© 2021. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

RESEARCH ARTICLE

Larval nutrition impacts survival to adulthood, body size and the allometric scaling of metabolic rate in adult honeybees

Elizabeth Nicholls*, Marta Rossi and Jeremy E. Niven

ABSTRACT

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

KEY WORDS: Diet, Protein, Development, Carbohydrate, Metabolic scaling, *Apis mellifera*

INTRODUCTION

The resting metabolic rate (RMR) of an organism can account for up to 50% of total energetic expenditure (Morgan et al., 1985) and is intrinsically linked to numerous aspects of physiological and behavioural functioning, from reproductive output to lifespan (Pettersen et al., 2018; Speakman, 2005). 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 et al., 1988; Brown et al., 2004; Chown et al., 2007; Gillooly et al., 2001; Glazier, 2005). Though the mechanism(s) underpinning allometric scaling of RMR remains highly debated (McNab, 1988; Savage et al., 2004; White and Seymour, 2003), 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 organism's diet will have considerable impact on the resources available for energetic expenditure, but the means by which diet affects RMR and the scaling of RMR remain 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 (Nespolo et al., 2005; Roces and Lighton, 1995). 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 et al., 2012). Yet, direct tests of the impact of the nutritional composition of developmental diets (i.e. diet quality) on adult metabolism are relatively rare outside of epidemiological studies.

Making *a priori* directional predictions about how the nutritional composition 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 nutritional 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 et al., 2011; Roeder and Behmer, 2014; Simpson and Raubenheimer, 2012). Optimal intake targets can be achieved through behaviours such as selective or compensatory feeding, or physiological/morphological means such as increasing gut length or food retention time (Felton, 1996; Behmer, 2009; Burton et al., 2011). Though insects have long been used as models to study the regulation of nutritional intake targets (Behmer, 2009), studies of the long-term impact of variation in nutrition over the course of development are somewhat lacking (Roeder and Behmer, 2014), and studies of the subsequent effects on adult metabolism are largely non-existent. A recent study found that adult stick insects exhibit developmental diet-dependent differences in RMR when

School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK.

*Author for correspondence (e.nicholls@sussex.ac.uk)

E.N., 0000-0002-3697-0223; J.E.N., 0000-0001-7786-5254

Received 5 February 2021; Accepted 14 June 2021

Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

reared from birth on leaves from plant species varying in their nutritional content and digestibility (Hill et al., 2020), but the impact of developmental diet on the scaling of RMR and body mass was not considered. Shorter term studies conducted in adult insects only are more common, and have typically observed a reduction in RMR in response to a nutritionally poor diet (Zanotto et al., 1997; Ayayee et al., 2018, 2020; but see Clark et al., 2016).

Bees meet all their nutritional demands via pollen and nectar collected from flowers (their main source of protein and carbohydrate, respectively), and unlike the nymphs and larvae of traditional models of insect nutrient regulation, such as locusts and caterpillars, bee larvae are entirely dependent on the provisioning choices of adult bees. This means bee larvae probably have very little opportunity to selectively regulate their intake of nutrients (but see Austin and Gilbert, 2021). Honeybees are unique among bees in that in-hive nurse bees process the pollen and nectar brought back to the nest by foragers, and convert it to a nutritional substance known as royal jelly, which they then regurgitate for larvae (Wright et al., 2018). Containing approximately 60% water, 15% protein, 20% carbohydrate and 5% fat, the exact macronutrient content of royal jelly can vary between colonies and over time (Ferioli et al., 2014; Garcia-Amoedo and De Almeida-Muradian, 2007; Howe et al., 1985). Furthermore, a recent study has demonstrated that nurse honeybees are unable to discriminate between pollen diets on the basis of nutritional quality (protein and/or lipid content) (Corby-Harris et al., 2018), meaning the proportion of macronutrients that individual larvae receive in their diet could vary, particularly in times or areas where the diversity of forage is limited (Donkersley et al., 2017). In addition, there is recent evidence to suggest that rising CO_2 levels associated with climate change are negatively affecting the nutritional composition of pollen and nectar provided by plants (Ziska et al., 2016). Given widespread concerns regarding the combined effects of habitat degradation and agricultural intensification on the availability of sufficiently diverse floral resources to meet the nutritional needs of adult bees and their offspring (Brodschneider and Crailsheim, 2010; Donkersley et al., 2017; Naug, 2009), and the fact that bees provide a pollination service vital to global food security, the question of how developmental diets impact on the metabolic function of adult bees is extremely apposite.

Here, we used *in vitro* rearing methods to tightly control honeybee larval diets independent of nurse bee behaviour, permitting an examination of the impact of diet nutritional composition on honeybee development and adult physiological function. Previous studies have shown that the ratio of protein to carbohydrate in honeybee larval diets can have significant impacts on larval survival (Helm et al., 2017), with unbalanced diets heavily skewed to either macronutrient resulting in poor growth and survival. To our knowledge, this is the first study to test the RMR of adult bees reared on different larval diets *in vitro*. By manipulating the ratio of royal jelly (protein) to sugars (carbohydrates), we aimed to determine the impact of specific macro-nutrients on adult RMR and scaling with body size.

MATERIALS AND METHODS

Honeybee (*Apis mellifera* L.) larvae were obtained from full-sized colonies housed on the University of Sussex campus, and reared in the laboratory using the *in vitro* method described by Schmehl et al. (2016). Briefly, 3 day old larvae were removed from the comb using a grafting tool, transferred to individual wells of a 48-well cell culture plate, and placed into an incubator fixed at 35°C, 94% relative humidity. Larvae were fed once per day for 5 days, and upon pupation transferred to a fresh cell culture plate. Survival was monitored daily until adult emergence.

Diet manipulation

A standard *in vitro* rearing diet (Table 1) of yeast (Sigma-Aldrich), royal jelly (The Raw Honey Shop, Brighton, UK) and sugars (glucose and fructose, Sigma-Aldrich) was manipulated to contain differing amounts of protein (by altering the amount of royal jelly) and/or carbohydrate (glucose and fructose), following the methods of Helm et al. (2017). Larvae were reared on one of five diets (Table 1, D1–5), where the amount of protein and carbohydrate was either increased or decreased relative to the diet described by Schmehl et al. (2016). Royal jelly was stored frozen at −20°C in 50 ml aliquots. Diets were freshly made every 2 days and stored at 4°C. Larvae were fed once per day for 5 days, and the volume of food varied according to the day of the experiment (days 1 and 2, 10 µl; day 3, 20 µl; day 4, 30 µl; day 5, 40 µl; and day 6, 50 µl). Between 60 and 78 larvae were assigned to each treatment group (N=371 larvae in total; D1 N=78; D2 N=60; D3 N=78, D4 N=78; D5 N=77). Bees were reared in two cohorts, grafted on 30 September 2019 and 20 October 2019. Royal jelly nutritional values (Table 1) were obtained by the supplier (The Raw Honey Shop) using the international standard for royal jelly (ISO 12824:2016). From these values we calculated the proportion of protein (P), carbohydrate (C) and lipids [based on the amount of 10-hydroxy-2decenoic acid (10-HDA), a major fatty acid found in royal jelly] and the ratio of protein:carbohydrate (P:C) in each of the five diets (Table 1).

Measuring RMR

To determine how larval diet affects adult metabolism, the RMR of adult bees was measured on the day of emergence (between 14 and 17 days from the day of grafting) using flow-through respirometry, with CO_2 production used as a measure of metabolic rate. Emerging adults were first individually weighed to the nearest milligram using a precision balance (Mettler Toledo). Bees were then restrained using a small cylinder of metal mesh to allow gas exchange, before being placed into a 2 ml plastic chamber. Air scrubbed of CO_2 and H_2O was then pumped through the chamber at a consistent rate of 100 ml min^{-1} via a mass flow controller (GFC17, Aalborg, Orangeburg, NY, USA), before passing through an infrared CO_2–

Table 1. Nutritional composition of diets fed to honeybee larvae

						% Diet component						
Diet	P	C	*N*	Yeast	Royal jelly	Glucose	Fructose	Water	Total P	Total C	Total L	P:C ratio
D1	med	med	78	1.0	51.0	4.1	8.2	35.7	8.2	18.5	0.8	1:2.3
D2	med	high	60	1.0	48.1	5.8	11.5	33.7	7.7	23.2	0.8	1:3.0
D3	med	low	78	1.1	54.3	2.2	4.3	38.0	8.7	13.1	0.8	1:1.5
D4	high	med	78	0.9	57.5	3.5	7.1	31.0	9.2	17.6	0.9	1:1.9
D5	low	med	77	1.2	42.2	4.8	9.6	42.2	6.8	19.5	0.7	1:2.9

P, protein; C, carbohydrate; L, lipid (10-hydroxy-2-decenoic acid); med, medium.

Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

H_2O analyser (Li7000, Li-Cor) which captured data on CO_2 production, relative to an empty control chamber (Nicholls et al., 2017; Perl and Niven, 2018). The temperature in the room was held constant at 25±2°C and recordings lasted for 20 min per bee. The first 5 min of the recording were treated as a settling period for the bee to adjust to the experimental set up and were excluded from analysis. During recording, the plastic chamber was covered to ensure it was dark, which reduced bee movement. The order in which bees from different diet treatment groups were measured was randomised. After recording, bees were frozen to immobilise them, and digital callipers were used to measure the intertegular span (defined as the distance between the points at which the wings attach to the thorax) in millimetres, a proxy measure for body size (Cane, 1987).

Data analysis

Respirometry data were analysed using OriginPro software (Origin 2016, OriginLab Corporation, Northampton, MA, USA). Volumes of CO_2 were baseline corrected and temperature was normalised using the Q_{10} correction for temperature differences. To calculate the rate of CO_2 production per bee, the volume of CO_2 (ppm) was converted to CO_2 fraction and multiplied by the flow rate (100 ml min^{-1}). The integral of CO_2 min^{-1} versus time (min) was calculated for a stable 15 min period of the recording, and divided by this time to give a rate of µl CO_2 h^{-1}.

All statistical analyses were conducted in R 3.6.2 (https://www.R-project.org). To examine how diet quality impacts larval survival, Kaplan–Meier survival analysis was performed using the survfit function from the 'survival' package. The log-rank test was used to test for differences in survival between diet treatments with a Bonferroni correction for multiple comparisons. Linear and mixed effect models were performed by restricted maximum likelihood (REML) estimation using the lmer and glmer function from the 'lme4' package to test the impact of diet treatment on the time to adult emergence (days), wet body mass (mg), body size (using intertegular distance as a proxy measure; mm), body condition (body mass/body size; mg mm^{-1}) and CO_2 production (µl CO_2 h^{-1}). The continuous variables body mass, body size, body condition and CO_2 production were log transformed. Date of grafting was included as a random effect. For all models, D2 was used as the reference category because bees in this treatment had the best survival. Significance of the fixed effects was determined using Satterthwaite's method for estimation of degrees of freedom by using the anova function from the 'lmerTest' package. Estimated marginal means (emm) and pairwise comparisons were obtained using the 'lsmeans' package and the *P*-value adjusted with the Tukey method. To test for differences in variance, we used the Brown–Forsythe test for non-normal data. All plots were made using the 'ggplot2' package.

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; Tables S1 and S2; Kaplan–Meier log-rank test, χ^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–D1 *P*=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 (Tables S1 and S2), 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; χ^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 days, D4 15.3±0.97 days, D5 15.7±0.96 days).

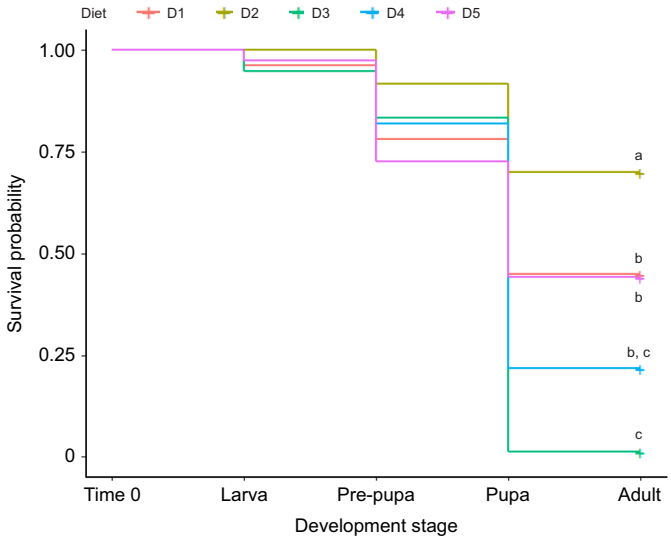

Fig. 1. Nutritional composition of larval diets affects survival to adulthood in honeybees. Dietary content can be found in Table 1. Crosses indicate the proportion of individuals in each diet treatment that reached adulthood (censored data). Differing letters indicate statistically significant differences in survival between diet treatments (*P*<0.05, Kaplan–Meier analysis). The number of larvae in each treatment group at time 0 is as follows: D1 *N*=78, D2 *N*=60, D3 *N*=78, D4 *N*=78, D5 *N*=77.

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, P:C 1:1.9), 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 (P:C Table 2; Table S3; estimate±s.e. −9.23±4.32 mg, d.f.=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 between bees reared on 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 mm, D2 3.04±0.08 mm, D5 2.97 ±0.08 mm). The variance in body size was also lowest in bees reared on D4, significantly lower than in 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; χ^2_1=12.01 *P*<0.001), but

Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

Table 2. Effect of larval diet on the time to adult emergence, body mass on emergence, body size and body condition

	Time to emergence (days)		Body mass (mg)		Body size (mm)		Body condition (mg mm^{-1})	
Diet comparison	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

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], for time to emergence, body mass, body size and body condition, respectively (see Table S3 for the complete outcome of the models). Significant differences in estimated marginal means between the diet treatment groups listed in the first column are shown in bold (see Table 1 for dietary content). *P*-values were adjusted using the Tukey method. The number of bees measured in each treatment is as follows: D1 *N*=28, D2 *N*=33, D3 *N*=0, D4 *N*=10, D5 *N*=30.

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^{-1}) than those reared on D1 (emm 29.5 ±0.82 mg mm^{-1}; estimate±s.e. 3.40±1.14 mg mm^{-1}, *P*=0.004) or

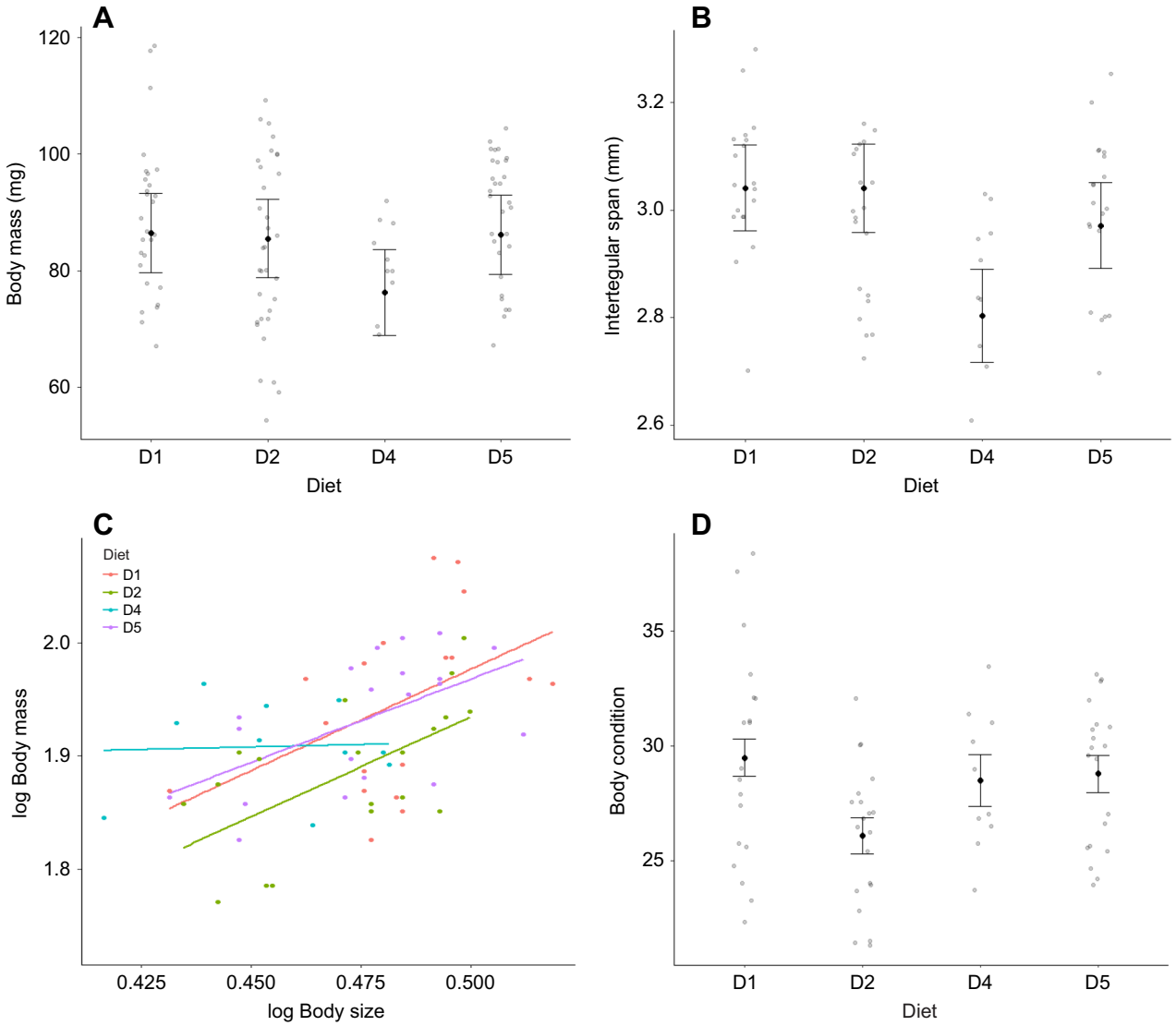

Fig. 2. Larval diet affects adult body mass, size and condition. (A) Body mass, (B) body size, (C) scaling of body mass (mg) and body size (mm), and (D) body condition (body mass/body size) of adult bees in each diet treatment. Dietary content can be found in Table 1. Grey circles in A, B and D are the individual data points, black circles represent the estimated marginal mean and whiskers are the standard error of the mean. The number of bees tested in each treatment is as follows: D1 *N*=28, D2 *N*=33, D3 *N*=0, D4 *N*=10, D5 *N*=30. Note, only one bee reared on D3 survived to adulthood, so bees from this group are not represented.

Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

D5 (emm 28.8±0.80 mg mm^{-1}; estimate±s.e. 2.70±1.13 mg mm^{-1}, 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 RMR 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 with 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±s.e. −1.00±0.47, d.f.=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; Tables S5, S6; $F_{1,63.72}$=2.67, P=0.107).

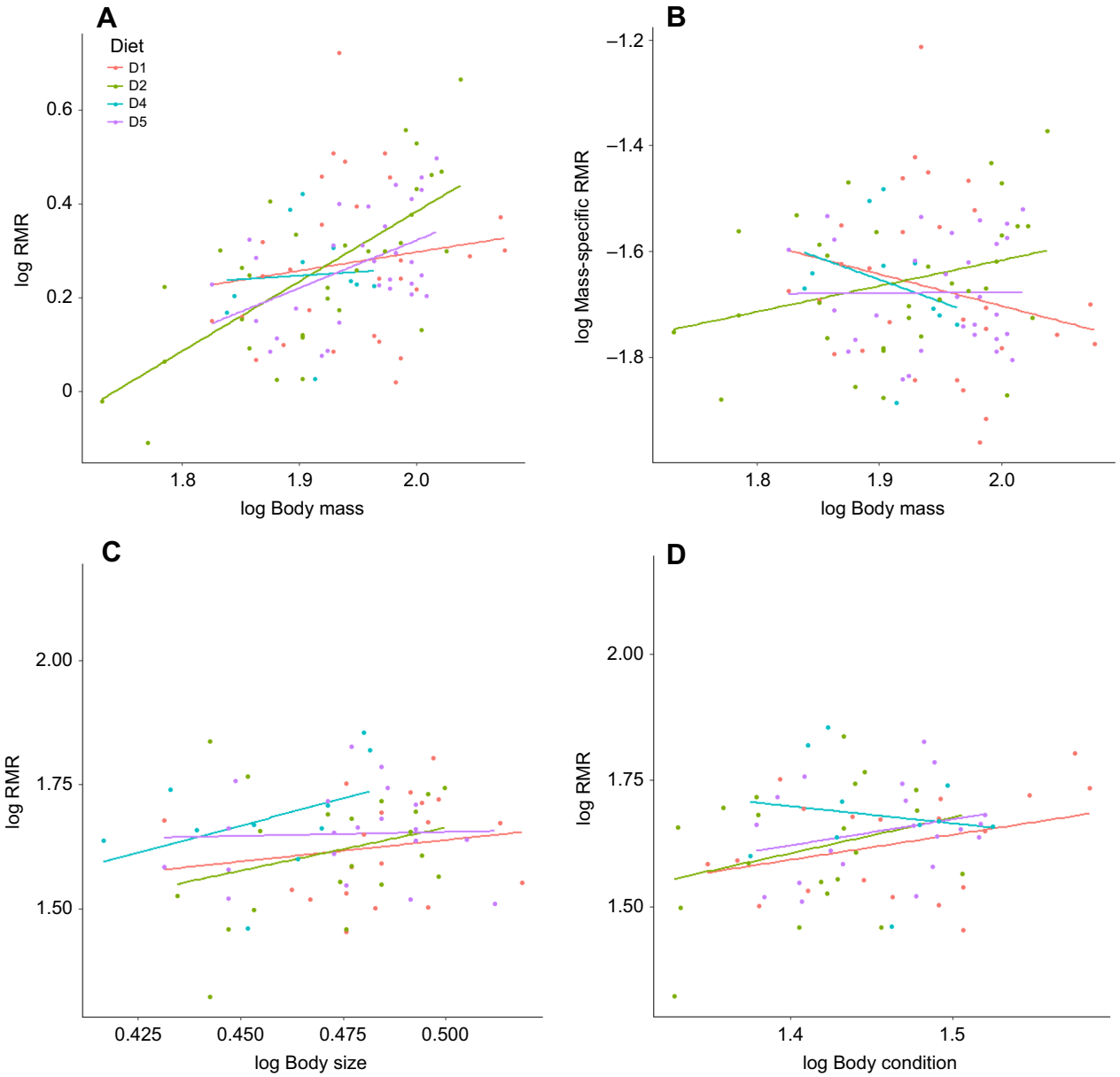

Fig. 3. Larval diet affects the allometric scaling of adult resting metabolic rate (RMR). (A) Scaling between CO_2 production (RMR, µl CO_2 h^{-1}) and body mass (mg), (B) mass-specific RMR (µl CO_2 h^{-1} mg^{-1}) and body mass (mg), (C) RMR (µl CO_2 h^{-1}) and body size (intertegular distance, mm) and (D) RMR (µl CO_2 h^{-1}) and body condition (body mass/body size, mg mm^{-1}). Dietary content can be found in Table 1. The number of bees tested in each treatment is as follows: D1 N=28, D2 N=33, D3 N=0, D4 N=10, D5 N=30.

Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

Table 3. Scaling relationship between body mass (mg) and RMR (μl CO_2 h^{-1}) for adult bees reared on larval diets of varying nutritional composition

Diet	*N*	log Slope ±s.e.	log Intercept ±s.e.	Regression equation
D1	28	0.397±0.52	2.155±2.33	R^2=0.022, $F_{1,26}$=0.585, P=0.451
D2	33	1.489±0.28	−2.674±1.22	R^2=0.486, $F_{1,31}$=29.27, P<0.001
D4	10	0.160±0.94	3.168±4.14	R^2=0.003, $F_{1,8}$=0.029, P=0.870
D5	30	1.016±0.36	−0.636±1.61	R^2=0.223, $F_{1,28}$=8.035, P=0.008

Dietary content can be found in Table 1. Slopes and intercepts (±s.e.) were calculated via least-squares regression.

DISCUSSION

Many organisms experience nutritionally sub-optimal diets during development, but very few studies have directly examined the impact of the nutritional composition of developmental diets 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, which is likely to limit 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 lifespan in adult bees (Brodschneider and Crailsheim, 2010; Daly et al., 1995; Eishchen et al., 1982). 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.

Larvae reared on a high carbohydrate diet had the highest survival to adulthood (D2, P:C 1:3), significantly higher than bees in all other treatment groups. Nearly all bees reared on the low carbohydrate diet failed to eclose (D3, P:C 1:1.5), and bees reared on the high protein diet (D4, P:C 1:1.9) also showed poor survival to adulthood. However, the absolute amount of protein and carbohydrate consumed over the course of development appears to be more important for survival than the ratio of macronutrients contained within the diet; although the low protein diet (D5, P:C 1:2.9) had a similar ratio of protein to carbohydrate as the high carbohydrate diet (D2, P:C 1:3), survival was significantly worse. The high carbohydrate diet (D2) contained 23.2% carbohydrate and 7.7% protein, whereas the low protein diet (D5) contained just 19.5% carbohydrate and 6.8% protein. The amount of food fed to larvae each day was fixed, so individuals were unable to compensate for imbalances in the macronutrient content of the diet by eating more food. Helm et al. (2017) also observed the highest survival in bees reared on a medium protein and high carbohydrate diet, and poor survival for bees reared on high protein diets, though survival was only recorded to the pupal stage. They concluded that there was an interaction between protein and carbohydrate on larval development, fitting with the idea of both the ratio and absolute amounts of protein and carbohydrate being important. Bees reared on the high carbohydrate diet, the best for survival, also took significantly longer to emerge as adults compared with bees in all other diet groups. This contrasts with previous studies in insects which have typically observed slower development on lower quality diets (Angell et al., 2020; Johnson et al., 1992).

The impact of high levels of dietary protein, both the absolute amount and relative content, upon survival has been demonstrated for bees as well as many other organisms (Cook and Behmer, 2010; Dussutour and Simpson, 2009, 2012; Le Couteur et al., 2015; Lee et al., 2008; Pirk et al., 2010; Solon-Biet et al., 2015). For example, the survival to adulthood, larval development and size of solitary Megachilid bees is best on a high carbohydrate diet (Austin and Gilbert, 2021). The absolute quantity rather than the ratio of dietary macronutrients has also been shown to impact survival in soldier flies (Barragan-Fonseca et al., 2019). However, the mechanism underpinning the deleterious effect of consuming large volumes of protein on lifespan is poorly understood (Wright, 1995; Westerterp et al., 1999; Halton and Hu, 2004; Arganda et al., 2017).

Diet also had a significant effect on emerging adult bees' body mass, size and condition, which fits with previous studies linking the quality of pollen and nectar in larval diets to emergent adult bee size (Burkle and Irwin, 2009; Roulston and Cane, 2002). To our knowledge, this is the first study to demonstrate experimentally that the specific macro-nutrient composition of the larval diet affects body mass, size and condition in worker honeybees, which are typically considered to exhibit limited variation in body size compared with other bee species such as bumblebees (Goulson et al., 2002) or solitary bees. Perhaps unsurprisingly, bees reared on the worst diet for survival, the high protein diet (D3, P:C 1:1.5), were the smallest and lightest on emergence. However, bees reared on this poor diet also had the narrowest range of body sizes, while those reared on the high carbohydrate diet had the best survival rate and the widest variation in body mass, suggesting that diets that increase survival also allow for a greater range of body sizes to emerge. Bees reared on the high carbohydrate diet had significantly lower body condition scores than bees reared on the diet containing a moderate amount of protein and carbohydrate. Diet-dependent variation in worker body size can have implications for both individual and colony functioning. Kerr and Hebling (1964) found that worker weight can affect the age at which worker honeybees make the transition from in-hive tasks to foraging, and in bumblebees and other bees, body size has been shown to correlate positively with foraging range (Greenleaf et al., 2007) and the weight of pollen and nectar loads that can be collected and transported back to the nest (Goulson et al., 2002; Kerr et al., 2019; Ramalho et al., 1998). Smaller bees have also been shown to be less effective at pollinating flowers (Jauker et al., 2012; Willmer and Finlayson, 2014). Thus, consuming inadequate amounts of macronutrients during development leads to both lower survival and body mass in adult worker bees, with potential consequences for the age structure and foraging efficiency of the colony, as well as wider ecological implications for the delivery of pollination.

Studies examining the impact of developmental diet on adult metabolism and metabolic scaling are rare, particularly in insects. It is unclear whether nutritionally poor diets lead to an increase or decrease in the RMR, given that this is likely to depend on the specific behavioural and/or physiological response(s) of an organism to an unbalanced diet (Burton et al., 2011). For example, organisms might be expected to reduce their metabolic

Journal of Experimental Biology (2021) 224, jeb242393. doi:10.1242/jeb.242393

Table 4. Effect of larval diet and body mass (mg) on RMR, and diet and body mass on mass-specific RMR

	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

Dietary content can be found in Table 1. Models used were [log(RMR)~diet*log(body mass)+(1|grafting cohort)] and [log(mass-specific RMR)~diet*log(body mass)+(1|grafting cohort)], to test the effect of larval diet and body mass (mg) on RMR, and mass-specific RMR, respectively. Significant *P*-values are in bold. The number of bees tested in each treatment is as follows: D1 *N*=28, D2 *N*=33, D3 *N*=0, D4 *N*=10, D5 *N*=30.

rates in response to a diet of poor nutritional quality to minimise energetic expenditure (McNab, 1986). However, physiological adaptations to imbalanced diets, such as increasing gut length, may be metabolically costly (Yang and Joern, 1994). The few studies that have examined the impact of manipulating the nutritional composition of diets have generally found that nutritionally poor diets elevate average RMR (Zanotto et al., 1997; Ayayee et al., 2018, 2020; but see Clark et al., 2016). Typically, these studies considered short-term impacts of diet on metabolism during either adulthood or a single juvenile stage, ignoring the impact of the nutritional composition of diets on the scaling of RMR with body mass or size.

We showed positive allometric scaling of RMR across all treatment groups, as is typical for insects (e.g. Niven and Scharlemann, 2005), and that larval diet has a long-term impact on metabolic scaling in adult bees. However, there were substantial differences in the slope of the allometric scaling relationship of RMR depending on diet. Bees reared on the high carbohydrate diet (D2, P:C 1:3) showed positive allometry, with larger bees exhibiting higher RMRs, which is unusual (Gillooly et al., 2001; Glazier, 2005; McNab, 1988; Naya et al., 2007; Roces and Lighton, 1995; Savage et al., 2004; Terblanche et al., 2008; White and Seymour, 2003). In comparison, the RMR of bees reared on a diet containing a moderate amount of protein and carbohydrate (D1, P:C, 1:2.3) showed isometry. Diets 2 and 5 have similar P:C ratios (1:3 and 1:2.9, respectively), and though the absolute amount of protein and carbohydrate differs between these two diets (D2 7.7% P, 23.2% C; D5 6.8% P, 19.5% C), significantly affecting larval survival, the scaling relationship between body mass and metabolic rate did not vary between adult bees in these diet treatments. Bees reared on the high carbohydrate diet (D2) also exhibited an unusual increase in mass-specific RMR with body mass, while bees reared on all other diets displayed a more typical decelerating or isometric relationship between mass-specific RMR and body mass. Neither body size (intertegular span) nor body condition scaled with RMR. This discrepancy with body mass is somewhat unexpected, given that we recorded RMR immediately following emergence, before additional feeding could strongly influence the bees' mass. That body size and body mass may scale quite differently with RMR is highly relevant for scaling studies that use body size as a proxy for mass.

In contrast to our finding that the nutritional content of larval diets affects the scaling of body mass and RMR, Karowe and Martin (1989) observed that while consumption of nutritionally poor diets by larvae of the moth *Spodoptera eridania* led to an elevated RMR, the slopes of the positive scaling relationships between RMR and body mass were unaffected by diet treatment. However, in this study, only protein quality was manipulated and metabolic rates were measured only from larvae. Scaling relationships may change during ontogeny and could therefore be differentially affected by diet (Frappell, 2008; Killen et al., 2007). Consuming algal diets with unbalanced phosphorous:carbon ratios has been shown to change the scaling relationship between RMR and body mass in *Daphnia*, though this finding was based on a pooled dataset across four closely related species (Jeyasingh, 2007). Therefore, to our knowledge, ours is the first study to demonstrate that an allometric scaling relationship can be altered by developmental diet within a single invertebrate species, significantly contributing to our understanding of the mechanistic basis of variation in the allometry of RMR (Vaca and White, 2010).

Here, we considered only differences in protein and carbohydrate content of the diets, given the number of studies demonstrating that insect herbivores tightly control their intake of these two nutrients (Behmer, 2009). The royal jelly used in our study contained ~1.56% lipids (slightly lower than the average 5% cited by Wright

et al., 2018), which would have also varied between diet treatments in a similar way to protein, accounting for between 0.7% and 0.9% of each diet. Lipids are increasingly being recognised as an important component of larval nutrition, with bees appearing to regulate their intake of fats at the level of both the colony and individual foragers (Vaudo et al., 2016a,b, 2020). Therefore, variation in the lipid content of the larval diet may also have had an impact on adult metabolism. Royal jelly also contains various micronutrients such as vitamins and sterols, which are important for hormone production and cannot be synthesised by bees themselves (Wright et al., 2018). Hill et al. (2020) observed changes in average RMR in stick insects reared from birth on leaves of three different plant species, though effects on metabolic scaling were not reported. The macro-nutrient content of leaves from the three plant species did not show much variation, but the concentration and digestibility of micronutrients did. This suggests that in future studies, additional nutritional components other than the macro-nutrients protein and carbohydrate should also be considered in the context of dietary impacts on metabolism.

Conclusions

There is increasing evidence that habitat fragmentation and farming intensification are reducing both the quantity and diversity of floral resources available for bees and other pollinators (Donkersley et al., 2017; Trinkl et al., 2020), which is of considerable concern given the global importance of insect pollination to ecosystem functioning and food security. Here, we show that the nutritional composition of larval diets impacts the metabolic functioning of adult worker bees, with diets more optimal for survival resulting in a higher metabolic rate per unit of body mass. As foraging bees already experience extremely high metabolic demands, differences in the quality of larval nutrition could impact metabolic function, which may negatively influence the foraging efficiency of workers. This could affect the accumulation of pollen and nectar stores available for brood rearing and overwintering, with consequences for overall colony success.

Acknowledgements

We thank Francis Ratnieks and Luciano Scandian for providing access to honeybee colonies and frames of larvae.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.N., J.E.N.; Methodology: E.N., M.R.; Formal analysis: E.N., M.R.; Investigation: M.R.; Writing - original draft: E.N.; Writing - review & editing: M.R., J.E.N.; Visualization: E.N., M.R.; Supervision: E.N.; Project administration: E.N.; Funding acquisition: E.N., J.E.N.

Funding

This research was made possible via a grant awarded to E.N. and J.E.N. from the C. B. Dennis Trust and a UK Research and Innovation Future Leaders Fellowship awarded to E.N. (MR/T021691/1).

Data availability

Data are available from the Dryad digital repository (Nicholls et al., 2021): dryad.jh9w0vt9v

References

Anderson, J. F. (1993). Respiratory energetics of two Florida harvestmen. *Comp. Biochem. Physiol. Part A Physiol.* **105**, 67-72. doi:10.1016/0300-9629(93)90174-3

Angell, C. S., Oudin, M. J., Rode, N. O., Mautz, B. S., Bonduriansky, R. and Rundle, H. D. (2020). Development time mediates the effect of larval diet on ageing and mating success of male antler flies in the wild. *Proc. R. Soc. B Biol. Sci.* **287**, 20201876. doi:10.1098/rspb.2020.1876

Arganda, S., Bouchebti, S., Bazazi, S., Le Hesran, S., Puga, C., Latil, G., Simpson, S. J. and Dussutour, A. (2017). Parsing the life-shortening effects of dietary protein: Effects of individual amino acids. *Proc. R. Soc. B Biol. Sci.* **284**, 20162052. doi:10.1098/rspb.2016.2052

Austin, A. J. and Gilbert, J. D. (2021). Solitary bee larvae prioritize carbohydrate over protein in parentally provided pollen. *Funct. Ecol.* **35**, 1069-1080

Ayayee, P. A., Ondrejech, A., Keeney, G. and Munõz-Garcia, A. (2018). The role of gut microbiota in the regulation of standard metabolic rate in female *Periplaneta americana*. *PeerJ* **6**, e4717. doi:10.7717/peerj.4717

Ayayee, P. A., Kinney, G., Yarnes, C., Larsen, T., Custer, G. F., Van Diepen, L. T. A. and Muñoz-Garcia, A. (2020). Role of the gut microbiome in mediating standard metabolic rate after dietary shifts in the viviparous cockroach, *Diploptera punctata*. *J. Exp. Biol.* **223**, jeb218271. doi:10.1242/jeb.218271

Barragan-Fonseca, K. B., Gort, G., Dicke, M. and van Loon, J. J. A. (2019). Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly *Hermetia illucens*. *Physiol. Entomol.* **44**, 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*. *Physiol. Zool.* **61**, 57-68. doi:10.1086/physzool.61.1.30163737

Behmer, S. T. (2009). Insect herbivore nutrient regulation. *Annu. Rev. Entomol.* **54**, 165-187. doi:10.1146/annurev.ento.54.110807.090537

Brodschneider, R. and Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie* **41**, 278-294. doi:10.1051/apido/2010012

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771-1789. doi:10.1890/03-9000

Burkle, L. and Irwin, R. (2009). Nectar sugar limits larval growth of solitary bees (Hymenoptera: Megachilidae). *Environ. Entomol.* **38**, 1293-1300. doi:10.1603/022.038.0441

Burton, T., Killen, S. S., Armstrong, J. D. and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B Biol. Sci.* **278**, 3465-3473. doi:10.1098/rspb.2011.1778

Cane, J. H. (1987). Estimation of bee size using intertegular span (Apoidea). *J. Kansas Entomol. Soc.* **60**, 145-147.

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. and Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282-290. doi:10.1111/j.1365-2435.2007.01245.x

Clark, R. M., Zera, A. J. and Behmer, S. T. (2016). Metabolic rate is canalized in the face of variable life history and nutritional environment. *Funct. Ecol.* **30**, 922-931. doi:10.1111/1365-2435.12574

Cook, S. C. and Behmer, S. T. (2010). Macronutrient regulation in the tropical terrestrial ant *Ectatomma ruidum* (Formicidae): a field study in costa rica. *Biotropica* **42**, 135-139. doi:10.1111/j.1744-7429.2009.00616.x

Corby-Harris, V., Snyder, L., Meador, C. and Ayotte, T. (2018). Honey bee (*Apis mellifera*) nurses do not consume pollens based on their nutritional quality. *PLoS ONE* **13**, e0191050. doi:10.1371/journal.pone.0191050

Daly, H. V., Danka, R. G., Hoelmer, K., Rinderer, T. E. and Buco, S. M. (1995). Honey bee morphometrics: Linearity of variables with respect to body size and classification tested with European worker bees reared by varying ratios of nurse bees. *J. Apic. Res.* **34**, 129-145. doi:10.1080/00218839.1995.11100898

Desai, M. and Hales, C. N. (1997). Role of fetal and infant growth in programming metabolism in later life. *Biol. Rev. Camb. Philos. Soc.* **72**, 329-348. doi:10.1017/S0006323196005026

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., Power, E. F., Wright, G. A. and Wilson, K. (2017). Nutritional composition of honey bee food stores vary with floral composition. *Oecologia* **185**, 749-761. doi:10.1007/s00442-017-3968-3

Dussutour, A. and Simpson, S. J. (2009). Communal nutrition in ants. *Curr. Biol.* **19**, 740-744. doi:10.1016/j.cub.2009.03.015

Dussutour, A. and Simpson, S. J. (2012). Ant workers die young and colonies collapse when fed a high-protein diet. *Proc. R. Soc. B Biol. Sci.* **279**, 2402-2408. doi:10.1098/rspb.2012.0051

Eishchen, F. A., Rothenbuhler, W. C. and Kulinčević, J. M. (1982). Length of life and dry weight of worker honeybees reared in colonies with different worker-larva ratios. *J. Apic. Res.* **21**, 19-25. doi:10.1080/00218839.1982.11100511

Felton, G. W. (1996). Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Arch. Insect Biochem. Physiol.* **32**, 107-130. doi:10.1002/(SICI)1520-6327(1996)32:1<107::AID-ARCH7>3.0.CO;2-X

Ferioli, F., Armaforte, E. and Caboni, M. F. (2014). Comparison of the lipid content, fatty acid profile and sterol composition in local Italian and commercial royal jelly samples. *J. Am. Oil Chem. Soc.* **91**, 875-884. doi:10.1007/s11746-014-2446-x

Frappell, P. B. (2008). Ontogeny and allometry of metabolic rate and ventilation in the marsupial: matching supply and demand from ectothermy to endothermy. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **150**, 181-188. doi:10.1016/j.cbpa.2008.02.017

Garcia-Amoedo, L. H. and De Almeida-Muradian, L. B. (2007). Physicochemical composition of pure and adulterated royal jelly. *Quim. Nova* **30**, 257-259. doi:10.1590/S0100-40422007000200002

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251. doi:10.1126/science.1061967

Glazier, D. S. (2005). Beyond the '3/4-power law': variation in the intra-and interspecific scaling of metabolic rate in animals. *Biol. Rev.* **80**, 611-662. doi:10.1017/S1464793105006834

Goulson, D., Peat, J., Stout, J. C., Tucker, J., Darvill, B., Derwent, L. C. and Hughes, W. O. H. (2002). Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Anim. Behav.* **64**, 123-130. doi:10.1006/anbe.2002.3041

Greenleaf, S. S., Williams, N. M., Winfree, R. and Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia* **153**, 589-596. doi:10.1007/s00442-007-0752-9

Hales, C. N. and Barker, D. J. P. (2001). The thrifty phenotype hypothesis: type 2 diabetes. *Br. Med. Bull.* **60**, 5-20. doi:10.1093/bmb/60.1.5

Halton, T. L. and Hu, F. B. (2004). The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J. Am. Coll. Nutr.* **23**, 373-385. doi:10.1080/07315724.2004.10719381

Helm, B. R., Slater, G. P., Rajamohan, A., Yocum, G. D., Greenlee, K. J. and Bowsher, J. H. (2017). The geometric framework for nutrition reveals interactions between protein and carbohydrate during larval growth in honey bees. *Biol. Open* **6**, 872-880. doi:10.1242/bio.022582

Hill, S. J., Silcocks, S. C. and Andrew, N. R. (2020). Impacts of temperature on metabolic rates of adult *Extatosoma tiaratum* reared on different host plant species. *Physiol. Entomol.* **45**, 7-15. doi:10.1111/phen.12310

Howe, S. R., Howe, S. R., Dimick, P. S. and Benton, A. W. (1985). Composition of freshly harvested and commercial royal jelly. *J. Apic. Res.* **24**, 52-61. doi:10.1080/00218839.1985.11100649

Jauker, F., Peter, F., Wolters, V. and Diekötter, T. (2012). Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia rufa* outbalance post-flowering disadvantages. *Basic Appl. Ecol.* **13**, 268-276. doi:10.1016/j.baae.2012.03.010

Jeyasingh, P. D. (2007). Plasticity in metabolic allometry: the role of dietary stoichiometry. *Ecol. Lett.* **10**, 282-289. doi:10.1111/j.1461-0248.2007.01023.x

Joern, A., Provin, T. and Behmer, S. T. (2012). Not just the usual suspects: insect herbivore populations and communities are associated with multiple plant nutrients. *Ecology* **93**, 1002-1015. doi:10.1890/11-1142.1

Johnson, J. A., Wofford, P. L. and Whitehand, L. C. (1992). Effect of diet and temperature on development rates, survival, and reproduction of the Indianmeal moth (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **85**, 561-566. doi:10.1093/jee/85.2.561

Karasov, W. H., Martínez Del Rio, C. and Caviedes-Vidal, E. (2011). Ecological physiology of diet and digestive systems. *Annu. Rev. Physiol.* **73**, 69-93. doi:10.1146/annurev-physiol-012110-142152

Karowe, D. N. and Martin, M. M. (1989). The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar *Spodoptera eridania* larvae (Lepidoptera: Noctuidae). *J. Insect Physiol.* **35**, 699-708. doi:10.1016/0022-1910(89)90089-9

Kerr, W. E. and Hebling, N. J. (1964). Influence of the weight of worker bees on division of labor. *Evolution* **18**, 267-270. doi:10.1111/j.1558-5646.1964.tb01599.x

Kerr, N. Z., Crone, E. E. and Williams, N. M. (2019). Integrating vital rates explains optimal worker size for resource return by bumblebee workers. *Funct. Ecol.* **33**, 467-478. doi:10.1111/1365-2435.13251

Killen, S. S., Costa, I., Brown, J. A. and Gamperl, A. K. (2007). Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proc. R. Soc. B Biol. Sci.* **274**, 431-438. doi:10.1098/rspb.2006.3741

Le Couteur, D. G., Tay, S. S., Solon-Biet, S., Bertolino, P., McMahon, A. C., Cogger, V. C., Colakoglu, F., Warren, A., Holmes, A. J., Pichaud, N. et al. (2015). The influence of macronutrients on splanchnic and hepatic lymphocytes in aging mice. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **70**, 1499-1507. doi:10.1093/gerona/glu196

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., Soran, N. and Raubenheimer, D. (2008). Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. *Proc. Natl. Acad. Sci. USA* **105**, 2498-2503. doi:10.1073/pnas.0710787105

McCarthy, I. D. (2000). Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. *J. Fish Biol.* **57**, 224-238. doi:10.1111/j.1095-8649.2000.tb00788.x

McNab, B. K. (1986). The influence of food habits on the energetics of eutherian mammals. *Ecol. Monogr.* **56**, 1-19. doi:10.2307/2937268

McNab, B. K. (1988). Complications inherent in scaling the basal rate of metabolism in mammals. *Q. Rev. Biol.* **63**, 25-54. doi:10.1086/415715

Moe, B., Brunvoll, S., Mork, D., Brobakk, T. E. and Bech, C. (2004). Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax aristotelis*). *J. Exp. Biol.* **207**, 4067-4076. doi:10.1242/jeb.01226

Morgan, K. R., Shelly, T. E. and Kimsey, L. S. (1985). Body temperature regulation, energy metabolism, and foraging in light-seeking and shade-seeking robber flies. *J. Comp. Physiol. B* **155**, 561-570. doi:10.1007/BF00694445

Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biol. Conserv.* **142**, 2369-2372. doi:10.1016/j.biocon.2009.04.007

Naya, D. E., Lardies, M. A. and Bozinovic, F. (2007). The effect of diet quality on physiological and life-history traits in the harvestman *Pachylus paessleri*. *J. Insect Physiol.* **53**, 132-138. doi:10.1016/j.jinsphys.2006.11.004

Nespolo, R. F., Castañeda, L. E. and Roff, D. A. (2005). The effect of fasting on activity and resting metabolism in the sand cricket, *Gryllus firmus*: A multivariate approach. *J. Insect Physiol.* **51**, 61-66. doi:10.1016/j.jinsphys.2004.11.005

Nicholls, E., Fowler, R., Niven, J. E., Gilbert, J. D. and Goulson, D. (2017). Larval exposure to field-realistic concentrations of clothianidin has no effect on development rate, over-winter survival or adult metabolic rate in a solitary bee, *Osmia bicornis*. *PeerJ* **5**, e3417. doi:10.7717/peerj.3417

Nicholls, E., Rossi, M. and Niven, J. (2021). Larval nutrition impacts the scaling of adult metabolic rate with body mass in honeybees. *Dryad, Dataset*. doi:10.5061/dryad.jh9w0vt9v

Niven, J. E. and Scharlemann, J. P. W. (2005). Do insect metabolic rates at rest and during flight scale with body mass? *Biol. Lett.* **1**, 346-349. doi:10.1098/rsbl.2005.0311

Nussear, K. E., Espinoza, R. E., Gubbins, C. M., Field, K. J. and Hayes, J. P. (1998). Diet quality does not affect resting metabolic rate or body temperatures selected by an herbivorous lizard. *J. Comp. Physiol. B* **168**, 183-189. doi:10.1007/s003600050135

Perl, C. D. and Niven, J. E. (2018). Metabolic rate scaling, ventilation patterns and respiratory water loss in red wood ants: activity drives ventilation changes, metabolic rate drives water loss. *J. Exp. Biol.* **221**, jeb182501. doi:10.1242/jeb.182501

Pettersen, A. K., Marshall, D. J. and White, C. R. (2018). Understanding variation in metabolic rate. *J. Exp. Biol.* **221**, jeb166876. doi:10.1242/jeb.166876

Pirk, C. W. W., Boodhoo, C., Human, H. and Nicolson, S. W. (2010). The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). *Apidologie* **41**, 62-72. doi:10.1051/apido/2009055

Ramalho, M., Imperatriz-Fonseca, V. L. and Giannini, T. C. (1998). Within-colony size variation of foragers and pollen load capacity in the stingless bee *Melipona quadrifasciata anthidioides* Lepeletier (Apidae, Hymenoptera). *Apidologie* **29**, 221-228. doi:10.1051/apido:19980302

Roark, A. M. and Bjorndal, K. A. (2009). Metabolic rate depression is induced by caloric restriction and correlates with rate of development and lifespan in a parthenogenetic insect. *Exp. Gerontol.* **44**, 413-419. doi:10.1016/j.exger.2009.03.004

Roces, F. and Lighten, J. R. B. (1995). Larger bites of leaf-cutting ants. *Nature* **373**, 392-392. doi:10.1038/373392a0

Roeder, K. A. and Behmer, S. T. (2014). Lifetime consequences of food protein-carbohydrate content for an insect herbivore. *Funct. Ecol.* **28**, 1135-1143. doi:10.1111/1365-2435.12262

Roulston, T. H. and Cane, J. H. (2002). The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evol. Ecol.* **16**, 49-65. doi:10.1023/A:1016048526475

Savage, V. M., Gillooly, J. F., Woodruff, W. H., West, G. B., Allen, A. P., Enquist, B. J. and Brown, J. H. (2004). The predominance of quarter-power scaling in biology. *Funct. Ecol.* **18**, 257-282. doi:10.1111/j.0269-8463.2004.00856.x

Schmehl, D. R., Tomé, H. V. V., Mortensen, A. N., Martins, G. F. and Ellis, J. D. (2016). Protocol for the *in vitro* rearing of honey bee (*Apis mellifera* L.) workers. *J. Apic. Res.* **55**, 113-129. doi:10.1080/00218839.2016.1203530

Simpson, S. J. and Raubenheimer, D. (2012). *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*, Princeton University Press.

Solon-Biet, S. M., Walters, K. A., Simanainen, U. K., McMahon, A. C., Ruohonen, K., Ballard, J. W. O., Raubenheimer, D., Handelsman, D. J., Le Couteur, D. G. and Simpson, S. J. (2015). Macronutrient balance, reproductive function, and lifespan in aging mice. *Proc. Natl. Acad. Sci. USA* **112**, 3481-3486. doi:10.1073/pnas.1422041112

Speakman, J. R. (2005). Body size, energy metabolism and lifespan. *J. Exp. Biol.* **208**, 1717-1730. doi:10.1242/jeb.01556

Terblanche, J. S., White, C. R., Blackburn, T. M., Marais, E. and Chown, S. L. (2008). Scaling of gas exchange cycle frequency in insects. *Biol. Lett.* **4**, 127-129. doi:10.1098/rsbl.2007.0522

Trinkl, M., Kaluza, B. F., Wallace, H., Heard, T. A., Keller, A. and Leonhardt, S. D. (2020). Floral species richness correlates with changes in the nutritional quality of larval diets in a stingless bee. *Insects* **11**, 125. doi:10.3390/insects11020125

Vaca, H. F. and White, C. R. (2010). Environmental modulation of metabolic allometry in ornate rainbowfish Rhadinocentrus ornatus. *Biol. Lett.* **6**, 136-138. doi:10.1098/rsbl.2009.0610

Vaudo, A. D., Stabler, D., Patch, H. M., Tooker, J. F., Grozinger, C. M. and Wright, G. A. (2016a). Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. *J. Exp. Biol.* **219**, 3962-3970. doi:10.1242/jeb.140772

Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F. and Grozinger, C. M. (2016b). Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proc. Natl. Acad. Sci. USA* **113**, E40435-Ee4042. doi:10.1073/pnas.1606101113

Vaudo, A. D., Tooker, J. F., Patch, H. M., Biddinger, D. J., Coccia, M., Crone, M. K., Fiely, M., Francis, J. S., Hines, H. M., Hodges, M. et al. (2020). Pollen protein: Lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects* **11**, 132. doi:10.3390/insects11020132

Wang, Y., Kaftanoglu, O., Brent, C. S., Page, R. E. and Amdam, G. V. (2016). Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). *J. Exp. Biol.* **219**, 949-959. doi:10.1242/jeb.130435

Westerterp, K. R., Wilson, S. A. J. and Rolland, V. (1999). Diet induced thermogenesis measured over 24h in a respiration chamber: effect of diet composition. *Int. J. Obes.* **23**, 287-292. doi:10.1038/sj.ijo.0800810

White, C. R. and Seymour, R. S. (2003). Mammalian basal metabolic rate is proportional to body mass2/3. *Proc. Natl. Acad. Sci. USA* **100**, 4046-4049. doi:10.1073/pnas.0436428100

Willmer, P. and Finlayson, K. (2014). Big bees do a better job: intraspecific size variation influences pollination effectiveness. *J. Pollinat. Ecol.* **14**, 244-254. doi:10.26786/1920-7603(2014)22

Wright, P. A. (1995). Nitrogen excretion: three end products, many physiological roles. *J. Exp. Biol.* **198**, 273-281. doi:10.1242/jeb.198.2.273

Wright, G. A., Nicolson, S. W. and Shafir, S. (2018). Nutritional physiology and ecology of honey bees. *Annu. Rev. Entomol.* **63**, 327-344. doi:10.1146/annurev-ento-020117-043423

Yang, Y. and Joern, A. (1994). Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. *Physiol. Entomol.* **19**, 75-82. doi:10.1111/j.1365-3032.1994.tb01077.x

Zanotto, F., Gouveia, S., Simpson, S. and Calder, D. (1997). Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *J. Exp. Biol.* **200**, 2437-2448. doi:10.1242/jeb.200.18.2437

Ziska, L. H., Pettis, J. S., Edwards, J., Hancock, J. E., Tomecek, M. B., Clark, A., Dukes, J. S., Loladze, I. and Polley, H. W. (2016). Rising atmospheric CO_2 is reducing the protein concentration of a floral pollen source essential for north American bees. *Proc. R. Soc. B Biol. Sci.* **283**, 20160414. doi:10.1098/rspb.2016.0414

Table S1. Log-rank test for differences in the survival curves of bees reared on different diets. Dietary content can be found in Table 1.

diet	*n*	observed	expected	(O-E)^2/E	(O-E)^2/V
D1	78	43	49.5	0.84	1.91
D2	60	18	43.0	14.57	32.38
D3	78	77	51.6	12.52	28.87
D4	78	61	51.3	1.82	4.17
D5	77	43	46.6	0.27	0.61

Table S2. Pairwise comparisons of survival between diet treatments using the log-rank test. Dietary content can be found in Table 1. Bonferroni method was used to adjust *p*-values for multiple comparisons. The number of surviving adults bees in each treatment is as follows: D1=35; D2=42; D3=1; D4=17; D5=34.

diet comparison	*p*-value
D1 – D2	**0.023**
D1 – D3	**<0.001**
D1 – D4	0.334
D1 – D5	1.000
D2 – D3	**<0.001**
D2 – D4	**<0.001**
D2 – D5	**0.013**
D3 – D4	0.143
D3 – D5	**0.002**
D4 – D5	0.842

Table S3. Effect of larval diet on the number of days to emergence, weight of adults on the day of emergence, body mass 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. Dietary content can be found in Table 1. The number of bees measured in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30.

		estimate ± s.e.	d.f.	*t*-value	*p*-value	variance ± s.d.
time to emergence (days)	**fixed effects**					
	Intercept (D2)	16.02 ± 0.96	1.01	16.68	**0.037**	
	D1	-0.49 ± 0.13	96.05	-3.98	**<0.001**	
	D4	-0.68 ± 0.18	96.06	-3.84	**<0.001**	
	D5	-0.36 ± 0.13	96.06	-2.89	**0.004**	
	random effects					
	grafting cohort					1.83 ± 1.35
	residual					0.22 ± 0.47
body mass (mg)	**fixed effects**					
	intercept (D2)	85.50 ± 6.72	1.11	12.73	**<0.038**	
	D1	0.91 ± 3.05	96.54	0.30	0.766	
	D4	-9.23 ± 4.32	96.61	-2.14	**0.035**	
	D5	0.68 ± 3.04	96.68	0.22	0.824	
	random effects					
	grafting cohort					82.15 ± 9.06
	residual					129.18 ±11.37
body size (intertegular distance; mm)	**fixed effects**					
	intercept (D2)	3.04 ± 0.08	1.27	37.04	**0.006**	
	D1	-0.00 ± 0.05	65.00	-0.00	0.998	
	D4	-0.24 ± 0.06	64.70	-3.91	**<0.001**	
	D5	-0.07 ± 0.05	64.88	-1.47	0.148	
	random effects					
	grafting cohort					0.01 ± 0.11
	residual					0.02 ± 0.13
body condition (mass/body size; mg/mm)	**fixed effects**					
	intercept (D2)	26.07 ± 0.80		32.63	**<0.001**	
	D1	3.40 ± 1.14		2.97	0.004	
	D4	2.40 ± 1.38		1.73	0.087	
	D5	2.70 ± 1.13		2.39	**0.019**	

Table S4. Brown-Forsythe test to compare variance in body mass (mg), body size (inter-tegular distance in mm) and body condition (body mass/body size) between treatments. Dietary content can be found in Table 1. The number of bees measured in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30.

	body mass			**body size**			**body condition**		
diet comparison	test statistic	df	*p*-value	test statistic	df	*p-value*	test statistic	df	*p*-value
D1 – D2	**8.40**	**1, 32**	**0.006**	3.52	1, 37	0.068	**7.22**	**1, 31**	**0.011**
D4 – D2	1.20	1, 25	0.285	3.95	1, 19	0.062	4.21	1, 18	0.054
D5 – D2	**6.30**	**1, 38**	**0.016**	0.26	1, 38	0.611	**7.74**	**1, 38**	**0.008**
D5 – D1	0.81	1, 32	0.374	1.77	1, 37	0.190	0.31	1, 31	0.584
D5 – D4	2.03	1, 25	0.167	**5.57**	**1, 19**	**0.026**	0.07	1, 19	0.801
D4 – D1	4.14	1, 27	0.052	**12.78**	**1, 18**	**0.002**	0.49	1, 26	0.489

Table S5. Effect of larval diet and body mass (mg) on body size, diet and body size (mm) on resting metabolic rate, and diet and body condition (body mass/body size; mg/mm) on resting metabolic rate. Models used were (log(Body size) ~ diet + log(Body mass) + (1|grafting cohort)), (log(RMR) ~ diet + log(Body size) + (1|grafting cohort)) and (log(RMR) ~ diet + log(Body condition) + (1|grafting cohort)) respectively. Dietary content can be found in Table 1. The number of bees measured in each treatment is as follows: D1=28; D2=33; D3=0; D4=10; D5=30.

		estimate ± s.e.	*t*-value	*p*-value	variance ± s.d.
body size (intertegular distance; mm)	**fixed effects**				
	Intercept (D2)	0.533 ± 0.17	3.181	**0.002**	
	D1	-0.007 ± 0.01	-0.503	0.598	
	D4	-0.070 ± 0.02	-3.660	**<0.001**	
	D5	-0.025 ± 0.01	-1.665	0.101	
	(log)Body Mass	0.130 ± 0.04	3.465	**<0.001**	
	random effects				
	grafting cohort				0.000 ± 0.02
	residual				0.002 ± 0.04
Resting metabolic rate (µL CO_2 per hour)	**fixed effects**				
	Intercept (D2)	2.903 ± 0.74	3.907	**<0.001**	
	D1	-0.051 ± 0.09	-0.605	0.548	
	D4	0.100 ± 0.12	0.848	0.406	
	D5	0.017 ± 0.09	0.197	0.845	
	(log)Body Size	0.791± 0.67	1.184	0.242	
	random effects				
	grafting cohort				0.006 ± 0.08
	residual				0.058 ± 0.24
Resting metabolic rate (µL CO_2 per hour)	**fixed effects**				
	Intercept (D2)	2.492 ± 0.79	3.144	**0.003**	
	D1	-0.080 ± 0.09	-0.922	0.360	
	D4	0.029 ± 0.11	0.268	0.790	
	D5	-0.021 ± 0.09	-0.237	0.814	
	(log)Body Condition	0.392 ± 0.24	1.633	0.107	
	random effects				
	grafting cohort				0.007 ± 0.08
	residual				0.057 ± 0.24

Table S6. Summary of the maximal and final models used in the metabolic rate analyses. All models were initially fitted according to the maximal model. All models also incorporated 'grafting cohort' as a random factor. Models were selected based on their AIC score.

response	maximal model	model compared	model comparisons (AIC score)	minimum adequate model (final model)	fixed effect significance
metabolic rate (MR)	(log(MR)~ diet * log(Body Mass) + (1|grafting cohort))	(log(MR)~ diet + log(Body Mass) + (1|grafting cohort))	Maximal AIC: 63.18 Comparison AIC: 65.92	(log(MR)~ diet * log(Body Mass) + (1|grafting cohort))	Diet: $F_{3, 92.43}$= 1.93, p=0.131 log(Body Mass): $F_{1, 92.48}$= 3.16, p=0.079 Diet x log(Body Mass): $F_{3\ 92.32}$= 2.04, p=0.114
mass-specific metabolic rate (MSMR)	(log(MSMR)~ diet * log(Body Mass) + (1|grafting cohort))	(log(MSMR)~ diet + log(Body Mass) + (1|grafting cohort))	Maximal AIC: 63.18 Comparison AIC: 65.92	(log(MSMR)~ diet * log(Body Mass) + (1|grafting cohort))	Diet: $F_{3, 92.43}$= 1.93, p=0.131 log(Body Mass): $F_{1, 92.48}$= 2.37, p=0.127 Diet x log(Body Mass): $F_{3\ 92.32}$= 2.04, p=0.114
metabolic rate	(log(MR)~ diet * log(Body Size) + (1|grafting cohort))	(log(MR)~ diet + log(Body Size) + (1|grafting cohort))	Maximal AIC: 14.15 Comparison AIC: 9.93	(log(MR)~ diet + log(Body Size) + (1|grafting cohort))	Diet: $F_{3, 34.32}$= 0.68, p=0.570 log(Body Size): $F_{1, 53.36}$= 2.37, p=0.242
metabolic rate	(log(MR)~ diet * log(Body Condition) + (1|grafting cohort))	(log(MR)~ diet + log(Body Condition) + (1|grafting cohort))	Maximal AIC: 13.45 Comparison AIC: 8.97	(log(MR)~ diet + log(Body Condition) + (1|grafting cohort))	Diet: $F_{3, 51.46}$= 0.56, p=0.643 log(Body Condition): $F_{1, 63.72}$= 2.67, p=0.107