspring hosts, (2) the host species, (3) the matching parent's morphological variable, and interactions between these predictors. Additionally, analysis using AIC (Akaike's Information Criterion) values were used for model selection to determine which of the previous linear models were best supported by the data. Parent-offspring regressions for both 'unilineal' and 'multilineal' *D. coccinellae* pairings were performed to (1) estimate the relationship between wasp body size morphology and their host environment, and its heritability, and (2) quantify the degree of phenotypic plasticity of body size morphology with change in host environment respectively. Within the 'multilineal' grouping of *D. coccinellae* lineages, parent-offspring pairs were further separated into parasitoid mother-daughter pairs which were reared from Smaller to Larger and Larger to Smaller host ladybeetles (Supplemental Data File).

To better understand the association between mother and daughter morphology, canonical correlation analysis (CCA) was conducted relating mother and daughter morphological variables (mass was not included because of missing values, which would have further reduced the sample size). Initially, the canonical correlation between mother and daughter morphology was assessed without accounting for host type. Then, to determine how much of the canonical correlation between mother and daughter was due to either mother or daughter host environment, partial CCA was conducted after accounting for: mother's host, daughter's host, combinations of mother's and daughter's host. Models that controlled for mother's host in mother's morphology and daughter's host in daughter's morphology, and mother's host in mothers morphology and combinations of mother's and daughter's hosts in daughter's morphology were also used. Partial CCA was done using the residuals from a

MANOVA using the mother or daughter morphology as the responses, and the mother, daughter, or interaction between mother and daughter host as the predictor. Large changes in the patterns of canonical correlation between mother and daughter when host was accounted for would indicate that the correlation was principally due to host-mediated effects (e.g. developmental environment, mother's investment decisions at oviposition), whereas stable patterns of correlation after host effects had been accounted for would be consistent with factors driven by the mother's state, independent of the host she developed in or oviposited on. All CCA analyses were conducted with the *yacca* package (version 1.4-2, Butts 2022).

Results

To establish that the three separate ladybeetle species do indeed provide parasitoid *D. coccinellae* larva with significantly different environmental conditions to develop within, we generated box plots and ran a two-sample t-test assuming unequal variances and a one-way ANOVA between the ladybeetle species across each body segment (Figure 3a-h, Table 1). Two-sample t-tests indicate that body segment measurements are non-significant between host beetle pairs, with substantial overlap between *C. maculata* and *H. convergens*. But, once *C. maculata* and *H. convergens* were grouped together under the 'Small' label and *C. septempunctata* being the 'Large' label (Figure 4a-h), all p-values for the t-test comparisons of body size across species categories were statistically significant (Alpha cutoff value at $p < 0.05$, Table 2, Supplementary Data File).

Although it is established that the developing *D. coccinellae* larvae are growing under conditions which differ significantly, Table 3, Figures 5a-f and the Supplementary Data File, only abdominal length, and head depth morphology significantly differ among emergent wasps, regardless if it is based on which host ladybeetle species or 'Small' vs 'Large' host ladybeetle body type the parasitoid wasp eclosed from.

The two strongest correlations of the morphological variables measured for both adult D. coccinellae body segments and the body segments of her host ladybeetle are correlations of: 0.51 between ladybeetle 'Abdominal Width (V)' and D. coccinellae Head Depth; and 0.45 between ladybeetle Abdominal Width (V) and D. coccinellae Thorax Length. The first canonical correlation reported in the analysis is 0.83, and it is the correlation between an axis through the beetle measurements with an axis through the wasp measurements. The majority of D. coccinellae and ladybeetle morpho-measurments are positively correlated. The first CC axis shows that there is an extreme negative outlier, in the direction of the Ventral Abdominal Width ladybeetle measurement of the host beetles. There does not seem to be distinction in the spread of the second CC axis.

Narrow sense heritability across each body segment measurement was captured by the slope of the line of best fit regression plots between parent-offspring pairs. (Figures 7a-f and 8a-f). The parent-offspring regressions in Figures 7a through 7f depict the experimental setup with both mother and daughter *D. coccinellae* egress from the same host species of coccinellid (termed as unilineal) and develop under similar host-environmental conditions [total df = 14]. The majority of unilineal parent-offspring regression plots indicate a slight negative slope or relationship, but the slope of parent-offspring relationship for body segment is not significantly different than a slope of zero and indicates that offspring body measurements are independent of their mother wasp's body segment size. This independence of the offspring body segment to parent body measurements are evident in: (Fig. 7a) fitted line plot of head length regression between unilineal parent-offspring pairs [regression slope = +0.030, p-value = 0.94], (Fig. 7b) fitted line plot of head depth regression between unilineal parent-offspring pairs [regression slope = -0.401 , p-value = 0.3], (Fig. 7c) fitted line plot of thorax length regression between unilineal parent-offspring pairs [regression slope = -0.065, p-value = 0.773], (Fig. 7d) fitted line plot of thorax depth regression between unilineal parent-offspring pairs [regression slope = +0.220, p-value = 0.541], (Fig. 7e) fitted line plot of wing length regression between unilineal parent-offspring pairs [regression slope = -0.208, p-value = 0.455], (Fig. 7f) fitted line plot of abdomen length regression between unilineal parent-offspring pairs [regression slope = +0.425, p-value = 0.33].

Alternately, Figures 8a through 8f display an experimental setup where mother and daughter *D. coccinellae* egress from different host species of coccinellid (termed as multilineal), thus developing under different environmental conditions. All six regression slopes for the measured body segments between multilineal parent-offspring pairs uniformly display a slight positive relationship [total df = 24]. Again, almost all of the regression slopes are nonsignificant and as such, there is a non-zero slope for each body segment regression indicating that body segment sizes are independent between daughter and mother *D. coccinellae* adult wasp. The independence, yet slight positive trend, of parent-offspring body measurements is apparent throughout: (Fig. 8a) fitted line plot of head length regression between multilineal parent-offspring pairs [regression slope = +0.295, p-value = 0.014], (Fig. 8b) fitted line plot of head depth regression between multilineal parent-offspring pairs [regression slope = +0.302, p-value = 0.081], (Fig. 8c) fitted line plot of thorax length regression between multilineal parent-offspring pairs [regression slope = +0.133, p-value = 0.302], (Fig. 8d) fitted line plot of thorax depth regression between multilineal parent-offspring pairs [regression slope = +0.238, p-value = 0.009], (Fig. 8e) fitted line plot of wing length regression between multilineal parent-offspring pairs [regression slope = +0.396, p-value = 0.021], (Fig. 8f) fitted line plot of abdomen length regression between multilineal parent-offspring pairs [regression slope = +0.313, p-value = 0.042].

Finally, both unilineal and multilineal parent-offspring pairs were combined into a total fitted-line regression analysis across all six body segment variables. The majority of regression slopes for the measured body segments are nonsignificant, yet the regression slopes uniformly show a slight positive trend. Again, the independence and slight positive trend of the parent-offspring body measurements is apparent throughout: (Fig. 9a) fitted line plot of head length regression between multilineal parent-offspring pairs [regression slope = +0.239, p-value = 0.078], (Fig. 9b) fitted line plot of head depth regression between multilineal parent-offspring pairs [regression slope = +0.115, p-value = 0.487], (Fig. 9c) fitted line plot of thorax length regression between multilineal parent-offspring pairs [regression slope = +0.049, p-value = 0.675], (Fig. 9d) fitted line plot of thorax depth regression between multilineal parent-offspring pairs [regression slope = +0.243, p-value = 0.03], (Fig. 9e) fitted line plot of wing length regression between multilineal parent-offspring pairs [regression slope = +0.174, p-value = 0.24], (Fig. 9f) fitted line plot of abdomen length regression between multilineal parent-offspring pairs [regression slope = +0.330, p-value = 0.037].

Using AIC values to determine which models are most appropriate for further analysis, we found that most models were not well supported by the data and the best supported model was the intercept only model. The exceptions to this were: offspring thorax depth with respect to parent thorax depth (Fig. 9d), offspring abdomen length with respect to parent abdomen length (Fig 9e), offspring head depth with respect to parent head depth with an interaction of the parent host species (Fig. 10b), and offspring head length with respect to parent head length (Fig. 9a).

For every CCA model only the first canonical axis was statistically significant (Rao's F approximation, p-values ranging from 0.002 to 0.02). All of the models yielded a qualitatively consistent pattern (Figure 11), in which the parent loadings were primarily positive and offspring loadings were negative except for wing length. The canonical correlation coefficients were also very consistent, ranging from a low of 0.76 for the model in which no host species were accounted for to a high of 0.8 for the model in which the combinations of mother and daughter host were accounted for in both the mother and daughter morphologies. The contrast between mother and daughter loadings increased in any model that daughter host was accounted for, with all of the mother's loadings becoming positive for those models.

Discussion

The unique life history strategies of *D. coccinellae* - thelytokous parthenogenesis, solitary behavior, and the ability to successfully oviposit in an uncharacteristically large range of host lady beetle species that span a wide spectrum of body sizes and shapes (Balduf, 1926; Ceryngier et al., 2012, 2018; Wright, 1979) present a great opportunity to understand the dynamics of phenotypic microevolution of size. This parasitoid attacks a group of predatory beetles that are widely used in biological control; our study highlights the importance of examining the genetic bases of ecological interactions underlying parasitoid-host relationships (Fei et al 2023, Rodrigues et al 2022, Sentis et al 2022).

Specifically, the diversity in host coccinellid morphology offers *D. coccinellae* (1) different host-parasitoid conflicts (Orr et al., 1992), (2) different environmental niches for their larvae to develop in, and (3) varying amounts of adipose tissue to feed upon. Therefore, we would predict that phenotypic plasticity in *D. coccinellae*'s ability to successfully parasitize its hosts offers the species a selective advantage at microevolutionary scales, while an occasional sexual reproductive cycle with a male (Shaw et al., 1999) offers an “escape” from Muller’s ratchet (i.e. irreversible accumulation of deleterious variants towards extinction). It has been well documented that variation in parasitoid wasp morphology is strongly associated with variation in body size and morphology of host species (Belshaw *et al.,* 2003; Symonds and Elgar, 2013). Furthermore, previous research indicates that the environmental variation in host lady beetle body size strongly influences the body size phenotype of each emergent *D. coccinellae*, with each next clonal generation being capable of significant size changes relative to the parent (Vansant *et al.*, 2019).

In this study, we utilize a common-garden, reciprocal transplant experiment over multiple generations to investigate the variation in body size morphology of emergent *D. coccinellae* conditioned on (1) the clonal parent, and (2) its host. Our study clearly points to the independence of body size morphology and phenotypes of emergent *D. coccinellae* and its clonal parent, further bolstering the idea of a plastic response to maintain size variation in the species at microevolutionary scales. As *D. coccinellae* reproduces through thelytoky, the process of asexual reproduction in which dipoid female parasitoids are born from unfertilized eggs, it can reasonably be expected that body size morphometric traits would exhibit strong correlation as estimated using parent-offspring regressions, as there is no source of additional genetic variation to affect relatedness through sexual reproduction and recombination or dominance (Slobodchikoff and Daly, 1971; Heimpel and De Boer, 2008). Yet, anything but a strong relationship is observed in our results. Across both the unilineal and multilineal parent-offspring regressions, most of the relationships return non-significant linear slopes, which imply that there is no difference from regression slopes of zero, indicating that there is extremely low heritable variation of size. This was an interesting finding, as we considered thelytokous parthenogenesis to be such a strong constrictor on genetic variation, that the significant shift in body size would have been expected to be at least partially evident in body size plasticity. This experiment also points to how low heritability could emerge from intense selection (here artificial). It is possible that an adult *D. coccinellae* can feasibly jump to a different species of host ladybeetle than that of their mother, given the available distribution of phenotypic variation in body size across one generation. Yet, repeated host shifts in a rapid succession of a few generations may introduce too intense an artificial selection pressure for this trait plasticity to endure, limiting the variation in body size variation of the following *D. coccinellae* generations. Therefore, as a result of negligible additive genetic variance in body size morphometric traits, we would also predict that there is little trait variability for natural selection to act on/work with, thereby minimizing the trait's ability to evolve. This is further complemented by the lack of significant differences in body size morphometric traits in emergent *D. coccinellae* among all host types as observed in our experiment. Further analyses of our controlled reciprocal transplant experiments, to quantify the fecundity of *D. coccinellae* females, perhaps differentially across different hosts would help predict the fitness consequences of natural selection on plastic size in these predominantly asexual species.

Additionally, of potential interest then is the differential efficacy of parasitization of small *D. coccinellae* on smaller versus larger coccinellid hosts. It has been predicted that host manipulation via “bodyguard” behavior (Maure et al., 2011, Maure et al., 2013) to protect *D. coccinellae* pupae from predators is presumably under selection for larger hosts, to possibly repel larger predatory species, e.g., crickets or carabid beetles. This hypothesis can also be tested by studying the fecundity, survival, duration of “bodyguard” behavior, and parasitization rates of emergent *D. coccinellae* across different Coccinellid hosts, while controlling for host size. It has also been noted that the sex of the coccinellid host, and prey availability in the field could also influence variability in size of adults (Belnavis, 1988), which were not controlled in our study.

Multivariate comparison of mother and daughter morphology yielded evidence that mothers produce offspring that differ from them, independent of the host species. Mother’s loadings on the first canonical correlation axis were positive, while their daughter's morphology had negative loadings, except for their wing lengths. This suggests that across all microevolutionary scenarios in our experiment, large mothers produce small daughters with long wings, while small mothers produce large daughters with short wings. A small body size with long wings is consistent with better dispersal ability (summarized in Johannson et al., 2009), and it is possible that large females are preferentially producing daughters that will disperse greater distances. Smaller mothers that produce large daughters with short wings may be maximizing the survival probability of their daughters at the expense of their potential dispersal distances. This pattern of increase in size of koinobionts such as Braconid wasps has also been previously reported to be correlated with increased longevity and fecundity (Boivin 2010). Since this pattern is independent of both mother and daughter host species, it is likely that it is mediated by the mother’s state. Our observations therefore offer partial support for Darwin’s fecundity-advantage model, but limited by energy availability from the host environment (Shine 1988). The mechanism for producing these changes is unknown, but facultative changes in the size of eggs, modified fecundity on energy availability, or epigenetic regulation of gene expression are some combination of the above are possible. The phenotypic variance caused by mothers producing daughters who are genetically identical but are morphologically different would further reduce the narrow sense heritability of traits, and could explain negative slope estimates for some of the traits.

Finally, our results also bring into question the micro- and macroevolutionary consequences of the evolution and maintenance of thelytokous parthenogenesis from ancestral arrhenotoky in these species. A recent study on the *D. coccinellae* genome by Sethuraman et al., 2022 pointed to an early divergence, accelerated rates of genome evolution via manifold duplications and gene loss along the *D. coccinellae* lineage. Significant duplication events were reported in transposase activity and stress response gene families, while significant gene losses were reported among olfactory/odorant receptors and viral-coevolution genes. We surmise that these duplication (and loss) events contribute to standing genomic variation in *D. coccinellae* that permit plasticity of size despite parthenogenetic reproduction and alternating reproductive trade-offs depending on host availability and host-associated energy limitations, independent of maternal genetics.

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Figures and Tables

Figure 1 Body segment morphometric traits measured from adult *D. coccinellae* parasitoid wasps, shown in lateral view with a millimeter scale on the stage. These traits were selected based on the morphometric segments outlined in Vansant *et al.*, 2019.

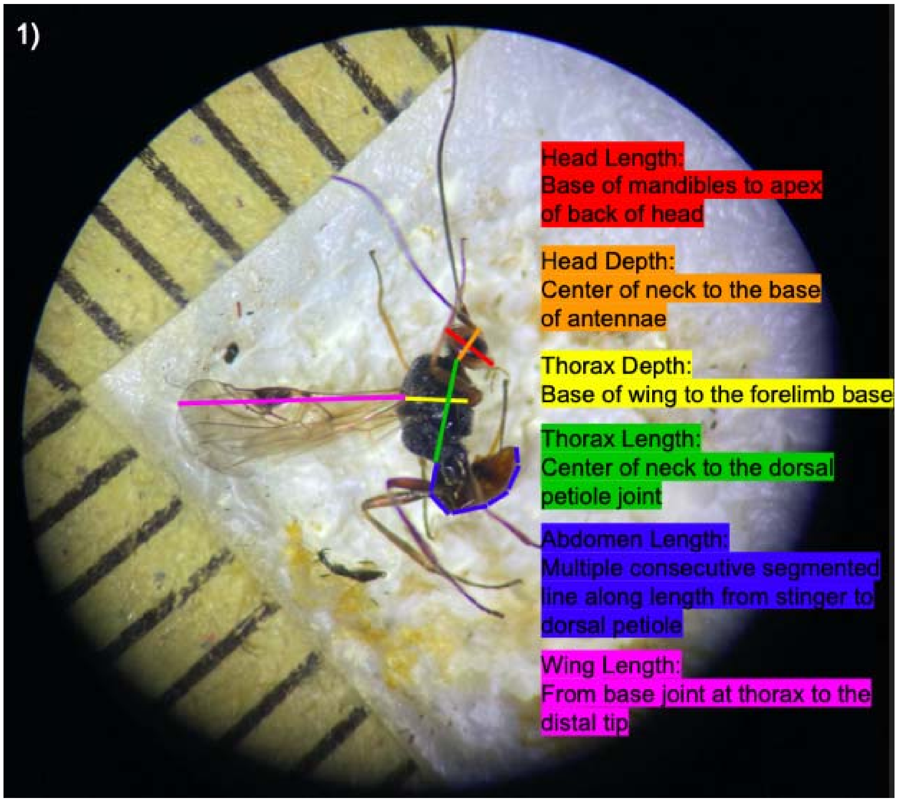
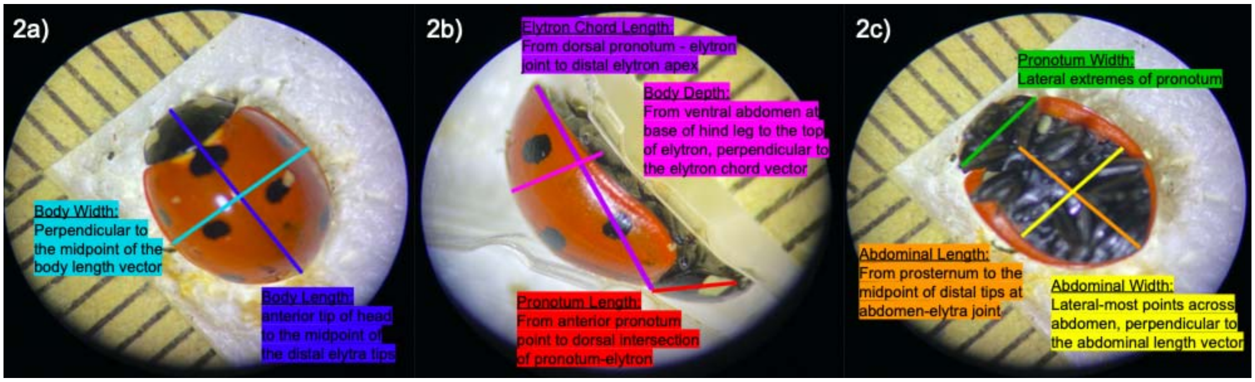
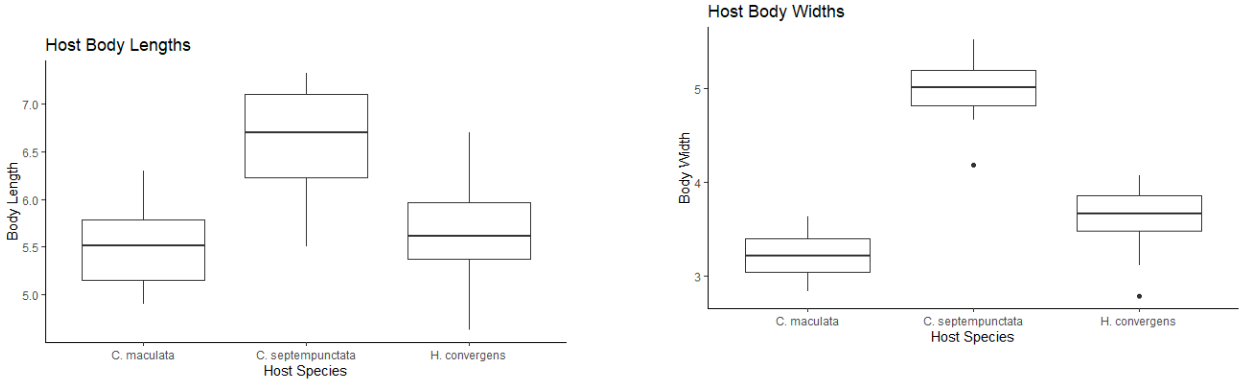


Figure 2 Body segment morphometric traits measured from host lady beetles which the parasitoid *D. coccinellae* egressed from. Shown from dorsal (Fig. 2a), lateral (Fig. 2b), and ventral (Fig. 2c) perspectives, with a millimeter scale on the stage. These traits were selected based on the morphometric segments outlined in Vansant *et al.*, 2019.



Full Text: 2a) "Body Width: Perpendicular to the midpoint of the body length vector", "Body Length: anterior tip of head to the midpoint of the distal elytra tips." 2b) "Elytron Chord Length: from dorsal pronotum – elytron join to distal elytron apex," "Body Depth: From ventral abdomen at base of hind leg to the top of elytron, perpendicular to the elytron chord vector", and "Pronotum Length: From anterior pronotum point to dorsal intersection of pronotum-elytron." 2c) "pronotum Width: Lateral extremes of pronotum," "Abdominal Length: From prosternum to the midpoint of distal tips at abdomen-elytra joint", and "Abdominal Width: Lateral-most points across abdomen, perpendicular to the abdominal length vector."

Figure 3a-3h Boxplots of morphometric variables measured for *Coleomegilla maculata*, *Hippodamia convergens*, and *Coccinella septempunctata* host ladybeetles across the dorsal, lateral, and ventral image viewpoints from R.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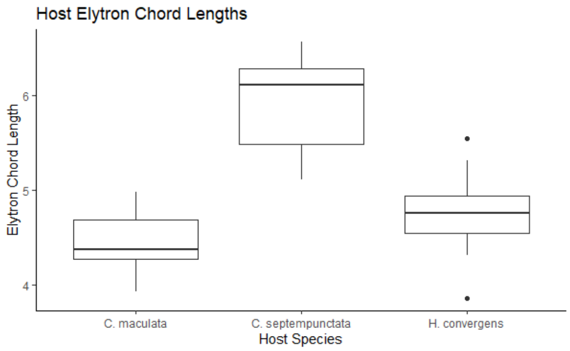
3b)

3c)



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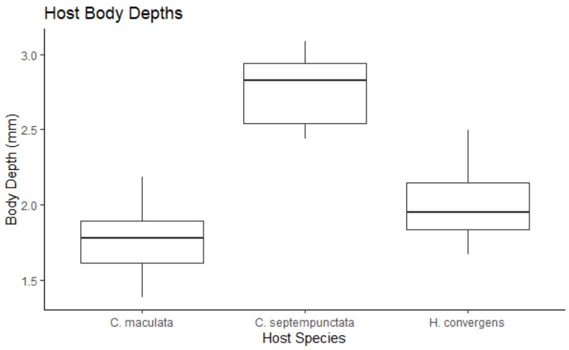

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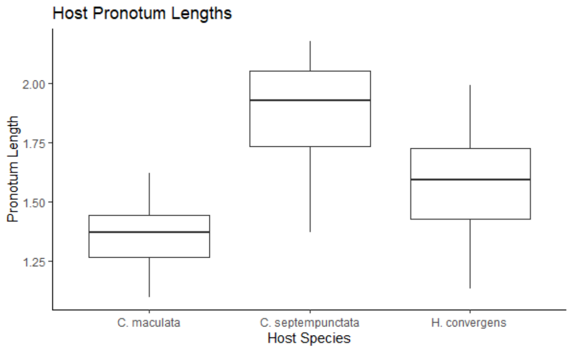

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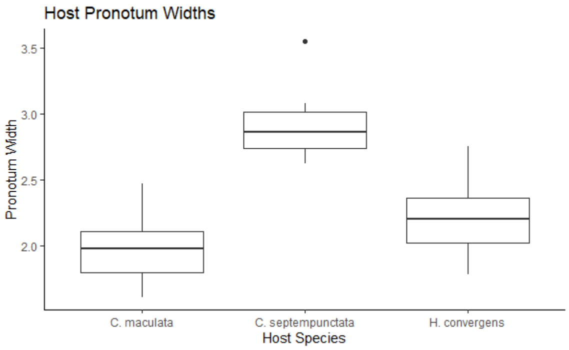

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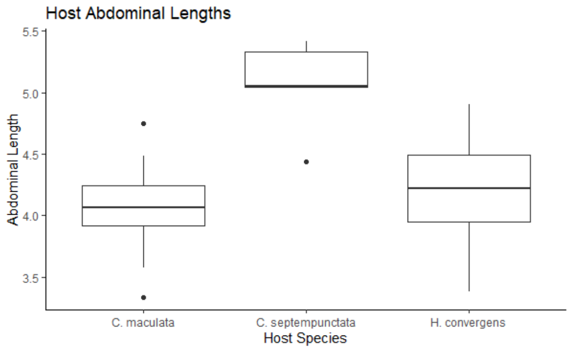

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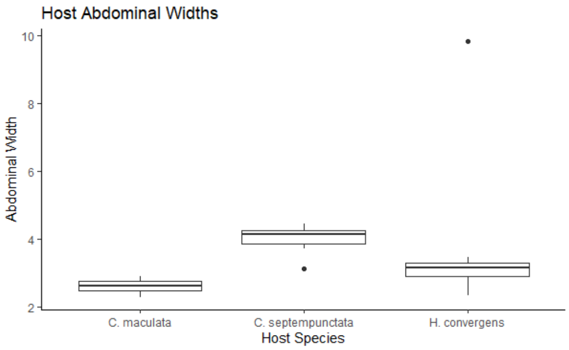
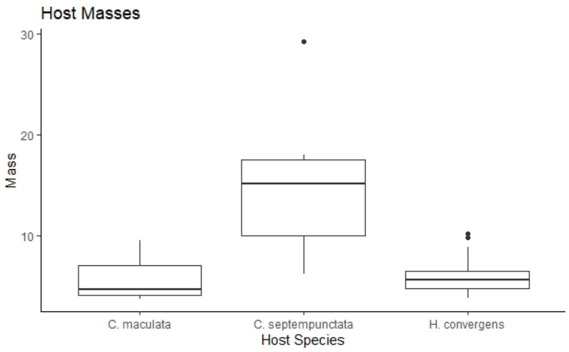
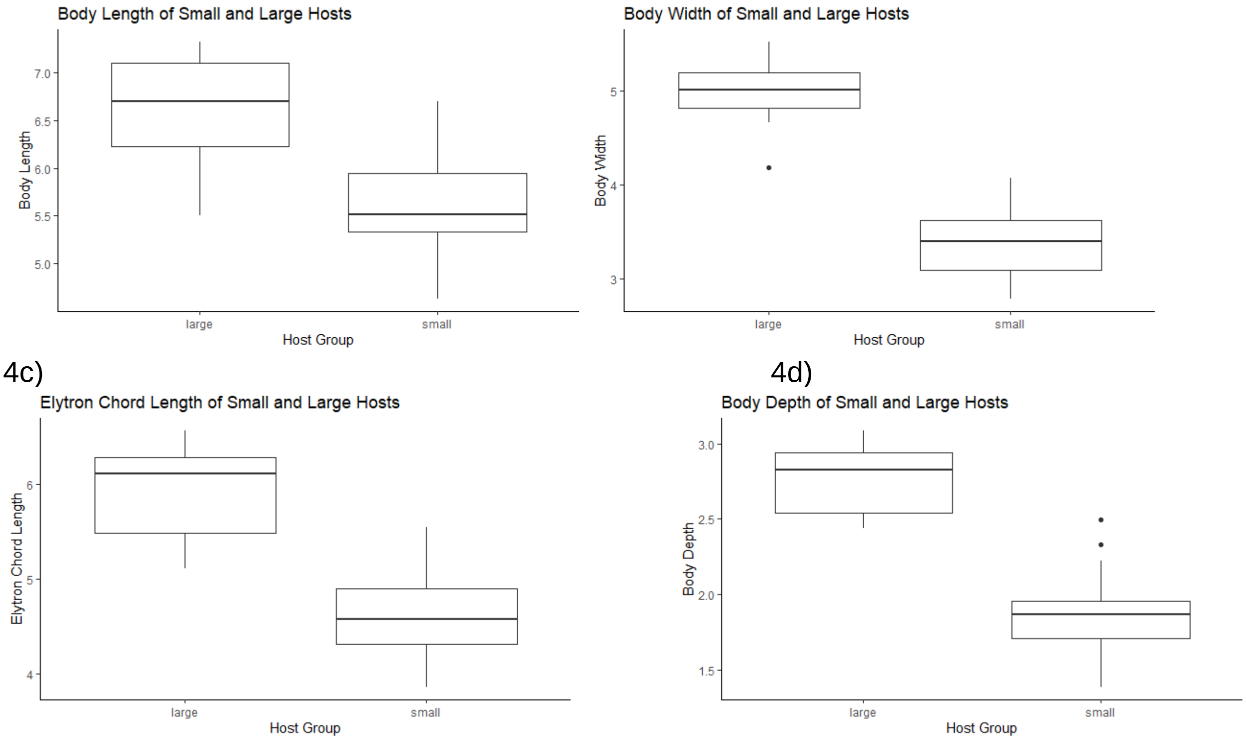




3i)

Figure 4a-4h Boxplots of morphometric variables measured for ‘Small’ (combined *Coleomegilla maculata* and *Hippodamia convergens*), and ‘Large’ (*Coccinella septempunctata*) host ladybeetles across the dorsal, lateral, and ventral image viewpoints from Minitab and MS Excel.



4a)

4b)

4c)

4d)

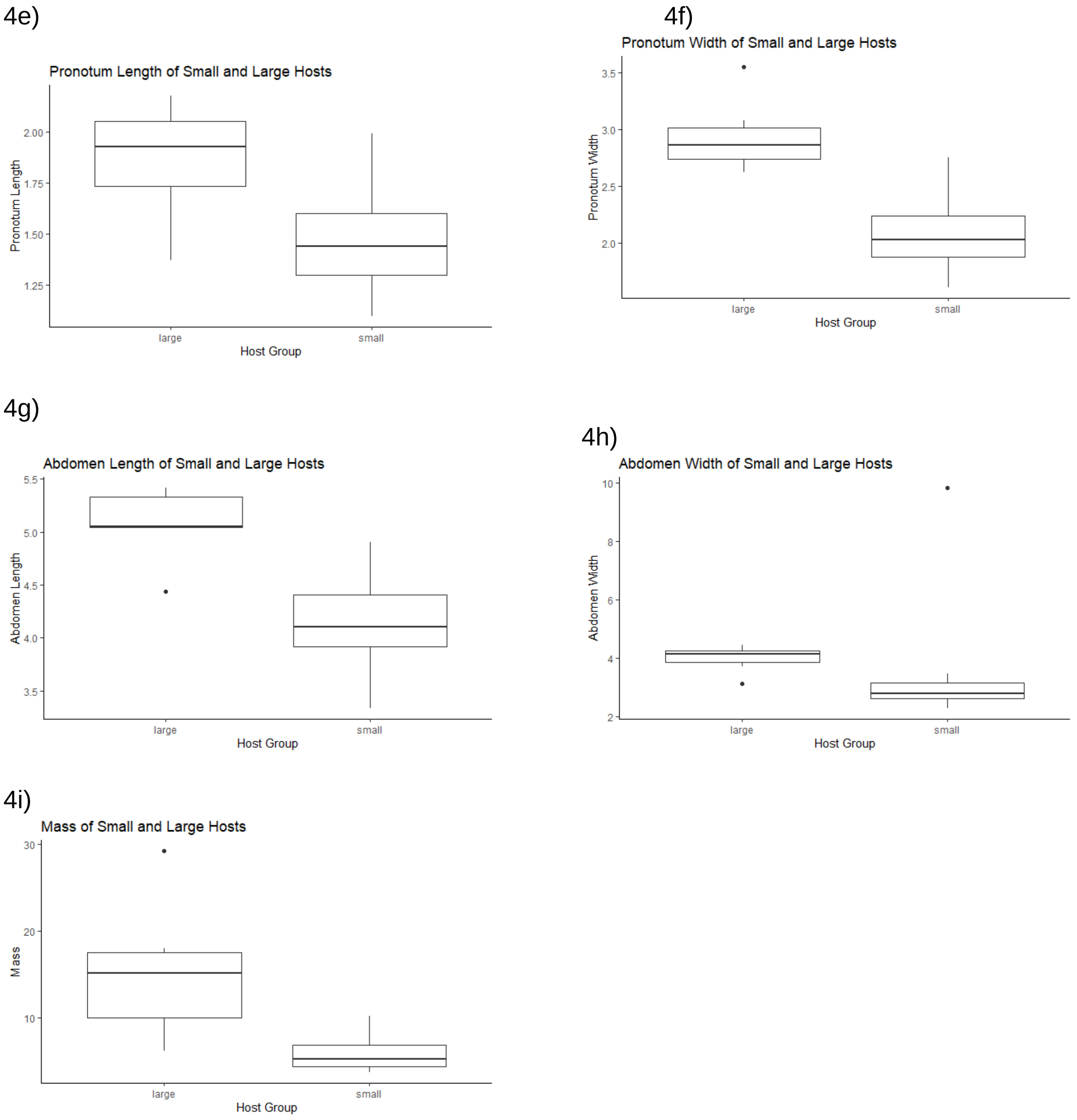
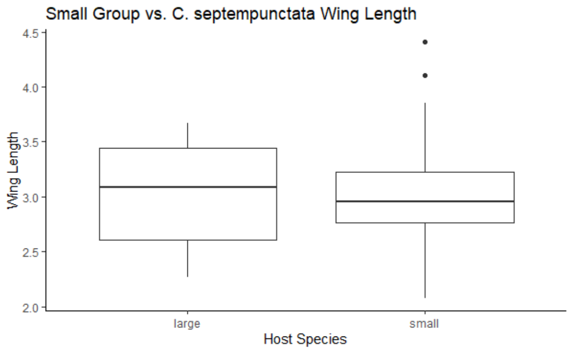


Figure 5 Boxplots for morphometric variables measured for *D. coccinellae*, separated by which host ladybeetle species the parasitoid egressed from (‘Small’ and ‘Large’ host categories are the same for the host ladybeetle analyses). Analyzed in R.

5a)



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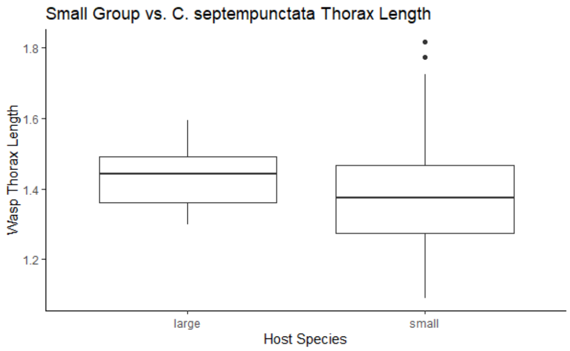

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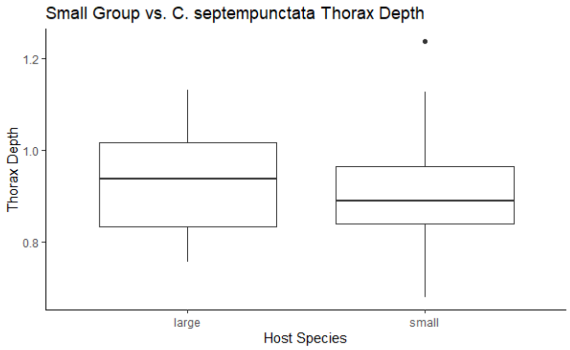
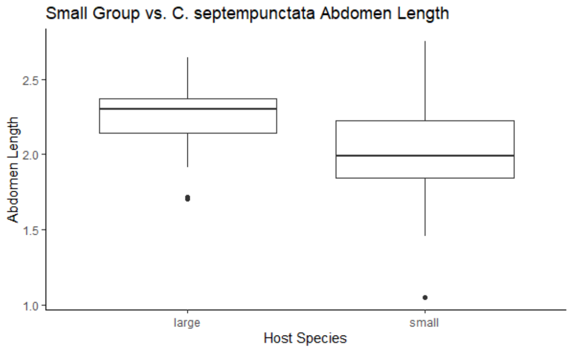
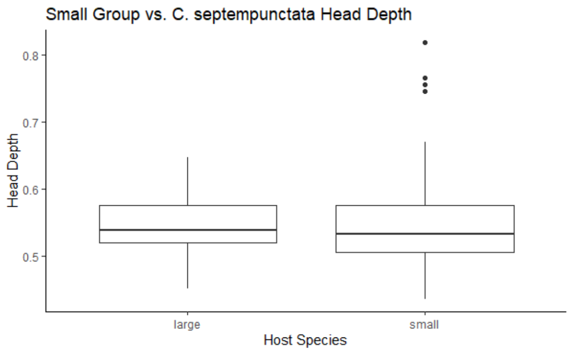


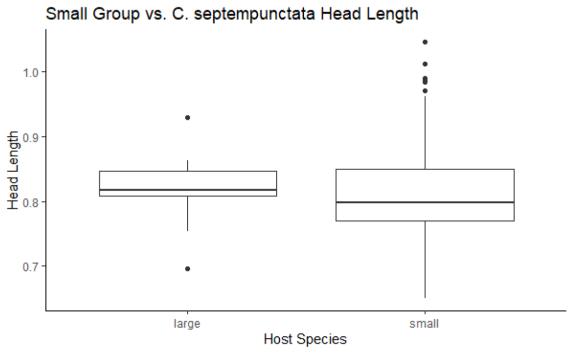
5d)



5e)



5f)



5g)

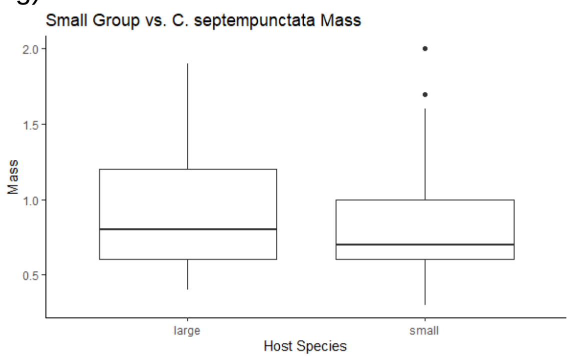


Figure 6 Canonical Correlation Analysis depicting the positive correlation between *D. coccinellae* morphological variables and host ladybeetle morphological variables. This plot was generated using MS Excel and MobaXterm.

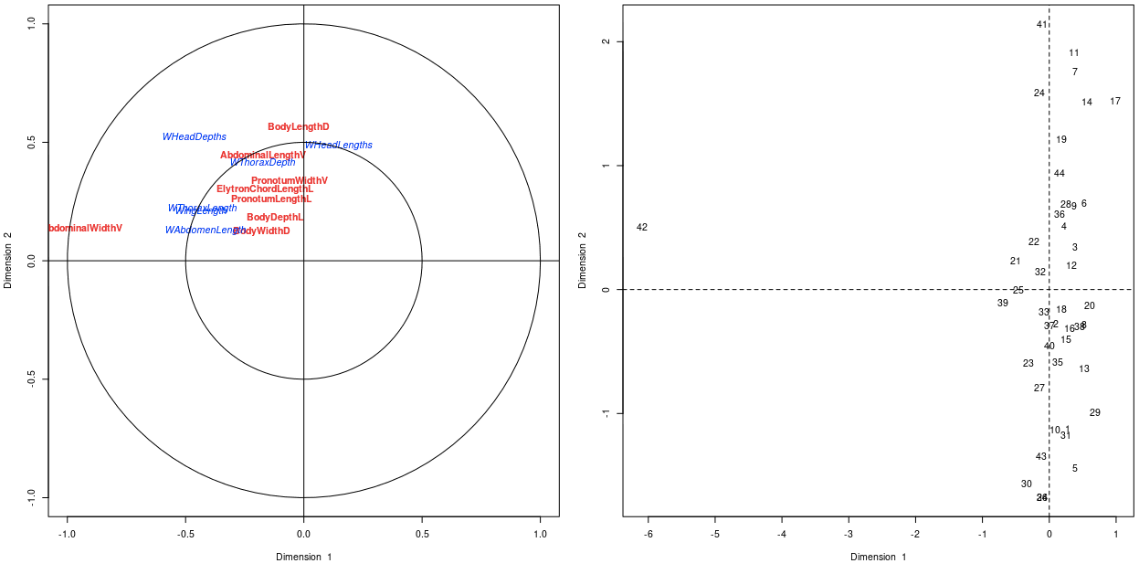
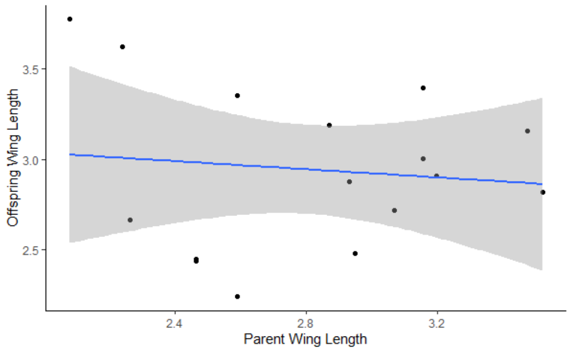


Figure 7 For each parent-offspring pair, mother *Dinocampus coccinellae* wasps egressed from the same host coccinellid species (‘unilineal’) as her daughter clone. All fitted line plots display parent-offspring regressions of the six measured body segments: head depth, head length, thorax length, thorax depth, wing length, and abdomen length [total df = 14]. (Fig. 7a) fitted line plot of head length regression between unilineal parent-offspring pairs [regression slope = +0.030, p-value = 0.94], (Fig. 7b) fitted line plot of head depth regression between unilineal parent-offspring pairs [regression slope = -0.401 , p-value = 0.3], (Fig. 7c) fitted line plot of thorax length regression between unilineal parent-offspring pairs [regression slope = -0.065, p-value = 0.773], (Fig. 7d) fitted line plot of thorax depth regression between unilineal parent-offspring pairs [regression slope = +0.220, p-value = 0.541], (Fig. 7e) fitted line plot of wing length regression between unilineal parent-offspring pairs [regression slope = -0.208, p-value = 0.455], (Fig. 7f) fitted line plot of abdomen length regression between unilineal parent-offspring pairs [regression slope = +0.425, p-value = 0.33].

7a)



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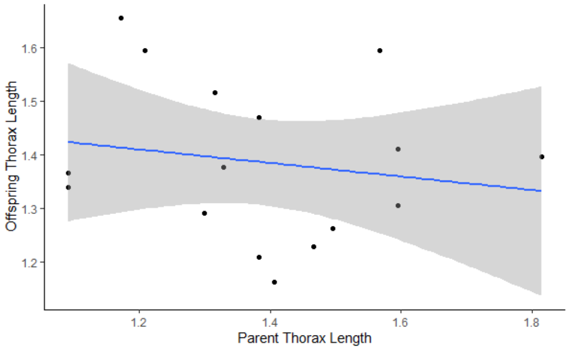

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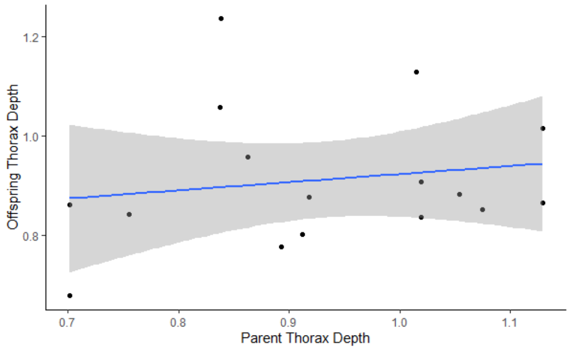

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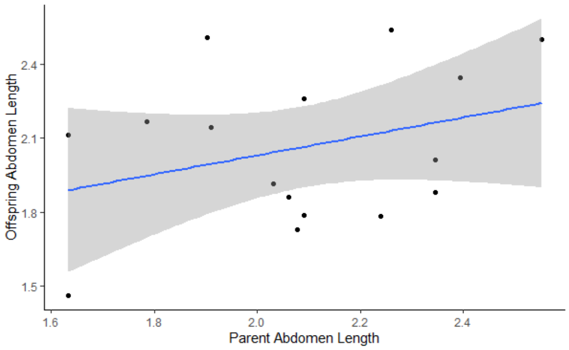

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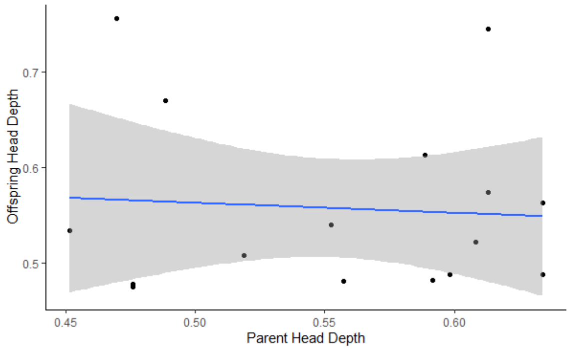

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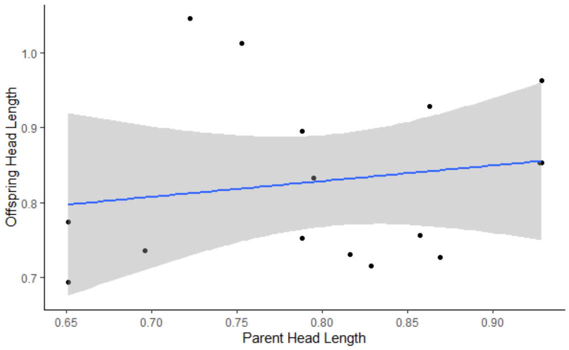


7g)

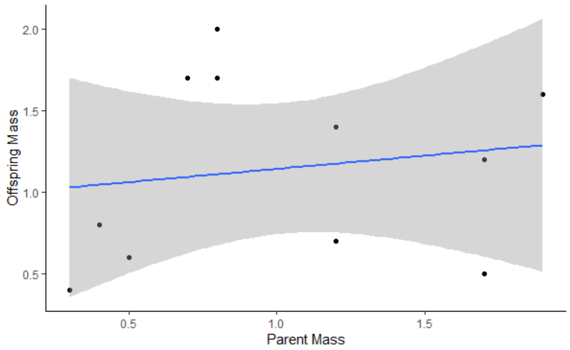
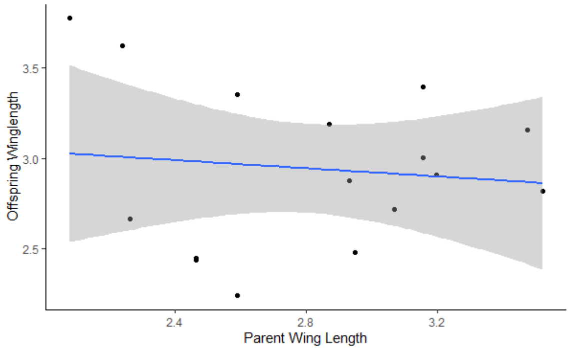
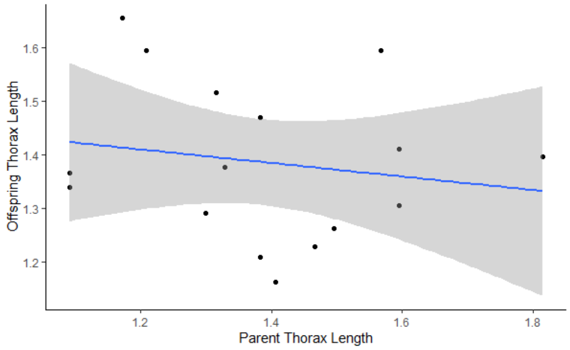


Figure 8 For each parent offspring pair, mother *Dinocampus coccinellae* wasps egress from a different species ('multilineal') of host coccinellid as her daughter clone. All fitted line plots display parent-offspring regressions of the 6 measured body segments: head depth, head length, thorax length, thorax depth, wing length, and abdomen length [total df =24]. (Fig. 8a) fitted line plot of head length regression between multilineal parent-offspring pairs [regression slope = +0.295, p-value = 0.014], (Fig. 8b) fitted line plot of head depth regression between multilineal parent-offspring pairs [regression slope = +0.302, p-value = 0.081], (Fig. 8c) fitted line plot of thorax length regression between multilineal parent-offspring pairs [regression slope = +0.133, p-value = 0.302], (Fig. 8d) fitted line plot of thorax depth regression between multilineal parent-offspring pairs [regression slope = +0.238, p-value = 0.009], (Fig. 8e) fitted line plot of wing length regression between multilineal parent-offspring pairs [regression slope = +0.396, p-value = 0.021], (Fig. 8f) fitted line plot of abdomen length regression between multilineal parent-offspring pairs [regression slope = +0.313, p-value = 0.042].

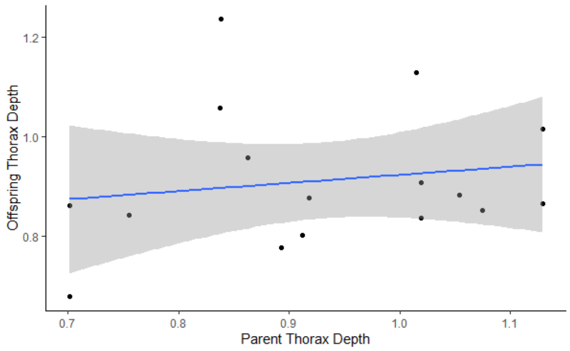
8a)



8b)



8c)



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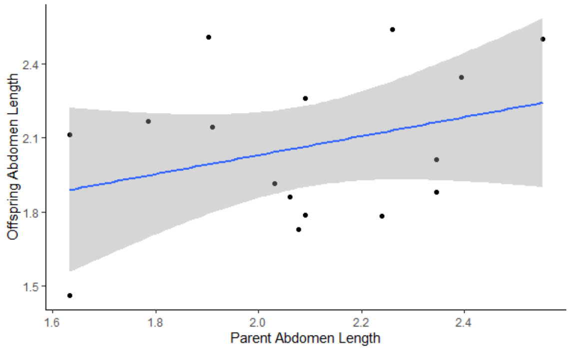

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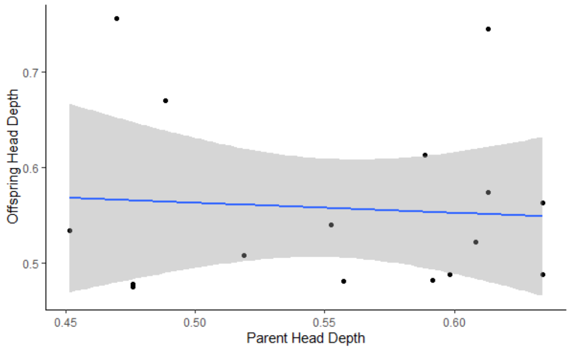

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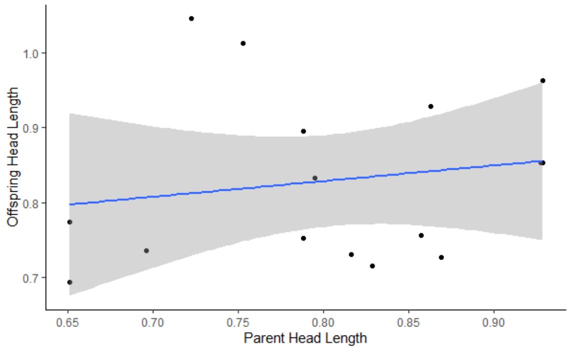
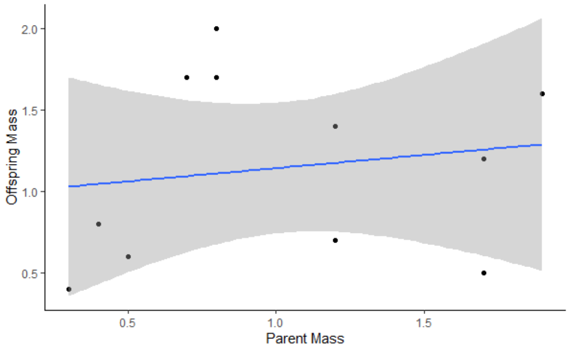
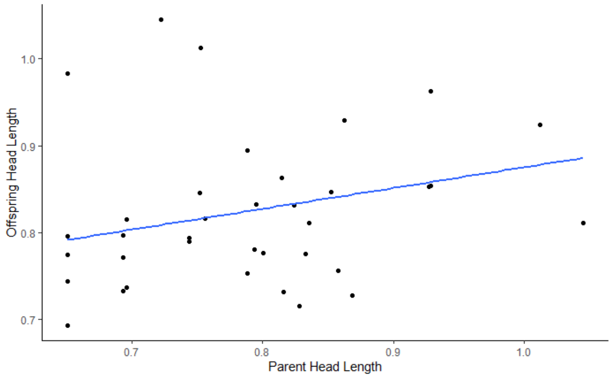




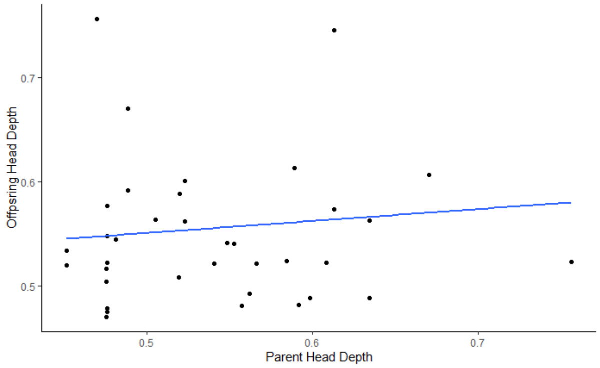
8g)

Figure 9 Displaying the total data collected for all parent-offspring pairings. All fitted line plots display parent-offspring regressions of the 6 measured body segments: head depth, head length, thorax length, thorax depth, wing length, and abdomen length [total df =39]. (Fig. 9a) fitted line plot of head length regression between multilineal parent-offspring pairs [regression slope = +0.239, p-value = 0.078], (Fig. 9b) fitted line plot of head depth regression between multilineal parent-offspring pairs [regression slope = +0.115, p-value = 0.487], (Fig. 9c) fitted line plot of thorax length regression between multilineal parent-offspring pairs [regression slope = +0.049, p-value = 0.675], (Fig. 9d) fitted line plot of thorax depth regression between multilineal parent-offspring pairs [regression slope = +0.243, p-value = 0.03], (Fig. 9e) fitted line plot of wing length regression between multilineal parent-offspring pairs [regression slope = +0.174, p-value = 0.24], (Fig. 9f) fitted line plot of abdomen length regression between multilineal parent-offspring pairs [regression slope = +0.330, p-value = 0.037].

9a)



9b)



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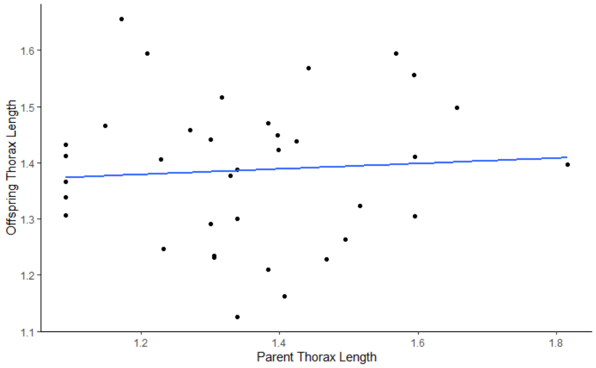

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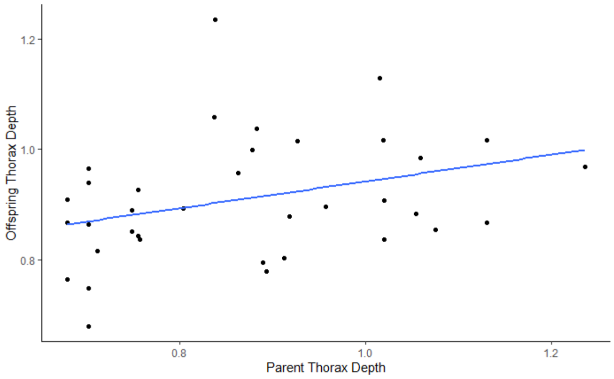

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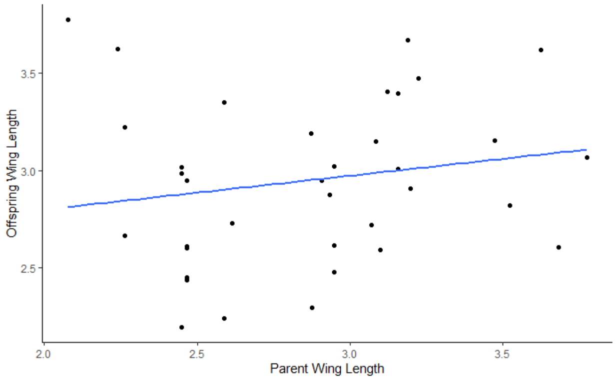


9f)

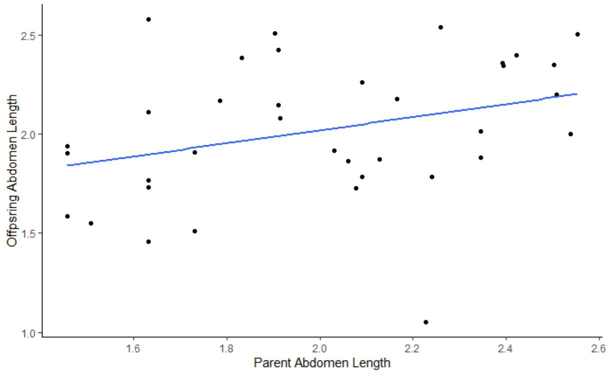
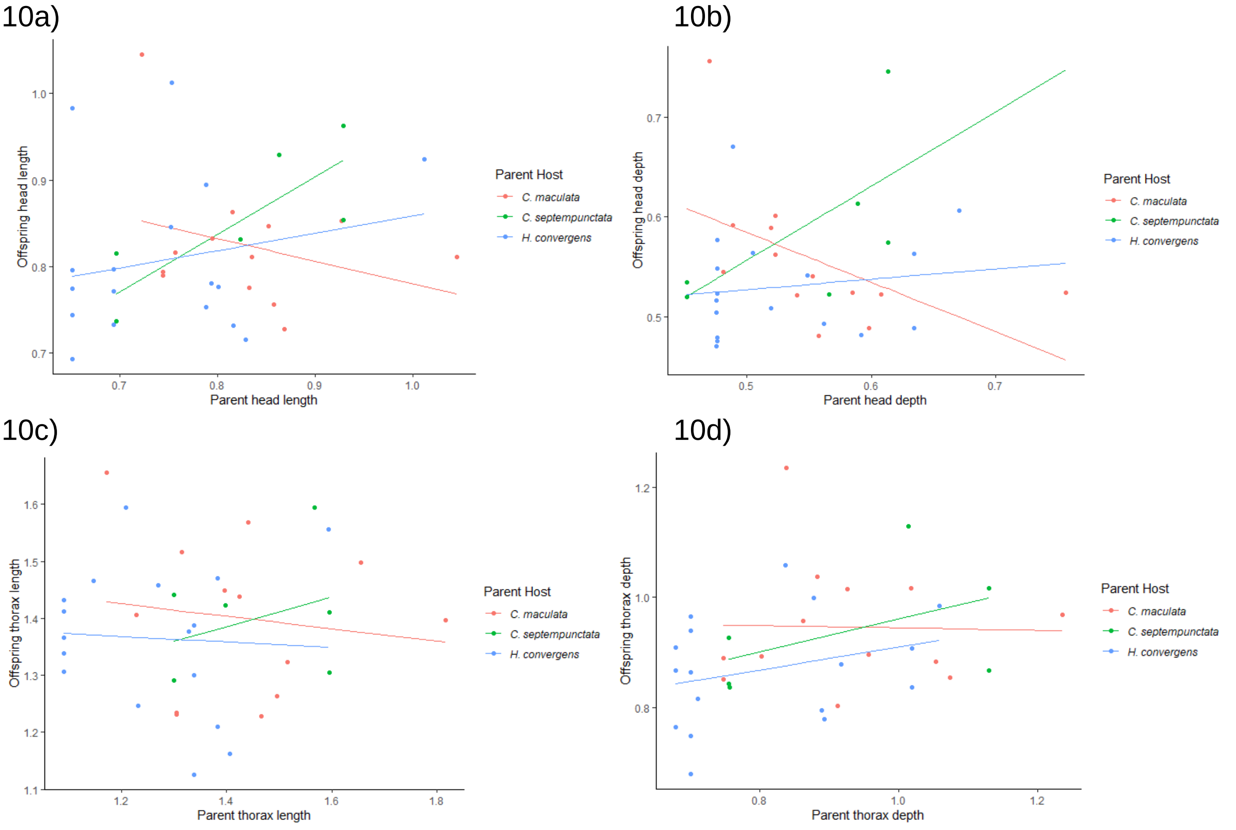
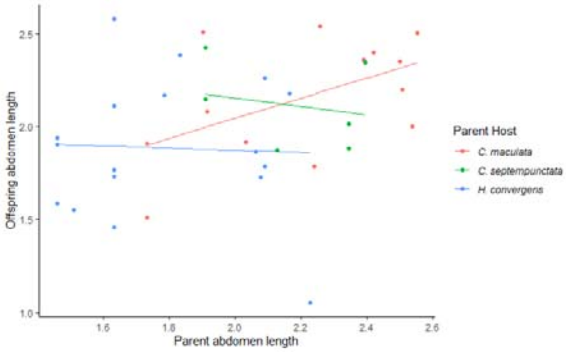
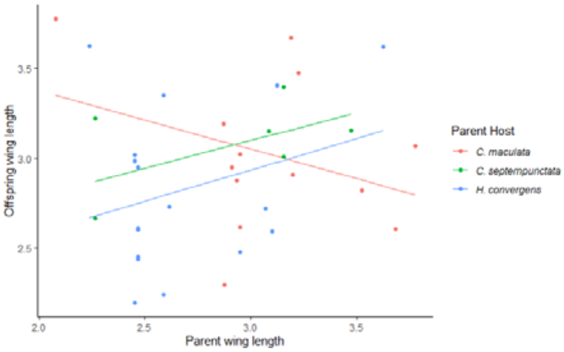


Figure 10 Displaying the total data collected for all parent-offspring pairings with interaction of the parent host species. All fitted line plots display parent-offspring regressions of 6 measured body segments, separated by parent host species [df=39]. (Fig. 10a) fitted line plot of head length regression between multilineal parent-offspring pairs, differentiating between parent host species [C. maculata: slope = -0.2602, p-value = 0.3343; C. septempunctata: slope = 0.9229, p-value = 0.018; H. convergens: slope = 0.4605, p-value = 0.1840], (Fig. 10b) fitted line plot of head depth regression between multilineal parent-offspring pairs, differentiating between parent host species [C. maculata: slope = -0.4978, p-value = 0.042; C. septempunctata: slope = 1.2439, p-value = 0.0017; H. convergens: slope = 0.6007, p-value = 0.075], (Fig. 10c) fitted line plot of thorax length regression between multilineal parent-offspring pairs, differentiating between parent host species [C. maculata: slope = -0.1086, p-value = 0.62; C. septempunctata: slope = 0.3702, p-value = 0.35; H. convergens: slope = 0.0598, p-value = 0.85], (Fig. 10d) fitted line plot of thorax depth regression between multilineal parent-offspring pairs, differentiating between parent host species [C. maculata: slope = -0.019, p-value = 0.931; C. septempunctata: slope = 0.3173, p-value = 0.3; H. convergens: slope = 0.2272, p-value = 0.45], (Fig. 10e) fitted line plot of abdomen length regression between multilineal parent-offspring pairs, differentiating between parent host species [C. maculata: slope = 0.5363, p-value = 0.08; C. septempunctata: slope = -0.7693, p-value = 0.19; H. convergens: slope = -0.5935, p-value = 0.16], (Fig. 10f) fitted line plot of wing length regression between multilineal parent-offspring pairs, differentiating between parent host species [C. maculata: slope = -0.3259, p-value = 0.22; C. septempunctata: slope = 0.6327, p-value = 0.1; H. convergens: slope = 0.6748, p-value = 0.08].



10e)


Offspring abdomen length
2.5
2.0
1.5
1.0
1.6
1.8
2.0
2.2
2.4
2.6
Parent abdomen length
Parent Host
C. maculata
C. septempunctata
H. convergens


Offspring wing length
3.5
3.0
2.5
2.0
2.5
3.0
3.5
Parent wing length
Parent Host
C. maculata
C. septempunctata
H. convergens

10f)

Figure 11. Summary of the results of canonical correlation analysis between mother and daughter morphology. Each model is shown as a row, labeled along the y-axis. Points give variable loadings (correlation between variable and CCA1 axis), and are labeled by variable abbreviation. The canonical correlation coefficient for CCA1 is given the the right of the points.

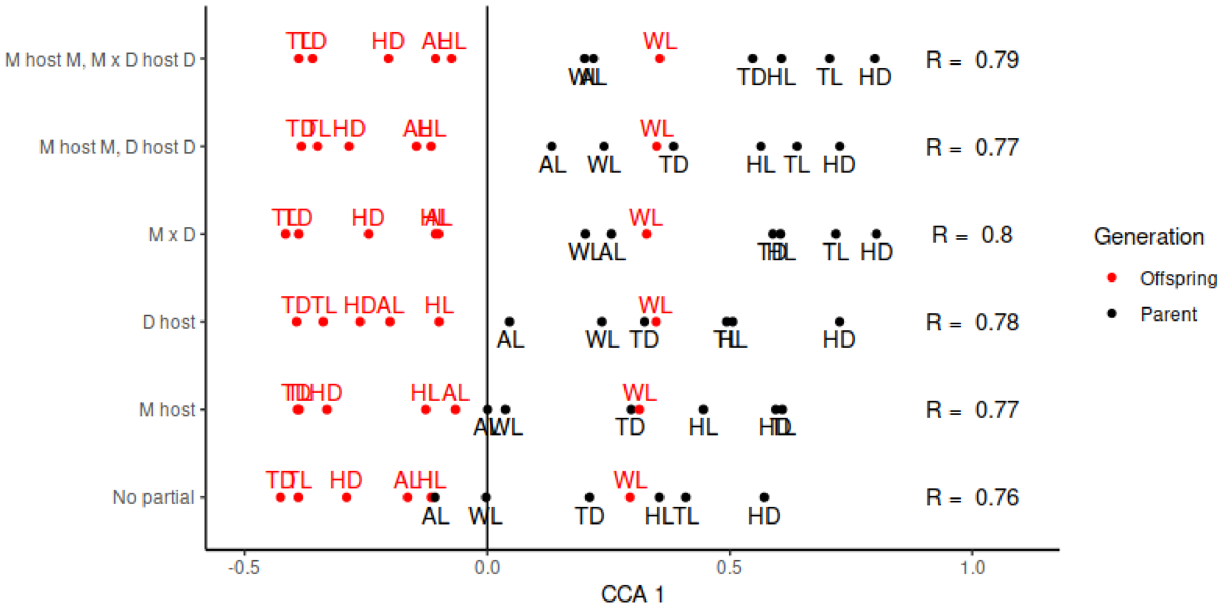


Table 1 P-values of two-tailed t-tests between the lady beetle body segment measurements across all three host lady beetle species: *Coleomegilla maculata (C.mac), Hippodamia convergens (H.con),* and *Coccinella septempunctata (C7).* (D) = Dorsal, (V) = Ventral, (L) = Lateral images from which the measurements were taken from. The alpha level is 0.05 for both ANOVA and T-test Analysis (Supplementary Datasheet).

	Body Length(D)	Body Width (D)	Elytron Chord Length (L)	Body Depth (L)	Pronotum Length (L)	Pronotum Width (V)	Abdominal Length (V)	Abdominal Width (V)
C.mac : *C7*	0.001	1.882E-06	0.000	0.000	0.001	0.000	1.076E-08	9.755E-06
C.mac : *H.con*	0.268	9.583E-06	0.002	0.001	0.000	0.003	0.180	0.041
H.con : *C7*	0.003	5.988E-06	0.001	0.000	0.008	0.001	5.551E-08	0.143

Table 1 Suggested

P-values of ANOVA and post-hoc tukey tests between the lady beetle morphological measurements across all three host species.

	Body Length (D)	Body Width (D)	Elytron Chord Length (L)	Body Depth (L)	Pronotum Length (L)	Pronotum Width (V)	Abdominal Length (V)	Abdominal Width (V)	Mass
ANOVA	3.4 E-05	2 E-15	5.38 E-10	1.95 E-12	3.16 E-06	7.71 E-10	2.1 E-07	6.63 E-03	3.89 E-08
C.mac : *C7*	2.66 E-05	0.000	0.000	0.000	0.000	0.000	0.000	.0118	0.000
C.mac : *H.con*	0.701	6.536 E-04	.0385	5.517 E-03	5.339 E-03	.0150	.471	.0467	.887
H.con : *C7*	2.2 E-04	0.000	0.000	0.000	6.590 E-03	0.000	0.000	.472	0.000

Table 2 P-values of two-tailed t-tests between the ladybeetle body segment measurements across small and large beetles. Both *Coleomegilla maculata (C.mac)* and *Hippodamia convergens (H.con)* host beetles are clubbed into the 'Small' category, with *Coccinella septempunctata (C7)* kept separate into the 'Large' category. The alpha level is 0.05 for both ANOVA and T-test Analysis (Supplementary Datasheet).

	Body Length(D)	Body Width (D)	Elytron Chord Length (L)	Body Depth (L)	Pronotum Length (L)	Pronotum Width (V)	Abdominal Length (V)	Abdominal Width (V)
Small : Large	0.002	4.353E -06	0.000	0.000	0.001	0.001	2.851E-08	0.000

Table 3 P-values of two-tailed t-tests between the *D. coccinellae* body segment measurements, by which beetle she eclosed from. The alpha level is 0.05 for both ANOVA and T-test Analysis (Supplementary Datasheet).

	Wing Length	Thorax Length	Thorax Depth	Abdomen Length	Head Length	Head Depth
C.mac : *C7*	0.567	0.198	0.546	0.026	0.895	0.692
C.mac : *H.con*	0.938	0.214	0.162	0.554	0.553	0.028
H.con : *C7*	0.565	0.019	0.136	0.012	0.693	0.075

Table 3 Suggest

P-values of ANOVA and post-hoc tukey tests between the *D. coccinellae* morphological measurements and which beetle she eclosed from.

	Wing Length	Thorax Length	Thorax Depth	Abdomen Length	Head Length	Head Depth	Mas**s**
ANOVA	0.946	0.162	0.261	0.097	0.0658	0.823	0.531
C.mac : *C7*	0.961	0.658	0.920	0.228	0.818	0.993	0.503
C.mac : *H.con*	0.994	0.371	0.345	0.684	0.0524	0.848	0.981
H.con C7	.941	0.172	0.357	0.079	0.505	0.870	0.667