Within-host competition drives energy allocation trade-offs in an insect parasitoid

J. Keaton Wilson Corresp. 1, Laura Ruiz 2, Goggy Davidowitz 1

1 Department of Entomology, University of Arizona, Tucson, Arizona, United States
2 Department of Neuroscience, University of Arizona, Tucson, Arizona, United States

Corresponding Author: J. Keaton Wilson
Email address: keatonwilson@me.com

Organismal body size is an important biological trait that has broad impacts across scales of biological organization, from cells to ecosystems. Size is also deeply embedded in life history theory, as the size of an individual is one factor that governs the amount of available resources an individual is able to allocate to different structures and systems. A large body of work examining resource allocation across body sizes (allometry) has demonstrated patterns of allocation to different organismal systems and morphologies, and extrapolated rules governing biological structure and organization. However, the full scope of evolutionary and ecological ramifications of these patterns have yet to be realized. Here, we show that density-dependent larval competition in a natural population of insect parasitoids (Drino rhoeo: Tachinidae) results in a wide range of body sizes (largest flies are more than 6 times larger (by mass) than the smallest flies). We describe strong patterns of trade-offs between different body structures linked to dispersal and reproduction that point to life history strategies that differ between both males and females and individuals of different sizes. By better understanding the mechanisms that generate natural variation in body size and subsequent effects on the evolution of life history strategies, we gain better insight into the evolutionary and ecological impacts of insect parasitoids in tri-trophic systems.
WITHIN-HOST COMPETITION DRIVES ENERGY ALLOCATION TRADE-OFFS IN AN INSECT PARASITOID

J. Keaton Wilson*,a, Laura Ruizb & Goggy Davidowitza

a Department of Entomology, University of Arizona, 1140 E South Campus Drive, Forbes 410, PO Box 210036 Tucson, AZ 85721

b Department of Neuroscience, University of Arizona, 1040 E 4th Street, PO Box 210077, Tucson AZ 85721

*Corresponding author - keatonwilson@me.com
Abstract

Organismal body size is an important biological trait that has broad impacts across scales of biological organization, from cells to ecosystems. Size is also deeply embedded in life history theory, as the size of an individual is one factor that governs the amount of available resources an individual is able to allocate to different structures and systems. A large body of work examining resource allocation across body sizes (allometry) has demonstrated patterns of allocation to different organismal systems and morphologies, and extrapolated rules governing biological structure and organization. However, the full scope of evolutionary and ecological ramifications of these patterns have yet to be realized. Here, we show that density-dependent larval competition in a natural population of insect parasitoids (*Drino rhoeo*; Tachinidae) results in a wide range of body sizes (largest flies are more than 6 times larger (by mass) than the smallest flies). We describe strong patterns of trade-offs between different body structures linked to dispersal and reproduction that point to life history strategies that differ between both males and females and individuals of different sizes. By better understanding the mechanisms that generate natural variation in body size and subsequent effects on the evolution of life history strategies, we gain better insight into the evolutionary and ecological impacts of insect parasitoids in tri-trophic systems.
Introduction

Body size is a biological trait that spans over 21 orders of magnitude (West, Brown, and Enquist 1999) with important impacts on chemical and physiological processes and inter- and intra-specific interactions in communities and ecosystems. Size can shape physiological processes like metabolism (West, Brown, and Enquist 1997) and is the outcome of complex genetic and developmental mechanisms (Davidowitz et al. 2003, 2012, 2016). Furthermore, biologists have long examined patterns of body size over large spatial scales (Blackburn, Gaston, and Loder 1999; Mousseau 1997), and examined how body size can affect complex community food webs (Jonsson, Cohen, and Carpenter 2005). Examining the relative size of different body parts (allometric scaling relationships (Gould 1966)) has become an important analytical tool alongside measurements of body size in diverse research topics including sexual selection (Emlen and Nijhout 2000; McCullough and Emlen 2013) physiological adaptation (Lane et al. 2018) and animal communication (Templeton, Greene, and Davis 2005). Together, body size and allometric scaling relationships not only provide a framework for conserved scaling rules that underlie developmental, physiological, and evolutionary processes (West, Brown, and Enquist 1997), but provide an opportunity to examine life history trade-offs, particularly in organisms like insects, where different body structures can act as proxies for investment into different life history strategies (reproduction, dispersal, information-acquisition and cognition).

At its core, life history theory seeks to determine the constraints on selection. Why doesn’t selection drive all fitness related traits to high levels and how do organisms differ in rules that govern trade-offs (Stearns 1989; Fairbairn and Roff 2006)? Life history trade-offs (defined as life history traits that are negatively associated with each other; Zera and Harshman 2001) have been studied across a wide variety of taxa (Zera and Harshman 2001), including extensive work on insects, particularly *Drosophila* (Reznick 1985) and crickets (Mole and Zera 1994; Zera and Denno 1997), to determine constraints and mechanisms that govern optimal fitness strategies (Reznick 1985). Work in wing polymorphic crickets has demonstrated a strong trade-off between flight capability and fecundity (Roff 1986; Zera, Potts, and Kobus 1998), as well as the importance of nutrient acquisition in shaping trade-offs among traits (Zera and Brink 2000). Though there are many clear examples of trade-offs, results are often mixed as some traits
demonstrate little or no trade-offs (McCullough and Emlen 2013; Fernández and Reynolds 2000; Tigreros and Davidowitz 2019). While measurements of allometry involve size (mass) of particular structures, these measurements can prove problematic when addressing life history trade-offs, because allocation costs can be masked in low-mass but energy-dense tissues (Zera and Harshman 2001). Energy provides a common currency with which to compare allocation to different tissues because of energetic differences among molecular building-blocks (i.e. an abdomen and thorax of similar weight may not accurately reflect a similar energetic allocation of resources, as the abdomen may contain a high proportion of energetically dense lipids). Here, we use a combination of weight and energy measurements to examine allocation to different body structures in a parasitoid fly (*Drino rhoeo*).

Tachinid flies (Diptera: Tachinidae) are internal parasitoids of arthropods and typically attack larval stages of herbivorous insects (Stireman, O'Hara, and Wood 2006). Tachinids are an understudied yet ecologically important group with approximately 10,000 described species that likely have powerful roles in shaping communities of plants and insects (Stireman, O'Hara, and Wood 2006; Wilson and Woods 2015). *Drino rhoeo* is a good model for examining trade-offs in resource allocation and patterns in body size because it is a gregarious parasitoid (multiple larvae develop together within a single host) with large variation in cohort size (here meaning groups of *D. rhoeo* larvae developing inside a single host, typically 8-50 larvae within a given host (Wilson and Woods 2015)). This variation means that there is likely competition for host resources at high densities, which can lead to naturally-occurring variation in nutrient acquisition available to larvae, resulting in population-level variation in body size and trait size. (Zera and Harshman 2001).

*Drino rhoeo* attacks caterpillars of the hawkmoth *Manduca sexta* and *Manduca quinquemaculata* (Bernays and Woods 2000; Mira and Bernays 2002; Wilson and Woods 2015) frequently at our field site in southeastern Arizona (near Portal and the Chiricahua mountains). Female flies target fourth- and fifth-instar caterpillars, laying eggs on the surface of the caterpillar after which fly larvae shortly (within 20 minutes) emerge and burrow into the caterpillar hemocoel where they grow and develop. Developing tachinid larvae typically completely consume the bodies of their hosts, resulting in host-death. Previous work has demonstrated that *D. rhoeo* can have a strong impact on the growth and development of hosts post-parasitization, affecting growth, weight and feeding habits (Wilson and Woods 2015) which may, in turn, affect the amount of resources available to the parasitoids.
Here, we use data from a natural population of *D. rhoeo* to address three main questions: (1) does larval competition and host quality drive variation in adult parasitoid body size, (2) what are the energy allocation strategies to different parasitoid body structures (heads, abdomens, thoraces, wings and legs) and how do they vary with parasitoid body size and sex and (3) are there allocation trade-offs among body structures that act as proxies for different life history strategies? To our knowledge, this if the first study that examines larval resource competition in tachinids with an emphasis on energy allocation among different body structures relative to size (resource competition has been shown by others: Allen, Geoff and Hunt 2001; Welch 2006, Lehmann 2008) and to extend these effects to patterns of life history trade-offs.

**Methods**

**Host and parasitoid collection**

Thirty-two *M. sexta* larvae in the 4th or 5th (final) instar were collected from the field near Portal, Arizona (~ 40 km radius) in August of 2017. Past work with *Drino rhoeo* showed that parasitization rate was high (~ 44%) in the field for *M. sexta* in 4th and 5th-instar stages (Wilson and Woods 2015), so we collected a mixture of individuals that appeared healthy, and some that appeared to have been attacked by parasitoids (e.g. dried gut fluids on the skin, melanized spots where fly larvae burrowed inside the caterpillar [Woods and Wilson, unpublished]). Larvae were raised together in a large plastic bin and fed cuttings from local *Datura wrightii*, their main host plant. Once larvae began to wander (Dominick and Truman 1984), they were placed in individual plastic cups (13 cm x 12 cm x 14 cm) filled with soil. Larvae were allowed to burrow and begin pupation and were transported back to the University of Arizona in Tucson where they were kept in an experimental greenhouse for the duration of their development.

**Head-capsule width as a measure of host quality**

Here, we define host larval quality as the energy potential of a host to developing parasitoid larvae. Larval mass is not a good measure of larval quality, because growth, feeding and development are all affected by parasitism (Wilson and Woods 2015), so we measured head-capsule width of hosts (all 5th-instar) as a proxy to estimate larval quality to developing flies. We examine the relationship between mass and head-capsule width in more detail in the Results section.
Fly emergence, weights and calorimetry

Seven of the thirty-two caterpillars (22%) collected were parasitized by *Drino rhoeo* and had successful fly emergence. Flies were allowed to emerge in the small plastic cups containing individual caterpillars described above. Once initial fly emergence was observed, we waited 48 hours to allow all individuals to successfully emerge from the soil, and then placed the cups in a -20 ºC freezer. Once flies were frozen, we transferred them to individually-labeled vials and scored sex using a dissecting scope to determine the presence of sexual patches on the ventral portion of the last abdominal segment on males. Files were sectioned into different body structures (head, abdomen, thorax, wings and legs) using a combination of scalpel, probe and forceps under a dissecting scope. Sectioned body structures were moved to small metal containers and placed in a drying oven at ~ 45º C for 48 hours before being placed back into vials and frozen again at -20 ºC until further processing. Dried individual body sections were weighed on a microbalance (Mettler Toledo XS3DU), with legs and wings being weighed together. In total, we collected 104 individual flies from the 7 hosts, though not all flies were used in all subsequent analyses because of some loss of body structures during the weighing or bombing process.

We used a Parr 6200 bomb calorimeter to determine energy content of heads, thoraces and abdomens. Because our calorimeter is designed to be used on larger tissue, we generated calibration curves for predicting caloric content based on tissue type and weight to be able to extrapolate to small weights (and respectively small energy content) that are below the threshold of detection for our calorimeter. Individual structures were mixed into different groups ranging from 1 to 10 structures from individual flies per bin (depending on the size of the tissue: more heads were needed to get to measurable weights than abdomens, because heads are smaller than abdomens) and separated by sex. We also varied the number of structures in binned groups to achieve enough variation in weight to generate accurate calibration curves. Binned tissue samples were weighed and placed in a crucible. We added 0.7 mL of mineral oil to samples as a heat spike (to increase the total energy content to a level readable by the machine). This method of measuring small samples is a standard procedure and the Parr calorimeter software automatically accounts for the mineral oil spike.

Zera and Harshman (2001) emphasize that to establish a physiological tradeoff among body functions, there is a need to establish that a specific resource (such as a specific lipid, protein or carbohydrate) is used by both functions which requires tracking the common resource in both
functions. In this study we are interested in total amount of resources allocated among functions and not a specific resource. The use of energy as the common currency encompasses all resources allocated to a function and allows for identifying physiological allocation tradeoffs among functions. We note that this method cannot measure the cost of building a structure, just the energy content of the resources that are in the structure.

We created tissue-specific calibration curves to generate estimates of the calorimetric content of different tissues based on weight. We focused on the three main body segments (heads, thoraces and abdomens). To generate curves, we fit linear regression models for each tissue type that were forced through the origin. All models had good predictive power (p < 0.05 for all models), though the predictive power for estimating the energetic content of heads ($R^2 = 0.58$) was less than for thoraces ($R^2 = 0.90$) and abdomens ($R^2 = 0.91$) because of smaller sample sizes (it takes many more fly-heads to generate the measurable weights than it does thoraces or abdomens) and more variation in measurements at smaller weights. We did not have enough flies to generate separate calibration curves for males and females, so all bins consisted of flies from a single sex, and calibration curves are tissue-specific but not sex specific, which might result in additional variation in extrapolations if there were large differences between the weight-energy correlations of different sexes.

Head capsule and host tissue calorimetry

To determine the relationship between head-capsule width and the usable energetic content of hosts, we used late 5th-instar *M. sexta* caterpillars from our colony in Tucson, AZ. We measured head-capsule width and then froze caterpillars in a -20º C freezer. We then thawed and separated caterpillar structures into two groups: skin and head-capsule and internal tissues that would be available to developing parasitoids (this included hemolymph, tracheae, muscle, but not gut tissue or any remaining food). We dried samples for each caterpillar in a drying oven at ~ 45º C for a minimum of 48 hours. We crushed dried tissue samples and split samples that were too large for our calorimeter into three sub-samples before bombing. We used a bomb calorimeter (Parr 6200 - methods described above) with a 600 µL mineral oil spike to measure the energetic content of each sub-sample before combining for further analysis.
Data Analyses

All analyses were performed in R (Version 3.5.0 “Joy in Playing”, www.r-project.org). We used linear mixed effects models (nlme) for modeling the relationship between adult fly size, host quality, cohort size and sex and AIC scores for model comparison and selection. Additional packages (effects, piecewiseSEM) were used to generate population level trend lines and marginal and conditional R² values. In spite of our limited sample size of hosts (n = 7), we included host as a random effect (with random intercepts) to help control for variance in conditions among hosts. Additionally, we used ordinary least squares (OLS) regression for examining allometric relationships between body weight and tissue weight and energetic content and AIC scores for model comparison and selection (Supplementary Table 1). Though some researchers have advocated the use of RMA regression (reduced-major axis regression), recent work has shown that OLS regression is better suited in many cases, especially those similar to ours where there is comparatively little measurement error (Kilmer and Rodríguez 2017). In comparisons of relative mass and energy allocation, polynomial models were fit where appropriate. All data and code are archived and available on Zenodo (doi:10.5281/zenodo.3356991).

Results

Cohort size, host quality and adult fly weight

On average, adult fly body size did not differ between males and females (males = 8.74 ± 3.58 mg, females = 8.51 ± 3.73 mg; F₁,₉₀ = 0.091, p = 0.7641). On average, adult fly dry weight decreased with increasing cohort size (Figure 1a), with an average fly from the largest cohort (38 flies) weighing 36% of an average fly from the smallest cohort (2 flies). Additionally, adult fly dry weight increased with increasing host quality (head-capsule width; Figure 1b). Overall, the best model that explained adult fly weight was one that included cohort size and host quality additively (Table 1-2) with host included as a random effect (random intercepts). We also performed a multiple linear regression on a reduced number of data points (n = 7) that were average fly-weight values for each cohort, to confirm the biological pattern we show here and reaffirm that the linear-mixed effects model framework is accounting for any pseudo-replication of sampling multiple flies within a single host. This analysis showed that similar significant effects of cohort size (p = 0.05) and host head capsule width (p = 0.03) with good predictive power (p = 0.006, R² = 0.76). Head-capsule width is a frequently-used proxy for body size, and
typically has strong positive correlations with body size (Smock 1980; Potter, Davidowitz, and
Woods 2011; D’Amico, Davidowitz, and Nijhout 2001) - it is also the best measurement of host-
quality in this system because of the complex interactions between parasitism, host-feeding and
body size (Wilson and Woods 2015). We found some support that head-capsule width is
positively associated with wandering-weight (F_{1,5} = 6.961, p = 0.046, R^2 = 0.498) in M. sexta
caterpillars in the field, despite a relatively small sample size and that caterpillars were
parasitized by varying numbers of tachinid larvae. Additionally, in a series of measurements on
lab-reared M. sexta, we found that pre-wander weight was positively correlated with the caloric
content of hosts excluding skin and the gut (F_{1,13} = 54.01, p < 0.0001, R^2 = 0.79). Together,
these data suggest that head-capsule width functions as a good proxy for both body size and
caloric content available to developing tachinids.

Size-relative allocation to different body structures

Overall, flies had relatively smaller heads as size increased, with no difference between males
and females (t = 1.102, p = 0.273), though the best fit model for these data was a 2nd-order
polynomial, with a slight increase in investment in heads at large body sizes (F_{2,89} = 51.8, p <
0.001, R^2 = 0.53; Figure 2a, Supplementary Table 1). Normalized wing weight (scaled to
individual body size – we use this definition of normalized throughout) decreased with body size
(F_{2,89} = 10.74, p = 0.001, R^2 = 0.09), with no differences between the sexes (t = -0.768, p =
0.444; Figure 2b). Normalized leg weight decreased linearly with body size (F_{2,80} = 5.169, p =
0.008, R^2 = 0.50), and while there was a significant effect of sex (t = -2.617, p = 0.01), the effect
size was small, with little difference between males and females, except only at small body
sizes (Figure 2c). Normalized thorax weight was best fit with a polynomial model that included
sex as an additive effect (F_{3,88} = 35.98, p < 0.001, R^2 = 0.54) - relative thorax weights were
highest in medium-sized flies, and were higher overall in males (t = 7.909, p < 0.001), with the
greatest difference between males and females occurring in medium-sized flies (Figure 2d).
Finally, normalized abdomen weight increased linearly with fly weight in both males and females
(F_{2,89} = 65.18, p < 0.001, R^2 = 0.56), though females showed increased relative investment
across all body weights (t = -8.399, p < 0.001; Figure 2e).

Energy tradeoffs and comparisons among body segments

Body segments differed in their average energy content with abdomens being the most energy
dense at 4.36 ± 0.44 calories/mg, followed by thoraces at 4.05 ± 0.33 calories/mg, followed by
heads at 1.96 ± 0.17 calories/mg. We compared the percentage of calories devoted to each body segment for male and female flies across body sizes and found strong potential trade-offs between thoraces and abdomens in both males and females, though the pattern is strongest for male flies of moderate size (Figure 3a). We note here that while a negative correlation between two traits has often been used as evidence for trade-offs (e.g. Zera and Harshman 2001; Stearns 1989), the interactions among traits are complex, and indirect effects may impact negative correlations. We also compared the relative amount of energy devoted to one segment after controlling for the total energy content of the three main body segments. Both male and female flies showed a strong negative correlation between the relative amount of energy invested in thoraces and abdomens ($F_{1,99} = 1983$, $p < 0.001$, $R^2 = 0.952$; Figure 3b), with no difference between males and females ($p = 0.209$). There was no significant pattern of trade-offs between heads and thoraces or heads and abdomens for male or female flies ($F_{1,99} = 1.225$, $p = 0.271$; $F_{1,99} = 1.187$, $p = 0.279$).

### Discussion

*Drino rhoeo* show clear indications of strong density-dependent larval competition (Figure 2), similar to other gregarious insect parasitoids (Taylor 1988; Harvey 2000; Allen and Hunt 2001). This competition (in combination with variation in host quality; Figure 2b, Table 1), leads to a wide range of body sizes that is naturally occurring, and is not sexually dimorphic (Figure 1). In spite of a low sample size of hosts ($n=7$), we found strong relationships between cohort size and adult fly weight, as well as moderate impacts of host quality on adult fly weight. The evolutionary and ecological implications of density-dependent larval competition in insect parasitoids have been examined only in hymenopteran parasitoids previously (Nicol and Mackauer 1999; Milonas 2005; Sykes et al. 2007). Our results presented here pave the way for further work examining tradeoffs between immune function and larval competition, female tachinid oviposition strategies, and optimal brood size in non-hymenopteran parasitoids.

Allometric scaling relationships of the mass of tissues provide insight into how investment in structures change across body sizes and sexes. Flies invested in relatively larger wings, legs, and heads at smaller body sizes while investing in relatively larger abdomens at high body sizes and large thoraces at moderate body sizes (Figure 2). Male and female flies differed markedly in their allocation to thoraces and abdomens with males investing relatively more in thoraces and females investing relatively more in abdomens (Figure 2d-e). Fly legs, wings and heads showed
hypoallometric scaling relationships (scaling coefficients of 0.709, 0.559, 0.68, respectively; Supplemental Figure 1), while thoraces for both males and female flies were isometric (scaling coefficient of 0.986). Abdomens for both males and females were hyperallometric (scaling coefficient of 1.236), mirroring previous work in other insects (Wickman and Karlsson 1989).

Both male and female flies showed strong trade-offs between abdomens and thoraces in both mass (Figure 2) and energetic content (Figure 3b). Though this trade-off is consistent across body sizes, relative allocation to each structure is markedly different between males and females and across body sizes (Figure 3a). As a group, the relative investment into abdomens or thoraces falls along a clear gradient – with females devoting energy to abdomens at the expense of thoraces, while the opposite is true for males (Figure 3b). Though the majority of flies follow this allocation rule, a few small male flies in our study demonstrate a different allocation strategy – less investment overall to abdomens and thoraces while devoting more energy to the head (Figure 3b). Investment in thoraces (a proxy for dispersal, though many insects are capable of resorbing wing musculature as adults (Stjernholm, Karlsson, and Boggs 2005; Boggs 2009)) is favored at small and moderate body sizes, while equal investment in thoraces and abdomens (reproduction and storage) is favored at large body sizes. These patterns are magnified in male flies, where some individuals invest up to 80% of the total energetic content of the three main body segments into their thoraces, and dampened in females. There is evidence in other insect systems that increased allocation to thoraces is correlated with higher flight performance (Berwaerts, Van Dyck, and Aerts 2002; Karlsson and Johansson 2008), though the energy allocation to thoraces we present here may also represent a minimum threshold required to produce functional flight. Researchers have also shown that increased allocation to abdomens is correlated with increased fecundity (Wickman and Karlsson 1989; Griffith 1994; Preziosi et al. 1996). Here, flies demonstrate strategies that closely match predictions made by life history theory for income-breeding insects (Davis et al. 2016) where dispersal and mobility are favored in poor quality environments (tachinids are likely income breeders that feed on nectar and pollen (Gilbert 1998; Tooker, Hauser, and Hanks 2006) and can ameliorate larval nutritional deficits as adults by being able to find high-quality resource sites) while a bigger relative investment in reproduction or energetic storage can be allocated in high quality larval environments (Boggs 2009). These allocation patterns are likely magnified in males and dampened in females because of heightened energy requirements for reproductive investment and oviposition success (Reznick 1985).
Conclusions

In conclusion, we show that larval competition and variation in host quality generate a range of body sizes in a population of insect parasitoids which in turn leads to different patterns of allocation to thoraces and abdomens across body sizes and between sexes. These strategies point to strong trade-offs between body segments tied to reproduction and dispersal. By better understanding the mechanisms that drive allocation to different tissues across body sizes and between sexes, particularly in under-studied species with potentially large ecological effects, we gain deeper insight into the evolution and ecology of tri-trophic systems and the underlying drivers of life history strategies.

Acknowledgements

We’d like to thank the director of the Southwestern Research Station and station employees for their help, as well as Cristina Francois, Lennie Park, Natasha Tigreros and Meck Slagle for their input on these data, analyses and presentation. We’d also like to thank Heather Costa for her support in the lab and for input on data, analyses and presentation.

References

Allen GR, Hunt J. 2001. Larval Competition, Adult Fitness, and Reproductive Strategies in the Acoustically Orienting Ormiine Homotria Aleni (Diptera: Tachinidae). Journal of Insect Behavior 14 (3): 283–97.

Bernays EA, Woods, HA. 2000. Foraging in nature by larvae of Manduca Sexta - Influenced by an endogenous Oscillation." Journal of Insect Physiology 46 (5): 825–36.

Berwaerts, K, H Van Dyck, P Aerts. 2002. Does Flight Morphology Relate to Flight Performance? An Experimental Test with the Butterfly Pararge Aegeria. Functional Ecology 16 (4): 484–91.

Blackburn, TM, K,. Gaston, and N Loder. 1999. Geographic Gradients in body size: a clarification of Bergmann's Rule Diversity & Distributions 5 (4): 165–74.

Boggs, CL. 2009. Uderstanding insect life histories and senescence through a resource allocation lens. Functional Ecology 23 (1): 27–37.

D'Amico, LJ, Davidowitz, G, Nijhout, HF. 2001. The Developmental and Physiological Basis of Body Size Evolution in an Insect. Proceedings. Biological Sciences 268 (1476): 1589–93.

Davidowitz, G, D'Amico, LJ, Nijhout, HF. 2003. Critical Weight in the Development of Insect
Davidowitz G, Nijhout HF, Roff, DA. 2012. Predicting the response to simultaneous selection: genetic architecture and physiological constraints. *Evolution* 66:2916-2928.

Davidowitz, G, Roff, DA, Nijhout, HF. 2016. Synergism and antagonism of proximate mechanisms enable and constrain the response to simultaneous selection on body size and development time: an empirical test using experimental evolution. *The American Naturalist* 188: 499-520.

Davis RB, Javoš, J., Kaasik, A, Õunap, E, Tammaru, T. 2016. An ordination of life-histories using morphological proxies: capital vs income breeding in insects. - *Ecology*, 97: 2112-2124.

Dominick OS, Truman JW. 1984. The Physiology of Wandering Behaviour in *Manduca Sexta*. I. Temporal Organization and the Influence of the Internal and External Environments. *The Journal of Experimental Biology* 110: 35–51.

Emlen DJ, Nijhout, HF. 2000. The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology* 45: 661–708.

Fairbairn DJ, Roff, DA. 2006. The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage. *Heredity* 97 (5): 319–28.

Fernández RJ, Reynolds, JF. 2000. Potential Growth and Drought Tolerance of Eight Desert Grasses: Lack of a Trade-Off? *Oecologia* 123 (1): 90–98.

Gilbert F. 1998. Functional, Evolutionary and Ecological Aspects of Feeding-Related Mouthpart Specializations in Parasitoid Flies. *Biological Journal of the Linnean Society. Linnean Society of London* 63 (4): 495–535.

Gould SJ. 1966. Allometry and Size in Ontogeny and Phylogeny. *Biological Reviews of the Cambridge Philosophical Society* 41 (4): 587–640.

Griffith H. 1994. Body Elongation and Decreased Reproductive Output within a Restricted Clade of Lizards (Reptilia: Scincidae). *Journal of Zoology* 233 (4): 541–50.

Harvey JA. 2000. Dynamic Effects of Parasitism by an Endoparasitoid Wasp on the Development of Two Host Species: Implications for Host Quality and Parasitoid Fitness. *Ecological Entomology* 25: 267–78.

Jonsson T, Cohen JE, Carpenter SR. 2005. Food Webs, Body Size, and Species Abundance in Ecological Community Description. In *Advances in Ecological Research*, 1–84.

Karlsson B., Johansson A. 2008. Seasonal Polyphenism and Developmental Trade-Offs between Flight Ability and Egg Laying in a Pierid Butterfly. *Proceedings of the Royal Society B: Biological Sciences* 275 (1647): 2131–36.
Kilmer JT, Rodriguez, RL. 2017. Ordinary Least Squares Regression Is Indicated for Studies of Allometry. *Journal of Evolutionary Biology* 30 (1): 4–12.

Lane SJ, Moran AL, Shishido CM, Tobalske BW, Woods HA. 2018. Cuticular Gas Exchange by Antarctic Sea Spiders. *The Journal of Experimental Biology* 221 (Pt 8). https://doi.org/10.1242/jeb.177568.

Lehmann, GU. (2008). How different host species influence parasitism patterns and larval competition of acoustically-orienting parasitoid flies (Tachinidae: Ormiini). *Animal Behavior: New Research*, 93-132.

McCullough EL, Emlen DJ. 2013. Evaluating the Costs of a Sexually Selected Weapon: Big Horns at a Small Price. *Animal Behaviour* 86 (5): 977–85.

Milonas PG. 2005. Influence of Initial Egg Density and Host Size on the Development of the Gregarious Parasitoid *Bracon hebetor* on Three Different Host Species. *Biocontrol* 50 (3): 415–28.

Mira A, Bernays EA. 2002. Trade-Offs in Host Use by *Manduca Sexta*: Plant Characters vs Natural Enemies. *Oikos* 97 (3): 387–97.

Mole S, Zera AJ. 1994. Differential Resource Consumption Obviates a Potential Flight-Fecundity Trade-Off in the Sand Cricket (*Gryllus firmus*). *Functional Ecology* 8 (5): 573.

Mousseau TA. 1997. “Ectotherms Follow the Converse to Bergmann’s Rule. *Evolution; International Journal of Organic Evolution* 51 (2): 630.

Nicol CMY, Mackauer M. 1999. The Scaling of Body Size and Mass in a Host-Parasitoid Association: Influence of Host Species and Stage. *Entomologia Experimentalis et Applicata* 90 (1): 83–92.

Potter KA, Davidowitz G, Woods HA. 2011. Cross-Stage Consequences of Egg Temperature in the Insect *Manduca Sexta*. *Functional Ecology*. https://doi.org/10.1111/j.1365-2435.2010.01807.x.

Preziosi, RF, Fairbairn DJ, Roff DA, Brennan JM. 1996. “Body Size and Fecundity in the Waterstrider *Aquarius remigis*: A Test of Darwin’s Fecundity Advantage Hypothesis. *Oecologia* 108 (3): 424–31.

Reznick, D. 1985. Costs of Reproduction: An Evaluation of the Empirical Evidence. *Oikos* 44 (2): 257.

Roff, DA. 1986. The Evolution of Wing Dimorphism in Insects. *Evolution; International Journal of Organic Evolution* 40 (5): 1009.

Smock, LA. 1980. Relationships between Body Size and Biomass of Aquatic Insects.” *Freshwater Biology*. https://doi.org/10.1111/j.1365-2427.1980.tb01211.x.
Stearns SC. 1989. Trade-Offs in Life-History Evolution. *Functional Ecology* 3 (3): 259.

Stireman JO, O’Hara JE, Wood zfm. 2006. Tachinidae: Evolution, Behavior, and Ecology.” *Annual Review of Entomology* 51 (January): 525–55.

Stjernholm F, Karlsson B, Boggs CL. 2005. Age-Related Changes in Thoracic Mass: Possible Reallocation of Resources to Reproduction in Butterflies. *Biological Journal of the Linnean Society. Linnean Society of London* 86 (3): 363–80.

Sykes EM, Innocent TM, Pen I, Shuker DM, West SA. 2007. Asymmetric Larval Competition in the Parasitoid Wasp *Nasonia vitripennis*: A Role in Sex Allocation? *Behavioral Ecology and Sociobiology* 61 (11): 1751–58.

StammaruT, Haukioja E. 1996. Capital Breeders and Income Breeders among Lepidoptera: Consequences to Population Dynamics. *Oikos* 77 (3): 561.

Taylor AD. 1988. Host Effects on Larval Competition in the Gregarious Parasitoid Bracon Hebetor. *The Journal of Animal Ecology* 57 (1): 163.

Templeton CN Greene E, Davis K. 2005. Allometry of Alarm Calls: Black-Capped Chickadees Encode Information about Predator Size. *Science* 308 (5730): 1934–37.

Tigreros N, Davidowitz G. 2019. Flight-Fecundity Tradeoffs in Wing-Monomorphic Insects.” *Advances in Insect Physiology*. https://doi.org/10.1016/bs.aiip.2019.02.001.

Tooker JF, Hauser M, Hanks LM. 2006. Floral Host Plants of Syrphidae and Tachinidae (Diptera) of Central Illinois. *Annals of the Entomological Society of America* 99 (1): 96–112.

Welch CH. 2006. Intraspecific competition for resources by *Ormia depleta* (Diptera: Tachinidae) larvae. *Florida Entomologist* 89(4): 497-502.

West GB, Brown JH, Enquist BJ. 1997. A General Model for the Origin of Allometric Scaling Laws in Biology. *Science* 276 (5309): 122–26.

West GB Brown JH, Enquis BJ. 1999. The Fourth Dimension of Life: Fractal Geometry and Allometric Scaling of Organisms. *Science* 284 (5420): 1677–79.

Wickman P, Karlsson B. 1989. “Abdomen Size, Body Size and the Reproductive Effort of Insects. *Oikos* 56 (2): 209.

Wilson, JK, Woods HA. 2015. Protection via Parasitism: *Datura* Odors Attract Parasitoid Flies, Which Inhibit *Manduca* Larvae from Feeding and Growing but May Not Help Plants.” *Oecologia* 179 (4): 1159–71.

Zera AJ, Brink T. 2000. Nutrient Absorption and Utilization by Wing and Flight Muscle Morphs of the Cricket *Gryllus firmus*: Implications for the Trade-off between Flight Capability and Early Reproduction. *Journal of Insect Physiology* 46 (8): 1207–18.

Zera AJ, Denno RF. 1997. Physiology and Ecology of Dispersal Polymorphism in Insects.
Figure Captions

Figure 1: Adult Drino rhoeo weight as a function of competitive larval environment (cohort size) and host quality. (a) The negative relationship between increasing larval competition and adult size. (b) The positive relationship between host quality (head-capsule width) and adult fly weight. In both panels, green points represent male flies and red points female flies. Black lines represent the trendline for the best fit linear mixed effects model that included cohort size and head-capsule width as fixed effects and host as a random effect. Gray bands represent the 95% confidence interval. Raw data is jittered horizontally slightly to aid in visualization, and larger black points represent the predicted (based on the best fit models) adult fly weight for each host.

Figure 2: Relative weight investment in different adult Drino rhoeo body structures as a function of body size. Different panels show relationships of relative weight investment of (a) heads, (b) wings, (c) legs, (d) thoraces and (e) abdomens. Males flies are represented by green points whereas females are represented by red points. Solid lines depict the trendlines for the best fit model for each body structure whereas gray bands represent the 95% confidence interval. In panels with two lines depicted (d-e), there was a significant difference between males and females.

Figure 3: Energetic trade-offs in allocation to different body segments of adult Drino rhoeo. (a) The percent calories of heads, abdomens and thoraces for male and female flies as a function of body size. Abdomens (red) and thoraces (blue) show significant differences between males (triangles) and females (circles), while there was no difference between males.
and females in energy allocation to heads (green). (b) Energy allocation trade-off between abdomens and thoraces for *Drino rhoeo*. Individual abdomen and thorax energy content is normalized for total energy content of all three main body segments and plotted against each other. Female flies are in red and male flies are in green, while body size is represented by the size of the circle. Marginal density plots depict the differences in the allocation of resources to thoraces and abdomens between males and females.

Supplemental Figure 1: Allometric scaling relationships of the mass of thoraces, abdomens, heads, legs and wings of adult *Drino rhoeo* flies. In both panels, the dotted line depicts the 1:1 line. The top panel (a) depicts thoraces and abdomens, with trendlines and 95% confidence intervals plotted from OLS regression models that included the effects of sex, additively. (b) Depiction of the scaling of heads, legs and wings with trendlines and 95% confidence intervals plotted from OLS regression models. In these tissues, there was no significant difference between males and females.
Figure 1

Adult *Drino rhoeo* weight as a function of competitive larval environment (cohort size) and host quality

(a) The negative relationship between increasing larval competition and adult size. (b) The positive relationship between host quality (head-capsule width) and adult fly weight. In both panels, green points represent male flies and red points female flies. Black lines represent the trendline for the best fit linear mixed effects model that included cohort size and head-capsule width as fixed effects and host as a random effect. Gray bands represent the 95% confidence interval. Raw data is jittered horizontally slightly to aid in visualization, and larger black points represent the predicted adult fly weight for each host.
Figure 2

Relative weight investment in different adult *Drino rhoeo* body structures as a function of body size.

Different panels show relationships of relative weight investment of (a) heads, (b) wings, (c) legs, (d) thoraces and (e) abdomens. Males flies are represented by green dots whereas females are represented by red dots. Solid lines depict the trendlines for the best fit model for each body structure whereas gray bands represent the 95% confidence interval. In panels with two lines depicted (d-e), there was a significant difference between males and females.
Figure 3

Energetic trade-offs in allocation to different body segments of adult *Drino rhoeo*.

(a) The percent calories of heads, abdomens and thoraces for male and female flies as a function of body size. Abdomens (red) and thoraces (blue) show significant differences between males (triangles) and females (circles), while there was no difference between males and females in energy allocation to heads (green). (b) Energy allocation trade-off between abdomens and thoraces for *Drino rhoeo*. Individual abdomen and thorax energy content is normalized for total energetic content of all three main body segments and plotted against each other. Female flies are in red and male flies are in green, while body size is represented by the size of the circle. Marginal density plots depict the differences in the allocation of resources to thoraces and abdomens between males and females.
Table 1 (on next page)

Model selection for factors affecting adult fly weight
Table 1. Model Selection for Factors Affecting Adult Fly Weight

| Model                      | Marginal R² | Conditional R² | AIC  |
|----------------------------|-------------|----------------|------|
| Weight ~ cohort size       | 0.338       | 0.716          | 408  |
| Model                                      | R²  | Adjusted R² | AIC  |
|--------------------------------------------|-----|-------------|------|
| Weight ~ cohort size + head-capsule width  | 0.462 | 0.683      | 401  |
| Weight ~ cohort size * head-capsule width  | 0.446 | 0.706      | 402  |
| Weight ~ cohort size*head-capsule width*Sex| 0.438 | 0.699      | 407  |
Table 2 (on next page)

Linear mixed effects model predicting fly weight as a function of cohort size and head-capsule width
Table 2. Linear mixed effects model predicting fly weight as a function of cohort size and head-capsule width

| Parameter          | Estimate | Standard Error | d.f. | t-value | p-value |
|--------------------|----------|----------------|------|---------|---------|
| Intercept          | -47.205  | 0.1909         | 85   | -1.723  | 0.09    |
| Cohort size        | -0.218   | 0.2727         | 4    | -3.000  | 0.04    |
| Head Capsule Width | 9.972    | 0.2466         | 4    | 2.226   | 0.09    |

Random Effects (Host – random intercepts)

| Parameter | Intercept | Residual |
|-----------|-----------|----------|
| StdDev    | 0.1947    | 1.935    |