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# Body and Wing Allometries Reveal Flight-Fecundity Tradeoff in Response to Larval Provisioning in *Osmia lignaria* (Hymenoptera: Megachilidae)

Bryan R. Helm,<sup>1</sup> Maxwell A. Baldwin,<sup>1</sup> Joseph P. Rinehart,<sup>2</sup> George D. Yocum,<sup>2,</sup> Kendra J. Greenlee,<sup>1,</sup> and Julia H. Bowsher<sup>1,3,</sup>

<sup>1</sup>Department of Biological Sciences, North Dakota State University, Fargo, ND, 58102 USA, <sup>2</sup>Insect Genetics and Biochemistry, Edward T. Schafer Agricultural Research Center, USDA-ARS, Fargo, ND, 58102 USA, and <sup>3</sup>Corresponding author, e-mail: julia. bowsher@ndsu.edu

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# Abstract

Variation in body size has important implications for physical performance and fitness. For insects, adult size and morphology are determined by larval growth and metamorphosis. Female blue orchard bees, *Osmia lignaria*, (Say) provision a finite quantity of food to their offspring. In this study, we asked how provision-dependent variation in size changes adult morphology. We performed a diet manipulation in which some larvae were starved in the final instar and some were given unlimited food. We examined the consequences on adult morphology in two ways. First, allometric relationships between major body regions (head, thorax, abdomen) and total body mass were measured to determine relative growth of these structures. Second, morphometrics that are critical for flight (wing area, wing loading, and extra flight power index) were quantified. Head and thorax mass had hyperallometric relationships with body size, indicating these parts become disproportionately large in adults when larvae are given copious provisions. However, abdominal mass and wing area increased hypoallometrically with body size. Thus, large adults had disproportionately lighter abdomens and smaller wing areas than smaller adults. Though both males and females followed these general patterns, allometric patterns were affected by sex. For flight metrics, small adults had reduced wing loading and an increased extra flight power index. These results suggest that diet quantity alters development in ways that affect the morphometric trait relationships in adult *O. lignaria* and may lead to functional differences in performance.

Key words: body size, allometry, flight morphometrics, solitary bee, Osmia lignaria

Life history theory predicts that organisms will alter their developmental trajectories to optimize individual fitness when faced with varying environmental conditions (Caswell 1983, Stearns and Koella 1986, Roff 1992, Arendt 1997, Nylin and Gotthard 1998, Boggs 2009). Such developmental variation often alters the timing of major life history events, such as stage transitions and reproductive age. But varying conditions also alter the growth of the organism, resulting in intraspecific variation in suites of life history (Atkinson 1994, Kingsolver and Huey 2008, Teder et al. 2014) and morphological traits (West-Eberhard 2005, Gokhale and Shingleton 2015, Mirth et al. 2016, Stillwell et al. 2016). The consequences of such variation are central for understanding individual performance, population dynamics, and ecological interactions (Caswell 1983, Peters 1983, West-Eberhard 2003, Chown and Gaston 2010).

For insects, nutritional variation during larval growth is one of the primary environmental factors driving trait plasticity in adult phenotypes (Arendt 1997, Teder et al. 2014). This is especially true for holometabolous species because adult traits develop during metamorphosis and require resources that are derived entirely from larval nutrition (Boggs 2009). Generally, higher-quality nutritional resources result in larger adult size and excess resources that enhance fecundity (Forrest 1987, Zera and Harshman 2001, Kingsolver and Huey 2008). However, the acquisition and accumulation of resources may also impose downstream constraints on the phenotype, such as when fecundity-flight tradeoffs occur (Tigreros and Davidowitz 2019). An abundance of larval nutritional resources generates larger, heavier individuals compared to those raised in nutrient-limited conditions, but extra mass imposes a cost for adult flight (Wickman and Karlsson 1989). Thus, we hypothesize that there will be increased allocation toward flight structures when larval resources are abundant to offset the cost of additional mass.

In the solitary bee Osmia lignaria Say (Hymenoptera: Megachilidae), mothers determine the amount of food each offspring consumes during the larval stage, and hence their adult body size, by the amount of

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/ licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com provision she places in the brood cell (Bosch and Vincens 2005, Bosch 2008). Each larva consumes its provision, remains a prepupa in the summer, and then completes metamorphosis to the adult stage in the fall (Torchio 1989). Adults overwinter in their nest cells and emerge the following spring (Torchio 1989, Kemp and Bosch 2000, Bosch et al. 2010, Sgolastra et al. 2012). Larval provisioning is an important source of life history and morphological variation for *O. lignaria*. Heritability of body size is low (Tepedino et al. 1984), and provision quantity is a primary indicator of adult size after development. Females are generally larger than males (Torchio and Tepedino 1980) but small adults of both sexes have been observed in nature when floral resources are scarce (Bosch 2008).

Because of the potentially counterbalancing tradeoffs that occur during development and among adult traits, we asked how adult phenotype is affected by developmentally induced size variation in *O. lignaria.* Altering larval provisions can induce a 10-fold mass difference between the largest and smallest *O. lignaria* adults (Helm et al. 2017). This provided an opportunity to examine how growth and allocation among body parts change with respect to nutritional provisioning in this solitary bee pollinator. We experimentally generated a range of adult sizes by either removing food or providing unlimited food during the final larval instar, so that we may infer allocative tradeoffs among fecundity (abdominal mass), flight (thoracic mass, wing area), and head mass. We also examined wing size and calculated flight morphometric measures that may also affect flight performance when adult bees vary in size.

# **Materials and Methods**

#### **Bees and Rearing Protocols**

Freshly capped *O. lignaria* nests were purchased from Crown Bees (http://crownbees.com, Woodinville, WA), and mailed overnight to Fargo, ND. Larvae were extracted from nests and reared in 24-well plates. Larvae were fed diets that were a homogenized mix of provisions from many brood cells. When larvae were in the fifth and final instar and had surpassed the minimum viable weight of approximately 53 mg (Helm et al. 2017), they were assigned to a treatment. For the 'fed' treatment, unlimited food was provided, which was approximately double what had been provisioned by the mother. Fed larvae were monitored to ensure they did not run out of food prior to pupation. For the 'starved' treatment, all food was removed. Individuals were tracked through pupation and metamorphosis (for details, see Helm et al. 2017). Adults were then removed from their cocoons, sexed, weighed, and frozen at -20°C.

### Measurement of Body Parts

Head, thorax, abdomen, and wings were disarticulated prior to morphometric measurements. Legs and antennae were removed so that the mass of each major body part was used for analysis without appendages. Head, thorax, and abdomen were dried to a constant weight in a 50°C drying oven and subsequently weighed using a calibrated microbalance (Mettler Toledo UMT2, Mettler Toledo, Columbus, OH). Wings were mounted onto glass slides in Euparal mounting medium (BioQuip, Compton, CA), covered, and then pressed flat prior to imaging. Images of wings were collected using a digital camera (Moticam 10, Motic, Richmond, BC, Canada) affixed to a dissection microscope (Olympus SZH10, Olympus America Inc., Melville, NY). Before each image set, an image of a micrometer was taken and used to calibrate measurements across wing images. Images were imported into Matlab 2015a and wing areas were calculated using the Image Analysis Toolbox.

# Data Analysis

All statistical analyses were performed with R version 4.0.1 (R Core Team 2020) in RStudio (2020, version 1.3.959). The effects of body mass, feeding treatment, and sex on morphological measurements were tested using linear models. Type II ANOVA tables were made from model objects using the Anova function in the *car* package (Fox and Weisberg 2019; version 3.0-10). All interactions were tested. The models were quality checked for deviations from normality, dispersion, and outliers using the package *DHARMa* (Hartig 2020; version 0.3.3.0). Morphometric measures were log<sub>10</sub> transformed because doing so improved the fit of the models. Tukey's HSD was used to compare differences between treatments.

Allometric scaling analyses were performed for each morphometric trait and for each combination of treatment and sex. Each trait was modeled as the power law function,  $\gamma = \beta_0 M^{\beta_1}$ , where  $\gamma$ was the trait of interest (head mass, thorax mass, abdomen mass, wing area), M was body mass,  $\beta_0$  was the scaling intercept, and  $\beta_1$  was the scaling coefficient. This function was linearized by logtransformation to,  $\log_{10} y = \log_{10} \beta_0 + \beta_1 \log_{10} M$ , and values were fitted for each parameter as standardized major axis regressions using the R package smatr (Warton and Weber 2002, Warton et al. 2006; version 3.4-8). For each morphometric trait, scaling relationships were analyzed across all individuals and for each feeding treatment and each sex separately (Table 2). Fitted scaling coefficient values for head, thorax, and abdomen were tested for isometry ( $\beta_1 = 1.0$ ) using by testing for a correlation between standard and fitted values using the slope.test function in the smatr package. Fitted scaling coefficients values for wing area were tested for the predicted relationship of  $\beta_1 = 2/3$  (Calder 1996). The models were quality checked using the package DHARMa (Hartig 2020; version 0.3.3.0). Data were visualized using ggplot2 (Wickham 2016; version 3.3.3).

#### Wing Loading and Extra Power Index

For each individual, wing loading (WL) was calculated as the ratio of body mass to the sum of the area of both wings:

$$WL = M_{Body} \swarrow_{A_{Wings}}$$

The extra power index (EPI) was calculated to estimate the excess power that could be generated above that which is required for steady, level flight (for assumptions and derivation, see Hepburn et al. 1998, Seidelmann 2014), using the equation:

$$\mathrm{EPI} = -\sqrt{\frac{\left(\frac{M_{\mathrm{Thorax}} \swarrow M_{\mathrm{Body}}}{M_{\mathrm{Body}} \swarrow A_{\mathrm{Wings}}}\right)^2}{M_{\mathrm{Body}} \swarrow A_{\mathrm{Wings}}}}$$

WL and EPI were then compared between feeding treatments and between sexes using type II analysis of variance with a fully crossed model.

# Results

#### Head Scaling Allometry

Head mass was positively correlated with body mass and was significantly affected by feeding treatment and sex (Table 1 [A]; Supp Table S1 [online only]). Head mass scaled hyperallometrically with body mass across all adults ( $\beta_1 = 1.06$ ), regardless of sex or feeding treatment (Fig. 1A). This global pattern was driven by hyperallometric scaling in the fed treatment ( $\beta_1 = 1.36$ , P < 0.0001). Isometric scaling was not rejected for starved individuals (P = 0.8927). For females

Table 1. Type II ANOVA tables for the effects of body mass, feeding treatment, and sex on head mass (A), thoracic mass (B), abdominal mass (C), and wing area (D)

Model term	df	Sum of squares	Mean square	F	Р
		A. Head mass	5		
Dry mass	1	0.202	0.202	52.2	<0.0001****
Treatment	1	0.0185	0.0185	6.50	0.013*
Sex	1	0.0328	0.0328	11.5	0.0012**
Dry mass * Treatment	1	2.73E-03	2.73E-03	0.958	0.33
Dry mass * Sex	1	1.23E-03	1.23E-03	0.431	0.514
Treatment * Sex	1	1.32E-04	1.32E-04	0.0463	0.830
Dry mass * Treatment * Sex	1	0	0	0	0.998
Residual	63	0.180	2.85E-03		
		B. Thoracic ma	ISS		
Dry mass	1	0.528	0.528	84.1	<0.0001****
Treatment	1	0.0117	0.0117	1.86	0.177
Sex	1	2.18E-03	2.18E-03	0.346	0.558
Dry mass * Treatment	1	0.0276	0.0276	4.40	0.040*
Dry mass * Sex	1	0.0641	0.0641	10.2	<0.01**
Treatment * Sex	1	0.0692	0.0692	11.0	<0.01**
Dry mass * Treatment * Sex	1	0.08093	0.0160	12.9	<0.001***
Residual	63	0.396	6.28E-03		
		C. Abdominal m	ass		
Dry mass	1	0.309	0.289	242.1	<0.0001****
Treatment	1	0	0	2.0E-04	0.990
Sex	1	7.16E-03	7.16E-03	5.61	0.020*
Dry mass * Treatment	1	5.73E-03	5.73E-03	0.449	0.505
Dry mass * Sex	1	5.72E-03	5.72E-03	0.448	0.506
Treatment * Sex	1	1.66E-03	1.66E-03	1.30	0.258
Dry mass * Treatment * Sex	1	2.41E-03	2.41E-03	1.89	0.174
Residual	63	0.0804	1.28E-03		
		D. Wing area			
Dry mass	1	0.0501	0.0501	23.1	<0.0001****
Treatment	1	6.13E-03	6.13E-03	2.83	$0.0974^{\dagger}$
Sex	1	7.28E-04	7.28E-04	0.336	0.564
Dry mass * Treatment	1	5.0E-06	5.0E-06	0.0025	0.960
Dry mass * Sex	1	1.36E-03	1.36E-03	0.627	0.431
Treatment * Sex	1	1.59E-03	1.59E-03	0.732	0.395
Dry mass * Treatment * Sex	1	2.22E-04	2.22E-04	0.102	0.75
Residual	63	0.136	2.16E-03		

Type II analysis of variance (ANOVA) performed using 'car' package in R. Dry mass was log<sub>10</sub> transformed prior to analysis.

 $\text{Statistical significance values: } ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ^{\dagger}P < 0.10. \\ \text{Statistical significance values: } ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ^{\dagger}P < 0.10. \\ \text{Statistical significance values: } *****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ^{\dagger}P < 0.10. \\ \text{Statistical significance values: } *****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ^{\dagger}P < 0.10. \\ \text{Statistical significance values: } *****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ^{\dagger}P < 0.10. \\ \text{Statistical significance values: } *****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ^{\dagger}P < 0.10. \\ \text{Statistical significance values: } *****P < 0.001, **P < 0.001$ 

across both treatments, head size was hyperallometric ( $\beta_1 = 1.09$ , P = 0.0442). For males across both treatments, head size was hypoallometric ( $\beta_1 = 0.93$ , P = 0.0263). Isometric scaling ( $\beta_1 = 1$ ) was not rejected for each treatment and sex combination separately (Table 2 [A]). This indicated that head mass was disproportionately large in well-provisioned individuals as compared to smaller, underfed individuals, and females had disproportionately larger heads than males (Fig. 1A).

### Thoracic Scaling Allometry

Thorax mass had a positive allometry with body mass. Neither feeding treatment nor sex had significant effects on thoracic scaling (Table 1 [B]), but interaction terms among feeding treatment, sex, and body mass were statistically significant, suggesting scaling exponents differed

among feeding treatments and sex (Table 1 [B]). Among all individuals, thorax mass scaled hyperallometrically with body mass (Fig. 1B; Table 2 [B]). Interestingly, starved males and fed females scaled hyperallometrically, although isometric relationships were not rejected for starved females or for fed males (Table 2 [B]). Starved males had a steep slope ( $\beta_1 = 3.95$ ) which was driven by one male with an especially small thorax (Fig. 1B). A residual test on the model indicated this outlier was significant (P = 0.0193). We chose to keep this individual in the dataset because its outlier nature was due to a real difference in morphology and not due to a mismeasurement. The differences in thorax mass among treatment groups and by sex likely explained the significance of interaction terms without significant main effects (Table 1 [B]), although it is notable that all groups had scaling exponents above 1 (Table 2 [B]). In summary, large individuals had disproportionately large thoraces compared to small individuals (Fig. 1B).



Fig. 1. Allometric scaling of head mass (A), thoracic mass (B), abdominal mass (C), and wing area (D). Predicted relationships are indicated by dashed lines. Global slopes are indicated by solid lines. Slopes of each subgroup are indicated by solid lines in the color corresponding to treatment. Linear relationships in wing loading (E), and the extra power index (F) are represented with a solid line and the gray shading indicates the 95% confidence interval.

## Abdominal Scaling Allometry

Abdominal mass was significantly affected by body mass and sex, but there was no significant effect of feeding treatment (Fig. 1C; Table 1 [C]). Abdomen mass scaled hypoallometrically with body mass among all individuals; although isometry was not rejected for each feeding treatment and sex separately (Table 2 [C]). We tested for isometry for each sex, combining the two treatments. The relationship for females and males was not significantly different from isometric (females, P = 0.3638; males, P = 0.4893). Thus, hypoallometry of the abdomen was supported only when both sexes were combined. Feeding treatment did not significantly affect abdomen mass (Table 1 [C]), This indicated that large individuals had disproportionately small abdomens relative to body mass compared to small individuals, and females trended toward larger abdomens than males for their body mass (Table 1 [C]).

#### Wing Area Scaling Allometry

Wing area was significantly affected by body mass, although no other experimental factors were statistically significant (Table 1 [D]). Wing area was tested against an expected slope of 2/3. Among all individuals, wing area scaled hypoallometrically with body mass (Table 2 [D]). However, fed males had a hyperallometric wing area scaling. No other sex- or treatment-specific allometries differed from the expected 2/3 relationship (Table 2 [D]). In summary, larger individuals had disproportionately small wing areas compared to smaller individuals (Fig. 1D).

## Wing Loading

WL was significantly affected by body mass, treatment, and sex (Table 3 [A]). None of the interaction terms were significant. Fed individuals had higher WL than starved individuals (Fig. 1E: Tukey HSD,  $\Delta = 0.97$ , P < 0.0001). Females had higher WL than males (Fig. 1E: Tukey HSD,  $\Delta = 0.537$ , P < 0.0001). Even when accounting for overall body mass, fed females had the highest values for WL.

#### **Extra Power Index**

The EPI was significantly affected by the three-way interaction between body mass, treatment, and sex (Table 3 [B]). None of the variables were significant on their own. The model was significantly affected by an outlier (P = 0.0193), likely due to the male with the small thorax. This outlier countered the overall trend (Fig. 1F). When a Tukey HSD was run on treatment, starved individuals had a significantly higher EPI than fed (Tukey HSD,  $\Delta = 0.0765$ , P < 0.0001), but there was no significant difference between sexes (Tukey HSD,  $\Delta = 0.0037$ , P = 0.788). Overall, EPI tended to decrease with body mass, but the significant pattern was an interaction between body mass, sex, and treatment (Fig. 1F).

# Discussion

Allometries of adult O. *lignaria* revealed complex responses to larval nutrition—a likely consequence of counterbalancing allocative

Table 2. Scaling allo	metries of head ma	ss (A), thoracic mas	s (B), abdominal	mass (C), and	wing area (D	) relative to bo	dy mass for	r male
and female adult O.	lignaria that had for	od removed either ea	arly (Starved) or v	were provided	unlimited foo	d (Fed) during	the larval st	age

			959	% CI		95%	% CI			Test of is $(\beta_1 =$	ometry 1)
Model term	п	β <sub>o</sub>	Lower	Upper	$\beta_1$	Lower	Upper	$R^2$	Р	r	Р
					A. Head	scaling					
All	71	-0.880	-0.965	-0.794	1.06	1.01	1.12	0.95	<0.0001	0.276	0.0198
Starved											
Male <sup>a</sup>	11	-0.872	-1.56	-0.188	1.09	0.604	1.95	0.32	0.0678	0.0990	0.772
Female	9	-0.822	-1.60	-0.0415	1.03	0.578	1.83	0.536	0.0249	0.0419	0.915
Fed											
Male	24	-0.877	-1.13	-0.444	1.03	0.788	1.35	0.616	< 0.0001	0.0524	0.808
Female	27	-1.16	-1.75	-0.585	1.24	0.954	1.61	0.584	< 0.0001	0.320	0.104
					B. Thoraci	c scaling					
										Test of is	ometry
										(β <sub>1</sub> =	: 1)
All	71	-0.858	-0.981	-0.734	1.19	1.11	1.28	0.92	< 0.0001	0.528	< 0.0001
Starved											
Male <sup>a</sup>	11	-3.63	-5.57	-1.69	3.95	2.47	6.29	0.590	< 0.01	0.945	< 0.0001
Female	9	-0.805	-1.58	-0.0269	1.19	0.723	1.97	0.658	< 0.01	0.293	0.445
Fed											
Male	24	-0.728	-1.08	-0.372	1.09	0.880	1.35	0.766	< 0.0001	0.173	0.420
Female	27	-0.998	-1.43	-0.564	1.27	1.05	1.54	0.774	< 0.0001	0.454	0.0174
					C. Abdomir	nal scaling					
										Test of is	ometry
										$(\beta_1 =$	: 1)
All	71	-0.184	-0.240	-0.129	0.953	0.918	0.990	0.975	< 0.0001	-0.289	0.0146
Starved											
Male <sup>a</sup>	11	-0.171	-0.627	0.285	0.941	0.593	1.49	0.599	< 0.01	-0.0971	0.776
Female	9	-0.411	-0.961	0.139	1.12	0.763	1.65	0.806	< 0.01	0.251	0.516
Fed											
Male	24	-0.396	-0.603	-0.189	1.11	0.984	1.25	0.924	< 0.0001	0.358	0.0855
Female	27	-0.481	-0.882	-0.0805	1.11	0.905	1.36	0.749	< 0.0001	0.207	0.300
					D. Wing are	ea scaling					
										Test of is $(\beta_1 =$	ometry 2/3)
All	71	0.832	0.770	0.893	0.536	0.497	0.577	0.902	<0.0001	-0.558	<0.0001
Starved											
Male <sup>a</sup>	11	0.832	0.587	1.08	0.552	0.361	0.844	0.663	0.00227	-0.312	0.351
Female	9	0.642	0.0011	1.28	0.679	0.338	1.37	0.2.84	0.140	0.0210	0.957
Fed	/	0.012	0.0011	1.20	0.072	0.000	1.07	0.201	0.110	0.0210	0.207
Male	24	0.0115	-0.625	0.648	1.07	0.736	1.57	0.232	0.0172	0 492	0.0147
Female	27	0.518	0.0571	0.979	0.712	0.496	1.023	0.193	0.0220	0.07322	0.717

All regression statistics were performed using the package 'smatr' for R version 3.1.1. Slopes ( $\beta_1$ ) and intercepts ( $\beta_0$ ) of allometries were calculated by standardized major axis regression, and 95% confidence intervals (CIs) for each each parameter were calculated. Tests of isometry were performed using likelihood ratio tests.

<sup>a</sup>Two males from the starved treatment were excluded from analysis because they had extreme deviations in single morphometric measures, these individuals are not included in reported sample sizes.

decisions during metamorphic development. Under fed conditions, the head and thorax grew disproportionally large relative to body mass, indicating increased allocation toward these tissues and their adult function. The overall trends in allocation were likely driven by two aspects of *O. lignaria* biology: females are larger than males and the sexes are dimorphic for many aspects of behavior and reproduction. *Osmia lignaria* has a sexually dimorphic maximum body size and even under unlimited feeding females grow larger than males (Helm et al. 2017). In this study, the length of the larval growth period in the fed treatment was not significantly different between the sexes (F = 0.5688, P = 0.4543), suggesting that sexually dimorphic growth rates account for body size differences. Overall

Table 3. Type II analysis of variance tables for the effects of body mass, feeding treatment, and sex on wing loading (A) and the extra power index (B)

Model term	df	Sum of squares	F	Р
A	. Wing	loading		
Dry mass	1	0.393	7.34	0.00866**
Treatment	1	0.311	5.81	0.0189*
Sex	1	0.336	6.28	0.0148*
Dry mass * Treatment	1	0.0110	0.206	0.652
Dry mass * Sex	1	0.0104	0.194	0.661
Treatment * Sex	1	0.0149	0.278	0.600
Dry mass * Treatment * Sex		0.0271	0.507	0.479
Residual	63	3.37		
B. E.	xtra po	wer index		
Dry mass	1	0.00469	1.523	0.222
Treatment	1	0.000936	0.3040	0.583
Sex	1	0.00428	1.39	0.243
Dry mass * Treatment	1	0.00101	0.328	0.569
Dry mass * Sex	1	0.000268	0.0870	0.769
Treatment * Sex	1	0.000384	0.125	0.725
Dry mass * Treatment * Sex	1	0.0238	7.70	0.00725**
Residual	63	0.194		

\*\*P < 0.01, \*\*\*P < 0.05.

differences in male and female body size accompany different behaviors and functional morphology. The allocation of resources to the head and thorax and away from the abdomen was predominately a function of overall body size, although there were sex-specific differences. Females in the fed treatment had a high investment in the thorax ( $\beta_1 = 1.27$  compared to 1.19 for the starved females), which corresponds to more muscles to support flight. Females in general allocate more resources to the head and abdomen than males, even after accounting for overall differences in body size. These patterns fit the biology of *O. lignaria*, a species in which female reproduction depends upon flight.

The observed patterns in allocation may reflect an adaptive strategy in O. lignaria, a species in which females depend upon flight for nest construction and brood provisioning. For other insects, excess resources are frequently invested into fecundity, causing hyperallometry of abdominal structures despite the increased flight cost of additional mass (Wickman and Karlsson 1989). However, in O. lignaria, abdominal growth was hypoallometric, indicating a proportional decrease in mass of reproductive tissues as body size increased. Females use flight and jaw muscles heavily during nest construction and foraging (Bosch and Kemp 2001). The thorax became proportionally larger in females individuals, indicating that the additional resources were diverted from abdomen to thoracic flight muscles. Female O. lignaria are capable of laying and provisioning only ~20 eggs in their lifespan because nest cell construction and provisioning require substantial energy and time investment (Bosch and Kemp 2001). Osmia lignaria males live for only a few days and mate with siblings emerging from the same nest, although flight may be used for dispersal in some cases. In females, allocation to tissues that boost flight capability and nest building may yield higher returns for fitness than allocating toward reproductive tissues directly.

When insects develop under limited resources, flight and fecundity are usually negatively associated (Tigreros and Davidowitz 2019). Wing polymorphic insects exemplify this tradeoff because winged morphs invest in wing growth for dispersal, whereas the wingless morph allocates those resources toward reproduction (Roff 1986, 1990; Roff and Fairbairn 1991). In Lepidoptera, food restriction causes an allocation to the wings and thorax and a decrease in WL (Tigreros and Davidowitz 2019). In *O. lignaria*, investment in the abdomen was maintained across body size. Investment in the thorax increased with body size but investment in wing area decreased. This pattern is similar to the butterfly *Pieris rapae* which maintained allocation to the thorax while decreasing investment in wing size (Tigreros et al. 2013). Allocation strategies in *O. lignaria* suggest flight and fecundity allocation are counterbalanced with respect to larval provisioning.

Wing-body morphometrics of O. *lignaria* add additional support for a tradeoff between fecundity and flight. Large O. *lignaria* adults had smaller wing areas with respect to body mass than small adults. This corresponded with reduced WL for small adults and increased WL for large adults. However, large adults had exaggerated growth of the thorax as compared to small adults. While this might suggest that the flight–fecundity tradeoff is mitigated by enhanced growth of flight musculature, small adults had a higher flight EPI than large adults, indicating that these individuals generate more excess power during flight or that less power is required for hovering flight. Thus, the maxim that 'bigger is better' (Kingsolver and Huey 2008) is not necessarily true when examining the complex relationships among traits related to flight performance.

Among different species of insects, the relationships between wing area, WL, and wing beat frequency have been thoroughly described (Byrne et al. 1988, Tercel et al. 2018). Insects that have low WL typically have reduced wing beat frequencies-for example, the fluttering flight of butterflies. Insects that have high WL generally have higher wing beat frequencies, such as the hovering, hummingbird-like flight of hawkmoths (Byrne et al. 1988). However, wing beat frequency is often negatively correlated with body mass because wing area increases with size (Tercel et al. 2018). Orchid bees have higher WL, increased flight costs, but lower wing beat frequencies as body mass increases among Euglossine orchid bee species (Dillon and Dudley 2004, Darveau et al. 2005). Relationships between flight-associated morphometrics have a high degree of conservation within phylogenetic groups (Tercel et al. 2018), such that O. lignaria are expected to have similar relationships among wing musculature, wing area/load, and flight biomechanics.

#### Conclusions

When comparing large and small adult O. lignaria, we observe the ends of a continuum of phenotypic variation that arises from environmental conditions during development. These results raise the question of whether there exists a two-way relationship between adult morphology and larval environment. In Drosophila melanogaster, variation in larval nutrition, temperature, and rearing density results in distinct adult wing and body allometries (Frazier et al. 2008, Shingleton et al. 2009, Bhan et al. 2014). Adult morphologies represent many traits that must work as integrated units for survival and reproduction (Nijhout and Wheeler 1996, Shingleton et al. 2007, Nijhout and Callier 2015). In turn, variation in adult performance may select for larval growth programs that are successful with respect to larval environmental conditions (Hoffmann et al. 2007). In support of that idea, adult Osmia with small body sizes have been observed in poor foraging years (Bosch 2008), indicating that the body sizes in this study included a range possible in nature. An important question remaining from this study is whether the observed

flight and body allometries affect flight performance and behavioral strategies for *O. lignaria* in nature.

#### Supplementary Data

Supplementary data are available at Journal of Insect Science online.

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# **Author Contributions**

BRH contributed to all aspects of the study and prepared the manuscript. MAB helped conduct measurements of morphometric traits, assisted with data analysis, and contributed to interpretation of data. JPR, GDY, KJG, and JHB assisted with drafting the article and revised for intellectual content.

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