1   **Larval nutrition impacts survival to adulthood, body size, and the**

2   **allometric scaling of metabolic rate in adult honeybees**

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7   **Key Words:** Diet Quality, Nutrition, Development, Metabolic Rate, Metabolic Scaling, Honeybee

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Summary statement

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9 We show, for the first time, that the nutritional quality of insect larval diets affects the scaling of

10 metabolic rate with body mass in newly emerged adult honeybees.

Abstract

12 Resting metabolic rate (RMR) is a fundamental physiological measure linked to numerous aspects of
13 organismal function, including lifespan. Although dietary restriction in insects during larval
14 growth/development affects adult RMR, the impact of larval diet *quality* on adult RMR has not been
15 studied. Using *in vitro* rearing to control larval diet quality, we determined the effect of dietary protein
16 and carbohydrate on honeybee survival-to-adulthood, time-to-eclosion, body mass/size and adult RMR.
17 High carbohydrate larval diets increased survival-to-adulthood and time-to-eclosion compared to both
18 low carbohydrate and high protein diets. Upon emergence, bees reared on the high protein diet were
19 smaller and lighter than those reared on other diets, whilst those raised on the high carbohydrate diet
20 varied more in body mass. Newly emerged adult bees' reared on the high carbohydrate diet showed a
21 significantly steeper increase in allometric scaling of RMR compared to those reared on other diets.
22 This suggests that diet quality influences survival-to-adulthood, time-to-eclosion, and the allometric
23 scaling of RMR. Given that agricultural intensification and increasing urbanisation have led to a
24 decrease in both forage availability and dietary diversity for bees, our results are critical to improving
25 understanding of the impacts of poor developmental nutrition on bee growth/development and
26 physiology.

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1. Introduction

The resting metabolic rate (RMR) of an organism can account for up to 50% of total energetic expenditure (Morgan, Shelly and Kimsey, 1985) and is intrinsically linked to numerous aspects of physiological and behavioural functioning, from reproductive output to lifespan (Speakman, 2005; Pettersen, Marshall and White, 2018). Despite this, surprisingly little is understood about the drivers of variation in RMR between organisms, particularly at the intra-specific level where consistent individual differences in RMR are frequently observed (McCarthy, 2000; Burton *et al.*, 2011). Both across and within many diverse taxa, RMR has been shown to scale allometrically with body size, with larger individuals having higher metabolic rates, and smaller individuals typically having higher mass-specific metabolic rates (Bartholomew, Lighton and Feener, 1988; Gillooly *et al.*, 2001; Brown *et al.*, 2004; Glazier, 2005; Chown *et al.*, 2007). Though the mechanism(s) underpinning allometric scaling of RMR remain highly debated (McNab, 1988; White and Seymour, 2003; Savage *et al.*, 2004), scaling exponents have been shown to be affected by several intrinsic and extrinsic factors, including activity level, temperature and diet (Glazier, 2005).

Metabolism is fuelled by food and therefore it is to be expected that an organisms' diet will have considerable impact on the resources available for energetic expenditure, yet the mechanism(s) by which diet affect RMR and the scaling of RMR remains poorly understood. As highlighted by Naya et al. (2007), in the short term (i.e. hours to days), diet may affect metabolism simply as a result of the energetic processes involved in digestion and absorption of nutrients (Roces and Lighton, 1995; Nespolo, Castañeda and Roff, 2005). In the longer term (i.e. weeks to months), the availability of certain nutrients in an organisms' diet may affect developmental processes such as organ growth or maintenance processes such as tissue repair (Anderson, 1993; Yang and Joern, 1994). In a number of taxa, including humans, restricting food during developmental stages has been shown to have long-term effects on adult metabolism (Desai and Hales, 1997; Moe *et al.*, 2004; Roark and Bjorndal, 2009), potentially allowing organisms to adapt to food scarcity in later life (Hales and Barker, 2001; Wang *et al.*, 2016). In many instances, however, organisms are more likely to experience a scarcity of particular nutrients, such as protein or carbohydrates, rather than a complete lack of food, and may be forced to provision their young with sub-optimal, unbalanced diets (Joern, Provin and Behmer, 2012). Yet direct tests of the impact of the nutritional *quality* of developmental diets on adult metabolism are relatively rare outside of epidemiological studies.

Making *a priori* directional predictions about how the nutritional quality of developmental diets might be expected to affect adult metabolic rates is challenging, because theoretical arguments can be made for both positive and negative associations between diet quality and RMR (McNab, 1986; Nussear *et al.*, 1998). Nutritional studies have shown that when offered diets of varying composition, organisms defend an optimal intake target of key macronutrients, in particular protein and carbohydrates which provide amino acids and energy vital for survival, growth and reproduction (Karasov, Martínez Del Rio and Caviedes-Vidal, 2011; Simpson and Raubenheimer, 2012; Roeder and Behmer, 2014). Optimal

66 intake targets can be achieved through behaviours such as selective or compensatory feeding, or

67 physiological/morphological means such as increasing gut length or food retention time (Felton, 1996;

68 Behmer, 2009; Burton *et al.*, 2011). Though insects have long been used as models to study the

69 regulation of nutritional intake targets (Behmer, 2009) studies of the long-term impact of variation in

70 nutrition over the course of development are somewhat lacking (Roeder and Behmer, 2014), and studies

71 of the subsequent effects on adult metabolism are largely non-existent. A recent study found that adult

72 stick insects exhibit developmental diet dependent differences in RMR when reared from birth on leaves

73 from plant species varying in their nutritional content and digestibility (Hill, Silcocks and Andrew,

74 2020), but the impact of developmental diet on the scaling of RMR and body mass was not considered.

75 Shorter term studies conducted in adult insects only are more common, and have typically observed a

76 reduction in RMR in response to a lower quality diet (Zanotto *et al.*, 1997; Ayayee *et al.*, 2018, 2020,

77 but see Clark, Zera and Behmer, 2016).

78   Bees meet all their nutritional demands *via* pollen and nectar collected from flowers (their main

79 source of protein and carbohydrate respectively), and unlike the nymphs and larvae of traditional

80 models of insect nutrient regulation, such as locusts and caterpillars, bee larvae are entirely dependent

81 on the provisioning choices of adult bees. This means bee larvae likely have very little opportunity to

82 selectively regulate their intake of nutrients (but see Austin and Gilbert, 2018). Honeybees are unique

83 among bees in that in-hive nurse bees process the pollen and nectar brought back to the nest by foragers,

84 and convert it to a nutritional substance known as royal jelly which they then regurgitate for larvae

85 (Wright, Nicolson and Shafir, 2018). Containing approximately 60% water, 15% protein, 20%

86 carbohydrates, and 5% fats, the exact macronutrient content of royal jelly can vary between colonies

87 and over time (Howe *et al.*, 1985; Garcia-Amoedo and De Almeida-Muradian, 2007; Ferioli, Armaforte

88 and Caboni, 2014). Furthermore, a recent study has demonstrated that nurse honeybees are unable to

89 discriminate between pollen diets on the basis of nutritional quality (protein and/or lipid content)

90 (Corby-Harris *et al.*, 2018), meaning the proportion of macronutrients that individual larvae receive in

91 their diet could vary, particularly in times or areas where the diversity of forage is limited (Donkersley

92 *et al.*, 2017). In addition, there is recent evidence to suggest that rising CO_2 levels associated with

93 climate change are negatively affecting the nutritional quality of pollen and nectar provided by plants

94 (Ziska *et al.*, 2016). Given widespread concerns regarding the combined effects of habitat degradation

95 and agricultural intensification on the availability of sufficiently diverse floral resources to meet the

96 nutritional needs of adult bees and their offspring (Naug, 2009; Brodschneider and Crailsheim, 2010;

97 Donkersley *et al.*, 2017), and the fact that bees provide a pollination service vital to global food security,

98 the question of how developmental diets impact on the metabolic function of adult bees is extremely

99 apposite.

100   Here we used *in vitro* rearing methods to tightly control honeybee larval diets independent of

101 nurse bee behaviour, permitting an examination of the impact of diet nutritional composition on

102 honeybee development and adult physiological function. Previous studies have shown that the ratio of

103 protein to carbohydrate in honeybee larval diets can have significant impacts on larval survival (Helm

104 *et al.*, 2017), with unbalanced diets heavily skewed to either macronutrient resulting in poor growth and

105 survival. To our knowledge this is the first study to test the RMR of adult bees reared on different larval

106 diets *in vitro*. By manipulating the ratio of royal jelly (protein) to sugars (carbohydrates), we aimed to

107 determine the impact of specific macro-nutrients on adult RMR and scaling with body size.

108

109 **2. Materials and Methods**

110 Honeybee (*Apis mellifera* L.) larvae were obtained from full-sized colonies housed on the

111 University of Sussex campus, and reared in the laboratory using the *in vitro* method described by

112 Schmehl *et al.* (2016). Briefly, three-day old larvae were removed from the comb using a grafting tool,

113 transferred to individual wells of a 48-well cell culture plate, and placed into an incubator fixed at 35°C,

114 94% relative humidity (RH). Larvae were fed once per day for five days, and upon pupation transferred

115 to a fresh cell culture plate. Survival was monitored daily until adult emergence.

116

117 *Diet manipulation*

118 A standard *in vitro* rearing diet (Table 1) of yeast (Sigma-Aldrich UK), royal jelly (The Raw

119 Honey Shop, Brighton) and sugars (glucose and fructose, Sigma-Aldrich UK) was manipulated to

120 contain differing amounts of protein (using royal jelly as a proxy) and/or carbohydrate (glucose and

121 fructose), following the methods of Helm *et al.* (2017). Larvae were reared on one of five diets (Table

122 1, D1-5), where the amount of protein and carbohydrate was either increased or decreased relative to

123 the diet described by Schmehl *et al.* (2016). Royal jelly was stored frozen at -20°C in 50 mL aliquots.

124 Diets were freshly made every two days and stored at 4°C. Larvae were fed once per day for five days,

125 and the volume of food varied according to the day of the experiment (Days 1 and 2 = 10 μL; Day 3 =

126 20 μL; Day 4 = 30 μL; Day 5 = 40 μL; and Day 6 = 50 μL). Between 60 and 78 larvae were assigned to

127 each treatment group (*N* = 371 larvae in total; D1=78; D2=60; D3=78, D4=78; D5=77). Bees were

128 reared in two cohorts, grafted on 30/9/2019 and 20/10/2019. Royal jelly nutritional values

129 (supplementary data) were obtained by the supplier (The Raw Honey Shop, Brighton) using the

130 international standard for royal jelly (ISO 12824:2016). From these values we calculated the proportion

131 and ratio of protein:carbohydrate (P:C) in each of the five diets (Table 1).

132 *Measuring resting metabolic rates*

133 To determine how larval diet affects adult metabolism, the RMR of adult bees was measured

134 on the day of emergence (between 14-17 days from the day of grafting) using flow-through

135 respirometry, with CO_2 production used as a measure of metabolic rate. Emerging adults were first

136 individually weighed to the nearest mg using a precision balance (Mettler Toledo, UK). Bees were then

137 restrained using a small cylinder of metal mesh to allow gas exchange, before being placed into a 2 mL

138 plastic chamber. Air scrubbed of CO_2 and H_2O was then pumped through the chamber at a consistent

139 rate of 100 mL min^{-1} via a mass flow controller (GFC17; Aalborg, NY, USA), before passing through
140 an infrared CO_2-H_2O analyser (Li7000, Li-Cor) which captured data on CO_2 production, relative to an
141 empty control chamber (Nicholls *et al.*, 2017; Perl and Niven, 2018). The temperature in the room was
142 held constant at 25°C (± 2°C) and recordings lasted for 20 minutes per bee. The first five minutes of
143 the recording were treated as a settling period for the bee to adjust to the experimental set up and were
144 excluded from analysis. During recording the plastic chamber was covered to ensure it was dark, which
145 reduced bee movement. The order in which bees from different diet treatment groups were measured
146 was randomised. After recording, bees were frozen to immobilise them, and digital callipers were used
147 to measure the intertegular span (defined as the distance between the points at which the wings attach
148 to the thorax) in mm, a proxy measure for body size (Cane, 1987).

149

150 *Data analysis*

151   Respirometry data was analysed using OriginPro software (Origin 2016, OriginLab
152 Corporation, Northampton, MA, USA). Volumes of CO_2 were baseline corrected and temperature
153 normalised using the Q10 correction for temperature differences. To calculate the rate of CO_2
154 production per bee, the volume of CO_2 (ppm) was converted to CO_2 fraction and multiplied by the flow
155 rate (100 mL min^{-1}). The integral of CO2 min^{-1} *versus* min was calculated for a stable 15-minute period
156 of the recording, and divided by this time to give a rate of μl CO_2 h^{-1}.

157   All statistical analyses were conducted in R 3.6.2 (R Core Team, 2019. https://www.R-
158 project.org). To examine how diet quality impacts larval survival, Kaplan-Meier survival analysis was
159 performed using the survfit function from the 'survival' package. The log-rank test was used to test for
160 differences in survival between diet treatments with a Bonferroni correction for multiple comparisons.
161 Linear and mixed effect models were performed by restricted maximum likelihood (REML) estimation
162 using the lmer and glmer function from the 'lme4' package to test the impact of diet treatment on the
163 time to adult emergence (days), wet body mass (mg), body size (using intertegular distance as a proxy
164 measure; mm), body condition (body mass/body size; mg/mm) and CO_2 production (μL CO_2 h^{-1}). The
165 continuous variables body mass, body size, body condition and CO_2 production were log transformed.
166 Date of grafting was included as a random effect. For all models, Diet 2 was used as the reference
167 category because bees in this treatment had the best survival. Significances of the fixed effects were
168 determined using Satterthwaite's method for estimation of degrees of freedom by using the anova
169 function from 'lmerTest'. Estimated marginal means (emm) and pairwise comparisons were obtained
170 using the 'lsmeans' package and the *p*-value adjusted with the Tukey method. To test for differences in
171 variance, we used the Brown-Forsythe test for non-normal data. All plots were made using the 'ggplot2'
172 package.

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3. Results

The ratio of P:C in larval diets affects honeybee development and survival

Diet had a significant effect on the survival of honeybees to adult emergence (Fig. 1, Table S1,2; Kaplan-Meier log-rank test, $\chi^2_4 = 54.7$, $p < 0.001$). Larvae reared on the high carbohydrate diet (D2), which had a P:C ratio of 1:3, had the best survival (70%), significantly higher than all other treatment groups (Fig. 1, Table S2; Log-rank test D2-D1p=0.023; D2-D3 p <0.001; D2-D4 p <0.001; D2-D5 p=0.013)). Bees reared on the high protein diet (D4, P:C 1:1.9) had very poor survival (22%), and only one bee reared on the low carbohydrate diet (D3, P:C 1:1.5) survived to adulthood (Fig. 1). Consequently, bees from D3 are excluded from subsequent analyses. Bees reared on the diet recommended by Schmehl *et al.* (2016) for rearing larvae (D1, P:C 1:2.3), and the low protein diet (D5, P:C 1:2.9) had similar levels of survival (Table S1,2), with just under half of all larvae reaching adulthood (~45%, Fig. 1). Diet also had a significant effect on development time (days to emergence) (Table 2, Table S3; $\chi^2_3 = 22.14$, $p < 0.001$), with bees reared on the high carbohydrate diet that maximised survival (D2) taking significantly longer to emerge (emm ± s.e. = 16.0 ± 0.96 days) than those in all other treatment groups (D1 = 15.5 ± 0.96; D4 = 15.3 ± 0.97; D5= 15.7 ± 0.96 days).

The ratio of P:C in larval diets affects adult body mass, size and condition

On emergence, bees reared on the high protein diet (D4), the second worst diet for survival, weighed approximately 10 mg less on average than those reared on all other diets (Fig. 2A), and were significantly lighter than those reared on the high carbohydrate diet D2 (Table 2, Table S3; estimate ± s.e. -9.23 ± 4.32 mg, df = 96.61, $p = 0.035$). Variance in body size also differed between diet treatments (Fig. 2A). There was a significant difference in the variance of body mass, both between bees reared on D2 and D1 (Table S4; Brown-Forsythe Test, $p = 0.007$), and D2 and D5 (Brown-Forsythe Test, $p = 0.016$) suggesting that the diet maximising survival (D2) allowed for a greater range of body masses.

Bees reared on the high protein diet (D4) were also significantly smaller (emm ± s.e. = 2.80 ± 0.09 mm) than bees in all other treatment groups, as measured by the intertegular span (Fig. 2B; Table 2, Table S3; D1= 3.04 ± 0.08, D2 = 3.04 ± 0.08, D5 = 2.97 ± 0.08 mm). The variance in body size was also lowest in bees reared on D4, significantly lower than bees reared on D1 (Table S4; Brown-Forsythe Test $p = 0.002$) or D5 ($p = 0.026$). As expected, there was a significant positive relationship between body mass and body size (Fig. 2C, Table S5; $\chi^2_1 = 12.01$, $p < 0.001$), but diet treatment had no significant effect on the relationship between body mass and body size.

Body condition scores (body mass/body size) also differed between diet treatments (Fig. 2D, Table 2, Table S3; $F_{3,65} = 3.354$, $p = 0.024$). Bees reared on the high carbohydrate diet (D2), had a significantly lower body condition score on average (emm = 26.1 ± 0.80 mg/mm) than those reared on D1 (emm = 29.5 ± 0.82 mg/mm; estimate ± s.e. 3.40 ± 1.14 mg/mm, $p = 0.004$) or D5 (emm = 28.8 ± 0.80 mg/mm; estimate ± s.e. 2.70 ± 1.13 mg/mm, $p = 0.020$). As with body mass, there was also a

significant difference in the variance of body condition scores between bees raised on D2 and D1 (Table S4; Brown-Forsythe Test $p = 0.011$) and D2 and D5 (Brown-Forsythe Test $p = 0.008$).

The ratio of P:C in larval diets affects the scaling of resting metabolic rate with body mass

Across all diet treatments, RMR (μL CO_2 h^{-1}) scaled positively with body mass (Fig. 3A, Table 3), and bees reared on the diet which maximised survival (D2) had a significantly steeper slope compared to those reared on D1 (Table 4). Diet also had a significant effect on the scaling of mass-specific RMR (RMR/body mass; Fig. 3B, Table 4). Bees reared on D2, the diet which maximised survival, showed a positive relationship between body mass and mass-specific RMR, whereas bees reared on all other diets exhibited a negative relationship (Fig. 3B). The difference in scaling between body mass and mass-specific RMR in bees reared on D1 and D2 was significant (Table 4; estimate $\pm$ s.e. = -1.00 $\pm$ 0.47, df = 92.03, $p = 0.035$). The nature of the scaling relationship between adult body mass and RMR differed considerably according to larval nutrition, with bees reared on D2 diet exhibiting positive allometry, bees reared on D5 diet exhibiting isometry and bees reared on D1 diet exhibiting negative allometry (Table 3). Body size was not a significant predictor of RMR (Fig. 3C; Table S5; $F_{1, 53.36} = 2.37$, $p = 0.242$), and while for most diet treatments there was a positive relationship between body condition and RMR, again this was not a significant predictor (Fig. 3D, Table S5; $F_{1, 63.72} = 2.67$, $p = 0.107$).

Discussion

Many organisms experience nutritionally sub-optimal diets during development, but very few studies have directly examined the impact of developmental diet quality on adult metabolism, particularly in insects. This question is of particular importance for bees, which as adult foragers face extremely high energetic demands, and as larvae experience a diet completely dependent on the provisioning choices of their mother and/or siblings, likely limiting their ability to self-regulate the intake of particular nutrients. Previous studies have shown that manipulating colony access to pollen results in reduced body size and life span in adult bees (Eishchen, Rothenbuhler and Kulinčević, 1982; Daly *et al.*, 1995; Brodschneider and Crailsheim, 2010). Because the exact nutritional content of larval diets is manipulated at the colony level through the brood tending behaviour of nurse bees, larval nutrition is unknown in such studies. By using *in vitro* rearing methods, we were able to tightly control the macro-nutrient content of larval honeybee diets, demonstrating that the protein and carbohydrate content of the honeybee larval diet has a significant impact on larval development time, survival to adulthood, and adult body mass, size and condition. Using flow-through respirometry to measure whole-organism metabolism, we have shown for the first time that the protein and carbohydrate content of the larval diet of a holometabolous insect can impact the scaling relationship between adult body mass and RMR.

246   Larvae reared on a high carbohydrate diet had the highest survival to adulthood (D2, P:C 1:3),
247   significantly higher than bees in all other treatment groups. Nearly all bees reared on the low
248   carbohydrate diet failed to eclose (D3, P:C 1:1.5), and bees reared on the high protein diet (D4, P:C
249   1:1.9) also showed poor survival to adulthood. However, the absolute *amount* of protein and
250   carbohydrate consumed over the course of development appears to be more important for survival than
251   the ratio of macronutrients contained within the diet; though the low protein diet (D5, P:C 1:2.9) had a
252   similar ratio of protein to carbohydrate as the high carbohydrate diet (D2), survival was significantly
253   worse. Helm *et al.* (2017) also observed the highest survival in bees reared on a medium protein and
254   high carbohydrate diet, and poor survival for bees reared on high protein diets, though survival was
255   only recorded to the pupal stage. They concluded that there was an interaction between protein and
256   carbohydrate on larval development, fitting with the idea of both the ratio and absolute amounts of
257   protein and carbohydrate being important. Across all treatment groups, we observed most deaths
258   occurring between pupation and adult eclosion, emphasising the importance of assessing survival to the
259   adulthood. Pupation is a highly metabolically active period (Roeder and Behmer, 2014), suggesting that
260   the impact of diet quality on the nutrient reserves available during this period may be the key to survival
261   to adulthood.

262   The impact of high levels of dietary protein, both the absolute amount and relative content,
263   upon survival has been demonstrated for bees as well as many other organisms (Lee *et al.*, 2008;
264   Dussutour and Simpson, 2009, 2012; Cook and Behmer, 2010; Pirk *et al.*, 2010; Le Couteur *et al.*, 2015;
265   Solon-Biet *et al.*, 2015). For example, the survival to adulthood, larval development, and size of solitary
266   Megachilid bees is best on a high carbohydrate diet (Austin and Gilbert, 2018). The absolute quantity
267   rather than the ratio of dietary macronutrients has also been shown to impact survival in soldier flies
268   (Barragan-Fonseca *et al.*, 2019). However, the mechanism underpinning the deleterious effect of
269   consuming large volumes of protein on lifespan is poorly understood. It may be that digestion of large
270   amounts of protein is very energetically costly (Westerterp, Wilson and Rolland, 1999; Halton and Hu,
271   2004), and produces toxic levels of nitrogen waste (Wright, 1995), though a recent study in adult ants
272   has shown that even feeding free amino acids that require little digestion leads to a reduction in life
273   span, leading the authors to suggest that excess amino acids may lead to an over-stimulation of the
274   nutrient pathways that regulate lifespan (Arganda *et al.*, 2017). Certain amino acids are seemingly more
275   toxic than others, notably methionine, serine, threonine and phenylalanine, suggesting that the precise
276   amino acid composition of an organisms' diet may be important for survival and longevity.

277   Bees reared on the high carbohydrate diet, the best for survival, also took significantly longer
278   to emerge as adults compared to bees in all other diet groups. This contrasts with previous studies in
279   insects which have typically observed slower development on *lower* quality diets (Johnson, Wofford
280   and Whitehand, 1992; Angell *et al.*, 2020). For example, Rodrigues *et al.* (2015) found that when
281   *Drosophila* larvae are given the choice between a diet that maximises survival, body size and fecundity,
282   *versus* a diet that maximises development rate, they preferentially feed on the latter, potentially as a

283   strategy to both avoid predation and maximise mating opportunities. Honeybees workers, in contrast,

284   have a rather unique life history, developing inside a well-defended colony with much less risk of

285   predation compared to other insects. Workers do not need to mate and reproduce, and there is little

286   competition between individuals for resources which are provided for them by foragers and nurses.

287   These factors may therefore reduce the pressure on honeybee larvae to develop quickly, particularly

288   considering that rapid development is often thought to lead to earlier or faster senescence (Monaghan,

289   Metcalfe and Torres, 2009). It remains to be determined how dietary macronutrients affect the

290   development rates of the reproductive castes, queens and drones, that may face different pressures on

291   developmental speed.

292   Diet also had a significant effect on emerging adult bees' body mass, size and condition, which

293   fits with previous studies linking the quality of pollen and nectar in larval diets to emergent adult bee

294   size (Roulston and Cane, 2002; Burkle and Irwin, 2009). To our knowledge, however, this is the first

295   study to demonstrate experimentally that the specific macro-nutrient composition of the larval diet

296   affects body mass, size and condition in worker honeybees, which are typically considered to exhibit

297   limited variation in body size compared to other bee species such as bumblebees (Goulson *et al.*, 2002)

298   or solitary bees. Perhaps unsurprisingly, bees reared on the worst diet for survival, the high protein diet,

299   were the smallest and lightest on emergence. However, bees reared on this poor diet also had the

300   narrowest range of body sizes, while those reared on the high carbohydrate diet had the best survival

301   rate and the widest variation in body mass, suggesting that diets that increase survival also allow for a

302   greater range of body sizes to emerge. Bees reared on the high carbohydrate diet had significantly lower

303   body condition scores than bees reared on the diet containing a moderate amount of protein and

304   carbohydrate. Diet-dependent variation in worker body size can have implications for both individual

305   and colony functioning. Kerr & Hebling (1964) found that worker weight can affect the age with which

306   worker honeybees make the transition from in-hive tasks to foraging, and in bumblebees and other bees,

307   body size has been shown to correlate positively with foraging range (Greenleaf *et al.*, 2007) and the

308   weight of pollen and nectar loads that can be collected and transported back to the nest (Ramalho,

309   Imperatriz-Fonseca and Giannini, 1998; Goulson *et al.*, 2002; Kerr, Crone and Williams, 2019). Smaller

310   bees have also been shown to be less effective at pollinating flowers (Jauker *et al.*, 2012; Willmer and

311   Finlayson, 2014). Thus, consuming inadequate amounts of macronutrients during development leads to

312   both lower survival and body mass in adult worker bees, with potential consequences for the age

313   structure and foraging efficiency of the colony, as well as wider ecological implications for the delivery

314   of pollination.

315   Studies examining the impact of developmental diet on adult metabolism and metabolic scaling

316   are rare, particularly in insects, and it is generally not agreed whether poor quality diets should lead to

317   an increase or decrease in the RMR of emerging adults, given that this is likely to depend on the specific

318   behavioural and/or physiological response(s) of an organism to an unbalanced diet (Burton *et al.*, 2011).

319   For example, organisms might be expected to reduce their metabolic rates in response to a low quality

320 diet to minimise energetic expenditure (McNab, 1986). However, physiological adaptations to

321 imbalanced diets, such as increasing gut length, may be metabolically costly (Yang and Joern, 1994).

322 The few studies that have examined the impact of manipulating diet nutritional quality have generally

323 found that nutritionally poor diets result in an elevation of the average RMR (14,33–35, but see 76).

324 Typically these studies have considered only the short term impacts of diet on metabolism, during either

325 adulthood or a single juvenile stage, and often do not report the impact of diet quality on the scaling of

326 RMR with body mass or size. Here we demonstrated that body mass scales positively with RMR across

327 all treatment groups, as is typical for insects (Niven and Scharlemann, 2005), and that larval diet has a

328 long-term impact on metabolic scaling in adult bees. However, there were substantial differences in the

329 slope of the scaling relationship between body mass and RMR depending on diet. Bees reared on the

330 high carbohydrate diet (D2) showed positive allometry, with larger bees exhibiting higher RMR, which

331 is very unusual [6-13]. In comparison, the RMR of bees reared on a diet containing a moderate amount

332 of protein and carbohydrate (D1) showed isometry, whilst that of bees reared on the low protein diet

333 (D5) exhibited a decelerating allometric relationship. Bees reared on the high carbohydrate diet (D2)

334 also exhibited an unusual increase in mass-specific RMR with body mass, while bees reared on all other

335 diets displayed a more typical decelerating or isometric relationship between mass-specific RMR and

336 body mass. Neither body size (intertegular span) nor body condition scaled with RMR. This discrepancy

337 with the finding for body mass is somewhat unexpected, given that we recorded RMR immediately

338 following emergence, before additional feeding could strongly influence the bees' mass. This is an

339 important finding given that body size is often used as a proxy for body mass but may in fact scale quite

340 differently with RMR. In contrast to our finding that diet quality affects the scaling of body mass and

341 RMR, Karowe & Martin (1989) observed that while consumption of lower quality diets by larvae of

342 the moth *Spodoptera eridania* led to an elevated RMR, the slopes of the positive scaling relationships

343 between RMR and body mass were unaffected by diet treatment. However, in this study only protein

344 quality was manipulated. Also metabolic rates were measured during the larval stage only, and in other

345 organisms scaling relationships have been shown to change during ontogeny and could therefore be

346 differentially affected by diet (Killen *et al.*, 2007; Frappell, 2008). Consuming algal diets with

347 unbalanced phosphorous:carbon ratios has been shown to change the scaling relationship between RMR

348 and body mass in *Daphnia*, though this finding was based on a pooled data set across four closely

349 related species (Jeyasingh, 2007). Therefore, our study is the first to demonstrate that the precise nature

350 of an allometric scaling relationship can be altered by developmental diet within a single invertebrate

351 species, significantly contributing to our understanding of the mechanistic basis of variation in the

352 allometry of RMR (Vaca and White, 2010).

353 The use of CO_2 production as a measure of RMR in the current study means that we were not

354 able to detect changes in respiratory quotient arising from potential shifts in the substrate used for

355 metabolism. For example, Clark *et al.* (2016) concluded that the lack of difference in RMR between

356 winged and flightless morphs of the cricket *Gryllus firmus,* was due to a shift in metabolic substrate

357   from carbohydrate to protein across diet treatments. Though we are unable to rule out this possibility,

358   if such a shift has also occurred in the adult bees measured in our study, given that we tested bees on

359   the day of emergence, prior to feeding, this would then suggest that substantial shifts in metabolic

360   substrate use can also occur in response to differences in larval nutrition.

361   Here we considered only differences in protein and carbohydrate content of the diets, given the

362   number of studies demonstrating that insect herbivores tightly control their intake of these two nutrients

363   (Behmer, 2009). Royal jelly also contains around 5% lipids, on average, which are increasingly being

364   recognised as an important component of larval nutrition, with bees appearing to regulate their intake

365   of fats at both the level of the colony and individual foragers (Vaudo *et al.*, 2016a; Vaudo *et al.*, 2016b;

366   Vaudo *et al.*, 2020). Therefore, variation in the lipid content of the larval diet may also have had an

367   impact on adult metabolism. Royal jelly also contains various micronutrients such as vitamins and

368   sterols, which are important for hormone production and cannot be synthesised by bees themselves

369   (Wright, Nicolson and Shafir, 2018). In one of the few other studies examining the impact of

370   developmental diet on adult insect metabolism, Hill *et al.* (2020) observed changes in average RMR in

371   stick insects reared from birth on leaves of three different plant species. Any effects on metabolic scaling

372   were not reported. The macro-nutrient content of leaves from the three plant species did not show much

373   variation, but the concentration and digestibility of micronutrients did. This suggests that in future

374   studies, additional nutritional components other than the macro-nutrients protein and carbohydrate

375   should also be considered in the context of dietary impacts on metabolism.

376

377   **Conclusions**

378   There is increasing evidence that habitat fragmentation and farming intensification are reducing

379   both the quantity and diversity of floral resources available for bees and other pollinators (Donkersley

380   *et al.*, 2017; Trinkl *et al.*, 2020), which is of considerable concern given the global importance of insect

381   pollination to ecosystem functioning and food security. Here we clearly demonstrate that the nutritional

382   quality of larval diets impacts the metabolic functioning of adult worker bees, with diets more optimal

383   for survival resulting in a higher metabolic rate per unit of body mass. As foraging bees already

384   experience extremely high metabolic demands, differences in the quality of larval nutrition could impact

385   the metabolic function which may negatively influence the foraging efficiency of workers.

386   Subsequently this could impact on the build-up of pollen and nectar stores available for brood rearing

387   and overwintering, with consequences for overall colony success.

388

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Author Contributions

EN conceived the study, designed the methods, performed statistical analysis and wrote the paper. MR collected data in the laboratory, performed statistical analysis and commented on the paper. JN conceived the study and commented on the paper.

Competing Interests

All authors declare no competing interests.

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References

Anderson, J. F. (1993) 'Respiratory energetics of two Florida harvestmen', *Comparative Biochemistry and Physiology -- Part A: Physiology*, 105(1), pp. 67–72. doi: 10.1016/0300-9629(93)90174-3.

Angell, C. S. *et al.* (2020) 'Development time mediates the effect of larval diet on ageing and mating success of male antler flies in the wild', *Proceedings of the Royal Society B*, 287(1938), p. 20201876. doi: 10.1098/rspb.2020.1876.

Arganda, S. *et al.* (2017) 'Parsing the life-shortening effects of dietary protein: Effects of individual amino acids', *Proceedings of the Royal Society B: Biological Sciences*, 284(1846), p. 20162052. doi: 10.1098/rspb.2016.2052.

Austin, A. J. and Gilbert, J. D. J. (2018) 'The geometry of dependence: Solitary bee larvae prioritize carbohydrate over protein in parentally provided pollen', *bioRxiv*, p. 397802. doi: 10.1101/397802.

Ayayee, P. A. *et al.* (2018) 'The role of gut microbiota in the regulation of standard metabolic rate in female *Periplaneta americana*', *PeerJ*, 6, p. e4717. doi: 10.7717/peerj.4717.

Ayayee, P. A. *et al.* (2020) 'Role of the gut microbiome in mediating standard metabolic rate after dietary shifts in the viviparous cockroach, *Diploptera punctata* ', *Journal of Experimental Biology*, 11. doi: 10.1242/jeb.218271.

Barragan-Fonseca, K. B. *et al.* (2019) 'Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly *Hermetia illucens*', *Physiological Entomology*, 44(2), pp. 148–159. doi: 10.1111/phen.12285.

Bartholomew, G. A., Lighton, J. R. B. and Feener, D. H. (1988) 'Energetics of trail running, load carriage, and emigration in the column-raiding army ant *Eciton hamatum*', *Physiological Zoology*, 61(1), pp. 57–68. doi: 10.1086/physzool.61.1.30163737.

Nicholls, Rossi & Niven 2020

422   Behmer, S. T. (2009) 'Insect herbivore nutrient regulation', *Annual Review of Entomology*, 54(1), pp.
423   165–187. doi: 10.1146/annurev.ento.54.110807.090537.

424   Brodschneider, R. and Crailsheim, K. (2010) 'Nutrition and health in honey bees', *Apidologie*, 41(3),
425   pp. 278–294.

426   Brown, J. H. *et al.* (2004) 'Toward a metabolic theory of ecology', *Ecology*, 85(7), pp. 1771–1789.
427   doi: 10.1890/03-9000.

428   Burkle, L. and Irwin, R. (2009) 'Nectar sugar limits larval growth of solitary bees (Hymenoptera:
429   Megachilidae)', *Environmental Entomology*, 38(4), pp. 1293–1300. doi: 10.1603/022.038.0441.

430   Burton, T *et al.* (2011) 'What causes intraspecific variation in resting metabolic rate and what are its
431   ecological consequences?', *Proceedings of the Royal Society B*, 278, pp. 3465–3473. doi:
432   10.1098/rspb.2011.1778.

433   Burton, T. *et al.* (2011) 'What causes intraspecific variation in resting metabolic rate and what are its
434   ecological consequences?', *Proceedings of the Royal Society B: Biological Sciences*, 278(1724), pp.
435   3465–3473. doi: 10.1098/rspb.2011.1778.

436   Cane, J. H. (1987) 'Estimation of bee size using intertegular span (Apoidea)', *Journal of the Kansas
437   Entomological Society*, 60, pp. 145–147.

438   Chown, S. L. *et al.* (2007) 'Scaling of insect metabolic rate is inconsistent with the nutrient supply
439   network model', *Functional Ecology*, 21(2), pp. 282–290. doi: 10.1111/j.1365-2435.2007.01245.x.

440   Clark, R. M., Zera, A. J. and Behmer, S. T. (2016) 'Metabolic rate is canalized in the face of variable
441   life history and nutritional environment', *Functional Ecology*, 30(6), pp. 922–931. doi: 10.1111/1365-
442   2435.12574.

443   Cook, S. C. and Behmer, S. T. (2010) 'Macronutrient regulation in the tropical terrestrial ant
444   *Ectatomma ruidum* (Formicidae): A field study in costa rica', *Biotropica*, 42(2), pp. 135–139. doi:
445   10.1111/j.1744-7429.2009.00616.x.

446   Corby-Harris, V. *et al.* (2018) 'Honey bee (*Apis mellifera*) nurses do not consume pollens based on
447   their nutritional quality', *PLoS ONE*, 13(1), p. e0191050. doi: 10.1371/journal.pone.0191050.

448   Le Couteur, D. G. *et al.* (2015) 'The influence of macronutrients on splanchnic and hepatic
449   lymphocytes in aging mice', *Journals of Gerontology - Series A Biological Sciences and Medical
450   Sciences*, 70(12), pp. 1499–1507. doi: 10.1093/gerona/glu196.

451   Daly, H. V. *et al.* (1995) 'Honey bee morphometrics: Linearity of variables with respect to body size
452   and classification tested with European worker bees reared by varying ratios of nurse bees', *Journal
453   of Apicultural Research*, 34(3), pp. 129–145. doi: 10.1080/00218839.1995.11100898.

Nicholls, Rossi & Niven 2020

454   Desai, M. and Hales, C. N. (1997) 'Role of fetal and infant growth in programming metabolism in

455   later life', *Biological Reviews*, 72(2), pp. 329–348. doi: 10.1111/j.1469-185X.1997.tb00016.x.

456   Donkersley, P. *et al.* (2017) 'Nutritional composition of honey bee food stores vary with floral

457   composition', *Oecologia*, 185(4), pp. 749–761. doi: 10.1007/s00442-017-3968-3.

458   Dussutour, A. and Simpson, S. J. (2009) 'Communal nutrition in ants', *Current Biology*, 19(9), pp.

459   740–744. doi: 10.1016/j.cub.2009.03.015.

460   Dussutour, A. and Simpson, S. J. (2012) 'Ant workers die young and colonies collapse when fed a

461   high-protein diet', *Proceedings of the Royal Society B: Biological Sciences*, 279(1737), pp. 2402–

462   2408. doi: 10.1098/rspb.2012.0051.

463   Eishchen, F. A., Rothenbuhler, W. C. and Kulinčević, J. M. (1982) 'Length of life and dry weight of

464   worker honeybees reared in colonies with different worker-larva ratios', *Journal of Apicultural*

465   *Research*, 21(1), pp. 19–25. doi: 10.1080/00218839.1982.11100511.

466   Felton, G. W. (1996) 'Nutritive quality of plant protein: Sources of variation and insect herbivore

467   responses', *Archives of Insect Biochemistry and Physiology*, 32(1), pp. 107–130. doi:

468   https://doi.org/10.1002/(SICI)1520-6327(1996)32:1<107::AID-ARCH7>3.0.CO;2-X.

469   Ferioli, F., Armaforte, E. and Caboni, M. F. (2014) 'Comparison of the lipid content, fatty acid profile

470   and sterol composition in local Italian and commercial royal jelly samples', *Journal of the American*

471   *Oil Chemists' Society*, 91(6), pp. 875–884. doi: 10.1007/s11746-014-2446-x.

472   Frappell, P. B. (2008) 'Ontogeny and allometry of metabolic rate and ventilation in the marsupial:

473   Matching supply and demand from ectothermy to endothermy', *Comparative Biochemistry and*

474   *Physiology - A Molecular and Integrative Physiology*, 150(2), pp. 181–188. doi:

475   10.1016/j.cbpa.2008.02.017.

476   Garcia-Amoedo, L. H. and De Almeida-Muradian, L. B. (2007) 'Physicochemical composition of

477   pure and adulterated royal jelly', *Quimica Nova*, 30(2), pp. 257–259. doi: 10.1590/S0100-

478   40422007000200002.

479   Gillooly, J. F. *et al.* (2001) 'Effects of size and temperature on metabolic rate', *Science*, 293(5538),

480   pp. 2248–2251. doi: 10.1126/science.1061967.

481   Glazier, D. S. (2005) 'Beyond the "3/4-power law": Variation in the intra- and interspecific scaling of

482   metabolic rate in animals', *Biological Reviews*, 80(4), pp. 611–662. doi:

483   10.1017/S1464793105006834.

484   Goulson, D. *et al.* (2002) 'Can alloethism in workers of the bumblebee, *Bombus terrestris*, be

485   explained in terms of foraging efficiency?', *Animal Behaviour*, 64(1), pp. 123–130. doi:

15

486   10.1006/anbe.2002.3041.

487   Greenleaf, S. S. *et al.* (2007) 'Bee foraging ranges and their relationship to body size', *Oecologia*,

488   153(3), pp. 589–596. doi: 10.1007/s00442-007-0752-9.

489   Hales, C. N. and Barker, D. J. P. (2001) 'The thrifty phenotype hypothesis: Type 2 diabetes', *British*

490   *Medical Bulletin*, 60(1), pp. 5–20. doi: 10.1093/bmb/60.1.5.

491   Halton, T. L. and Hu, F. B. (2004) 'The effects of high protein diets on thermogenesis, satiety and

492   weight loss: A critical review', *Journal of the American College of Nutrition*, 23(5), pp. 373–385. doi:

493   10.1080/07315724.2004.10719381.

494   Helm, B. R. *et al.* (2017) 'The geometric framework for nutrition reveals interactions between protein

495   and carbohydrate during larval growth in honey bees', *Biology Open*, 6(6), pp. 872–880. doi:

496   10.1242/bio.022582.

497   Hill, S. J., Silcocks, S. C. and Andrew, N. R. (2020) 'Impacts of temperature on metabolic rates of

498   adult *Extatosoma tiaratum* reared on different host plant species', *Physiological Entomology*, 45(1),

499   pp. 7–15. doi: 10.1111/phen.12310.

500   Howe, S. R. *et al.* (1985) 'Composition of freshly harvested and commercial royal ielly', *Journal of*

501   *Apicultural Research*, 24(1), pp. 52–61. doi: 10.1080/00218839.1985.11100649.

502   Jauker, F. *et al.* (2012) 'Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia*

503   *rufa* outbalance post-flowering disadvantages', *Basic and Applied Ecology*, 13, pp. 268–276. doi:

504   10.1016/j.baae.2012.03.010.

505   Jeyasingh, P. D. (2007) 'Plasticity in metabolic allometry: The role of dietary stoichiometry', *Ecology*

506   *Letters*, 10(4), pp. 282–289. doi: 10.1111/j.1461-0248.2007.01023.x.

507   Joern, A., Provin, T. and Behmer, S. T. (2012) 'Not just the usual suspects: Insect herbivore

508   populations and communities are associated with multiple plant nutrients', *Ecology*, 93(5), pp. 1002–

509   1015. doi: 10.1890/11-1142.1.

510   Johnson, J. A., Wofford, P. L. and Whitehand, L. C. (1992) 'Effect of diet and temperature on

511   development rates, survival, and reproduction of the Indian meal moth (Lepidoptera: Pyralidae)',

512   *Journal of Economic Entomology*, 85(2), pp. 561–566. doi: 10.1093/jee/85.2.561.

513   Karasov, W. H., Martínez Del Rio, C. and Caviedes-Vidal, E. (2011) 'Ecological physiology of diet

514   and digestive systems', *Annual Review of Physiology*, 73, pp. 69–93. doi: 10.1146/annurev-physiol-

515   012110-142152.

516   Karowe, D. N. and Martin, M. M. (1989) 'The effects of quantity and quality of diet nitrogen on the

517   growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar *Spodoptera*

518   *eridania* larvae (Lepidoptera: Noctuidae)', *Journal of Insect Physiology*, 35(9), pp. 699–708. doi:
519   10.1016/0022-1910(89)90089-9.

520   Kerr, N. Z., Crone, E. E. and Williams, N. M. (2019) 'Integrating vital rates explains optimal worker
521   size for resource return by bumblebee workers', *Functional Ecology*, 33(3), pp. 467–478. doi:
522   10.1111/1365-2435.13251.

523   Kerr, W. E. and Hebling, N. J. (1964) 'Influence of the weight of worker bees on division of labor',
524   *Evolution*, 1, pp. 267–270. doi: 10.2307/2406400.

525   Killen, S. S. *et al.* (2007) 'Little left in the tank: Metabolic scaling in marine teleosts and its
526   implications for aerobic scope', *Proceedings of the Royal Society B: Biological Sciences*, 274(1608),
527   pp. 431–438. doi: 10.1098/rspb.2006.3741.

528   Lee, K. P. *et al.* (2008) 'Lifespan and reproduction in *Drosophila*: New insights from nutritional
529   geometry', *Proceedings of the National Academy of Sciences*, 105(7), pp. 2498–2503. doi:
530   10.1073/pnas.0710787105.

531   McCarthy, I. D. (2000) 'Temporal repeatability of relative standard metabolic rate in juvenile Atlantic
532   salmon and its relation to life history variation', *Journal of Fish Biology*, 57(1), pp. 224–238. doi:
533   10.1006/jfbi.2000.1313.

534   McNab, B. K. (1986) 'The influence of food habits on the energetics of eutherian mammals',
535   *Ecological Monographs*, 56(1), pp. 1–19. doi: 10.2307/2937268.

536   McNab, B. K. (1988) 'Complications inherent in scaling the basal rate of metabolism in mammals',
537   *Quarterly Review of Biology*, 63(1), pp. 25–54. doi: 10.1086/415715.

538   Moe, B. *et al.* (2004) 'Developmental plasticity of physiology and morphology in diet-restricted
539   European shag nestlings (*Phalacrocorax aristotelis*)', *Journal of Experimental Biology*, 207(23), pp.
540   4067–4076. doi: 10.1242/jeb.01226.

541   Monaghan, P., Metcalfe, N. B. and Torres, R. (2009) 'Oxidative stress as a mediator of life history
542   trade-offs: Mechanisms, measurements and interpretation', *Ecology Letters*, 12(1), pp. 75–92. doi:
543   10.1111/j.1461-0248.2008.01258.x.

544   Morgan, K. R., Shelly, T. E. and Kimsey, L. S. (1985) 'Body temperature regulation, energy
545   metabolism, and foraging in light-seeking and shade-seeking robber flies', *Journal of Comparative
546   Physiology B*, 155(5), pp. 561–570. doi: 10.1007/BF00694445.

547   Naug, D. (2009) 'Nutritional stress due to habitat loss may explain recent honeybee colony collapses',
548   *Biological Conservation*, 142(10), pp. 2369–2372. doi: 10.1016/j.biocon.2009.04.007.

549   Naya, D. E., Lardies, M. A. and Bozinovic, F. (2007) 'The effect of diet quality on physiological and

550   life-history traits in the harvestman *Pachylus paessleri*', *Journal of Insect Physiology*, 53(2), pp. 132–
551   138. doi: 10.1016/j.jinsphys.2006.11.004.

552   Nespolo, R. F., Castañeda, L. E. and Roff, D. A. (2005) 'The effect of fasting on activity and resting
553   metabolism in the sand cricket, *Gryllus firmus*: A multivariate approach', *Journal of Insect*
554   *Physiology*, 51(1), pp. 61–66. doi: 10.1016/j.jinsphys.2004.11.005.

555   Nicholls, E. *et al.* (2017) 'Larval exposure to field-realistic concentrations of clothianidin has no
556   effect on development rate, over-winter survival or adult metabolic rate in a solitary bee, Osmia
557   bicornis', *PeerJ*. doi: 10.7717/peerj.3417.

558   Niven, J. E. and Scharlemann, J. P. W. (2005) 'Do insect metabolic rates at rest and during flight scale
559   with body mass?', *Biology Letters*, 1(3), pp. 346–349. doi: 10.1098/rsbl.2005.0311.

560   Nussear, K. E. *et al.* (1998) 'Diet quality does not affect resting metabolic rate or body temperatures
561   selected by an herbivorous lizard', *Journal of Comparative Physiology - B Biochemical, Systemic,*
562   *and Environmental Physiology*, 168(3), pp. 183–189. doi: 10.1007/s003600050135.

563   Perl, C. D. and Niven, J. E. (2018) 'Metabolic rate scaling, ventilation patterns and respiratory water
564   loss in red wood ants: Activity drives ventilation changes, metabolic rate drives water loss', *Journal*
565   *of Experimental Biology*, 221, p. jeb182501. doi: 10.1242/jeb.182501.

566   Pettersen, A. K., Marshall, D. J. and White, C. R. (2018) 'Understanding variation in metabolic rate',
567   *Journal of Experimental Biology*, 221, p. jeb166876. doi: 10.1242/jeb.166876.

568   Pirk, C. W. W. *et al.* (2010) 'The importance of protein type and protein to carbohydrate ratio for
569   survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*)', *Apidologie*, 41(1), pp.
570   62–72. doi: 10.1051/apido/2009055.

571   Ramalho, M., Imperatriz-Fonseca, V. L. and Giannini, T. C. (1998) 'Within-colony size variation of
572   foragers and pollen load capacity in the stingless bee *Melipona quadrifasciata anthidioides* Lepeletier
573   (Apidae, Hymenoptera)', *Apidologie*, 29(3), pp. 221–228. doi: 10.1051/apido:19980302.

574   Roark, A. M. and Bjorndal, K. A. (2009) 'Metabolic rate depression is induced by caloric restriction
575   and correlates with rate of development and lifespan in a parthenogenetic insect', *Experimental*
576   *Gerontology*, 44(6), pp. 413–419. doi: 10.1016/j.exger.2009.03.004.

577   Roces, F. and Lighton, J. R. B. (1995) 'Larger bites of leaf-cutting ants', *Nature*, 373(6513), pp. 392–
578   392. doi: 10.1038/373392a0.

579   Rodrigues, M. A. *et al.* (2015) '*Drosophila melanogaster* larvae make nutritional choices that
580   minimize developmental time', *Journal of Insect Physiology*, 81, pp. 69–80. doi:
581   10.1016/j.jinsphys.2015.07.002.

582   Roeder, K. A. and Behmer, S. T. (2014) 'Lifetime consequences of food protein-carbohydrate content
583   for an insect herbivore', *Functional Ecology*, 28(5), pp. 1135–1143. doi: 10.1111/1365-2435.12262.

584   Roulston, T. H. and Cane, J. H. (2002) 'The effect of pollen protein concentration on body size in the
585   sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes)', *Evolutionary Ecology*, 16(1), pp.
586   49–65. doi: 10.1023/A:1016048526475.

587   Savage, V. M. *et al.* (2004) 'The predominance of quarter-power scaling in biology', *Functional*
588   *Ecology*, 18(2), pp. 257–282. doi: 10.1111/j.0269-8463.2004.00856.x.

589   Schmehl, D. R. *et al.* (2016) 'Protocol for the *in vitro* rearing of honey bee (*Apis mellifera* L.)
590   workers', *Journal of Apicultural Research*, 55(2), pp. 113–129. doi:
591   10.1080/00218839.2016.1203530.

592   Simpson, S. J. and Raubenheimer, D. (2012) *The nature of nutrition: A unifying framework from*
593   *animal adaptation to human obesity*, *The Nature of Nutrition: A Unifying Framework from Animal*
594   *Adaptation to Human Obesity*. Princeton University Press. doi: 10.5860/choice.50-2662.

595   Solon-Biet, S. M. *et al.* (2015) 'Macronutrient balance, reproductive function, and lifespan in aging
596   mice', *Proceedings of the National Academy of Sciences*, 112(11), pp. 3481–3486. doi:
597   10.1073/pnas.1422041112.

598   Speakman, J. R. (2005) 'Body size, energy metabolism and lifespan', *Journal of Experimental*
599   *Biology*, 208(9), pp. 1717–1730. doi: 10.1242/jeb.01556.

600   Trinkl, M. *et al.* (2020) 'Floral species richness correlates with changes in the nutritional quality of
601   larval diets in a stingless bee', *Insects*, 11(2), p. 125. doi: 10.3390/insects11020125.

602   Vaca, H. F. and White, C. R. (2010) 'Environmental modulation of metabolic allometry in ornate
603   rainbowfish Rhadinocentrus ornatus', *Biology Letters*, 6(1), pp. 136–138. doi:
604   10.1098/rsbl.2009.0610.

605   Vaudo, A. D. *et al.* (2016) 'Bumble bees regulate their intake of essential protein and lipid pollen
606   macronutrients', *Journal of Experimental Biology*, 219(24), pp. 3962–3970. doi: 10.1242/jeb.140772.

607   Vaudo, Anthony D. *et al.* (2016) 'Macronutrient ratios in pollen shape bumble bee (*Bombus*
608   *impatiens*) foraging strategies and floral preferences', *Proceedings of the National Academy of*
609   *Sciences*, 113(28), pp. e40435–e4042. doi: 10.1073/pnas.1606101113.

610   Vaudo, A. D. *et al.* (2020) 'Pollen protein: Lipid macronutrient ratios may guide broad patterns of bee
611   species floral preferences', *Insects*, 11(2), p. 132. doi: 10.3390/insects11020132.

612   Wang, Y. *et al.* (2016) 'Starvation stress during larval development facilitates an adaptive response in
613   adult worker honey bees (*Apis mellifera* L.)', *Journal of Experimental Biology*, 219(7), pp. 949–959.

Nicholls, Rossi & Niven 2020

614   doi: 10.1242/jeb.130435.

615   Westerterp, K. R., Wilson, S. A. J. and Rolland, V. (1999) 'Diet induced thermogenesis measured

616   over 24 h in a respiration chamber: effect of diet composition', *International Journal of Obesity*,

617   23(3), pp. 287–292. doi: 10.1038/sj.ijo.0800810.

618   White, C. R. and Seymour, R. S. (2003) 'Mammalian basal metabolic rate is proportional to body

619   mass2/3', *Proceedings of the National Academy of Sciences*, 100(7), pp. 4046–4049. doi:

620   10.1073/pnas.0436428100.

621   Willmer, P. and Finlayson, K. (2014) 'Big bees do a better job: intraspecific size variation influences

622   pollination effectiveness', *Journal of Pollination Ecology*, 14, pp. 244–54. doi: 10.26786/1920-

623   7603(2014)22.

624   Wright, G. A., Nicolson, S. W. and Shafir, S. (2018) 'Nutritional physiology and ecology of honey

625   bees', *Annual Review of Entomology*, 63, pp. 327–344. doi: 10.1146/annurev-ento-020117-043423.

626   Wright, P. A. (1995) 'Nitrogen excretion: Three end products, many physiological roles', *Journal of

627   Experimental Biology*, 198(2), pp. 273–281.

628   Yang, Y. and Joern, A. (1994) 'Compensatory feeding in response to variable food quality by

629   *Melanoplus differentialis*', *Physiological Entomology*, 19(1), pp. 75–82. doi: 10.1111/j.1365-

630   3032.1994.tb01077.x.

631   Zanotto, F. P. *et al.* (1997) 'Nutritional homeostasis in locusts: Is there a mechanism for increased

632   energy expenditure during carbohydrate overfeeding?', *Journal of Experimental Biology*, 200(18), pp.

633   2437–2448.

634   Ziska, L. H. *et al.* (2016) 'Rising atmospheric CO2 is reducing the protein concentration of a floral

635   pollen source essential for north American bees', *Proceedings of the Royal Society B: Biological

636   Sciences*, 283(1828), p. 20160414. doi: 10.1098/rspb.2016.0414.

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Tables

diet	P	C	*n*	% diet component							P:C ratio
				yeast	royal jelly	glucose	fructose	water	total P	total C	
D1	med	med	78	1.0	51.0	4.1	8.2	35.7	8.2	18.5	1:2.3
D2	med	high	60	1.0	48.1	5.8	11.5	33.7	7.7	23.2	1:3.0
D3	med	low	78	1.1	54.3	2.2	4.3	38.0	8.7	13.1	1:1.5
D4	high	med	78	0.9	57.5	3.5	7.1	31.0	9.2	17.6	1:1.9
D5	low	med	77	1.2	42.2	4.8	9.6	42.2	6.8	19.5	1:2.9

Table 1| Composition of artificial diets fed to larvae in the P:C ratio diet manipulation experiment (P = Protein, C = Carbohydrate).

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diet	time to emergence (days)		body mass (mg)		body size (mm)		body condition (mg/mm)	
	estimate ± s.e.	*p*-value	estimate ± s.e.	*p*-value	estimate ± s.e.	*p*-value	estimate ± s.e.	*p*-value
D1 – D2	**-0.50 ± 0.13**	**<0.001**	0.91 ± 3.07	0.991	-0.00 ± 0.05	1.000	**3.40 ±1.14**	**0.021**
D4 – D2	**-0.63 ± 0.18**	**<0.001**	-9.23 ± 4.35	0.154	**-0.24 ± 0.06**	**0.002**	2.40 ±1.38	0.315
D5 – D2	**-0.36 ± 0.13**	**<0.025**	0.68 ± 4.22	0.996	-0.07 ± 0.05	0.500	2.70 ± 1.13	0.890
D4 – D1	-0.18 ± 0.17	0.714	-10.14 ± 3.07	0.083	**-0.24 ± 0.05**	**<0.001**	-1.00 ± 1.40	0.090
D5 – D1	0.14 ± 0.12	0.676	-0.23 ± 2.99	0.100	-0.07 ± 0.04	0.333	-0.70 ± 1.14	0.927
D5 – D4	0.32 ± 0.17	0.243	9.09 ± 4.17	0.088	**0.17 ± 0.05**	**0.008**	0.30 ± 1.38	0.996

Table 2| Least-square pairwise comparisons of the effect of diet treatment on the time to adult emergence, body mass on emergence, body size and body condition. Models applied were (days to emergence ~ diet + (1|grafting cohort)), (body mass ~ diet + (1|grafting cohort)), (body size ~ diet + (1|grafting cohort)) and (body condition ~ diet) respectively. *P*-values were adjusted using the Tukey method. (P:C ratio in diets: D1 = 1:2.3; D2 =1:3.0; D4 = 1:1.9; D5 = 1:2.9). The number of bees measured in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30. See Table S3 for the complete outcome of the models.

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diet	P:C	*n*	log slope ± s.e.	log intercept ± s.e.	regression equation
D1	1:2.3	28	0.397 ± 0.52	2.155 ± 2.33	$R^2 = 0.022$, $F_{1,26} = 0.585$, $p = 0.451$
D2	1:3.0	33	1.489 ± 0.28	-2.674 ± 1.22	$R^2 = 0.486$, $F_{1,31} = 29.27$, $p < 0.001$
D4	1:1.9	10	0.160 ± 0.94	3.168 ± 4.14	$R^2 = 0.003$, $F_{1,8} = 0.029$, $p = 0.870$
D5	1:2.9	30	1.016 ± 0.36	-0.636 ± 1.61	$R^2 = 0.223$, $F_{1,28} = 8.035$, $p = 0.008$

Table 3| Scaling relationship between body mass (mg) and RMR (μL CO2 h^{-1}) for adult bees reared on different larval diets. Slopes and intercepts ± s.e. calculated *via* least-squares regression.

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		estimate ± s.e.	d.f.	*t*-value	*p*-value	variance ± s.d.
RMR **(µL CO$_2$ h^{-1})**	**fixed effects**					
	Intercept (D2)	-1.21 ± 1.27	92.16	-0.95	0.343	
	D1	4.36 ± 2.10	92.05	2.08	**0.040**	
	D4	2.40 ± 4.63	92.46	0.52	0.610	
	D5	4.02 ± 2.33	92.58	1.73	0.088	
	(log)Body Mass	1.17 ± 0.28	92.86	4.09	**<0.001**	
	D1: (log)Body Mass	-1.00 ± 0.47	92.03	-2.14	**0.035**	
	D4: (log)Body Mass	-0.58 ± 1.05	92.43	-0.55	0.582	
	D5: (log)Body Mass	-0.93 ± 0.52	92.61	-1.79	0.077	
	random effects					
	grafting cohort					0.04 ± 0.20
	residual					0.08 ± 0.29
mass-specific RMR **(µL CO$_2$ mg^{-1} h^{-1})**	**fixed effects**					
	Intercept (D2)	-1.21 ± 1.27	92.16	-0.95	0.343	
	D1	4.36 ± 2.10	92.05	2.08	**0.040**	
	D4	2.40 ± 4.63	92.46	0.52	0.610	
	D5	4.02 ± 2.33	92.58	1.73	0.088	
	(log)Body Mass	0.17 ± 0.28	92.86	0.58	0.563	
	D1: (log)Body Mass	-1.00 ± 0.47	92.03	-2.14	**0.035**	
	D4: (log)Body Mass	-0.58 ± 1.05	92.43	-0.55	0.582	
	D5: (log)Body Mass	-0.93 ± 0.52	92.61	-1.79	0.077	
	random effects					
	grafting cohort					0.04 ± 0.20
	residual					0.08 ± 0.29

Table 4| Effect of larval diet and body mass (mg) on RMR, and diet and body mass on mass-specific RMR. Models used were (log(RMR) ~ diet * log(body mass) + (1|grafting cohort)) and (log(mass-specific RMR)) ~ diet * log(body mass) + (1|grafting cohort)) respectively. The number of bees tested in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30.

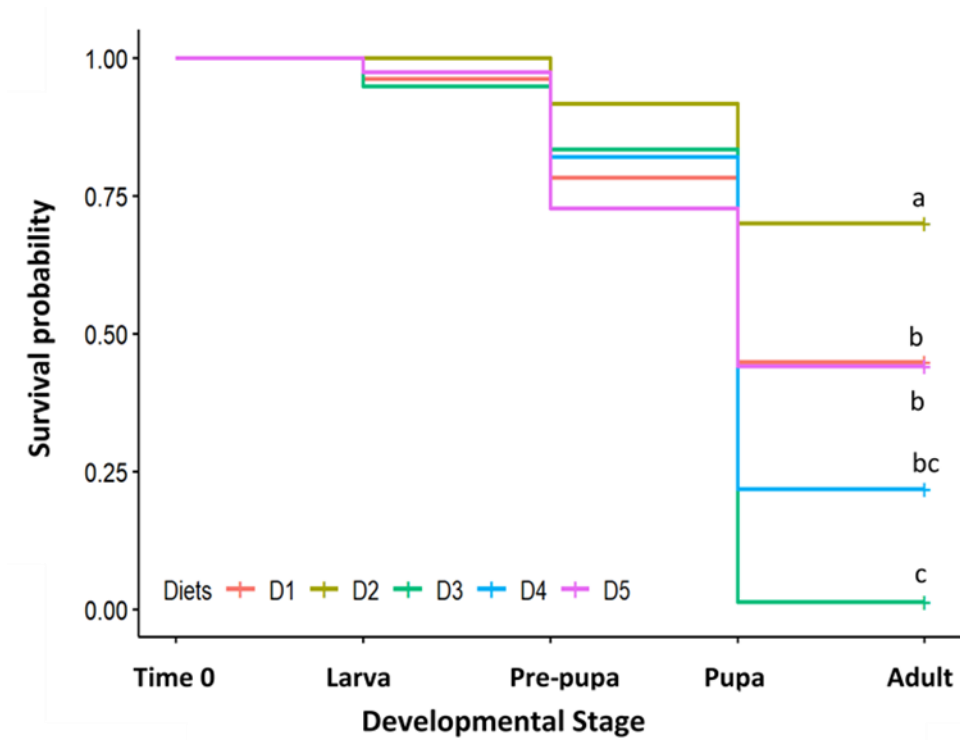Nicholls, Rossi & Niven 2020

Figures

Figure 1| Probability of survival of bees reared on different larval diets (P:C ratio in diets: D1 = 1:2.3; D2 =1:3.0; D3 = 1:1.5; D4 = 1:1.9; D5 = 1:2.9). The number of larvae in each treatment group at Time 0 is as follows: D1= 78; D2=60; D3=78; D4=78; D5=77. Crosses indicate the proportion of individuals in each diet treatment that reached adulthood (censored data). Letters indicate statistically significant differences in survival ($p < 0.05$, Kaplan-Meier analysis).
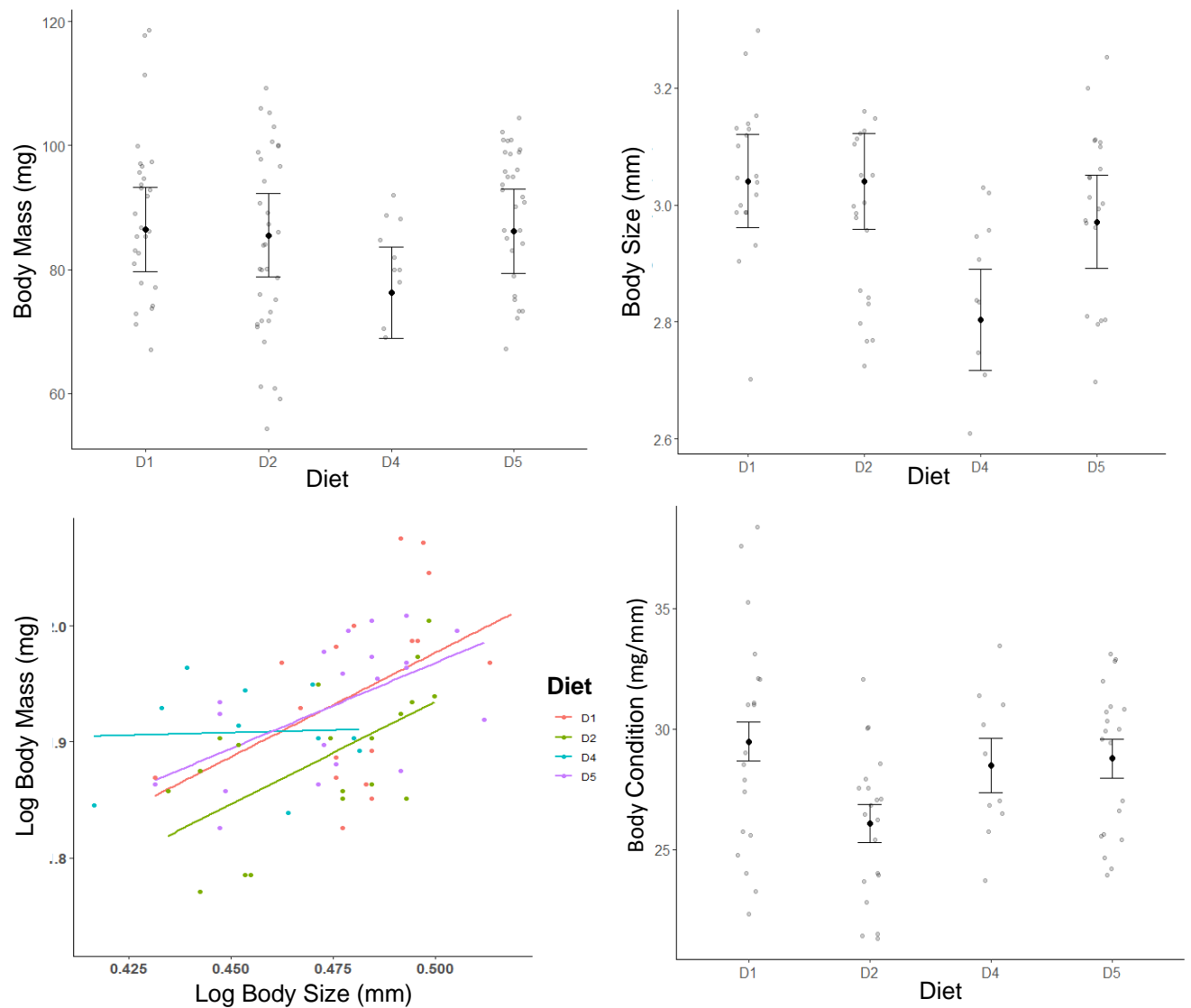
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Figure 2| Body mass (A) body size (B) the scaling of body mass and body size (C) and body condition (body mass/body size) (D) of adult bees in each diet treatment. Grey points are the individual data points, black points represent the estimated marginal mean and whiskers are the standard error of the mean. (P:C ratio in diets: D1= 1:2.3; D2 = 1:3.0; D4 = 1:1.9; D5 = 1:2.9). The number of bees measured in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30.
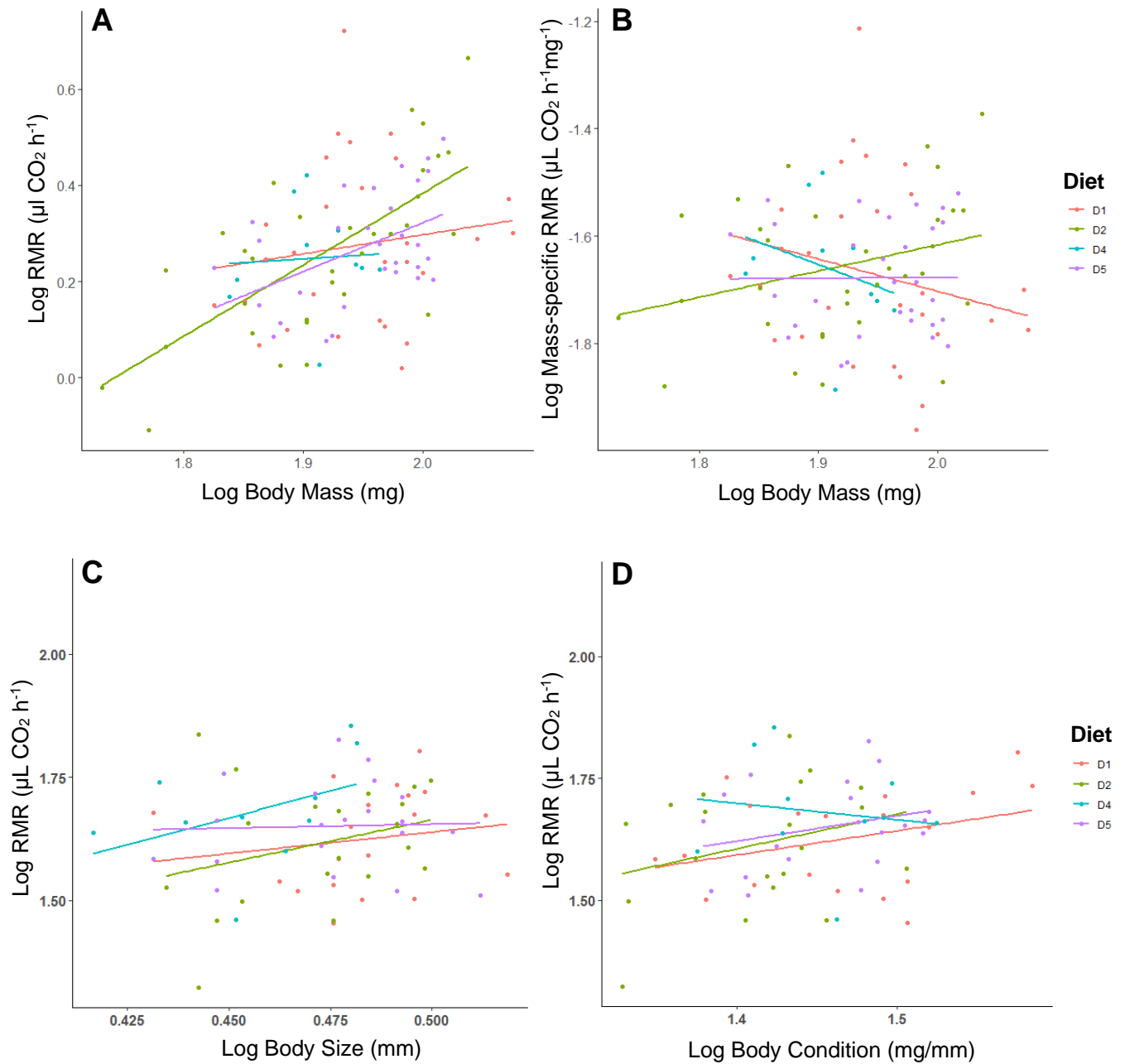
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Figure 3| Scaling between CO_2 production (RMR) and body mass (A), mass-specific RMR and body mass (B), RMR and body size (intertegular distance) (C) and RMR and body condition (body mass/body size) (D) for bees reared on larval diets differing in P:C ratio (D1 = 1:2.3; D2 =1:3.0; D3 = 1:1.5; D4 = 1:1.9; D5 = 1:2.9). The number of bees tested in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30.