

Food restriction alters energy allocation strategy during growth in tobacco hornworms (*Manduca sexta* larvae)

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Received: 16 February 2015 / Revised: 6 June 2015 / Accepted: 8 June 2015
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Abstract Growing animals must alter their energy budget in the face of environmental changes and prioritize the energy allocation to metabolism for life-sustaining requirements and energy deposition in new biomass growth. We hypothesize that when food availability is low, larvae of holometabolic insects with a short development stage (relative to the low food availability period) prioritize biomass growth at the expense of metabolism. Driven by this hypothesis, we develop a simple theoretical model, based on conservation of energy and allometric scaling laws, for understanding the dynamic energy budget of growing larvae under food restriction. We test the hypothesis by manipulative experiments on fifth instar hornworms at three temperatures. At each temperature, food restriction increases the scaling power of growth rate but decreases that of metabolic rate, as predicted by the hypothesis. During the fifth instar, the energy budgets of larvae change dynamically. The free-feeding larvae slightly decrease the energy allocated to growth as body mass increases and increase the energy allocated to life sustaining. The opposite trends were observed in food restricted larvae, indicating the predicted prioritization in the energy budget under food restriction. We compare the energy budgets of a few endothermic and ectothermic species and discuss how different life histories

lead to the differences in the energy budgets under food restriction.

Keywords Food restriction · Energy budget · Scaling law · Growth · *Manduca sexta*

Introduction

Life history hypothesis of energy allocation priority rules

Growing animals uptake food from the environment and partition the assimilated energy from food between two compartments: the energy deposited in the new biomass growth and the energy spent on metabolism for life-sustaining requirements, such as maintenance of existing biomass, biosynthesis, defense, and foraging (Brody 1945; Hou et al. 2008; Kooijman 2010). The former is the combustion energy stored in tissues, and the latter is dissipated as heat. The energy allocation strategy often exhibits phenotypic plasticity. In the face of low food availability, animals are able to adjust their energy budgets and prioritize the energy allocation to growth and metabolism (Glazier 2002; Hou et al. 2011b; Roff 2001). Here, we hypothesize that a species prioritizes metabolism at the expense of growth, if it either has a life span much longer than the food shortage period that is usually seasonal or is able to search for new food sources and actively end the food scarcity. In either case, the food scarcity period is temporary to such a species. Under food restriction, this kind of species would retard growth and allocate more energy to keep good health, because it can resume growth after the *temporary* food scarcity is over (compensatory growth (Dmitriev 2011; Mangel and Munch 2005)). Taking this strategy, reproduction is delayed, but due to the high investment in maintenance, the

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-015-1289-0) contains supplementary material, which is available to authorized users.

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animals will have low mortality and high-quality offspring, and therefore, the overall fitness will not be undermined.

In contrast, if the food scarcity is relatively long term to a species, then this species would prioritize growth at the expense of metabolism. We hypothesize that holometabolic insects with a short larval stage belong to this category. Larvae of holometabolic insects must grow and reach a threshold size to successfully pupate and then eclose, mate, and reproduce (Davidowitz et al. 2003; Nijhout 1975). Moreover, it is generally difficult for insect larvae to find a new or better food source (such as a host plant). With short larval stages and limited ability of finding new food sources, food scarcity for them is almost permanent. For such species, keeping fast growth under food restriction (FR) would be favored by selection. It is well known that fast growth comes with the costs of self-maintenance (Hou 2014; Mangel and Stamps 2001), such as impaired immune function (De Block and Stoks 2008), shorter life span (Metcalf and Monaghan 2001), poor performance (Morgan and Metcalf 2001), and presumably poor offspring quality. However, if these species suppress growth and allocate more energy to maintenance, they may still not be able to survive through the low-food period as it may be longer than their larval stage and cannot be ended by active foraging. By prioritizing growth, the animals will not only reach the size to pupate before the low-food season is over but also will have relatively large size for high fecundity (Honěk 1993).

The classifications above have exceptions. For example, several mealworm species (holometabolous) can take months to finish the larval stage, and such species may sacrifice growth for maintenance under food restriction. We need to emphasize that the key issue in our hypothesis is whether the species experiences food shortage that encompasses its developmental period, which depends on the species life history and habitat. Note, some species can enter diapause stage, during which the rates of food uptake and growth are nearly zero (Hahn and Denlinger 2011). In this paper, we are only interested in the cases where animals still allocate energy to grow under a limited but non-zero food supply, so the energy budget of diapausing species is not discussed.

Numerous efforts have been made to study how long-living species, such as endotherms, adjust their energy budgets under FR (see review in (Hou et al. 2011b)). But, as far as we know, no study has been conducted on the larvae of holometabolic insects. In this paper, we first develop a simple theoretical model, based on conservation of energy and allometric scaling laws, for understanding the dynamic energy budget of growing animals under FR. We then test the prediction derived from the hypothesis by manipulative experiments of FR on the fifth instar tobacco hornworms (the last instar of *Manduca sexta* larvae). Depending on the ambient temperature and food supply level, the fifth instar hornworms grow from ~1 to ~12 g in 5~10 days before pupation. It has also been shown that

hornworms' movement in the field is minimal (Bernays and Woods 2000). Thus, the short larval stage and limited capability of leaving the poor environment make hornworm a good model to test the hypothesis.

Allometric scaling model of energy budget in growing insect larvae

Many empirical and theoretical studies have been conducted for understanding the energy allocation strategy of growing animals. The basic energy budgets described in the studies are similar (Brody 1945; Hou et al. 2008; Kearney and White 2012; Kooijman 2010). During growth, in a unit time, the energy assimilated from food, F , is partitioned between the energy deposited in new biomass, S , which is proportional to growth rate, and metabolic energy, B , which is dissipated as heat, i.e., $F=S+B$. For growing insect larvae, the rates of assimilation, F , metabolic energy, B , and energy deposited in biomass, S , can be approximately expressed as scaling functions of body mass, m , i.e., $F=F_0m^f$, $B=B_0m^b$, and $S=S_0m^s$, where F_0 , B_0 , and S_0 are normalization constants, and f , b , and s are scaling powers (Greenlee and Harrison 2005; Sears et al. 2012). The rigorously mathematic form of the equation $F=S+B$ requires that F , B , and S have the same scaling powers, i.e., $f=s=b$. If the scaling powers of two of them are different, then the third one cannot be expressed as a power function of body mass. However, in biological studies, all of the allometric scaling laws are obtained from statistical fitting of empirical data and therefore are approximate. The numerical estimates show that even if the scaling powers are different, the numerical values of F calculated from the equation, $F=S+B$, can still be well fitted as a scaling law of m as $F=F_0m^f$ approximately (Figs. S1 and S2 in the Electronic Supplementary Material, ESM). This approximation is good (with the R^2 values of the fitting being very close to 1), when the difference between the scaling powers s and b is small. In our study, since the scaling powers of all three rates are between 0 and 1, the difference between any two of them cannot be larger than 1. Thus, we can safely apply this approximation and express three rates in the equation $F_0m^f \approx S_0m^s + B_0m^b$. The same approximation also holds for the endotherms, if only a short period of growth is considered, instead of a whole sigmoidal growth trajectory (Brody 1945; Hou et al. 2008).

Now, we divide both sides of this equation by the assimilation rate, $F=F_0m^f$, and have

$$1 = S/F + B/F \\ \approx (S_0/F_0)m^{s-f} + (B_0/F_0)m^{b-f} \quad (1)$$

where S/F and B/F are proportions of the energy assimilated from food that are allocated to growth and metabolism, respectively. In Eq. 1, if $s=f=b$, we have $1=S_0/F_0+B_0/F_0$, which means that the energy allocation proportions are constants, not

varying with body mass during growth. If $s \neq f \neq b$, then the proportion of energy allocated to growth and metabolism changes as body mass increases. Equation 1 imposes a constraint on the scaling powers: As m increases, the proportions, S/F and B/F , cannot both increase or both decrease, because the sum of them should be 1. So, the sign of $s-f$ and $b-f$ in Eq. 1 must be opposite, i.e., if $s < f$, then $b > f$, and vice versa.

We applied FR to hornworms by decreasing the coefficient, F_0 , but keeping the scaling power, f , the same. Previous studies have shown that after FR starts, the drop in growth rate is greater than that in metabolic rate in many growing animals (Hou et al. 2011b), including hornworms (Hayes et al. 2014). If hornworms prioritize growth over metabolism under FR, as we hypothesized, then our model makes three predictions.

- Prediction 1: Growth rate of hornworms under FR will increase with body mass more quickly than it does under the free-feeding condition to compensate for the initial drop in growth rate following FR. This means that the scaling power of growth rate (s) under FR will be higher than that under free feeding.
- Prediction 2: Since the sign of $s-f$ and $b-f$ in Eq. 1 must be opposite, if the scaling power of growth (s) increases under FR, then the power of metabolic rate (b) must decrease, compared to the free-feeding controls.
- Prediction 3: With an unchanged f , an increased s , and a decreased b under FR, the proportion of energy allocated to growth, $S/F \propto m^{s-f}$, increases more quickly with body mass, and the proportion of energy allocated to metabolism, $B/F \propto m^{b-f}$, increases more slowly or even decreases with body mass in the FR larvae compared to the free-feeding controls.

Materials and methods

Animal rearing

To test the predictions, we reared six cohorts of fifth instar hornworms with two levels of food supply, ad libitum (AL) and food restriction (FR), at three temperatures, 20, 25, and 30 °C in the summer of 2012 and 2013. One hundred and fifty hornworms (*Manduca sexta* larvae) were raised from eggs (Carolina Biological supply) on a long day cycle (17 h light: 7 h dark) at 25 °C. Animals were fed ad libitum and checked for molting each day until fifth instar. On the first day of the fifth instar, larvae were randomly separated into three incubators at 20, 25, and 30 °C. At each temperature, larvae were randomly separated into two cohorts with different food

supply levels (see below). There were six cohorts of larvae (two food levels \times three temperatures), each consisting of ~ 25 larvae. Each larva was reared in an individual transparent vial, 5 cm in diameter and 12 cm in length. At each temperature, cohorts with two food treatments were reared during the same period in the same incubator. This way, the environmental induced differences in growth and metabolism between two food treatments within a temperature are minimized.

Food supply levels and assimilation rate

At approximately the same time each day, the larvae were fed a wheat germ-based diet (hornworm medium bulk diet, Carolina Biological supply, NC). The dry and wet mass ratio of the diet is about 20 %. The energy content in the dry food, E_{food} , is 20,160 J/g. At each temperature, the cohorts with two food treatments were fed with the diet from the same batch, so that the potential slight variation in nutrient components among batches is eliminated for comparisons within one temperature. After larvae entered the fifth instar, two cohorts at each temperature were fed with two levels of food supply: AL and FR. The AL cohorts fed freely, and we measured the food intake of each larva daily to the nearest 1 mg on a digital microbalance (Perkin-Elmer AD6). During the experiment, no larva in the AL cohorts ran out of food. For FR cohorts, we measured the body mass of each individual to the nearest 0.1 mg. Based on the body mass, we fed individual larva with the amount of food calculated from the equation $F = 0.3 \times m^{0.75}$ at 20 °C, $F = 0.4 \times m^{0.70}$ at 25 °C, and $F = 0.5 \times m^{0.75}$ at 30 °C, where F and m are the mass of food amount and body, respectively, both in unit of grams. We designed these food restriction levels based on our previous results of food uptake rates of ad libitum larvae at each temperature. This way, the food uptake rate of the FR cohort at each temperature has roughly the same scaling power of the AL cohort at the same temperature, but the normalization coefficient, F_0 , is approximately 40 % of the AL cohort. So, FR larvae were fed 40 % of AL larvae with the same body mass at the same temperature. During the experiment, every larva in the FR cohorts finished the food every day, so the food intake is equal to the food supply.

We define the digestibility, D , as

$$D = \frac{F_{\text{dry}} \times E_{\text{food}} - \text{Dry feces} \times E_{\text{feces}}}{F_{\text{dry}} \times E_{\text{food}}} \times 100\%$$

where F_{dry} is the mass of dry food consumed by each larva during 24-h period, $F_{\text{dry}} = F_{\text{wet}} \times 20\%$, and E_{food} and E_{feces} are energy contents in dry food and dry feces, respectively, in unit of Joules/gram. To estimate digestibility, feces of five larvae from each cohort (20 from 25 °C-AL cohort) were collected each day and oven-dried at 65 °C for 72 h. In each cohort, feces samples were separated into two groups: feces produced in the first half period of fifth instar and feces in the second

half period. The energy content of the dry feces was measured by the oxygen bomb calorimeter (Grodzinski et al. 1975) (Parr 1108 combustion bomb). All samples were combusted to completion, and the temperature change of the water (2 l) was measured to the tenth of a degree. Assimilation rate (watts) was then estimated by

$$F = F_{\text{dry}} \times E_{\text{food}} \times D/86400 \quad (2)$$

where the factor, 86,400, converts the unit of days to seconds.

Growth rate

Body mass of 25 larvae in each cohort were measured at the same time every day from the first day of the fifth instar to the wandering stage to the nearest 0.1 mg on a digital microbalance (Perkin-Elmer AD6). The growth rate, in unit of watts, is defined as the increment of dry body mass from 1 day to the next multiplied by the energy content of the dry body tissue, i.e., $S = \Delta m \times E_{\text{tissue}}/86400$, where Δm , in unit of grams, is the increment of dry body mass during the 24-h period, and E_{tissue} is the energy content of dry tissue in unit of Joules/gram. To determine the dry and wet body mass ratio and the energy content of dry mass, 10 larvae were reared at 20, 30 °C-AL, 20, and 30 °C-FR in the fall of 2012. Two larvae from each cohort were killed every other day and were oven-dried at 65 °C for 72 h. The energy content of the dry body tissue was measured by the oxygen bomb calorimeter (Grodzinski et al. 1975) (Parr 1108 combustion bomb). We assumed that the dry/wet body mass ratio and the energy content of the dry mass in larvae that were reared in different seasons do not vary. Based on this assumption, we calculated the growth rate, using the data of the energy content and dry/wet mass ratio obtained from the killed larvae and the data of the daily wet mass increment obtained from the larvae reared until pupation.

Metabolic rate

We used the same method described in our previous publication to measure the metabolic rate of hornworm larvae (Hayes et al. 2014). On the first day of the fifth instar, six larvae from each cohort were randomly chosen for the respirometry measurement. The rates of O₂ consumption and CO₂ production, $V^*_{\text{O}_2}$ and $V^*_{\text{CO}_2}$, of the same larvae were measured for 7 to 10 min every day during the fifth instar until the wandering stage, using a flow-through respirometry system with an in-current flow measurement (Lighton 2008). A CA-10 CO₂ analyzer (Sable Systems International (SSI); Las Vegas, Nevada, USA) was calibrated before all trials using air running through a column of drierite/ascarite (II)/magnesium perchlorate. The analyzer was then spanned with a gas of known CO₂ concentration (1000 ppm CO₂ in air). The FA-10 Oxygen

analyzer (SSI) was calibrated using air free of CO₂ and water vapor and an assumed O₂ of 20.95 % (Lighton 2008). Baselines were taken before, in between, and after each trial by running air scrubbed of water and CO₂ through the system. Flow rate of the scrubbed air was set at 60 ml/min using an SS-4 subsampler (SSI). This air was then sent to the larva or baseline chamber. Between the CO₂ and O₂ analyzers, we scrubbed the CO₂ and water vapor produced by the larvae, so that the CO₂ and water concentration will not affect the measurement of O₂ (Lighton 2008). During the trials, temperature was controlled using a PELT5 temperature controller (SSI) that housed the respirometry and baseline chambers. Respirometry chambers for individual larvae were 60-cc syringe barrels fitted with rubber stoppers connected to intake and outlet tubing.

ExpeData software (SSI) was used to correct for the drift in CO₂ and O₂ concentration. The rates $V^*_{\text{O}_2}$ and $V^*_{\text{CO}_2}$ were calculated as $V^*_{\text{CO}_2} = fr \times [\text{CO}_2]/100$, and $V^*_{\text{O}_2} = fr \times (20.95 - [\text{O}_2]) / (100 - [\text{O}_2])$, where fr is the flow rate, and $[\text{CO}_2]$ and $[\text{O}_2]$ are the concentration of CO₂ and O₂ in the respirometry chamber (Lighton 2008). Each data point represents the average of the measurement taken during the time interval. The larval metabolic rate, B in unit of watts, was calculated as $B = (43.25 - 22.5 \times RER) \times V^*_{\text{CO}_2}/60$, where $RER = V^*_{\text{CO}_2}/V^*_{\text{O}_2}$ is the respiratory exchange ratio (Withers 1992).

Data analysis and statistics

Data on growth, food uptake, feces production, growth, and metabolism were collected and analyzed for larvae that survived to the wandering stage. The rates of food intake, feces production, and growth decrease considerably as the larvae approach times of pupation. Thus, we followed Sears et al. (2012) and restricted our analysis of these rates to the “free growth period” during which the increases in growth rate are positive (Esperk and Tammaru 2004). The growth rate of hornworms slows down and levels off toward the end of the fifth instar, making the growth trajectory a sigmoidal shape (Nijhout et al. 2006). But, during the free growth period, the growth rate increases monotonically and scales with body mass allometrically (Sears et al. 2012).

The rates of growth, S , assimilation, F , and metabolism, B , all in unit of watts, are expressed as scaling laws of dry body mass, m , in the form of $R = a \times m^d$, where R is the rate of interest, a is the scaling coefficient, S_0 , F_0 , and B_0 , and d is the scaling exponents, s , f , and b , as in Eq. 1. The scaling equation was logarithm transformed, $\text{Log}(R) = \text{Log}(a) + d \times \text{Log}(m)$, and the ordinary least square linear regression was used to estimate the scaling coefficients and exponents. Data on the rates of growth and metabolism of three cohorts, 20, 30 °C-AL, 30 °C-FR, are taken from our previous publication

for analysis and comparison (Hayes et al. 2014). We performed a full model ANCOVA with body mass as a covariate to test if there is significant interaction of two factors temperature \times food on the rates of growth and metabolism and separate ANCOVA using food supply level as a single factor to test if food restriction has significant effects on growth and metabolism within the same temperatures. Since we made multiple measurements on the same individuals repeatedly, we treated individual larvae as random factors to control for repeated measurements when performing ANCOVA. Statistical analyses were performed using SPSS 21.

Results

Assimilation rate

The energy content of feces between each cohort is not significantly different ($P > 0.05$). The average value is $E_{\text{feces}} = 14,786 \pm 616$ J/g dry mass. The digestibility weakly scales with body mass in two cohorts 20 °C-AL ($F_{1,21} = 7.106, P < 0.014$) and 25 °C-AL ($F_{1,74} = 6.336, P < 0.014$). For other cohorts ($P > 0.05$, see detailed statistics in Table 1), we calculated the average value of the digestibility over ontogeny. The scaling laws and average digestibilities are listed in Table 1. The FR cohorts at each temperature have slightly higher digestibility than the AL cohorts. The average values of the digestibilities of the AL cohorts are in agreement with previous studies (Reynolds and Nottingham 1985; Timmins et al. 1988).

Using the digestibility and Eq. 2, we estimated the assimilation rates as scaling laws of dry body mass of six cohorts (Fig. 1 and Table 2). The scaling power of the assimilation rate varies in a narrow range between cohorts reared at different food supply level and temperatures, from 0.63 for cohort 25 °C-AL to 0.83 for cohort 30 °C-AL. For the FR cohorts at each temperature, the scaling powers of the assimilation rates are the same as the powers of the food supply rate, because the digestibilities in these cohorts do not scale with body mass, and every hornworm finished supplied food every day; thus, the food intake rate equals the food supply rate. The assimilation rates of FR larvae are 43, 44, and 37 % of that of the AL fed larvae at 20, 25, at 30 °C, respectively.

Table 1 Digestibility of six food-temperature cohorts

Cohort	Digestibility
20 °C-AL	$0.744 \times m^{0.043}$ ($R^2 = 0.22; F_{1,21} = 7.106, P = 0.014$)
20 °C-FR	0.748 ± 0.092 ($N = 35; F_{1,33} = 0.112, P = 0.74$)
25 °C-AL	$0.717 \times m^{-0.047}$ ($R^2 = 0.07; F_{1,74} = 6.336, P = 0.013$)
25 °C-FR	0.80 ± 0.105 ($N = 23; F_{1,21} = 1.668, P = 0.21$)
30 °C-AL	0.74 ± 0.039 ($N = 20; F_{1,18} = 1.261, P = 0.28$)
30 °C-FR	0.80 ± 0.053 ($N = 26; F_{1,24} = 1.045, P = 0.32$)

Growth rates

The dry/wet body mass ratio is approximately 14 % in each cohort, similar to the results from previous study (Sears et al. 2012). Energy contents of dry body tissue of larvae reared at different temperature and food supply level do not vary significantly (ANOVA, $P > 0.6$ for all cohorts). So, we group the data and use the average value, $E_{\text{tissue}} = 23,693 \pm 656$ J/g dry mass, to calculate the growth rates. This average is close to the previously estimated value, 20,190 J/g (Hayes et al. 1992).

Multiplying the daily dry body mass increment by E_{tissue} , we estimated the growth rates in unit of watts as scaling laws of dry body mass in six cohorts (Fig. 2 and Table 2). Both temperature and food supply have positive effect on growth rate, in agreement with previous studies (Kingsolver and Woods 1997; Reynolds and Nottingham 1985; Timmins et al. 1988). Within the same temperatures, FR significantly reduces the normalization coefficient of growth rate, S_0 (Fig. 2 and Table 2; ANCOVA, $P < 0.001$ at all temperatures). FR increases the scaling power of growth rate at each temperature, although the increases are insignificant: from 0.82 to 0.86 at 20 °C (ANCOVA, $F_{1,158} = 0.14, P = 0.709$), from 0.51 to 0.86 at 25 °C ($F_{1,232} = 0.125, P = 0.724$), and from 0.67 to 0.77 at 30 °C ($F_{1,98} = 2.275, P = 0.137$).

Metabolic rates

Figure 3 and Table 2 show the metabolic rate as scaling laws of dry body mass in six cohorts. As predicted, within the same temperatures, food restriction causes a significant decrease in metabolic scaling powers: At 20 °C, b decreases from 0.83 to 0.19 (ANCOVA, $F_{1,132} = 38.654, P < 0.001$); at 25 °C, it decreases from 0.75 to 0.42 (ANCOVA, $F_{1,126} = 4.228, P = 0.042$), and at 30 °C, it decreases from 0.77 to 0.39 (ANCOVA, $F_{1,97} = 4.222, P = 0.044$). Food restriction also reduces the normalization coefficients of metabolic rate (ANCOVA, $F_{1,113} = 10.227, P < 0.002$ at 20 °C; $F_{1,123} = 1.277, P = 0.261$ at 25 °C, and $F_{1,95} = 17.707, P < 0.001$ at 30 °C).

Proportion of energy allocation

We used the scaling laws obtained in the previous sections (Table 2) to calculate the proportion of assimilated energy allocated to growth and metabolism, S/F and B/F , under both ad libitum AL and food restriction conditions at three temperatures. In Eq. 1, the sum of these two proportions must be one, i.e., $(S_0/F_0)m^{s-f} + (B_0/F_0)m^{b-f} = 1$. However, Eq. 1 requires all three rates, F , S , and B to be measured over the same time interval, e.g., per day. But in this study, both rates of food assimilation and growth were measured and averaged over the period of 1 day, whereas metabolic rates were measured and averaged over a time interval of 7–10 min. So, one must

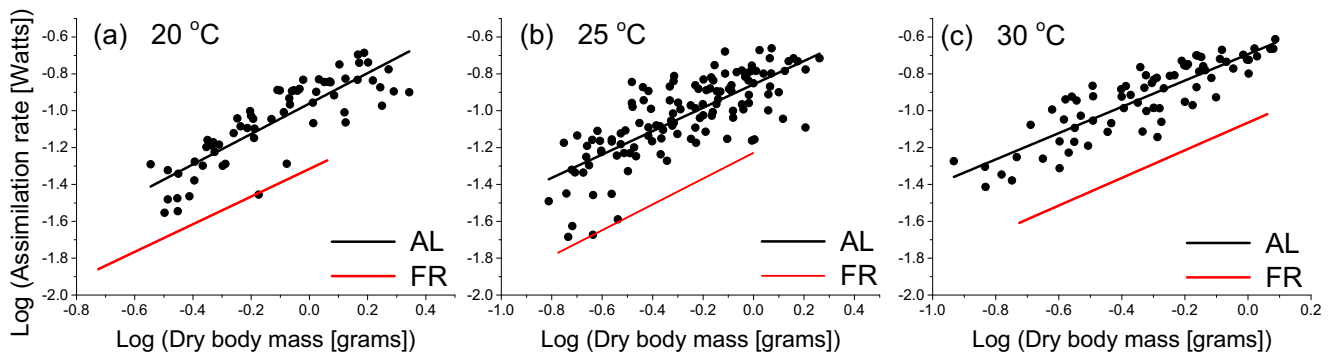


Fig. 1 Food assimilation rate in unit of watts of ad libitum (AL) and food restricted (FR) cohorts of hornworms (*M. sexta* larvae) at **a** 20, **b** 25, and **c** 30 °C. The assimilation rate is calculated from Eq. 2. In FR cohorts, every larva finished supplied food every day, so the food intake rate is exactly

equal to the supply rate, which was designed to be scaling power laws of body mass. Thus, in FR cohorts, the rates are plotted as *straight lines*, and data points are not shown. The fitted scaling laws of the AL cohorts are listed in Table 2

assume that the average value of the metabolic rate over the 7–10-min interval, as well as the rates of food assimilation and growth, are constants during the day in which they were measured, so that the “watt” values—energy per second—can be estimated. Nonetheless, for *M. sexta* larvae, such a fast growing animal, this assumption is invalid. Another way to accurately carry out Eq. 1 is to measure these rates of the same larvae multiple times every day, so that the changes in the rates during one day can be estimated. However, it was not practical for a study of ~150 larvae. This methodological problem introduces a systematic error in metabolic rate. When compared to growth and food assimilation rate, we assume that the value of metabolic rate, which is averaged over a 7–10 min period at the beginning of a day, is a constant over the whole day. However, since larvae keep growing during the rest of the day, their metabolic rate keeps increasing as body mass increases during the day. So, the value averaged over 7–10 min, which is used in Eq. 1, is smaller than the assumed constant. For this reason, the sum of S/F and B/F is smaller than 1.

We calculated the assimilation rate (F) using the equation $F=S+B$ and the measured values of S and B for each temperature-food cohort. We then plotted the calculated F against the measured F and performed the linear regression in Fig. 4. For the reasons we discussed above, the calculated F should be smaller than the measured F , and therefore, the linear regression would have a slope smaller than 1 (note, the slope in this case is the ratio of the calculated F and the measured F). The results well illustrate this point. The slopes of the six cohorts range from 0.67 to 0.97 (0.81 ± 0.125), suggesting that the sum of measured S and B is about 81 % of the measured F . The fittings are good in general, as the R^2 values are larger than 0.97 for five out of six groups of data. The data from AL-30 °C cohort has a R^2 value of 0.85, indicating that the measurement errors are relatively large in this cohort.

The inaccuracy of the metabolic rate assay, however, will not affect the scaling power of metabolic rate. Scaling power reflects the allometric relationship between the rate and body mass. As long as the body mass and the corresponding metabolic rate are measured at the same time, the scaling power

Table 2 Scaling laws of food assimilation, metabolism, and growth of ad libitum (AL) and food restricted (FR) hornworms (*M. sexta* larvae) reared at 20, 25, and 30 °C

Cohort	Metabolic rate (watts) $B=B_0 \times m^b$	Assimilation rate (watts) $F=F_0 \times m^f$	Growth rate (watts) $S=S_0 \times m^s$
20 °C-AL	$0.0159 \times m^{0.83}$ (95 % CI: 0.73, 0.93) $R^2=0.82$	$0.109 \times m^{0.83}$ (95 % CI: 0.70, 0.95) $R^2=0.73$	$0.0737 \times m^{0.82}$ (95 % CI: 0.69, 0.95) $R^2=0.71$
20 °C-FR	$0.0091 \times m^{0.19}$ (95 % CI: 0.010, 0.37) $R^2=0.05$	$0.0483 \times m^{0.75}$	$0.0315 \times m^{0.86}$ (95 % CI: 0.77, 0.94) $R^2=0.80$
25 °C-AL	$0.0246 \times m^{0.75}$ (95 % CI: 0.66, 0.85) $R^2=0.80$	$0.139 \times m^{0.63}$ (95 % CI: 0.54, 0.73) $R^2=0.58$	$0.0861 \times m^{0.51}$ (95 % CI: 0.40, 0.61) $R^2=0.43$
25 °C-FR	$0.0169 \times m^{0.42}$ (95 % CI: 0.27, 0.57) $R^2=0.32$	$0.0591 \times m^{0.70}$	$0.0399 \times m^{0.86}$ (95 % CI: 0.74, 0.98) $R^2=0.62$
30 °C-AL	$0.0257 \times m^{0.77}$ (95 % CI: 0.67, 0.86) $R^2=0.80$	$0.203 \times m^{0.71}$ (95 % CI: 0.62, 0.81) $R^2=0.75$	$0.126 \times m^{0.67}$ (95 % CI: 0.51, 0.83) $R^2=0.55$
30 °C-FR	$0.0167 \times m^{0.39}$ (95 % CI: 0.21, 0.57) $R^2=0.39$	$0.0862 \times m^{0.75}$	$0.0466 \times m^{0.77}$ (95 % CI: 0.66, 0.89) $R^2=0.80$

All m 's are dry mass in grams

The 95 % confidential interval refers to the power of the scaling laws

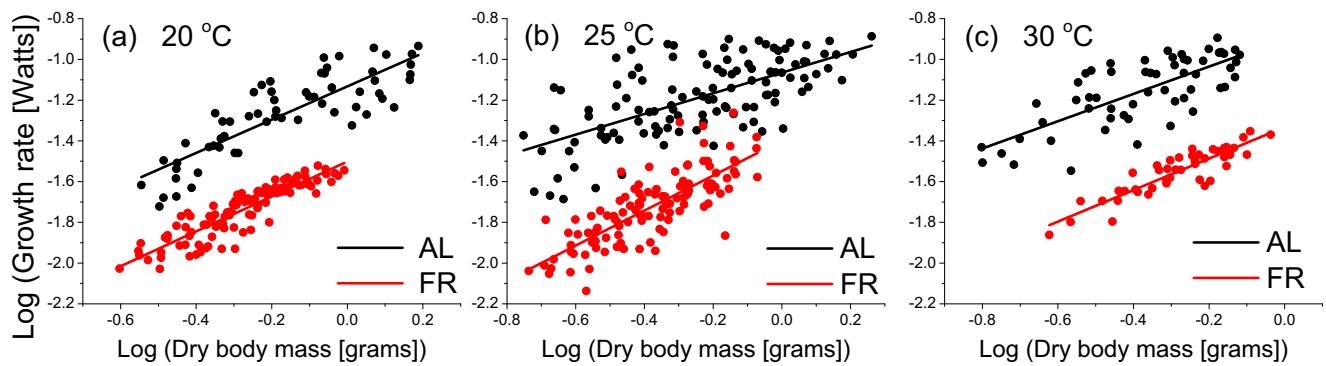


Fig. 2 Growth rate in unit of watts of ad libitum (AL) and food restricted (FR) cohorts of hornworms (*M. sexta* larvae) at **a** 20, **b** 25, and **c** 30 °C. The fitted scaling law of each cohort is listed in Table 2. (Data of cohorts

20 °C-AL, 30 °C-AL, and 30 °C-FR are from our previous publication (Hayes et al. 2014).)

will be accurate. In other words, if we had measured body mass and metabolic rate at multiple time points during a day, these points would all cluster closely around the same metabolic rate-body mass curve.

Although the accurate quantitative analysis of the proportion of energy allocation is impossible, we can still conduct a qualitative analysis, which will illustrate the salient feature of the larval energy budget and more importantly how food restriction alters the budget. In Fig. 5, we plotted the proportions, S/F and B/F , as a function of body mass during the fifth instar for both AL and FR cohorts. Under AL conditions, at each temperature, the allocation to metabolism is about 15 % of the assimilated energy at the beginning of the fifth instar and increases slightly throughout the fifth instar until the wandering stage. The energy allocation to growth at 20 °C is about 70 % at the beginning and decreases slightly throughout the fifth instar. At 25 and 30 °C, the allocation to growth decreases from 70~80 to ~60 % throughout the fifth instar.

Food restriction alters the energy allocation strategy of hornworms. The altered strategies under FR have the similar patterns at each temperature. When the FR starts, about 40 % assimilated energy is allocated to metabolism, and about 55 % is allocated to growth (Fig. 5). These proportions change as body mass increases during the fifth instar. The allocation to

growth increases as body mass at each temperature and finally reaches above 60 % before the end of free growing period, close to the value under AL. In contrast, the allocation to metabolism decreases to below 20 %, also close to the value under AL (Fig. 5).

Discussion

Species that prioritize metabolism over growth

Animals with different life histories take different energy allocation strategies to maximize their fitness under low food availability. We hypothesize that the strategies depend on whether food scarcity is relatively temporary to the species or not. In this study, we show that hornworms, larvae of a holometabolic insect with a short larval stage, prioritize growth over metabolism, so that they can reach a threshold body mass to pupate before food scarcity is over. In our experiments, all the FR larvae pupated, eclosed, and laid viable eggs. Thus, by taking this strategy, hornworms minimize the food restriction-induced harm to their fitness.

Many species, both endothermic and ectothermic, which have life span longer than the seasonal food scarcity, prioritize

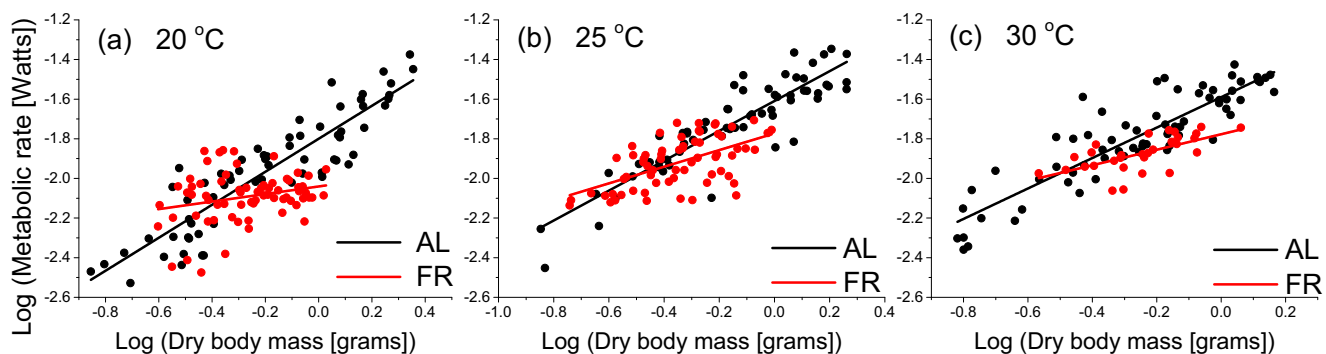


Fig. 3 Metabolic rate in unit of watts of ad libitum (AL) and food restricted (FR) cohorts of hornworms (*M. sexta* larvae) at **a** 20, **b** 25, and **c** 30 °C. The fitted scaling law of each cohort is listed in Table 2.

(Data of cohorts 20 °C-AL, 30 °C-AL, and 30 °C-FR are from our previous publication (Hayes et al. 2014).)

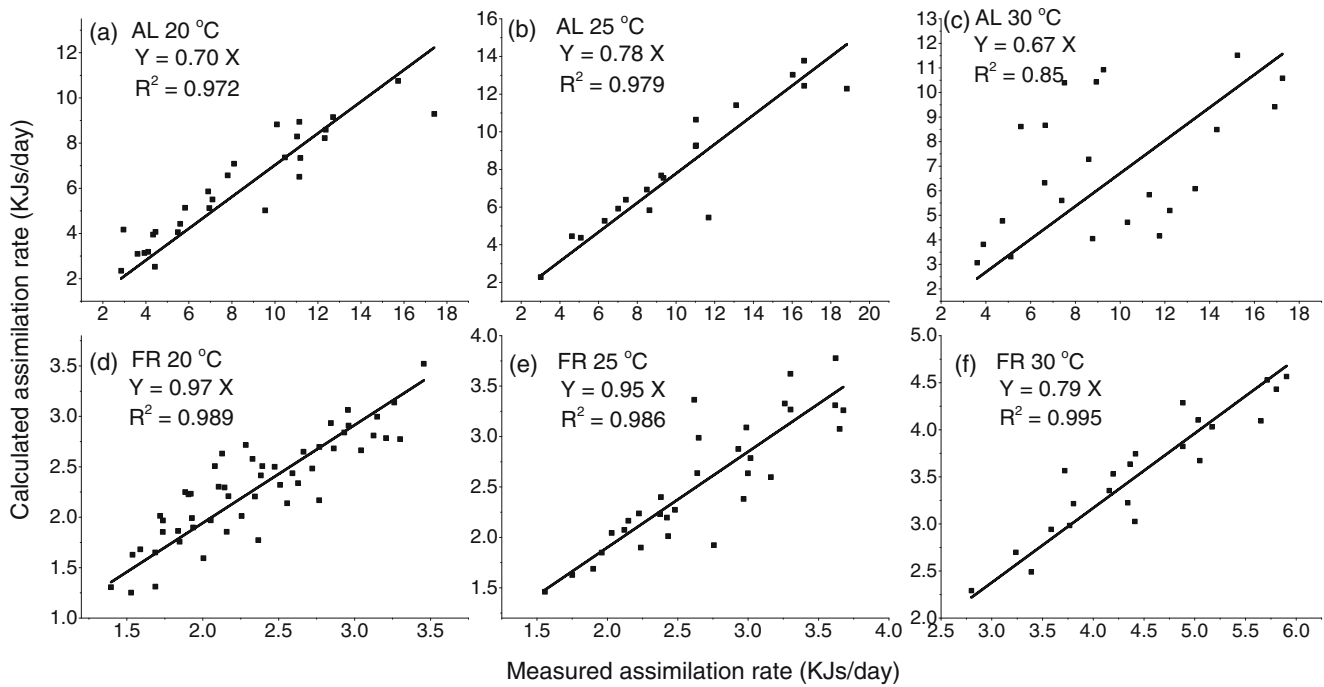


Fig. 4 The calculated assimilation rate (F) versus the empirical F . The values of F are calculated from the equation $F=S+B$, where S and B are measured growth rate and metabolic rate. The unit of these three rates are converted to Joules/day

metabolism over growth. Studies on rats (McCarter and Palmer 1992) have shown that when FR starts, the mass-specific metabolic rate decreases in the FR animals, but it quickly increases to the same level as the AL animals. The same FR-induced change in metabolic rate has been observed in several avian species (Kitaysky 1999; Konarzewski and Starck 2000; Rønning et al. 2009). The trend of changes in metabolic rate of FR mammals and birds is opposite of what we have observed in FR hornworms. Studies on “growth efficiency” also support the hypothesis. Growth efficiency is defined as body mass gain per unit of food intake and therefore is equivalent to and can be converted to the proportion of assimilated energy allocated to growth, S/F . Naim et al. (1980) have found that the growth efficiency in rats decreases at the beginning of FR, then increases for a short

period, but eventually decreases, also opposite of what has been seen in FR hornworms. The similar conclusion can be drawn from a few studies on birds growing under FR (Benyi and Habi 1998; Ocak and Erener 2005).

It has been found that some ectothermic species, such as amphipods, bivalves, and some cladocerans, also give priority to maintenance when food supply stresses directly or indirectly via effects of toxicants (Glazier and Calow 1992; Jokela 1997; Maltby 1994). Amphipods and bivalves usually live longer than the low food availability season. As to cladocerans, their life span is plastic. Numerous studies have shown that the life span of *Daphnia* can be extended by food restriction through suppressing reproduction (Pietrzak et al. 2010; Steinberg et al. 2010). Thus, unlike hornworms, which must grow to a threshold of body mass to pupate before food

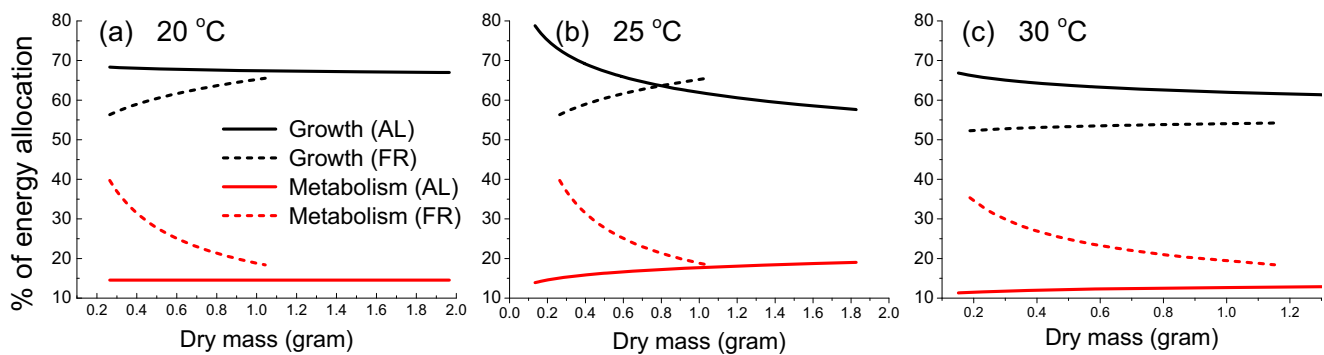


Fig. 5 Energy allocation of ad libitum (AL) and food restricted (FR) cohorts of hornworms (*M. sexta* larvae) at **a** 20, **b** 25, and **c** 30 °C. The black lines are proportions of assimilated energy allocated to growth

(solid: AL; dash: FR), and the red lines are proportions of metabolism (solid: AL, dash: FR). The allocation proportions are calculated from the scaling laws listed in Table 2

scarcity is over, these species can retard growth and postpone reproduction under food restriction.

Growing larvae have space for adaptive shift in energy budget

Although lepidopteran larvae allocate most of the assimilated energy to deposition in new biomass (S), still a significant amount is allocated to metabolism (B), which can serve as “spared resource” for an adaptive shift in energy budget. Here, we conduct a detailed analysis to illustrate it. The metabolic energy, B , can be further partitioned between three compartments, namely energy for synthesizing new biomass, B_{syn} , energy for maintaining existing biomass, B_{maint} , and energy for locomotion and other activities, B_{act} , i.e., $B = B_{\text{syn}} + B_{\text{maint}} + B_{\text{act}}$ (Hou et al. 2008). The term B_{syn} includes all the indirect costs of growth, such as assembling macromolecules from monomers, and is proportional to the direct energy deposition in new biomass (S). Combining the equation above and Eq. 1, we arrive at a complete energy budget,

$$F = S + B_{\text{syn}} + B_{\text{m}} + B_{\text{act}} \quad (3)$$

The first two terms in Eq. 3, S and B_{syn} , are energy allocated to growth (direct and indirect cost), and the last two terms, B_{maint} and B_{act} , are non-growth energy expenditures. Within the framework of Eq. 3, we can calculate the fractions of assimilated energy that hornworms allocate to growth and non-growth expenditures.

Sears et al. (2012) have estimated that it takes 1197 J to synthesize 1 g of dry biomass in the fifth instar hornworms. Recalling that the combustion energy of dry biomass in hornworm is 23,693 J/g, the ratio of indirect and direct cost of growth in fifth instar hornworm, B_{syn}/S , is 0.051. We have shown that when FR starts, 55 % of assimilated energy is allocated to the direct cost of growth (S , energy deposition in biomass), and 45 % is to metabolism (B). Using the ratio $B_{\text{syn}}/S = 0.051$, we conclude that $55\% \times 0.051 \approx 3\%$ of assimilated energy is allocated to indirect cost of growth, B_{syn} , which is included in B . Thus, when FR initiates, the energy for maintaining existing biomass and activity (the non-growth energy, $B_{\text{maint}} + B_{\text{act}} = B - B_{\text{syn}}$) is $45\% - 3\% = 42\%$, a considerable fraction, of the assimilated energy from food. Similarly, for ad libitum fed larvae, which allocate about 70 % assimilated energy to the direct cost of growth, the non-growth energy is about $30\% - 70\% \times 0.051 \approx 26\%$. This analysis shows, perhaps counterintuitively, that although the hornworm has been considered a “growing machine,” it still has plenty of “space” for channeling non-growth energy to growth, especially at the beginning of food restriction.

Our results show that FR alters the energy allocation strategy of hornworms. At each temperature, FR causes an increase in the scaling power of growth rate, but a decrease in

that of metabolic rate (Figs. 2 and 3, Table 2), agreeing with our first prediction. These results suggest that under FR, the hornworms prioritize growth over metabolism in their energy budget. The prioritization can also be seen from the FR-induced changes in the proportion of assimilated food energy allocated to growth and metabolism (Fig. 4). At each temperature, the ad libitum (AL) cohorts slightly decrease the energy allocated to growth as body mass increases during the fifth instar and increase the energy allocated to metabolism (Fig. 4). However, in the FR cohorts, as body mass increases, more and more assimilated energy is allocated to growth, whereas less and less is allocated to metabolism, agreeing with our second prediction.

Hornworm is known to have a critical weight about 6 g, at which the larvae no longer need to feed to pupate at a normal time (D'Amico et al. 2001; Davidowitz et al. 2003). If larvae no longer need to feed, would this affect their energy allocation strategies? We have two reasons to believe that it would not. First, the value of critical mass is empirically determined by complete starvation, under which larvae have no choice but to stop feeding. However, the larvae in our study did have food supply and kept growing. Since insects' fecundity is positively correlated to body size (Honěk 1993), hornworms need to reach a large body size before pupation as long as they have food supply, instead of stopping feeding at a merely minimum size for pupation. Second and quantitatively, we found that the scaling powers of growth and metabolic rates have no significant differences between larvae smaller and larger than the critical weight, 6 g (ANCOVA, $P = 0.836$ for growth, and 0.387 for metabolic rate), indicating that there is no shift in allocation strategy before and after critical weight. This analysis is based on the data from ad libitum fed larvae. For FR larvae, most of them were smaller than 6 g by the end of free growing period, so we do not have enough data point for the similar analysis. However, if critical weight would affect the energy allocation strategy in FR hornworms that nonetheless still have food supply to grow, it would also affect the strategy in AL larvae in a similar way. Our analysis on AL larvae rules out such an effect.

Consequences of different strategies in life history trade offs

Reaching a large body size at a certain age is important to organisms' fitness (Roff 2001; Stearns 1992). But, as discussed above, selection does not always favor fast growth when food supply is restricted. With the same goal of maximizing fitness, the different energy allocation strategies lead to profound differences in life history traits. Growth rate is obviously one of the traits being affected. Here, we discuss how different strategies alter the FR-induced energy trade offs and therefore affect animals' health maintenance and longevity. FR induces two types of energy trade offs. The first trade off

is between energy deposition in biomass growth (S , the direct cost of growth) and metabolism (B) via equation $F=S+B$. The second one is between biosynthesis (B_{syn} , the indirect cost of growth) and non-growth expenditures (maintenance, B_{maint} , and activity, B_{act}) via equation $B=B_{\text{syn}}+B_{\text{maint}}+B_{\text{act}}$. When animals that prioritize metabolism are under FR, their metabolism (B) keeps relatively high, and deposition in biomass (S) is largely suppressed (the first trade off). When S is reduced by FR, animals do not need to do as much biosynthesis work, so the indirect cost of growth (B_{syn}) is also reduced accordingly. With a high metabolism (B) and reduced biosynthesis (B_{syn}), the energy for maintaining existing biomass (B_{maint}) is increased (the second trade off; note: The energy for activity, B_{act} , is usually unchanged under FR, see review in (Hou et al. 2011b)). In other words, FR channels energy from biosynthetic work to health maintenance through these two trade offs. With increased B_{maint} , animals are able to achieve a better health under FR. Indeed, we have hypothesized that these two trade offs are the underlying mechanism for the well-known effect of FR on extending life span, assuming that better health is positively correlated to longevity (Hou et al. 2011a). Empirical data of life span extension from more than 200 FR studies on small rodents strongly support our quantitative predictions derived from this hypothesis (Hou 2013).

However, due to the different strategy, the holometabolic insect larvae may not benefit from FR as much as endotherms. Their strategy under FR is to maximize deposition in biomass (S) at the expense of metabolism (B). Consequently, the biosynthesis work (B_{syn}) is not suppressed as much as in mammals. Thus, we hypothesize that in this type of species, with suppressed B and not much suppressed B_{syn} , the energy for maintenance (B_{maint}) would not increase as much, and the effect of FR on health maintenance would not be as significant, as in the species that prioritize metabolism. We call for more comparative studies on species that take different strategies to test the hypothesis. Life span is a common proxy for health maintenance. However, in holometabolic insect, metamorphosis causes a series of physiological changes. It has been shown that these changes can offset the link between larval health maintenance and adult life span of holometabolous insects (Campero et al. 2008). So, to test the hypothesis, oxidative damages on macromolecules and resistance to oxidative insults are probably better surrogates for health maintenance.

Variation in metabolic scaling exponents induced by food restriction

An interesting result of this study is the broad variation in metabolic scaling (0.19–0.85) induced by the variation in food supply level (Table 2). Several theories have been proposed to explain the variation in metabolic scaling exponents. Both the dynamic energy budget theory (DEB) (Kearney and White

2012; Kooijman 2010) and the metabolic level boundaries hypothesis (MLB) (Glazier 2005) suggest that the variation stems from the fact that the metabolic scaling is the “complex result of diverse adaptations evolved in the context of both physicochemical and ecological constraints” (Glazier 2005), including oxygen and metabolite supply, temperature, food availability, predator, lifestyle, etc. Another theory, the resource transport network theory (RTN) (West and Brown 2005; West et al. 1997), assumes that the structure and dynamics of the transport network in organisms living in the normal and constant environment have evolved in such a way that the energy cost of transport is optimized to the minimum, which results in a 3/4 power for metabolic rate as a canonical value. Our results show that larvae living under the normal condition (free-feeding) have the metabolic scaling exponents close to 3/4, whereas when the environmental factor (food supply level) varies, the exponents vary broadly due to the “interrelated physiological and ecological traits,” agreeing with the suggestions of both Glazier (Glazier 2005) and West and Brown (2005).

Acknowledgments We gratefully acknowledge the careful reviews and suggestions of three anonymous reviewers. We would like to thank Dr. Rex Gerald for his help with measuring combustion energy content of larval tissue and feces, Dr. Toomas Tammaru and Dr. Douglas Glazier for their excellent suggestions that helped to develop the hypothesis, and Dr. Wenyun Zuo for her enlightening discussion.

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