2014

Energy tradeoffs between food assimilation, growth, metabolism and maintenance

Lihong Jiao

ENERGY TRADEOFFS BETWEEN FOOD ASSIMILATION, GROWTH, METABOLISM AND MAINTENANCE

by

LIHONG JIAO

A THESIS

Presented to the Graduate Faculty of the

MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN BIOLOGY

2014

Approved by

Chen Hou, Advisor
Yue-wern Huang
Robert Aronstam

PUBLICATION THESIS OPTION

This thesis has been prepared in the style utilized by the *Insect Science* and *American Naturalist*. Pages 5-18 has been published *Insect Science*. And pages 19-44 has been submitted to and is currently under review in the *American Naturalist*, including Appendices A, B and C. Page 45-63 will be revised and submitted to *Aging Cell*.

ABSTRACT

The effect of metabolic rate (MR) on organisms' health maintenance is a long-standing puzzle and empirical data on this issue is contradictory. A theoretical model was developed for understanding animal's energy budget under the food condition of *Ad libitum* (AL) and food restriction. This model offers a framework for understanding the role of MR and health maintenance mechanism from the perspective of energy tradeoff between food assimilation, growth, metabolism and maintenance. Hornworm (*Manduca sexta* larva) has been selected as an model to test the energetic tradeoff under different food supply and ambient temperatures. The changes in energy budget can reveal its health maintenance mechanism during growth. The experiments' results show that (1) under food restriction, high temperature can slow down the growth rate to compensate for the high metabolism; (2) the free-feeding larvae slightly decrease the energy allocated to growth as body mass increases, and increase the energy allocated to metabolism, while the food restricted larvae prioritize growth at the expense of metabolism; (3) during growth, the mainly reason of the accumulated damages is caused by the changes in biosynthesis instead of the changes in metabolic energy.

ACKNOWLEDGMENTS

I gratefully thank my advisor, Dr. Chen Hou for teaching me how to walk on water and his long term guidance of my research. And, I gratefully would like to thank Dr. Yue-wern Huang for offering technical support and his laboratory for my study and Dr. Robert Aronstam for his long term backup on my education. Also, I really appreciate Dr. Paul Nam's help with MDA measurement using HPLC system, instruments maintenance and valuable suggestions on method improvement and Dr. Rex Gerald for his help with measuring combustion energy content of larval tissue and feces.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

SECTION

1. GENERAL INTRODUCTION

The role of metabolic rate (MR) in animals health maintenance and longevity is unclear and empirical data on this issue is contradictory (Speakman et al. 2004). In general, inter-specific data from wild animals within the same taxon (McCoy and Gillooly 2008) show that, with a few exceptions, the ones with higher mass-specific MR have shorter lifespan. Under laboratory conditions, lowering body temperature and MR also have been shown to extend lifespan of both ectotherms (Klass 1977, Partridge, Piper and Mair 2005, Van Voorhies and Ward 1999) and endotherms (Conti et al. 2006) that were fed freely.

Based on the data from *Ad libitum* (AL) fed animals and the oxidative stress theory, it has been hypothesized (Rikke and Johnson 2004, Weindruch and Walford 1988) that lowering body temperature and metabolic rate (MR) is also one of the major mechanisms of food restriction (FR), which extends the lifespan of a broad diversity of organisms, while keeping them in a relatively healthy state (Masoro 2005, Weindruch and Walford 1988). However, numerous studies have shown that FR does not substantially decrease the mass-specific MR of mammals (see review in (Hou, Bolt and Bergman 2011d, Mccarter, Masoro and Yu 1985)). Studies on ectothermic species also found that while extending the lifespan, FR does not lower MR in them after body mass is corrected (Partridge et al. 2005, Houthoofd, Braeckman and Vanfleteren 2003, Mair et

al. 2003, Hulbert et al. 2004, Walker et al. 2005). These findings indicate that lowering MR is not crucial for FR to extend lifespan. Moreover, a few studies on mice (Liao et al. 2011b), houseflies (Cooper et al. 2004), parthenogenetic insects (Roark and Bjorndal 2009), nematodes (Houthoofd et al. 2003), and yeasts (Lin et al. 2002) have shown that under FR, MR seems to be positively correlated to health maintenance and lifespan.

The controversial correlation between metabolic rate (MR) and health maintenance has been a long-standing puzzle (Mccarter et al. 1985, Brys, Vanfleteren and Braeckman 2007, Speakman et al. 2004, Stuart and Brown 2006, Promislow and Haselkorn 2002, Hughes and Reynolds 2005). A theoretical model was developed grounded on empirical data for understanding animals' energy budget under the conditions of *Ad libitum* (AL) fed and food restriction (FR), as well as the underlying mechanisms of FR's effects (Hou et al. 2011d, Hou, Bolt and Bergman 2011c, Hou et al. 2008, Hou, Bolt and Bergman 2011b). The model suggests that the detailed energy tradeoff between growth, metabolism, and maintenance may be the key for understanding the role of MR and how FR enhances heath maintenance (Hou et al. 2011b).

The goal of this thesis is to unravel the relationship between food assimilation, growth rate, metabolic rate and health maintenance from the energetic perspective. Hornworm, *Manduca sexta*, grows from 1mg at the 1st instar stage to 15 grams at the fifth instar stage in 20 some days making it an ideal model to study animal's energetics during growth under laboratory condition. This thesis consists of three related projects to investigate that how hornworm adjusts its energy budget to adapt different food supply and environmental temperatures, and how the changes in energy budget affect its health maintenance.

In the first project, the energy tradeoffs ware studied in hornworms under food restriction. It has been well know that when fed ad libitum (AL), ectothermic animals usually grow faster and have higher metabolic rate at higher ambient temperature. However, food restriction (FR) condition, may impose an energy tradeoff between growth and metabolism. We measured the rates of growth and metabolism of four cohorts of 5th instar hornworms (Manduca sexta larvae) reared at two levels of food supply (AL and FR) and two temperatures (20 ^{0}C and 30 ^{0}C). Our results show that, compared to the cohorts reared at 20 ^{0}C, the ones reared at 30 ^{0}C have high metabolic rates under both AL and FR conditions, but a high growth rate under AL and a low growth rate under FR were observed. Our results indicate that for ectothermic animals under food restriction (FR), high temperature can lead to a high metabolic rate, but growth can slow down to compensate for the high metabolism.

Second, a simple theoretical model was developed, based on conservation of energy and allometric scaling laws, for understanding the dynamic energy budget of growing hornworms under food restriction. We test the model by manipulative experiments on 5th instar hornworms at three temperatures (20 ^{0}C, 25 ^{0}C and 30 ^{0}C). At each temperature, food restriction increases the scaling power of growth rate, but decreases that of metabolic rate, as predicted by the model. During the 5th 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 metabolism. The opposite trends were observed in food restricted larvae, indicating that insect larvae prioritize growth at the expense of metabolism.

Third, experiments have been conducted to investigate how the energy tradeoffs between growth, metabolism, and maintenance affect hornworm's health maintenance. Oxidative metabolism causes various forms of molecular and cellular damages that are associated with the health maintenance. During growth, a fraction of metabolic energy is allocated to new biomass synthesis. It has been shown that changes in biosynthesis also induce damage accumulation. However, all the existing studies only investigated the collective effects of metabolism and biosynthesis on damage accumulation during growth. It remains unclear how each of these biological processes plays a role in causing damage. A model was developed based on the first principle of energy conservation to disentangle the effects of changes in biosynthetic and metabolic rate on the total accumulated damage from an energetic perspective. The model predicts that during growth, the changes in damage are mainly caused by the changes in biosynthesis, whereas the consequences of the changes in metabolic energy are insignificant. We then test the model by experiments on the 5^{th} instar hornworms. We manipulated the biosynthesis and metabolism of hornworms by rearing them at different food supply levels, and assayed the phospholipid oxidative damage. The empirical results strongly support the predictions of the model.

PAPER

I. HIGH TEMPERATURE SLOWS DOWN GROWTH IN TOBACCO HORNWORMS (MANDUCA SEXTA LARVAE) UNDER FOOD RESTRICTION

Abstract

When fed *ad libitum* (AL), ectothermic animals usually grow faster and have higher metabolic rate at higher ambient temperature. However, if food supply is limited, there is an energy tradeoff between growth and metabolism. Here we hypothesize that for ectothermic animals under food restriction (FR), high temperature will lead to a high metabolic rate, but growth will slow down to compensate for the high metabolism. We measure the rates of growth and metabolism of four cohorts of 5^{th} instar hornworms (*Manduca sexta* larvae) reared at two levels of food supply (AL and FR) and two temperatures (20 and 30 ^{o}C). Our results show that, compared to the cohorts reared at 20 ^{o}C, the ones reared at 30 ^{o}C have high metabolic rates under both AL and FR conditions, but a high growth rate under AL and a low growth rate under FR, supporting this hypothesis.

INTRODUCTION

Ontogenetic growth, an energetically costly process, is fueled by metabolism (Wieser 1994). Understanding the relationship between growth and metabolism has been a central theme in ecological physiology (Sibly and Calow 1986, Karasov and del Rio 2007), and it requires a framework of animals' energy allocation strategy. During growth,

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 (Brody 1945, Hou et al. 2008, Kooijman 2000, van der Meer 2006) , i.e.,

$$F = S + B \tag{1}$$

For ectothermic animals, food availability and ambient temperature are two major environmental factors that largely influence their energy budget (Lee and Roh 2010, Atkinson 1994, Zuo et al. 2012, Miller et al. 2009). When ectothermic animals are fed with unlimited food (*ad libitum*, AL), high temperature induces an increased metabolic rate, *B* (Gillooly et al. 2001). Along with metabolism, the growth rate increases with temperatures (Gillooly et al. 2002, Zuo et al. 2012, Atkinson 1994). Thus, under AL condition the rates of metabolism and growth are positively correlated. The temperature-induced increase in the rates of metabolism and growth is known as the Q10 effect, referring to the increase in the growth and metabolic rate for a 10^{o}C increase in temperature, and usually takes on values between 2 and 3 (Gillooly et al. 2001), but sometime below 2 (Hack 1997, Chappell 1983). The increased energy requirements are met by the increased food uptake rate until the capacity of an animal's digestive system reaches its limit (Hammond and Diamond 1997). However, the correlation between metabolism and growth may not always be positive when temperature increases (Diamond and Kingsolver 2010, Clissold, Coggan and Simpson 2013). When the food availability is limited and lower than AL level, Eq. 1 ($F = S + B$) suggests an energy tradeoff between growth, *S*, and metabolism, *B* (Hou et al. 2011d). For a given body mass, if *F* is limited, then any change in either *S* or *B*, due to environmental factors such

as temperature, must cause a change in the other in the opposite direction. Since the metabolic rate of ectothermic species increases with ambient temperature, we hypothesize that in ectothermic animals fed with a fixed food supply lower than the AL level, high temperature will lead to a reduced growth rate. We use the 5th instar tobacco hornworms (*Manduca sexta* larvae) as a model to test this hypothesis. The 5th instar hornworm grows from 1~2 grams to 7~15 grams in 6~10 days depending on the temperature and food level, making it an ideal model to study growth (Kingsolver and Woods 1997, Reynolds and Nottingham 1985).

MATERIAL AND METHODS

Animal Rearing. In the summer of 2012, we raised approximately 100 tobacco hornworms (*Manduca sexta* larvae) from eggs obtained from Carolina Biological supply (NC) *ad libitum* on a long day cycle (17 hours light:7 hours dark) at 25°C until the 5th instar. On the first day of the 5th instar, we randomly separated the larvae into two incubators, which were set at temperatures 20°C and 30°C respectively. At each temperature, we fed the larvae at two food supply levels, *ad libitum* (AL) and food restriction (FR) (see below). We therefore had four cohorts of larvae (2 temperatures × 2 food level), which were labeled as 20°C-AL, 20°C-FR, 30°C-AL, and 30°C-FR. Each cohort consisted of ~ 25 larvae. Each larva was reared in an individual plastic clear vial (diameter: 5 cm; length: 12 cm).

Growth Rate. We measured the body mass of each larva in every cohort at approximately the same time every day from the first day of the 5th instar to the nearest

0.1 mg, using a digital microbalance (Perkin-Elmer AD6). We define the growth rate, in unit of gram/day, as the increment of body mass from one day to the next.

Food Supply Levels. After weighing the larval body mass, we fed the larvae with a wheat germ-based diet (hornworm medium bulk diet, Carolina Biological supply, NC). The AL cohorts fed freely, and we measured the food uptake rate of every larva every day. During the experiment, no larva in the AL cohorts ran out of food. For both FR cohorts at 20 ^{o}C and 30 ^{o}C, we fed each larva with the amount of food calculated from the equation $F = 0.5 \times m^{0.75}$, where F is the amount of food and m is the body mass, both in units of grams. Food supply was weighted to the nearest 1 mg. Our previous data on food uptake rate of AL larvae suggest that this food restriction level is well below AL for larvae reared at both 20 ^{o}C and 30 ^{o}C. The data from this study also confirm this. In this study, the food uptake rate of AL-fed cohorts scale with body mass as $F = 1.313 \times m^{0.74}$ (R^2 = 0.76) at 30^{o}C and $F = 0.622 \times m^{0.78}$ (R^2 = 0.71) at 20^{o}C. We used the same equation, $F = 0.5 m^{0.75}$, to feed both 20°C-FR and 30°C-FR cohort, because the food restriction level needs to be the same at both temperatures to test the hypothesis. During the experiments, every larva in the FR cohorts completely finished its food every day.

The higher temperature causes higher water loss in food. Although FR larvae at both temperatures obtain the same amount calories every day, the water content in diet affects the growth and metabolic rate of hornworms. Martin and Van't Hof (1988) have shown that the growth efficiency (body mass gain per food intake) is 12% lower, and metabolic rate is 16% higher, in the hornworms fed on a diet containing 65% water compared to the ones on an 82% water diet. To measure the water evaporation, at each temperature we prepared five food samples with the similar mass and shape as the food

given to the larvae, and placed the samples in the vials that the larvae were reared in. We then calculate the percentage of water loss in diet after 12 hours and 24 hours.

Metabolic Rate. We used equipment from Sable Systems International (SSI; Las Vegas, Nevada, USA) to perform the flow-through respirometry with an incurrent flow measurement (Lighton 2008). Before all trials, we calibrated a CA-10 CO_2 analyzer (SSI) with air run through an ascarite column and then spanned it with a gas of known CO_2 concentration (1,000 p.p.m. CO_2 in N_2 ± 1). We then calibrated an FA-10 Oxygen analyzer (SSI) with water and CO_2 scrubbed air at 20.95% (Lighton 2008). A baseline measurement was taken before, between, and after each experimental trial by running air scrubbed of water and CO_2 through an empty chamber and then into the respirometry system. We set flow rate at 60 ml min^{-1} using an SS-4 subsampler (SSI). This air was then sent to the larva or baseline chamber. Between the CO_2 and O_2 analyzers, we scrubbed the CO_2 produced by the larvae by a column of ascarite magnesium perchlorate so that the CO_2 concentration will not affect the measurement of O_2. Temperature was controlled using a pelt-5 temperature controller (SSI) that houses the respirometry and baseline chambers. Respirometry chambers for individual larvae were 60-cc syringe barrels fitted with rubber stoppers. We randomly chose six larvae from each cohort on the first day of the 5^{th} instar, and used the same individuals for the respirometry measurement every day until the wandering stage. The rates of O_2 consumption and CO_2 production, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, of each larva were measured for 7-10 minutes time interval every day after their body mass was measured.

We used SSI ExpeData software (SSI) to correct for drifts in CO_2 and O_2 concentration. The rates $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated as $\dot{V}_{CO_2} = FR \times [CO_2]/100$, and

$\dot{V}_{O_2} = \mathrm{FR} \times (20.95 - [O_2]) / (100 - [O_2])$, where FR is the flow rate, and $[CO_2]$ and $[O_2]$ are the concentration of CO_2 and O_2 in the respirometry chamber (Lighton 2008). Each data point represents the average of the measurement taken during the time interval. The larval metabolic rate (in unit of watts) was calculated as $B = (43.25 - 22.5 \times RER) \times \dot{V}_{CO_2} / 60$, where $RER = \dot{V}_{CO_2} / \dot{V}_{O_2}$ is the respiratory exchange ratio (Blaxter 1989, Withers 1992).

Data Analysis and Statistics. Data on metabolic rate (*B*) was collected and analyzed every day for the same six larvae in each cohort from the first day of the 5th instar to the wandering stage. The data on food intake (*F*) and growth (*S*) was collected from all the larvae in each cohort that were alive at the end of the experiment. Mortality rate was between 10~20% among cohorts, so the data on *F* and *S* were from 20~23 individuals in each cohort every day. Larvae decrease their food intake and growth rate considerably as they approach the peak mass (Sears et al. 2012, Esperk and Tammaru 2004). Thus we followed Sears et al. (2012) and restricted our analysis of the rates of food intake (*F*) and growth (*S*) to the "free growth period", during which the increase in growth rate is positive. All three rates, *F*, *S*, and *B*, are expressed as scaling power laws of body mass (Sears et al. 2012, Greenlee and Harrison 2005), in the form of $R = a \times m^d$, where *R* is the rate of interest, *a* is the scaling coefficient, *d* is the scaling power, and *m* is the body mass. The scaling equation was logarithm transformed, $Log(R) = Log(a) + d \times Log(m)$, and the ordinary least square linear regression was used to estimate the scaling coefficients and powers. Statistical analyses were performed using SPSS 20. We performed a full model ANCOVA with body mass as a covariate to test if there is significant interaction of two factors temperature×food on the rates of growth and

metabolism. We then conducted separate ANCOVA using temperature as a single factor to test if within the same diet regime temperatures have significant effects on growth and metabolism.

RESULTS

Metabolic Rate. For AL cohorts, the metabolic rate scales with body mass as $B_{30\,^{o}\text{C-AL}} = 0.00568 \times m^{0.77}$ (R^2 = 0.80) at 30 ^{o}C, and $B_{20\,^{o}\text{C-AL}} = 0.00309 \times m^{0.83}$ (R^2 = 0.82) at 20 ^{o}C (Fig. 1A). For food restricted (FR) cohorts, the metabolic rate scales with body mass as $B_{30\,^{o}\text{C-FR}} = 0.00775 \times m^{0.39}$ (R^2 = 0.39) at 30 ^{o}C and $B_{20\,^{o}\text{C-FR}} = 0.00467 \times m^{0.46}$ (R^2 = 0.43) at 20 ^{o}C (Fig. 1B).

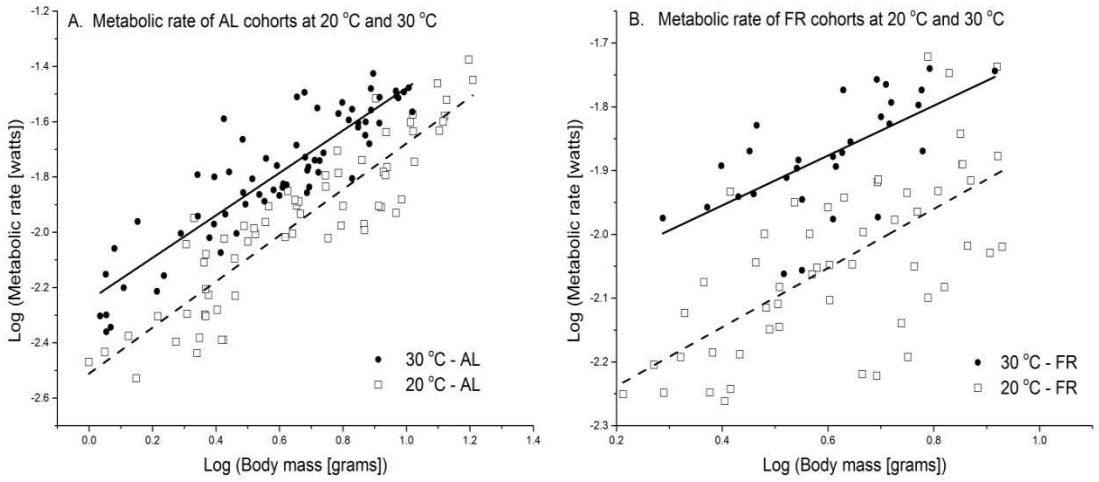

Figure 1. The Effects of Temperature on Metabolic Rates in Ad Libitum (AL) and Food Restricted (FR) M. Sexta Larvae. Within the same diet regime, the slopes of metabolic rate are the same at different temperatures (ANCOVA, $P > 0.05$), but the intercept is higher at the higher temperature (ANCOVA, $P < 0.05$). There is no interaction of temperature×food (ANCOVA, $F_{1,210} = 2.507$, $P = 0.115$).

The full model ANCOVA shows that there is no significant interaction of temperature×food on metabolic rate ($F_{1,210}$ = 0.135, P = 0.714). Within the same diet regime, different temperatures have no significant effect on the slopes of the metabolic rates (ANCOVA, $F_{1,131}$ = 1.574 and P = 0.212 for AL cohorts; $F_{1,82}$ = 0.009 and P = 0.598 for FR cohorts). But within the same diet regime, the intercept of the metabolic rate significantly increases at high temperature. In AL cohorts, $B_{30\,^{o}\text{C-AL}}$ is about 1.70-fold higher than $B_{20\,^{o}\text{C-AL}}$ (Q10 =1.70, ANCOVA, $F_{1,131}$ = 126.31, P < 0.001); and in FR cohorts, $B_{30\,^{o}\text{C-FR}}$ is 1.50-fold higher than $B_{20\,^{o}\text{C-FR}}$ (Q10 = 1.5; ANCOVA, $F_{1,82}$=69.39, P < 0.001).

Growth Rate. For growth rate, there was a significant interaction of temperature×food (ANCOVA, $F_{1,258}$ = 122.042, P < 0.001). Within the same diet regime (AL or FR), temperature has no significant effect on the slope of growth rate (ANCOVA, $F_{1,117}$ = 0.556 and P = 0.457 for AL cohorts; $F_{1,143}$ = 1.824 and P = 0.179 for FR cohorts). For AL-fed animals, Fig. 2A shows that the growth rate of the cohort 30 ^{o}C-AL scales with body mass as $S_{30\,^{o}\text{C-AL}} = 0.909 \times m^{0.64}$ (R^2 = 0.51), 2.43-fold higher than the cohort 20 ^{o}C-AL $S_{20\,^{o}\text{C-AL}} = 0.386 \times m^{0.62}$ (R^2 = 0.71) (ANCOVA, $F_{1,117}$=118.063, P < 0.001). However, opposite to what is observed in the AL-fed cohorts, Fig. 2B shows that the growth rate of the 20 ^{o}C-FR cohort, scaling as $S_{20\,^{o}\text{C-FR}} = 0.323 \times m^{0.68}$ (R^2 = 0.87), is 1.07-fold higher than the 30 ^{0}C-FR cohort (ANCOVA, ANCOVA, $F_{1,143}$=10.61, P < 0.001), which scales as $S_{30\,^{o}\text{C-FR}} = 0.265 \times m^{0.77}$ (R^2 = 0.80).

The percentages of water loss after 12 hours are 3.11% ± 0.66% and 6.55% ± 2.10% at 20 ^{o}C and 30 ^{o}C, respectively. After 24 hours, the water losses are 4.43% ±

0.42% and 9.81% ± 2.41% at 20 ^{o}C and 30 ^{o}C respectively. The sample size is five at each temperature.

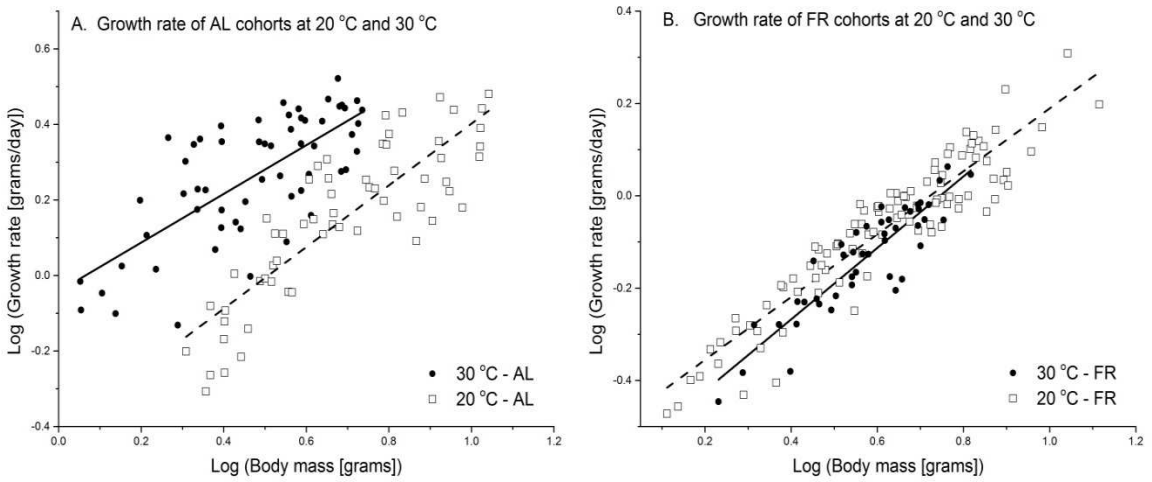

Figure 2. The Effects of Temperature on Growth Rates in Ad Libitum (AL) and Food Restricted (FR) M. Sexta Larvae. With the same regime, the slopes of growth rate are the same at different temperatures (ANCOVA, $P > 0.05$). The intercept is higher at higher temperature under AL (Panel A), whereas it is lower at higher temperature under FR (ANCOVA, $P < 0.05$) (Panel B). There is a significant interaction of temperature×food (ANCOVA, $F_{1,258} = 122.042$, $P < 0.001$).

DISCUSSION

In this study we are interested in how increasing temperature affects the rates of growth and metabolism of food restricted hornworms fed with the same food supply level. In *ad libitume* (AL) larvae, 10 ^{o}C increase in temperature leads to a 1.7-fold increase in metabolic rate (Fig. 1), in agreement with the general Q10 effect (Gillooly et al. 2001, Chappell 1983, Hack 1997). With the increasing temperature, the larvae increase food uptake by 2-fold, obtaining more energy to meet the increased metabolic requirement. The similar temperature-induced increase in the food uptake rate in AL *M.*

sexta larvae has been observed previously (Kingsolver and Woods 1997, Reynolds and Nottingham 1985). In AL larvae, the higher temperature also leads to a higher growth rate (Fig.1b) as expected (Gillooly et al. 2002, Atkinson 1994).

In food restricted (FR) larvae, the 10 ^{o}C increase in temperature also causes an increase in metabolic rate to a lower degree—1.5-fold. However, under FR condition, the high temperature induces a 1.08-fold lower growth rate. Statistically, there is a significant temperature×diet interaction for growth rate (ANCOVA, $F_{1,258} = 122.042$, $P = 0.000$), so that rising temperature increases growth under AL condition, but decreases it under FR condition. The interaction of temperature and diet is insignificant for metabolism (ANCOVA, $F_{1,210} = 2.507$, $P = 0.115$), and rising temperature increases metabolic rate regardless of diet regimes. Our hypothesis predicts the insignificant temperature×diet interaction for metabolism, as well as, the significant interaction for growth. The metabolic rate of ectotherms always increases with the ambient temperature (Gillooly et al. 2001). The higher metabolic rate comes with a high cost in terms of resources and energy from food. With a fixed food supply, it is inevitable that less resource and energy is available for growth. Thus, this tradeoff results in a slower growth rate at higher temperature (Fig. 2).

The tradeoff between growth and metabolism and the consequential suppression of growth at high temperature may also be enhanced by the prolonged starvation time at high temperature. The higher metabolism leads to faster food intake. We do not have accurate data on feeding behavior to conduct a rigorous statistical comparison on the feeding times between FR cohorts at different temperatures. But, FR larvae at 30 ^{o}C finished their food less than 8~10 hours on average, whereas the ones at 20 ^{o}C spent more

than 17~18 hours. So, FR larvae at 30 ^{o}C experienced a longer starvation time than the ones at 20 ^{o}C during every 24-hour period. Prolonged starvation may cause mobilization of reserves accumulated in fat bodies, and mass loss. Thus, the retarded net growth in the FR larvae at 30 ^{o}C (body mass gain – body mass loss during the 24-hour period) is aggravated by the longer starvation. In this case, the tradeoff between growth and metabolism reach an extreme degree, i.e., larvae not only allocate less energy to growth, but also have to mobilize bio-tissue (negative growth) to provide energy to match the increased metabolism when the energy from food is limited.

The differences in the growth rate between the FR cohorts at two temperatures are not likely caused by the difference in water losses in food at the different temperatures. Our results show that the FR larvae at 30 ^{o}C finished food less than 8~10 hours, and the water loss in 12 hours at 30 ^{o}C is 6.55% ± 2.10%; the FR larvae at 20 ^{o}C spent 17~18 hours on feeding, and the water loss at 20 ^{o}C in 24 hours is 4.43% ± 0.42% . Thus, the difference between the water losses in the food that was consumed by the larvae at both temperatures is about 6% – 4% ≈ 2 %. In Martin and Van't Hof's study on hornworms (1988),17% difference in water contents in diet causes 12% and 16% differences in growth and metabolism, respectively. So, we believe that the 2% difference in our study is negligible.

The energy tradeoff between growth and metabolism has been observed in other insect species. Lee and Roh (2010) analysed the interactive effects of temperature and nutrition on growth rate in the final instar beet armyworm (caterpillar of *Spodoptera exigua*), which were reared at one of three temperatures (18, 26, and 34 °C), and received one of six diets differing in their ratio of protein and carbohydrate (P:C). They found that

for rates of food intake and growth there was a significant temperature × diet interaction, so that the difference in these rates between temperatures was greatest on moderate P:C diets and least on the most extreme diets (extremely high and low P:C), which are considered severe deficiencies of energy and protein respectively. The authors stated "the mechanisms remains to be elucidated but severe energy and protein deficiency resulting from eating these diets seem likely." We believe that the tradeoff between growth and metabolism revealed by our study can explain Lee and Roh's results. At balanced diet (moderate P:C diet), the food intake rates of armyworm are relatively high at all temperatures, which is similar to free-feeding in our study. Thus, growth increases with temperature, as also seen in our study, and the authors observed large differences in growth rate between temperatures. When diet has deficiency of either energy or protein (imbalanced P:C ratio), the food intake of armyworm is low at all temperatures, similar to the food restriction in this study. Because of the high metabolism at high temperature, relatively less resources and energy was allocated to growth in armyworms at high temperature, so that growth is suppressed at high temperature, and authors observed the smallest difference in growth rate between temperatures. The authors proposed: "this situation is expected to be aggravated when metabolic rate increases as a function of temperature," but they did not measure the metabolic rate of the caterpillars. By measuring rates of growth and metabolism, our study explicitly reveals the tradeoff between them, and therefore supports Lee and Roh's speculation.

With a different purpose, a study of Miller et al. (2009) indirectly showed the tradeoff in locusts fed *ad libitum* (AL). The authors measured thermal preferences in migratory locust (Locusta migratoria) and investigated growth efficiency (conversion of

ingesta to body mass) at different temperature and diet regimes. Locusts were fed with diets of high-protein, high-carbohydrate, or a choice between both. The authors found that locusts placed in a thermal gradient selected temperatures near 38°C, maximizing rates of weight gain. But at this temperature protein and carbohydrate were poorly converted to body mass, compared to the intermediate temperature (32°C). The authors concluded "body temperature preference thus yielded maximal growth rates at the expense of efficient nutrient utilization." Within the framework developed in our study, the growth efficiency (or nutrient utilization efficiency) is equivalent to *S*/*F*, the ratio of growth to food intake, which is equal to $(F-B)/F$ by the virtue of Eq. 1. The observation that growth is higher, but the efficiency is lower at higher temperature in free-feeding locust indicates that as temperature increases, the percentage increase in metabolic rate, *B*, is faster than the percentage increases in food intake rate, *F*, so that the ratio $(F-B)/F$ is lower at the high temperature. The temperature induced mismatches between the rates of metabolism and food intake (faster increase in *B* but slower increase in *F* as temperature increases) have been seen in many free-feeding ectotherms (Lemoine and Burkepile 2012, Kearney and White 2012). Analysing the mechanisms underlying the mismatch is beyond the scope of this paper, and we refer to the recent publication of Lemoine and Burkepile (2012) for detailed discussion. In our study, the growth rate and growth efficiency in free-feeding larvae both increase as temperature. Using our data on the rates of growth and food intake of free-feeding larvae at 20 and 30 ^{o}C, we found that the growth efficiency (*S*/*F*) is about 57% at 20 ^{o}C on average, and increases to 61% at 30 ^{o}C, opposite to Miller et al's study on locusts. The reason that we did not observe the mismatch between the rates of metabolism and food intake is because it usually occurs at

extremely high temperatures. In a study on hornworms, Kingsolver and Woods (1997) investigated the thermal sensitivity of growth and feeding with a temperature range from 14 to 42 ^{o}C. When temperature is above 34 ^{o}C (higher than that in our study), the mismatch was observed. In Miller et al's study (2009), the temperature, at which mismatch was seen, was 38 ^{o}C, also higher than that in our study.

In conclusion, through a simple experiment we show that due to the tradeoff between growth and metabolism, when food supply is fixed and below *ad libitum* level, growth rate is negatively correlated to ambient temperature in hornworm, opposite of what has been observed in free fed insect larvae.

II. FOOD RESTRICTION-INDUCED ALTERNATION OF ENERGY ALLOCATION STRATEGY DURING ONTOGENY: A CASE STUDY OF TOBACCO HORNWORMS (MANDUCA SEXTA LARVAE)

Abstract

Growing animals must alter their energy budget in the face of environmental changes, and prioritize the energy allocation to metabolism and growth. We hypothesize that when food availability is low, larvae of holometabolic insects with a short development stage prioritize 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 5^{th} 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 5^{th} 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 metabolism. The opposite trends were observed in food restricted larvae, indicating the predicted prioritization in the energy budget under food restriction. This is the first study that uses the allometric scaling laws to reveal the dynamic changes of growing animals' 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.

INTRODUCTION

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 requirement, such as maintenance of existing biomass, biosynthesis, defense and forage (Brody 1945, Kooijman 2010, Hou et al. 2008). The former is the combustion energy stored in bio-tissues, and the latter is dissipated as heat. The energy allocation strategy often exhibits phenotypic plasticity. In the face of environmental changes, such as fluctuating quantity and quality of diet, animals are able to adjust their energy budgets and prioritize the energy allocation to growth and metabolism (Schoener 1971, Hou et al. 2011d, Roff 2001). Generally an animal's body mass is positively correlated to its fecundity (Charnov 1993, Honěk 1993), so, when all else kept equal (such as temperature, predation risk), maximizing growth and body mass would maximize animal's fitness. However, here we argue that when the food supply is low, allocating relatively more energy to growth may not be favored by selection in some animals. We hypothesize that animals with different life histories take three different strategies: (i) prioritizing metabolism at the expense of growth, (ii) prioritizing growth at the expense of metabolism, and (iii) equally suppressing both metabolism and growth.

Endotherms may take strategy (i) for three reasons. First, they need to invest a certain amount of energy to metabolism to keep the body temperature homeostasis. Empirical data show that even under severe food restriction (FR), body temperature is only lowered by 2-3 ^{o}C in mice, and ~0.5 ^{o}C in larger mammals (see review in (Hou et al. 2011d). Second, the non-hibernating species need to allocate energy to foraging when

facing food scarcity. In fact, mammals under FR keep the same activity level as their *ad libitum* (AL) fed counterparts (see review in (Hou et al. 2011d)). Third and perhaps the more important, the low food availability period is relatively temporary to endotherms. This is because their lifespans are usually longer than the season of low food availability, and they are able to search for new food sources, actively ending the food scarcity. Taking all these reason into account, if endotherms under FR retard growth, and allocate more energy to metabolism to maintain the existing biomass and keep good health, they can resume growth after the *temporary* food scarcity is over (compensatory growth (Mangel and Munch 2005, Broekhuizen et al. 1994, Dmitriew 2011)). This way their reproduction is delayed, but due to the high investment in maintenance, they have low mortality and high-quality offspring, and therefore the overall fitness will not be undermined.

A hypothesize is that ectotherms with a short development period, such as holometabolic insects with short larval stage, may take strategy (ii). Larvae of holometabolic insects must grow and reach a threshold size to successfully pupate, and then eclose, mate and reproduce (Davidowitz, D'Amico and Nijhout 2003, Nijhout 1975). Moreover, most insect larvae are not able to leave the poor environment (such as a host plant), searching for new sources. With short larval stages and inability to leave the poor environment, food scarcity for them is almost permanent, instead of temporary. 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 can not be ended by active foraging. In contrast, keeping fast growth under FR at the cost of low maintenance would be favored by selection, because this way 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).

Strategy (iii) may be taken by ectotherms with a long development period, such as hemimetabolic insects whose larval stage lasts several months. This is because, unlike endotherms they do not need to keep a high metabolic rate in order to maintain body temperature homeostasis, but unlike ectotherms with short development, they can resume growth after the low-food supply period, and therefore do not have to keep a high growth rate under FR.

Note, some species can enter diapause stage, during which the rates of food uptake and growth are nearly zero (Hahn and Denlinger 2011, Koštál 2006). In this paper, we only focus on 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 endotherms adjust their energy budgets under food restriction (FR). But as far as we know, no study has been conducted on the larvae of holometabolic insects, which may take strategy (ii). In this paper, a simple theoretical model was first developed, based on conservation of energy and allometric scaling laws, for understanding the dynamic energy budget of growing animals under FR. Then the prediction derived from the hypothesis by manipulative experiments of FR was tested on the 5^{th} instar tobacco hornworms (the last instar of *Manduca sexta* larvae). Depending on the ambient temperature and food supply level, the 5^{th} instar hornworms grow from ~1 gram to ~12 grams in 5~10 days before pupation. Its short

larval stage and incapability 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, Kooijman 2000, Kearney and White 2012). 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., $F = F_0 m^f$, $B = B_0 m^b$, and $S = S_0 m^s$, where F_0, B_0, and S_0 are normalization constants, and *f*, *b*, and *s* are scaling powers (Sears et al. 2012, Greenlee and Harrison 2005). The rigorously mathematic form of 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 scaling law. However, in biological studies, all of the allometric scaling powers are obtained from statistical fitting of empirical data. The numerical simulations show that if the scaling powers and the normalization coefficients of *B* and *S* vary, the numerical values of *F* generated by the equation $F = B_0 m^b + S_0 m^s$ can be well fitted as a scaling function with high r^2 values (Fig. S1 in Appendix A). So, although the powers may be different, these three rates can still be expressed as scaling

functions of body mass approximately as $F_0 m^f \approx S_0 m^s + B_0 m^b$. 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 both sides of this equation are divided by the assimilation rate, $F = F_0 m^f$, and have

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

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 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.

Now food restriction (FR) is applied to animals by decreasing the coefficient, F_0, but keeping the scaling power, f, the same. When FR starts, rates of both growth and metabolism must decrease as a response to the suddenly lowered food supply. This means that both coefficients (the intercepts), S_0 and B_0, decrease (Fig. 1A). If animals prioritize one rate over the other under FR (strategy i and ii), then the only way to increase the energy allocation to the prioritized rate is to increase its scaling power (Fig. 1A). With a fixed scaling power, *f*, and an increased power of the prioritized rate, Eq. 1 predicts that the scaling power of the non-prioritized rate must be decreased in FR animals, compared

to the *ad libitum* (AL) controls (Fig. 1A). In animals that do not prioritize either rate (strategy iii), the scaling powers of them will keep unchanged under FR (Fig. 1A).

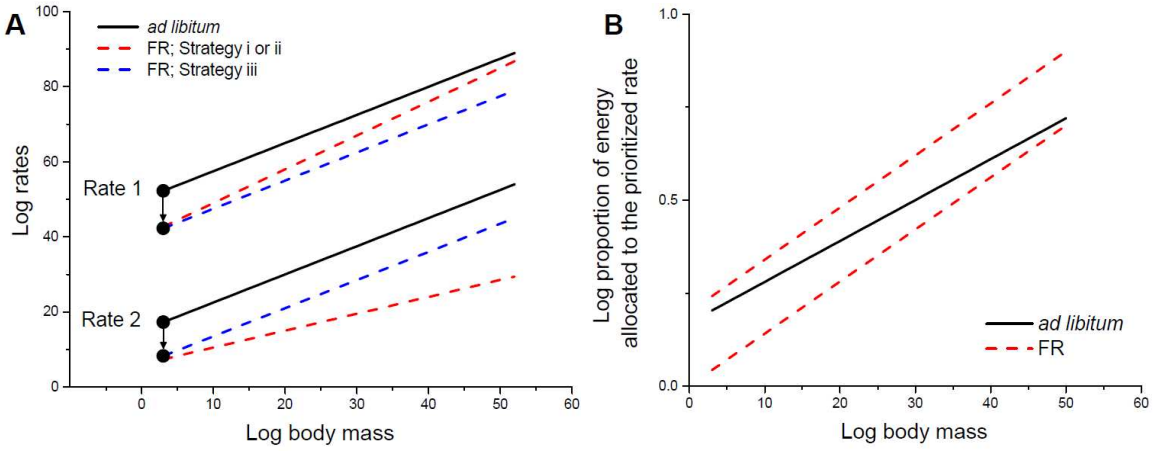

Figure 1. Schematics of Predictions by Eq.1. (A) Rates of interest as scaling laws of body mass under AL and FR condition. When FR initiates, both rates drop to lower values (dots on the left ends of the curves). For strategy i and ii takers, if Rate 1 is prioritized under FR, then the slope of Rate 1 will become steeper (the red dashed line of Rate 1 in the figure). Consequently, Eq. 1 predicts that the slope of the other rate (the red dashed line of Rate 2 in the figure) will become shallower. For strategy iii takers (blue dashed lines in the figure), the slopes of the rates remain the same under FR. (B) Proportion of assimilated energy allocated to the prioritized rate. Under FR, the slope of the proportion for the prioritized rate increases. But, as shown in Fig. 1A, the intercepts of both rates decrease. The degree of decreasing in the prioritized rate (S_0 or B_0) may be larger or smaller than that in food supply (F_0). Thus, the value of the proportion (S/F or B/F) under FR may be lower or higher than that under AL condition.

The hypothesis predicts that FR increases the scaling power of the prioritized rate, and decreases the other one. Since the scaling power of food supply, f, is fixed, Eq. 1 also predicts that the scaling power of the energy allocation proportion in the prioritized rate, either $s - f$ or $b - f$, will increase. However, Eq. 1 does not make predictions on the

values of the energy allocation proportions, S/F and B/F. This is because while the intercept of food supply, F_0, decreases under food restriction, the intercepts of the rates, S_0 and B_0, may decrease at different degrees in different animals. So, the intercepts of the proportions under food restriction, S_0/F_0, and B_0/F_0, can be either larger or smaller than those under *ad libitum* (AL, Fig. 1B). This means that even if the allometric trend of the proportion, i.e., the scaling power, in food restricted animals may be higher or lower than that in the free feeding animals, the overall value of the proportion may still be smaller or larger in the food restricted animals (Fig. 1B).

To test the predictions, six cohorts of 5^{th} instar hornworms were reared with two levels of food supply, *ad libitum* (AL) and food restriction (FR), at three temperatures, 20 ^{o}C, 25 ^{o}C, and 30 ^{o}C (see Method). At each temperature, we kept the scaling power of the food supply the same in the AL and FR cohorts, and lowered the normalization coefficients of it by approximately 60% (see Method). Under these conditions, we predict that the growth scaling power, *s*, will be larger, and the metabolic scaling power, *b*, will be smaller in FR larvae, compared to the AL controls (prediction based on Fig. 1A), which indicate that under FR, hornworms allocate more and more energy to growth as body mass increases during the 5^{th} instar period. We also predict that, compared to the AL controls, the proportion of the assimilated energy allocated to growth, S/F, increases faster, and the proportion of metabolism, B/F, increases slower (shallower slope) or even decreases (negative slope) during the ontogeny of the FR larvae (prediction based on Fig. 1B).

METHODS

Animal Rearing. In the summer of 2012 and 2013, approximately 150 hornworms (*Manduca sexta* larvae) were raised from eggs (Carolina Biological supply) on a long day cycle (17 hours light: 7 hours dark) at 25°C. Animals were fed *ad libitum* and checked for molting each day until 5th instar. On the first day of the 5th instar, larvae were randomly separated into three incubators at 20 °C, 25 °C 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 (2 food levels × 3 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 eliminated.

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 20160 Joules/gram. 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 entering the 5th instar, two cohorts at each temperature were fed with two levels of food supply: *ad libitum* (AL) and food restriction (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 ^{o}C, $F = 0.4 \times m^{0.70}$ at 25 ^{o}C, and $F = 0.5 \times m^{0.75}$ at 30 ^{o}C, where F and m are the mass of food amount and body, both in unit of grams. These food restriction levels were designed based on previous results of food uptake rates of *ad libitum* (AL) 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.

The digestibility is defined, D, as $D = \dfrac{F_{\text{dry}} \times E_{\text{food}} - \text{Dry feces} \times E_{\text{feces}}}{F_{\text{dry}} \times E_{\text{food}}} \times 100\%$,

where F_{dry} is the mass of dry food consumed by each larva during 24-hr 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 were collected each day and oven-dried at 65°C for 72 hours. In each cohort, feces samples were separated into two groups: feces produced in the first half period of 5$^{\text{th}}$ instar, and feces in the second half period. The energy content of the dry feces was measured by the oxygen bomb calorimeter (Grodzinski, Klekowski and Duncan 1975) (Parr 1108 combustion bomb). All samples were combusted to completion and the temperature change of the water (2 liters) 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, 86400, converts the unit of day to second.

Growth Rate. Body mass of 25 larvae in each cohort were measured at the same time every day from the first day of the 5$^{\text{th}}$ 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 one 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-hr 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°C-AL, 30°C-AL, 20°C-FR, 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 hours. 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, the growth rate was calculated, 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. The same method described in our previous publication was used to measure the metabolic rate of hornworm larvae (Hayes et al. 2014). The details are available in the Appendix B. The larval metabolic rate, B in unit of watts, was calculated as $B = (43.25 - 22.5 \times RER) \times \dot{V}_{CO_2} / 60$, where $RER = \dot{V}_{CO_2} / \dot{V}_{O_2}$ is the respiratory exchange ratio (Blaxter 1989, 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 is positive (Esperk and Tammaru 2004). The growth rate of hornworms slows down and levels off towards the end of the 5^{th} instar, making the growth trajectory a sigmoidal shape (Nijhout, Davidowitz and Roff 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, $Log(R) = Log(a) + d \times Log(m)$, 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 ^{o}C-AL, 30 ^{o}C-AL, 30 ^{o}C-FR, are taken from our previous publication for analysis and comparison (Hayes et al. 2014). A full model ANCOVA was performed with body mass as a covariate to test if there is significant interaction of two factors temperature×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 multiple measurements were made on the same individuals repeatedly, individual larvae were treated as random

factors to control for repeated measurements when performing ANCOVA. The random factors were excluded from the model if their effects are insignificant ($P > 0.05$).

RESULTS

Assimilation Rate. The digestibility of each cohort is listed in Table S1 in the Appendix C. Using the digestibility and Eq. 2, we estimate the assimilation rates as scaling laws of dry body mass of six cohorts (Fig. 2 and Table 1). 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 ^{o}C-AL to 0.83 for cohort 30 ^{o}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 the ones of the AL fed larvae at 20 °C, 25 ^{o}C, at 30 ^{o}C, respectively.

Growth Rates. The combustion energy content of dry mass, E_{tissue} (=23693 ± 656 Joules/gram dry mass), of each cohort is analyzed in Appendix C. 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 (Figure 3 and Table 1). Both temperature and food supply have positive effect on growth rate, in agreement with previous studies (Reynolds and Nottingham 1985, Kingsolver and Woods 1997, Timmins et al. 1988).

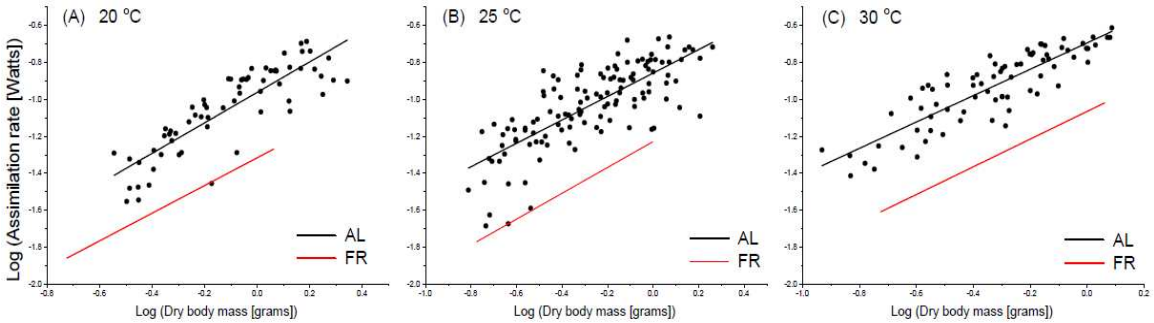

Figure 2. Food Assimilation Rate in Unit of Watts of Ad Libitum (AL) and Food Restricted (FR) Cohorts of Hornworms at Different Temperatures. The assimilation rate is calculated from Eq.2. In FR cohorts, every larva finished supplied food every day, so the food intake rate is exactly equal to the supply rate, which was designed to be scaling power laws of body mass. Thus, in FR cohorts the rates are plotted as straight lines. The fitted scaling laws of the AL cohorts are listed in Table 1.

Table 1. Scaling Laws of Food Assimilation, Metabolism and Growth of Ad Libitum (AL) and Food Restricted (FR) Hornworms Reared at Different Temperatures.

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) $R^2 = 0.80$

Within the same temperatures, food restriction (FR) significantly reduces the normalization coefficient of growth rate, S_0 (Fig. 3. and Table 1; 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 ^{o}C (ANCOVA, $F_{1,158} = 0.14$, $P = 0.709$), from 0.51 to 0.86 at 25 ^{o}C ($F_{1,232} = 0.125$, $P = 0.724$), and from 0.67 to 0.77 at 30 ^{o}C ($F_{1,98} = 2.275$, $P = 0.137$).

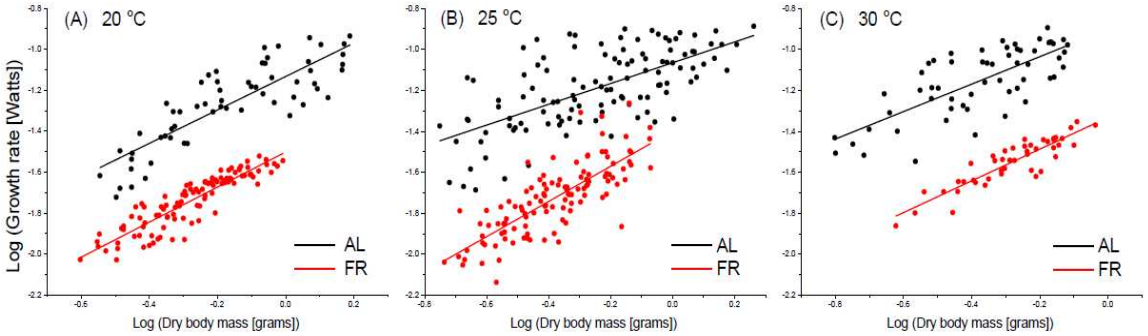

Figure 3. Growth Rate in Unit of Watts of Ad Libitum (AL) and Food Restricted (FR) Cohorts of Hornworms at Different Temperatures. The fitted scaling law of each cohort is listed in Table 1. (Data of cohorts 20 ^{o}C-AL, 30 ^{o}C-AL, and 30 ^{o}C-FR are from our previous publication (Hayes et al. 2014).)

Metabolic Rates. Figure 4 and Table 1 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 ^{o}C, it decrease 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 ^{o}C; $F_{1,123} = 1.277$, $P = 0.261$ at 25 ^{o}C, and $F_{1,95} = 17.707$, $P < 0.001$ at 30 ^{o}C).

Proportion of Energy Allocation. Now we use the scaling laws obtained in the previous sections (Table 1) 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 (FR) conditions at three temperatures. In Eq. 1, the sum of these two proportions must be

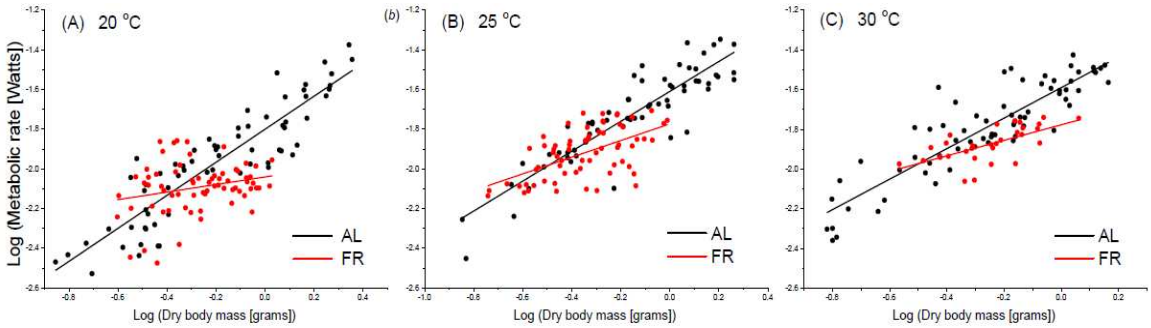

Figure 4. Metabolic Rate in Unit of Watts of Ad Libitum (AL) and Food Restricted (FR) Cohorts of Hornworms at Different Temperatures. The fitted scaling law of each cohort is listed in Table 1. (Data of cohorts 20 ^{o}C-AL, 30 ^{o}C-AL, and 30 ^{o}C-FR are from our previous publication (Hayes et al. 2014).)

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 are measured and averaged over the period of one day, whereas metabolic rates were measured and averaged over a 7~10-minute interval. So, one must assume that the average value of the metabolic rate over the 7~10-minute 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 more than 100 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 minutes 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 minutes, 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. Nonetheless, this problem 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 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 plot the proportions, S/F and B/F, as a function of body mass during the 5^{th} 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

5th instar, and increases slightly throughout the 5th instar until the wandering stage. The energy allocation to growth at 20 °C is about 70% at the beginning, and decreases slightly throughout the 5th instar. At 25 °C and 30 °C, the allocation to growth decreases from 70~80% to ~60% throughout the 5th instar. Note, the sum of the proportions of metabolism and growth is close to, but not equal to one, due to the reason discussed above.

Food restriction (FR) 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 did not keep constants during the 5th 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).

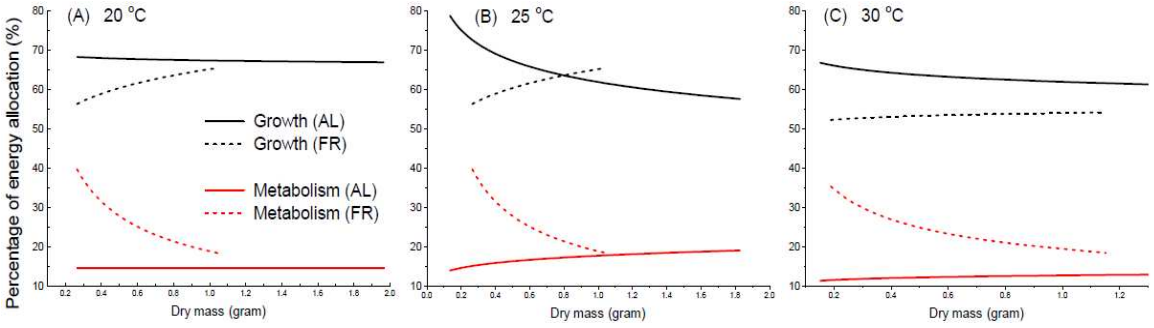

Figure 5. Energy Allocation of Ad Libitum (AL) and Food Restricted (FR) Cohorts of Hornworms at Different Temperatures. The black lines are proportion of assimilated energy allocated to growth (solid: AL; dash: FR), and the red lines are proportions of metabolism (solid: AL, dash: FR). The allocation proportions are calculated from the scaling laws listed in Table 1.

DISCUSSION

Growing Machine has 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 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., $B = B_{syn} + B_{maint} + B_{act}$ (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,

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

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 Joules to synthesize one gram of dry biotissue in the 5^{th} instar hornworms. Recalling that the combustion energy of dry biomass in hornworm is 23693 Joules/gram, the ratio of indirect and direct cost of growth in 5^{th} instar hornworm, B_{syn}/S, is 0.051. We have shown that when food restriction (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 $B_{syn}/S = 0.051$, 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, $B_{maint} + B_{act} = B - B_{syn}$) 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 counter-intuitively, 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 food restriction (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 (Fig. 3 and 4, Table 1), agreeing with our predictions in Fig. 1A. 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. 5). At each temperature, the *ad libitum* (AL) cohorts slightly decrease the energy allocated to growth as body mass increases during the 5^{th} instar, and increase the energy allocated to metabolism (Fig. 5). 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. These results support the predictions in Fig. 1B.

Hornworm is known to have a critical weight about 6 grams, at which the larvae no longer needs to feed to pupate at a normal time (Davidowitz et al. 2003, D'Amico, Davidowitz and Nijhout 2001). 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 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 maximize 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 gram (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 food restricted (FR) larvae, most of them were smaller than 6 gram 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.

Empirical Evidence for Strategies i and iii. In the introduction, we have hypothesized that animals with different life histories take different energy allocation strategies to maximize their fitness under low food availability. Endotherms prioritize metabolism to maintain the health (strategy i), and they can resume growth after the low-food period. The larvae of holometabolic insects with short larval stage prioritize growth so that they can reach a threshold body mass to successfully pupate before food scarcity is over (strategy ii). Ectotherms with long development stage may equally suppress both

metabolism and growth (strategy iii). In our experiments, all the FR larvae pupated, eclosed, and laid viable eggs. Thus, by taking strategy (ii), i.e., keeping high growth rate at the expense of metabolism, hornworms minimize the food restriction-induced harm to their fitness.

For the other two strategies, the available data generally support the hypothesis. Mammals and birds prioritize metabolism at the expense of growth under FR. The studies on rats by McCarter and his workers (McCarter and McGee 1989, 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 trend of changes in metabolic rate of FR rats is opposite of what we have observed in FR hornworms. Studies on "growth efficiency" also support the hypothesis. This 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, although these studies only reported either the FR-induced changes in growth efficiency, or the changes in metabolic scaling powers, but not both. It was found that Japanese quail (Ocak and Erener 2005) and broiler chicken (Benyi and Habi 1998) lower their growth efficiency under FR. In alcid chicks, including tufted puffin, horned puffin, crested auklet, and parakeet auklet, FR increased the metabolic scaling power (Kitaysky 1999). The same change has also been observed in Japanese quail (Rønning et al. 2009). In sand martin, the metabolic scaling power is the same in FR animals as in the AL

counterparts (Brzęk and Konarzewski 2001). In a study of song thrush chicks (Konarzewski and Starck 2000), although the scaling powers were not reported, the mass-corrected metabolic rate was found to be higher in the FR animal. Among the studies we have found on how bird chicks respond to food restriction, only in European shag was the metabolic scaling power found to be lower in the FR chicks (Moe et al. 2004). Due to the lack of data on food assimilation rates in these studies, we cannot estimate the exact changes in proportion of metabolism in FR animals. However, as discussed above, an increase in metabolic scaling power in FR animals suggests that the FR animals increase the energy allocation to metabolism as body mass increases, opposite of what has been shown in hornworms.

Most studies on ectothermic animals' energy budget under low food supply focus on non-growing animals (e.g., (Naya and Bozinovic 2006, Trzcionka et al. 2008, Devi, Prabhakara Rao and Prasada Rao 1986, Marsden, Newell and Ahsanullah 1973, Hagerman 1970, Rossetto et al. , Armitage and Wall 1982), or the growth and metabolism of a population, instead of individuals (e.g., (Verity 1985, Bohrer and Lampert 1988). However, limited data on growing ectothermic animals support our hypothesis. A non-diapausing nematode species, *Caenorhabditis briggsae*, takes strategies ii. *C. briggsae*'s larval stage is about five days, and they do not enter dauer stage when food resource is low (Schiemer 1982). Thus, the length of their development stage is similar to hornworms, and their energy budget under FR is also similar to hornworms. Schiemer (1982) found that FR decreases the metabolic scaling power in *C. briggsae,* and the growth efficiency in FR *C. briggsae* keeps increasing during the larval stage, whereas that of AL *C. briggsae* decreases near the end of larval stage. The similar

changes in metabolism and growth were observed in hornworms here. In contrast, the Indian stick insect, a hemimetabolic insect species, takes strategy iii. The Indian stick insect has a long juvenile stage that lasts 3-8 months (Roark and Bjorndal 2009). With the long juvenile stage, Indian stick insect can potentially resume growth after the low-food supply period, and therefore do not have to prioritize growth under FR. So, Eq. 1 predicts that the scaling powers of growth and metabolism will not change under FR, whereas the coefficients of the rates will be lowered. Indeed, Roark and Bjorndal (2009) have shown that under FR, the coefficient of the metabolic rate (intercept), B_0, is lowered, but the scaling power, b, keeps the same as the AL counterparts. The authors did not report the proportions of assimilated energy allocated to growth and metabolism, but the unchanged metabolic scaling power in FR animals suggests that the FR Indian stick insect may keep the trend of energy allocation to metabolism the same as their AL counterparts as body mass increases, and the overall proportion S/F and B/F may be the same in AL and FR individuals.

Consequences of Different Strategies in Life History Tradeoffs. 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 (FR). 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 focus on how different strategies alter the FR-induced energy tradeoffs, and therefore affect animals' health maintenance and longevity. FR induces two types of energy tradeoffs. The first tradeoff 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 $B = B_{syn} + B_{maint} + B_{act}$. When endotherms (strategy i takers) are under FR, their metabolism (B) keeps relatively high, and deposition in biomass (S) is largely suppressed (the first tradeoff). 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 tradeoff; Note: the energy for activity, B_{act}, in endotherms is usually unchanged under FR, see review in (Hou et al. 2011d)). In other words, FR channels energy from biosynthesis work to health maintenance through these two tradeoffs. With increased B_{maint}, endotherms are able to achieve a better health under FR. Indeed, we have hypothesized that these two tradeoffs are the underlying mechanism for the well-known effect of FR on extending lifespan in mammals, assuming better health is positively correlated to longevity (Hou, Bolt and Bergman 2011a, Hou et al. 2011c). Empirical data of lifespan extension from more than 100 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, in terms of health maintenance, as much as endotherms. The strategy ii takers try to maximize deposition in biomass (S) at the expense of metabolism (B) under FR. Consequently, the biosynthesis work (B_{syn}) is not suppressed as much as in endotherms. Thus, with suppressed B and not much suppressed B_{syn}, the energy for maintenance (B_{maint}) in strategy ii takers does not increase as much as it does in

endotherms. Similarly, in the strategy iii takers B_{maint} does not increase as much as in endotherms either, because they equally suppress growth and metabolism. No empirical study has investigated the effect of FR on health maintenance in strategy ii takers during their larval development. However, in one of the strategy iii takers, Indian stick insect, Roark and Bjorndal (2009) have shown that FR failed to extend its lifespan, indicating that FR fails to channel energy from biosynthesis work to maintenance due to this strategy. We call for more comparative studies, especially on strategy ii and iii takers, to test the hypothesis that with the same level of food restriction, the strategy i takers benefit more in terms of health maintenance and longevity than the strategy iii takers, which in turn benefit more than the strategy ii takers.

III. ENGERY TRADEOFFS BETWEEN GROWTH, METABOLISM, AND MAINTENANCE IN HORNWORMS (MANDUCA SEXTA LARVAE)

INTRODUCTION

The deleterious productions of oxidative metabolism, such as reactive oxygen species (ROS), cause various forms of damages on macromolecules, cells, and tissues, which in turn undermine organism's health maintenance and longevity (Barja 2004, Lombard et al. 2005, Hulbert et al. 2007, Balaban, Nemoto and Finkel 2005, Sohal and Weindruch 1996). To counteract the accumulation of damage, organisms have evolved highly efficient repairing mechanisms, such as oxidant scavenging and damage repair (Beckman and Ames 1998, Merry 2004, Monaghan, Metcalfe and Torres 2009). The repairing mechanisms require energy and resources. If the resource and energy that could be allocated to repairing are otherwise channeled to other biological process, then damage will inevitably accumulate despite the high repairing efficiency (Monaghan et al. 2009, Stearns 1992).

Biosynthesis during growth, one of the most intensively investigated biological processes that tradeoff with repairing, is positively correlated with oxidative damage level and other proxies of it, such as declined performance and shortened lifespan (Hou 2013, Metcalfe and Monaghan 2001, Rollo 2002, Metcalfe and Monaghan 2003, Mangel and Stamps 2001). Rapid growth leads to higher phospholipid peroxidation (Nussey et al. 2009), protein carbonyl content (Forster, Sohal and Sohal 2000), decreased antioxidant defenses in red blood cells (Alonso-Alvarez et al. 2007), declined locomotion ability (Mangel and Stamps 2001) and immune function (De Block and Stoks 2008), and higher

mortality rate and shortened lifespan (Inness and Metcalfe 2008, Mair et al. 2003, Merry 1995, Bartke 2005, Bartke 2003). A special type of rapid growth—catch up growth, referring to infants with low birth weight reaching to or exceeding the normal body weight later in life, increases the risk of adult-onset metabolic syndromes and short lifespan in human and laboratory rodents (Jennings et al. 1999, Eriksson et al. 1999, Ong et al. 2000, Ozanne and Hales 2004, Barker 2001, Lucas, Fewtrell and Cole 1999, Hales and Ozanne 2002, Langleyevans and Sculley 2006). In contrast, suppressed growth, usually induced by food restriction or genetic interference with growth hormone, keeps animals in a relatively youthful and healthy state, and largely extends lifespan in a broad diverse of species, indicating the up-regulations of somatic damage repairing in these animals (McCay, Crowell and Maynard 1935, Weindruch and Walford 1988, Masoro 2005, Sinclair 2005, Merry 2002, Brown-Borg et al. 1996, Brown-Borg 2003, Bartke 2005, Holehan and Merry 1986, Yu 1994, Heilbronn and Ravussin 2003, Mair and Dillin 2008).

Oxidative metabolism causes somatic damage accumulation. During growth, a fraction of metabolic energy is allocated to biosynthesizing new tissues. Thus, changes in biosynthetic rate also influences on damage accumulation. However, most of the studies did not disentangle the effects of them on somatic damage. Although biosynthesis is fueled by metabolism, the relation between them is not simply proportional. When one of them increases, the other can increase (Ricklefs 2003, West, Brown and Enquist 2001, Wieser 1994), decrease (Hayes et al. 2014, Steyermark 2002), or keep roughly the same (Brown, Nagy and Morafka 2005, Nagy 2000, Álvarez and Nicieza 2005, McCarter and Palmer 1992). Thus, the changes in damage level observed in the studies that

manipulated biosynthetic rate (growth rate) are collective results caused by the changes in both biosynthetic and metabolic rate. The goal of this paper is to unravel the effects of changes in biosynthetic and metabolic rate on the total change in damage accumulation from an energetic viewpoint. A simple theoretical model is developed based on the first principle of energy conservation and real physiological parameters. The model predicts that, if the repairing efficiency is high, then the changes in damage level caused by the changes in metabolic rate is negligible compared to that caused by the changes in biosynthetic rate. In other words, under the condition of highly efficient repairing, damage level is more sensitive to the changes in biosynthesis than that in metabolic rate. Then the model is tested by experiments on the 5^{th} instar tobacco hornworms (the last instar of *Manduca sexta* larvae). The growth of hornworms is manipulated by rearing them at different food supply levels. The lipid peroxidation is measured as an index of damage accumulation in larvae with different rates of growth and metabolism. In 7~10 days, hornworms grow from ~1 gram at the last molting to ~12 grams before pupation with a 10-fold increase in metabolic rate, making it a good model to test the predictions.

MODEL DEVELOPMENT

A theoretical model has been developed to estimate the effects of metabolic and biosynthetic rate on somatic damage (Hou 2014, Hou 2013, Hou et al. 2011c, Hou et al. 2011a). The quantitative predictions by the model are strongly supported by data from more than 200 empirical studies on small laboratory rodents and wild animals across a broad range of species (Hou 2013, Hou et al. 2011c). Here we briefly review the model and make four prediction based on it.

During growth, the total metabolic rate, B, is partitioned between the rates of energy allocated to maintaining existing biomass, B_{maint}, energy required to synthesize new biomass, and energy spent on other activities (such as foraging), B_{act}. (West et al. 2001, Hou et al. 2008, Brody 1945), i.e., $B = B_{\text{maint}} + B_{\text{syn}} + B_{\text{act}}$. The maintenance term, B_{maint}, includes the energy spent on the repairing mechanisms, such as oxidant scavenging and damage repair. The rate of energy allocated to biosynthesis (B_{syn}) can be expressed as $B_{syn} = E_{\text{m}} dm / dt$, where dm/dt is the growth rate (increase in body mass, m, per unit time, t), and E_{m} is the metabolic energy required to synthesize one unit of bio-tissue, such as the energy for assembling macromolecules from monomers. E_{m} is also called indirect cost of growth with the dimension of energy/mass (Hou et al. 2008, Brody 1945). The energy spent on activities, B_{act}, is usually a constant fraction of the total metabolic rate during growth (Hou et al. 2008, Nagy, Girard and Brown 1999), i.e., $B_{\text{act}} = c \times B$ where c is a dimensionless constant, indicating the activity level of the animal. For free-living mammals and birds, c is about 50%~70%. It is less than 20%~30% in caged animals (Nagy et al. 1999, Hou et al. 2008). Putting everything together gives the rate of energy allocated to repairing:

$$B_{\text{maint}} = (1-c)B - B_{\text{syn}} = (1-c)B - E_{\text{m}} dm / dt \quad (1)$$

Two assumptions are made for estimating the accumulation of oxidative damage. Assumption 1: Within a species, the rate of somatic damage accumulation, H, caused by deleterious products of oxidative metabolism, such as reactive oxygen species (ROS), is proportional to the rate of oxygen consumption (metabolic rate, B). The assumption is based on the observations that metabolic and ROS generation rate are proportional to each other (see review in (Hou 2013)). Thus, we have the rate of damage accumulation

(damaged mass/time), $H = \delta B$, where δ is a constant within a species, indicating the amount of damaged mass associated with one unit of metabolic energy. Here the damaged mass can be cell membrane, protein, DNA, or other macromolecules (Mangel and Munch 2005). Assumption 2: Repairing the damage requires metabolic energy. The rate of repair, R (repaired mass/time), is proportional to the energy available for maintenance (repairing damage), B_{maint}, with a coefficient η, i.e., $R = \eta B_{\text{maint}}$, where η is also a constant, indicating the amount of mass that can be repaired by one unit of metabolic energy.

The net damage, $H - R$, accumulates. The accumulated damage can be integrated as a function of time, i.e., $\int_0^t (H - R) d\tau$. Using Eq. 1 and Assumptions 1 and 2, we have the net somatic damage,

$$\begin{aligned} D(t) &= \int_0^t (\delta \times B - \eta \times B_{\text{maint}}) d\tau \\ &= [1 - (1 - c) \times \varepsilon] \int_0^t B d\tau + \varepsilon E_{\text{m}} \Delta m \Big|_0^t \\ &= [1 - (1 - c) \times \varepsilon] \times ME + \varepsilon \times SE \end{aligned} \tag{2}$$

where $\varepsilon = \eta/\delta$ is the effective repairing efficiency, indicating the ratio of repaired mass and damaged mass for one unit of energy; $ME = \int_0^t B d\tau$ is the total metabolic energy spent during growth; Δm is the increase of body mass during growth, and E_{m} is the energy required to synthesize one unit of biomass, so $SE = E_{\text{m}} \Delta m$ is the synthetic energy spent during growth. Thus, Eq. 2 decomposes the net damage in two terms. The first term, $D_B = [1 - (1 - c) \times \varepsilon] \times ME$, indicates how damage changes when metabolic rate changes; the second term, $D_{\text{syn}} = \varepsilon \times SE$, estimates the effect of biosynthesis on damage. Both terms are proportional to energy factors (ME and SE) with coefficients $1 - (1 - c) \times \varepsilon$ and ε respectively.

Now, we consider caged laboratory animals, whose activity level is nearly zero so that the constant c is negligible. In this case, the net somatic damage reduces to $D = D_B + D_{syn} = (1-\varepsilon) \times ME + \varepsilon \times SE$. The sensitivities of damage to the changes in metabolic and biosynthetic rate depend on the coefficients of these two terms, $1-\varepsilon$ and ε. For high repairing efficiency ε, the coefficient of D_B is much smaller than that of D_{syn}, i.e., $(1-\varepsilon) << \varepsilon$. This means that the damage accumulation is more sensitive to the biosynthetic term *SE* than to the metabolic term *ME*. In other words, *SE* will cause more damage than *ME*, if they increase the same amount. On the other hand, if $(1-\varepsilon)$ is close to ε, then both *ME* and *SE* will cause same degree of changes in damage level. Fig. 1 illustrates how repairing efficiency influences on damage accumulation when both metabolic and biosynthetic rates vary. For large efficiency (ε = 0.96 in Fig. 1A and 1B), increases in metabolic rate alone without changing biosynthetic rate will not cause a significant increase in damage level (Fig. 1A), whereas increases in biosynthesis with metabolic rate keeping the same will lead to a great increase in damage (Fig. 1B). In contrast, if repairing efficiency is small (ε = 0.5 in Fig 1C and 1D), increases in both metabolic and biosynthetic rate cause considerable increases in damage.

Based on the first principle of biochemistry and fitting of empirical data, the repairing efficiency ε has been estimated to be in the neighbourhood of 0.99 (Hou 2013, Hou et al. 2011c). For such a high efficiency, we predict that during growth, the changes in damage are mainly caused by the changes in biosynthesis rate (growth rate), whereas the consequence of the changes in metabolic rate are insignificant. We test this prediction by assaying the lipid peroxidation levels in groups of 5^{th} instar hornworms with different growth and metabolic rates. The variation in these rates can be induced by varying the

level of food supply (Hayes et al. 2014, Jiao et al. 2014) (see details in method section). We use plasma malondialdehyde (MDA) as a surrogate of somatic damage, which is a specific end-product of phospholipid oxidative damage, and has been commonly used as a biomarker of oxidative stress (Hall et al. 2010, Nussey et al. 2009). We assume that the level of MDA is proportional to the total somatic damage (*D*) with a factor *g*, as $MDA = g \times D$, and therefore Eq. 2 becomes

$$MDA = g \times (1 - \varepsilon) \times ME + g \times \varepsilon \times SE \quad (3)$$

We need to emphasize that damage accumulates over the entire growth, so a considerable fraction of MDA assayed in this study was accumulated during the first four instars of the larval lives, whereas the manipulations of growth and metabolic rate only started when the larvae entered the fifth instar. Thus, to test how manipulations of these rates influence the damage accumulation, we must remove the effects of *ME* and *SE* in the first four instars from the assayed MDA level. Previous and this study show that both *ME* and *SE*, the metabolic and synthetic energy spent during a period of growth, are linearly proportional to the body mass at the end of this period (see Fig. 2A, 2B, and (West, Brown and Enquist 2004)). We measured the body mass at the end of the 4^{th} instar of the larvae, and linearly regressed assayed MDA level on this mass. The residual of MDA after the removal of this mass is then considered the damage caused by *SE* and *ME* during the 5^{th} instar period—the manipulated period. The MDA level, *SE*, and *ME* are all linearly correlated to the final body mass at the end of the growth period, *M* (Fig. 2 and 3). This means that the final body mass has a confounding effect on these variables. Mass

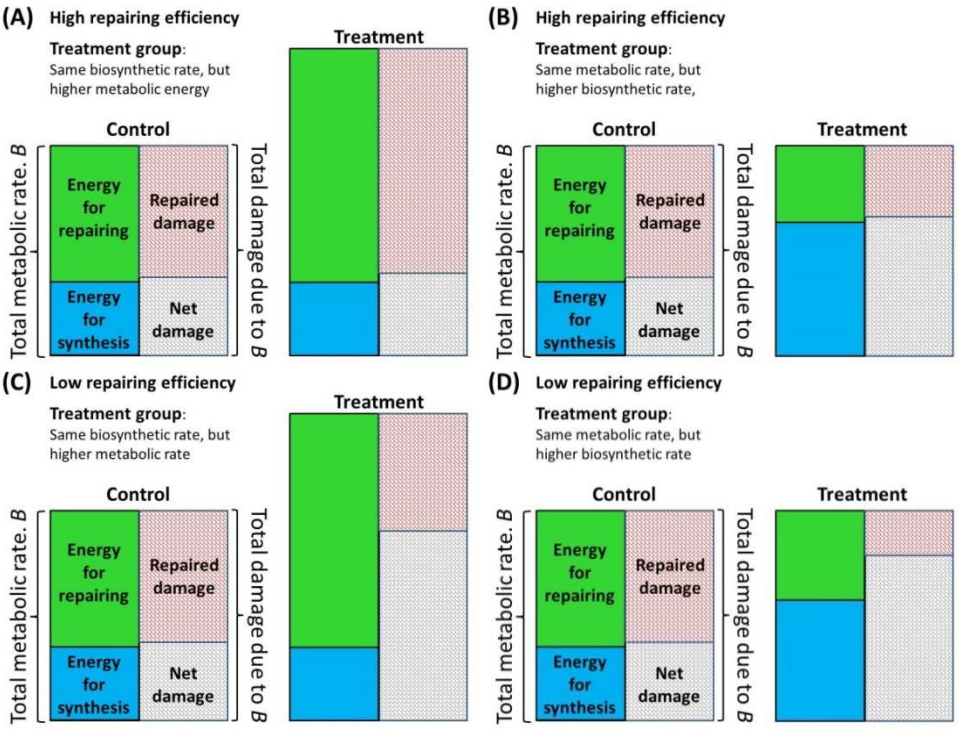

Figure 1. Conceptual Illustration of The Effects of Repairing Efficiency, Oxidative Metabolism, and Biosynthesis on Somatic Damage. Two groups of conceptual animals are compared: control group (left), and the treatment group (right) in each pannel. In each group, the solid green and solid blue boxes are energy allocated to maintenance and biosynthesis, B_{maint} and B_{syn} respectively. The sum of these two is the metabolic energy, $B = B_{\text{maint}} + B_{\text{syn}}$ (Eq. 1) under the condiction that activity level is negligible; The red shangled box represents the repaired damage, proportional to the energy for repairing (B_{maint}) with efficiency (ρ) as $R = \rho \times B_{\text{maint}}$; and the grey shangled box represents the net damage, which is the difference between the total damage caused by oxidative metabolism ($H = \eta B$) and repaired damage ($R = \rho B_{\text{maint}}$), as $\eta B - \rho B_{\text{maint}} = (\eta - \rho)B + \rho B_{\text{syn}}$ (Eq. 2). Pannels (A) and (B) show the cases of high reparing efficiency ($\varepsilon = \rho / \eta = 96\%$ in the figure). When repairing efficiency is high, i.e., ρ is close to η, the metabolic term $(\delta - \rho) \times B$ in the net damage is close to zero, regardless how metabolic rate (B) changes. The major contribution to the net damage comes from the biosynthetic term ρB_{syn}. So, in pannel (A) the treatment group with higher metabolic but same biosynthetic rate compared to control group has roughly the same net damage as the control, wherease in pannel (B) the treatment group with high biosynthetic but same metabolic rate has significantly higher net damage than the control group. Pannels (C) and (D) show the cases of low reparing rate ($\varepsilon = \rho / \eta = 50\%$ in the figure). When ρ is smaller than δ, then the metabolic term $(\delta - \rho) \times B$ makes signifant contribution to the net damage. Thus, both treatment groups in (C) and (D) have higher net damage than the control group.

residuals were calculated from the linear regression for these variables on final body mass. Then we regressed the mass residual of MDA on the mass residuals of *ME* and *SE*, as

$$MDA_{\text{residual}} = \alpha \times ME_{\text{residual}} + \beta \times SE_{\text{residual}} + \gamma \quad (4)$$

Where α, β, and γ are regression coefficients.

Comparing the result of regression with Eq. 3, we make four specific predictions. First, the constant term of the regression γ is nearly zero; Second, the regression coefficient of the metabolic term, α, is smaller than that of the biosynthetic term, β. Meanwhile, the metabolic term has a large P-value, indicating its insignificant contribution to the MDA level; Third, the ratio of the coefficients, α and β, gives $\alpha / \beta = (1-\varepsilon)/\varepsilon$. The repairing efficiency (ε) estimated from this equation is in the neighborhood of 0.99, which is the value estimated from the biochemistry principles (Hou et al. 2011c); and fourth, after the insignificant contribution of the metabolic term is removed, the MDA level is linearly proportional to the synthetic energy *SE*.

MATEIRALS AND METHODS

Animal Rear and Food Supply Levels. Approximately 80 hornworms were raised from eggs (Carolina Biological supply) on a long day cycle (17 hours light: 7 hours dark) at 25 ^{0}C. Animals were fed ad libitum and checked for molting each day until 5th instar. To prevent the worms becoming pupae, they were allowed to survive for 4 days and were collected blood samples on the fourth day of 5th instar. On the first day of the 5th instar, larvae were randomly separated to be treated under four different food restriction strategies: *ad libitum* (AL), long term food restriction (LFR), short term food

restriction (SFR), and catch up growth (CUG). Each cohort consisted of 20 larvae. AL and LFR group larvae were fed *ad libitum* and food restricted separately for the first three days of the 5th instar. Larvae in SFR group were fed *ad libitum* for the first two days and food restricted on the third day, while CUG group larvae were treated in the opposite way, food restricted on the first two days but fed *ad libitum* on the third day of 5th instar. Larvae which need to be food restricted were supplied 50% of *ad libitum* food following the equation: $F = 0.27 \times m + 0.44$, where F and m are the mass of food amount and body, both in unit of grams.

Synthetic Energy Spent During the 5th Instar. Body mass of each larva in every cohort was measured approximately at the same time every day from the first day of the 5th instar to the nearest 0.1 mg the 4th day of the 5th instar using a digital microbalance (Perkin-Elmer AD6). We define the growth rate, in unit of gram/day, as the increment of body mass from 1 day to the next. The energy for biosynthesis during 3 days growth, SE, in unit of Joules, is calculated as the increment of body mass from one day to the fourth day in 5th instar multiplied by the energy required to synthesis one unit tissue, i.e., $SE = \Delta m \times E_m$, where Δm, in unit of grams, is the increment of body mass during the 3 days period, and E_m = 168 Joules/gram is the energy required to synthesize one unit of biomass in the 5th instar hornworms (Sears et al. 2012).

Metabolic Energy Spent During the 5th Instar. The same method described in previous publication was used to measure the metabolic rate of hornworm larvae (Hayes et al. 2014). The rates of O_2 consumption and CO_2 production, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, of each larva were measured for 7-10 minutes time interval every day after their body mass was measured. The rates $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated as $\dot{V}_{CO_2} = \mathrm{FR} \times [CO_2]/100$, and

$\dot{V}_{O_2} = FR \times (20.95 - [O_2]) / (100 - [O_2])$, where FR is the flow rate, and [C O_2] and [O_2] are the concentration of C O_2 and O_2 in the respirometry chamber (Lighton, 2008). By assuming that each data point represents the average of the measurement taken during 24-hour period, the larval metabolic rate for each day (in unit of Joules) was calculated as $ME = (10.34\text{-}5.38 \times RQ) \times \dot{V}_{CO_2} \times 4.18 \times 24 \times 60$, where $RQ = \dot{V}_{CO_2} / \dot{V}_{O_2}$ is the respiratory exchange ratio (Blaxter, 1989, Withers, 1992). The metabolic energy consumption was defined as the sum of larval metabolic expenditure each day from the 2nd day to the 4th day in 5th instar. Since all treatments began on the 1st day in 5th instar, after 24 hours, the effect of the treatment can be measured. Thus, data collection of metabolic rate started on the 2nd day instead of the 1st day.

MDA Assay.

Chemical and reagents. All chemicals and reagents used were HPLC grade or analytical. Acetonitrile, Tetrhydrofuran (THF), and Trichloroacetic Acid (TCA) were purchased from Fisher Science (Fair Lawn, New Jersey, USA). 1,1,3,3-Tetraethoxypropane (TEP) butylated hydroxytoluene (BHT) and 2-Thiobarbituric acid (TBA) were obtained from Sigma–Aldrich (St. Louis, MO, USA); potassium phosphates, hydrochloric acid, sodium hydroxide, methanol, n-Butanol and ethanol from Fisher Science(Fair Lawn, New Jersey, USA); Ultra-pure water were used to prepare mobile phase and other aqueous solutions.

Blood samples preparation. Blood samples were collected in 3mL centrifuge tubes containing an EDTA solution as anticoagulant (Grotto et al. 2007, Hermans et al. 2005)by clipping the third proleg of Manduca sexta larvae. After centrifugation at 6000 ×

g for 10 min at 4^0C, the supernatant plasma were transferred to a new tube and stored at -70^0 C until MDA determination.

MDA standards. The stock MDA solution was prepared by adding 5 µL MDA standard (1,1,3,3, tetramethoxypropane) from freezer and 5 mL of 1/6N HCl into a screw cap Pyrex tube. After boiling this stock mixture for 5 min, set the mixture immediately on ice. The stock standard was further diluted in 10% TCA, 500 ppm BHT, Saturated TBA solution to gain the different MDA concentrations of 13, 27, 40.5 67.5 nM . These standard solutions were processed under the same condition as described in next section to get the standard calibration curve for the estimation of total MDA.

Total MDA. A step of alkaline hydrolysis of protein bound MDA (Pilz, Meineke and Gleiter 2000, Grotto et al. 2007, Hong et al. 2000, Moselhy et al. 2013) was processed by adding 25 µL of 3N NaOH into 100 µL worm plasma and incubating at 60 ◦C for 30 min in a water bath system. 100 µL of 500 ppm BHT solution was added into the mixture to prevent further oxidization. The hydrolyzed sample was adjusted with 1mL 0.1 N HCl and 1mL 10% TCA. After centrifugation at 3,000 rpm for 10 min, 500 µL of supernatant was removed into a pyrex boiling tube which contains 500 µL of TBA. The mixture solution was boiled for 10 min and then rapidly put on ice to cool down. After this, 500 µL of solution was transferred into a disposable glass tube containing 1 mL of n-butanol. Then the mixture was vortexed the mixture at least 30 seconds and centrifuged at 1000 rpm for 10 min. The top layer was filtered through a 0.45 µm Syringe filter into an auto injector vial. Immediately, 50 µL of plasma samples or standards were injected to an Alltima C18 column (5 µm, 250 × 4.6 mm) for HPLC analysis.

The HPLC analysis was performed using an Agilent 1100 Series system, equipped with degasser, pump, autosampler and fluorescence detector and system controller with a PC control grogram. The HPLC system was eluted with mobile phase consisting of 69.4% (v/v) Na-Phosphate buffer (5 mM, pH=7), 30% (v/v) Acetonitrile and 0.6% (v/v) THF at 1 mL/min flow rate . The fluorescence detector wavelength set as 515 nm (excitation) and 553 nm (emission). The sample run 7 min and the retention time of MDA-TBA was around 2.5 min.

MDA data collection. The data on MDA level after HPLC analysis is the concentration in blood of the hornworm in unit of nM/ mL. We assume that the blood volume is proportional to the whole body mass of each larva. Thus we multiplied the assayed MDA concentration by the larval body mass on the 4^{th} day for each larvae to represent the accumulated damage level during the 3 days period.

Data Analysis and Statistics. All data on growth were collected for larvae from the 1^{nd} day to the 4^{th} day at the 5^{th} instar stage, while the metabolism data were measured and analyzed starting on the 2^{nd} day instead of the 1^{st} day. The data of MDA level were determined by the HPLC system in two weeks after blood samples collection. Based on three days growth, 72 data points were obtained on growth, metabolism and MDA level to analyze the reasons of the accumulated damage. Statistical analyses were performed using SPSS 21. We did the mean comparisons among the 4 cohorts on the results of MDA level and growth rate by ANOVA test. An initial regression model of MDA containing M_0, the energy for body mass increment, SE, and metabolic energy, ME, was processed by multiple linear regression procedure. Since the damage on M_0 was failed to be detected by experiment, due to the tiny body size of larvae, we removed the M_0 effect

from the initial model by doing residual analysis. Meanwhile, the body mass on the 4th day considered as a confounding variable was excluded from terms, growth, metabolism and MDA, because the body mass on the 4th day is proportional to each of these terms. Otherwise, the correlation between dependent variable and independent variables can be regarded as a spurious relationship due to this confounding factor.

RESULTS

Metabolic and Synthetic Energy Spent During the 5th Instar Period. The different treatments of food supply induced broad variations in both synthetic energy and metabolic energy spent during the 5th instar. *SE* varies from *ca.* 200 Joules to *ca.*1000 Joules (Fig. 2A), and *ME* ranges from *ca.* 2500 Joules to *ca*. 9000 Joules. Figure 2 also shows that these two energies are linearly proportional to the body mass at the end of growth period, *M*.

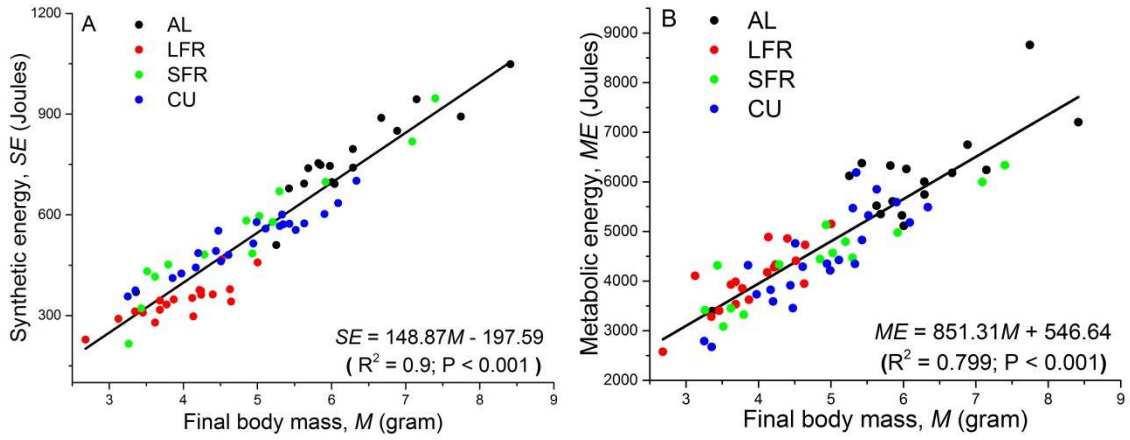

Figure 2. Linear Regressions of Synthetic Energy (A) and Metabolic Energy (B) on Final Body Mass.

Regression of Mass Residuals of MDA on Mass Residuals of SE and ME. Figure 3 shows that MDA accumulated in the 5th instar is linearly proportional to the final body mass, *M*.

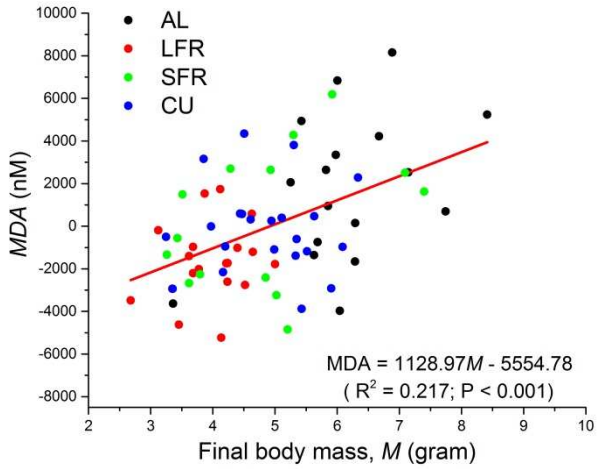

Figure 3. Linear Regression of MDA Level on Final Body Mass.

After removing the confounding effects of body mass, *M*, the regression of MDA on *SE* and *ME* yields $MDA_{\text{residual}} = \alpha \times ME_{\text{residual}} + \beta \times SE_{\text{residual}} + \gamma$. The statistics of the regression are given in Table 1.

Table 1. Statistic Results of Linear Regression of MDA Residual on The Residuals of Synthetic and Metabolic Energy.

Variables	Coefficients	Sig.	Partial correlation
Constant	$\gamma = -2.84 \times 10^{-12}$	1.000	
SE_{residual}	β = 9.958	0.06	0.225
ME_{residual}	α = 0.392	0.519	0.078

The regression results strongly support the first three predictions. First, the constant term is zero. Second, the coefficient of ME_{residual}, $\alpha = 0.392$, is 25-fold smaller than that of SE_{residual}, $\beta = 9.958$. Moreover, the P-value of ME_{residual} is 0.519, suggesting its insignificant effect on MDA level. Third, the ratio $\alpha/\beta = (1-\varepsilon)/\varepsilon = 25.4$ gives $\varepsilon = 0.962$, close to 0.99.

Figure 4 tests the fourth prediction. After the insignificant effect of *ME* is removed, the regression of MDA_{residual} on SE_{residual} yields $MDA_{\text{residual}} = 8.91 \times SE_{\text{residual}} + 8.32 \times 10^{-4}$ (Pearson's $r = 0.211$, P = 0.0745).

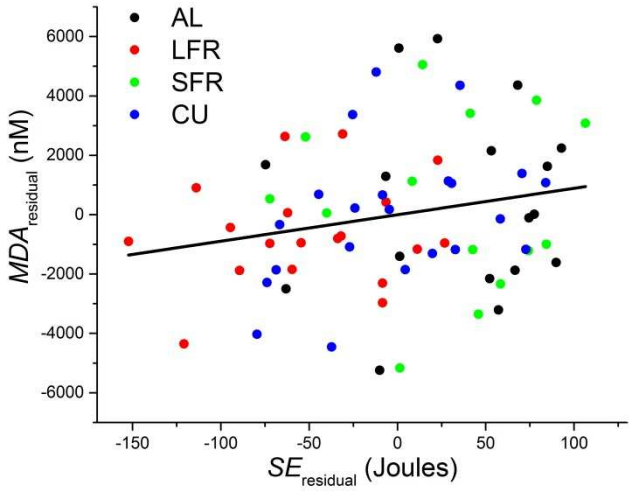

Figure 4. Linear Regression of Mass Residual of MDA on Mass Residual of Synthetic Energy.

DISCUSSION

The regression coefficient of the metabolic energy (*ME*) in MDA level, $\alpha = 0.392$, is much smaller than that of the synthetic energy (*SE*), $\beta = 9.958$. The ratio of these two

coefficients give the repairing efficiency, ε = 0.962. A concern rises regarding the measurement of metabolic energy, *ME*. In this study, we measured the larval metabolic rate once a day, and assumed that the measured rate is the average over 24 hours on the day it was measured. We then multiplied this value by 24 hours to obtain the metabolic energy spent during that day. However, for hornworm, such a fasting growing animal, this assumption is invalid. As body mass increases, metabolic rate also increase. So, the real metabolic energy spent during the day is larger than what we estimated. However, underestimate of *ME* does not weaken our conclusion. Instead, an accurately estimated *ME*, which would be larger than the one we used in this study, will lead to an even smaller regression coefficient, α, and therefore supports our prediction even more strongly.

The role of metabolic rate in animals health maintenance and longevity is unclear and empirical data on this issue is contradictory (Speakman et al. 2004). In general, inter-specific data from wild animals within the same taxon (McCoy and Gillooly 2008) show that, with a few exceptions, the ones with higher mass-specific metabolic rate have shorter lifespan. Under laboratory conditions, lowering body temperature and metabolic rate also have been shown to extend lifespan of both ectotherms (Klass 1977, Partridge et al. 2005, Van Voorhies and Ward 1999) and endotherms (Conti et al. 2006) that were fed freely. These empirical evidence support rate of living theory (Pearl 1928, Lints 1989), and the modern version of it, the oxidative stress theory (Barja 2004, Balaban et al. 2005), which suggests that the oxidative metabolism and its deleterious productions (e.g., reactive oxygen species, ROS) cause molecular and cellular damages that are associated with the health maintenance and process of aging.

Based on the data from *Ad libitum* (AL) fed animals and the oxidative stress theory, it has been hypothesized (Rikke and Johnson 2004, Weindruch and Walford 1988) that lowering body temperature and metabolic rate is also one of the major mechanisms of food restriction (FR), which extends the lifespan of a broad diversity of organisms, while keeping them in a relatively healthy state (Masoro 2005, Weindruch and Walford 1988). However, numerous studies have shown that FR does not substantially decrease the mass-specific metabolic rate of mammals (see review in (Hou et al. 2011d, Mccarter et al. 1985)). Studies on ectothermic species also found that while extending the lifespan, FR does not lower MR in them after body mass is corrected (Partridge et al. 2005, Houthoofd et al. 2003, Mair et al. 2003, Hulbert et al. 2004, Walker et al. 2005). These findings indicate that lowering metabolic rate is not crucial for FR to extend lifespan. Moreover, a few studies on mice (Liao et al. 2011a), houseflies (Cooper et al. 2004), parthenogenetic insects (Roark and Bjorndal 2009), nematodes (Houthoofd et al. 2003), and yeasts (Lin et al. 2002) have shown that under FR, metabolic rate seems to be positively correlated to health maintenance and lifespan. The controversial correlation between metabolic rate and longevity has been a long-standing puzzle (Mccarter et al. 1985, Brys et al. 2007, Speakman et al. 2004, Stuart and Brown 2006, Promislow and Haselkorn 2002, Hughes and Reynolds 2005).

The results from our study suggests that during growth, changes in metabolic rate actually do not lead to significant changes in somatic damage, and therefore will not have great effects on overall longevity. The key factor that influences on damage accumulation and longevity is biosynthesis rate. However, biosynthesis rate and metabolic rate are associated. As we reviewed in the introduction, they can be positively, negatively, or not

correlated. Thus, although change in metabolic rate does not directly lead to changes in damage, it can affect damage accumulation indirectly through its effects on biosynthesis. Here, we give an example taken from (Hou 2014). When animals are under food restriction (FR), the food assimilation rate is more or less fixed. Since assimilated energy from food is partitioned by metabolic energy and energy deposited in new-biomass, the fixed food assimilation imposes a tradeoff between metabolism and growth (Derting 1989; Hayes et al. 2014), i.e., high metabolic rate (either basal or activity) suppresses growth. Suppressed growth in turn will lead to a lower damage level. So, under FR, changes in metabolic rate do have an impact on damage accumulation, but this impact is exerted through its effect on growth.

In conclusion, it has shown that in hornworms the increase of metabolic rate does not cause significant increase in the phospholipid oxidative damage. The major contributor of the damage is biosynthesis.

SECTION

2. CONCLUSIONS

This thesis analyzed how hornworm adjusts its energy budget to adapt different food supply and environmental temperatures, and how the changes in energy budget affect its health maintenance. Three major findings were obtained from the experiments' results. First, under food restriction condition, high tempura can lead to high metabolism but slow down the growth rate; second, the larvae fed *Ad libitum* decrease the energy channeled to growth as body mass increases, and increase the energy allocated to metabolism, while the food restricted larvae showed an opposite trend by prioritization growth at the consumption of metabolism; last but not least, the major reason of the accumulated damages is due to the changes in biosynthesis instead of the changes in metabolic energy.

APPENDIX A.

Figure 1S

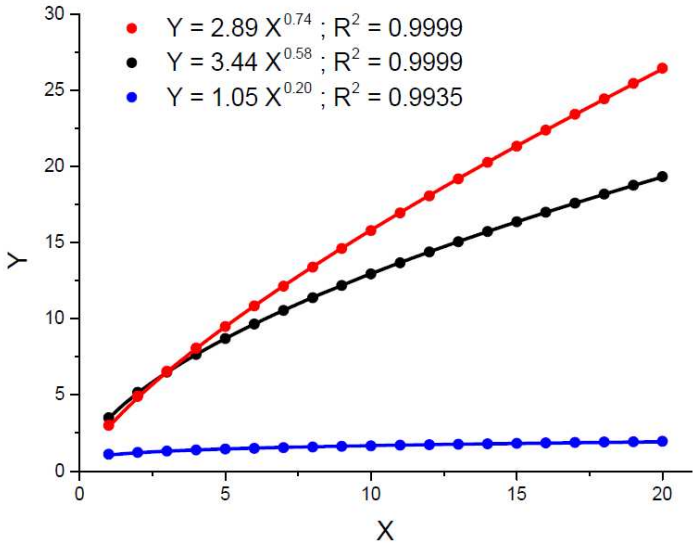

Figure 1S. An examples of fitting scaling exponents of *Y*.

Y is calculated from equation $Y = B_0 X^b + S_0 X^s$; Red dots: $Y = 2X^{0.8} + X^{0.5}$; Black dots: $Y = 3X^{0.6} + 0.5X^{0.3}$; Blue dots: $Y = 0.1X^{0.6} + X^{0.1}$. The scaling coefficients, B_0 and S_0, and the scaling exponents, *b* and *s*, are randomly chosen and are unitless.

APPENDIX B

Measurement of Metabolic Rate

We used the same method described in one of our previous publications (Hayes et al. 2014) to measure the metabolic rate of hornworm larvae. On the first day of the 5th instar, six larvae from each cohort were randomly chosen for the respirometry measurement. The rates of O_2 consumption and CO_2 production, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of the same larvae were measured for seven to ten minutes every day during the 5th instar until the wandering stage, using a flow-through respirometry system with an incurrent flow measurement (Lighton 2008). A CA-10 CO_2 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 CO_2 concentration (1,000 p.p.m. CO_2 in air). The FA-10 Oxygen analyzer (SSI) was calibrated using air free of CO_2 and water vapor and an assumed O_2 of 20.95% (Lighton 2008). Baselines were taken before, in between, and after each trial by running air scrubed of water and CO_2 through the system. Flow rate of the scrubbed air was set at 60 ml min^{-1} using an SS-4 subsampler (SSI). This air was then sent to the larva or baseline chamber. Between the CO_2 and O_2 analyzers, we scrubbed the CO_2 and water vapor produced by the larvae, so that the CO_2 and water concentration will not affect the measurement of O_2 (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 CO_2 and O_2 concentration. The rates $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated as $\dot{V}_{CO_2} = \mathrm{FR} \times [CO_2]/100$, and

$\dot{V}_{O_2} = \mathrm{FR} \times (20.95 - [O_2]) / (100 - [O_2])$, where FR is the flow rate, and $[CO_2]$ and $[O_2]$ are the concentration of CO_2 and O_2 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 $B = (43.25 - 22.5 \times RER) \times \dot{V}_{CO_2} / 60$, where $RER = \dot{V}_{CO_2} / \dot{V}_{O_2}$ is the respiratory exchange ratio (Blaxter 1989, Withers 1992).

APPENDIX C

Combustion Energy Content of Feces, Dry Body Tissue, and Digestibility

The energy content of feces between each cohort is not significantly different ($P > 0.05$). The average value is E_{feces} = 14786 ± 616 Joules/gram dry mass. The digestibility weakly scales with body mass in two cohorts 20 ^{o}C-AL and 25 ^{o}C-AL ($P < 0.05$). For other cohorts ($P > 0.05$), we calculated the average value of the digestibility over ontogeny. The scaling laws and average digestibilities are listed in Table S1. 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).

Table S1. Digestibility of Six Cohorts.

Cohort	Digestibility
20^{o}C-AL	$0.744 \times m^{0.043}$ (R^2 = 0.22; P = 0.014)
20^{o}C-FR	0.748 ± 0.092 (N = 35; P = 0.74)
25^{o}C-AL	$0.717 \times m^{-0.047}$ (R^2 = 0.07; P = 0.014)
25^{o}C-FR	0.80 ± 0.105 (N = 23; P = 0.21)
30^{o}C-AL	0.74 ± 0.039 (N = 20; P = 0.28)
30^{o}C-FR	0.80 ± 0.053 (N = 26; P = 0.32)

The dry/wet body mass ratio is approximately 14% in each cohort, similar to the results from previous study (Sears et al. 2012). Temperature and food supply level do not make significant difference in the ratio (ANOVA, $P > 0.05$).

Combustion energy contents of dry body tissue of larvae reared at different temperature and food supply level do not vary significantly (ANOVA, $P > 0.4$). We

oven-dried the body tissue, and used oxygen bomb calorimetry to assess the combustion energy content of dry tissue (E_{tissue}). If there was food residing in the larval guts, then the combustion energy of dried food may be included in the overall E_{tissue}. As such, the measured value of E_{tissue} may be different than the real one.

For food restricted larvae, it is not a concern. In our experiments, the food restricted (FR) larvae finished their food less than *ca.* 9 hours at 30 ^{o}C, and *ca*. 17 hours at 20 ^{o}C. Since we killed larvae for dry mass assay 24 hours after they were fed in the previous day, these FR larvae had experienced starvation time of 15 and 7 hours at 30 ^{o}C and 20 ^{o}C respectively. So, we can safely assume that there was no residual food in guts of FR larvae before they were killed and oven-dried. There was food in the guts of *ad libitum* (AL) larvae before they were killed. However, the average value of E_{tissue} in AL larvae is 23541±785 Joules/dry gram , slightly lower but very close to the one in FR larvae, 23845±523 Joules/dry gram. The slightly lower value in AL larvae may be attributed to the facts that the energy content of food is 20160 Joules/gram (method section), close but lower than that of body tissue, so the overall value is lower when residual food in AL larvae is included. Nonetheless, the insignificant difference between the values of FR and AL larvae (ANOVA, $P>0.446$) suggests that the residual food in AL larval guts has an insignificant effect on the overall E_{tissue}. So, we group the data and use the average value, E_{tissue} = 23693 ± 656 Joules/gram dry mass to calculate the growth rates.

BIBLIOGRAPHY

Alonso-Alvarez, C., S. Bertrand, B. Faivre & G. Sorci (2007) Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. Functional Ecology, 21**,** 873-879.

Álvarez, D. & A. G. Nicieza (2005) Is metabolic rate a reliable predictor of growth and survival of brown trout (Salmo trutta) in the wild? Canadian Journal of Fisheries and Aquatic Sciences, 62**,** 643-649.

Armitage, K. B. & T. J. Wall (1982) The effects of body size, starvation and temperature acclimation on oxygen consumption of the crayfish Orconectes nais. Comparative Biochemistry and Physiology Part A: Physiology, 73**,** 63-68.

Atkinson, D. 1994. Temperature and organism size: A biological law for ectotherms. In Advances in Ecological Research, Vol 25, 1-58.

Balaban, R., S. Nemoto & T. Finkel (2005) Mitochondria, Oxidants, and Aging. Cell, 120**,** 483-495.

Barja, G. (2004) Free radicals and aging. Trends in Neurosciences, 27**,** 595-600.

Barker, D. J. P. (2001) Fetal and infant origins of adult disease. Monatsschrift Kinderheilkunde, 149**,** S2-S6.

Bartke, A. (2003) Can Growth Hormone (GH) Accelerate Aging? Evidence from GH-Transgenic Mice. Neuroendocrinology, 78**,** 210-216.

Bartke, A. (2005) Role of the Growth Hormone/Insulin-Like Growth Factor System in Mammalian Aging. Endocrinology, 146**,** 3718-3723.

Beckman, K. B. & B. N. Ames (1998) The free radical theory of aging matures. Physiological Reviews, 78**,** 547-581.

Benyi, K. & H. Habi (1998) Effects of food restriction during the finishing period on the performance of broiler chickens. Br Poult Sci, 39**,** 423-5.

Blaxter, K. L. 1989. Energy Metabolism in Animals and Man. Cambridge, U.K.: Cambridge Univ. Press.

Bohrer, R. & W. Lampert (1988) Simultaneous measurement of the effect of food concentration on assimilation and respiration in Daphnia magna Straus. Functional Ecology**,** 463-471.

Brody, S. 1945. Bioenergetics and growth. New York: Reinhold.

Broekhuizen, N., W. S. C. Gurney, A. Jones & A. D. Bryant (1994) Modelling Compensatory Growth. Functional Ecology, 8**,** 770-782

Brown-Borg, H. M. (2003) Hormonal regulation of aging and life span. Trends Endocrin Met, 14**,** 151-153.

Brown-Borg, H. M., K. E. Borg, C. J. Meliska & A. Bartke (1996) Dwarf mice and the ageing process. Nature, 384**,** 33-33.

Brown, T. K., K. A. Nagy & D. J. Morafka (2005) Costs of Growth in Tortoises. Journal of Herpetology, 39**,** 19-23.

Brys, K., J. R. Vanfleteren & B. P. Braeckman (2007) Testing the rate-of-living/oxidative damage theory of aging in the nematode model Caenorhabditis elegans. Experimental Gerontology, 42**,** 845-851.

Brzęk, P. & M. Konarzewski (2001) Effect of food shortage on the physiology and competitive abilities of sand martin (Riparia riparia) nestlings. Journal of Experimental Biology, 204**,** 3065-3074.

Chappell, M. (1983) Metabolism and thermoregulation in desert and montane grasshoppers. Oecologia, 56**,** 126-131.

Charnov, E. L. 1993. Life history invariants: some explorations of symmetry in evolutionary ecology. U.S.A.: Oxford University Press.

Clissold, F. J., N. Coggan & S. J. Simpson (2013) Insect herbivores can choose microclimates to achieve nutritional homeostasis. The Journal of Experimental Biology, 216**,** 2089-2096.

Conti, B., M. Sanchez-Alavez, R. Winsky-Sommerer, M. C. Morale, J. Lucero, S. Brownell, V. Fabre, S. Huitron-Resendiz, S. Henriksen, E. P. Zorrilla, L. de Lecea & T. Bartfai (2006) Transgenic Mice with a Reduced Core Body Temperature Have an Increased Life Span. Science, 314**,** 825-828.

Cooper, T. M., R. J. Mockett, B. H. Sohal, R. S. Sohal & W. C. Orr (2004) Effect of caloric restriction on life span of the housefly, Musca domestica. FASEB J, 18**,** 1591-3.

D'Amico, L. J., G. Davidowitz & H. F. Nijhout (2001) The developmental and physiological basis of body size evolution in an insect. Proceedings of the Royal Society of London. Series B: Biological Sciences, 268**,** 1589-1593.

Davidowitz, G., L. J. D'Amico & H. F. Nijhout (2003) Critical weight in the development of insect body size. Evolution & Development, 5**,** 188-197.

De Block, M. & R. Stoks (2008) Short-term larval food stress and associated compensatory growth reduce adult immune function in a damselfly. Ecological Entomology, 33**,** 796-801.

Devi, V., Y. Prabhakara Rao & D. G. V. Prasada Rao (1986) Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropodMorula granulata (Duclos). Proceedings: Animal Sciences, 95**,** 539-547.

Diamond, S. E. & J. G. Kingsolver (2010) Environmental dependence of thermal reaction norms: host plant quality can reverse the temperature-size rule. The American Naturalist, 175**,** 1-10.

Dmitriew, C. M. (2011) The evolution of growth trajectories: what limits growth rate? Biological Reviews, 86**,** 97-116.

Eriksson, J. G., T. Forsén, J. Tuomilehto, P. D. Winter, C. Osmond & D. J. P. Barker (1999) Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ, 318**,** 427-431.

Esperk, T. & T. Tammaru (2004) Does the 'investment principle'model explain moulting strategies in lepidopteran larvae? Physiological Entomology, 29**,** 56-66.

Forster, M. J., B. H. Sohal & R. S. Sohal (2000) Reversible Effects of Long-Term Caloric Restriction on Protein Oxidative Damage. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 55**,** B522-B529.

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

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage & J. H. Brown (2002) Effects of size and temperature on developmental time. Nature, 417**,** 70-73.

Greenlee, K. J. & J. F. Harrison (2005) Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, Manduca sexta. Journal of Experimental Biology, 208**,** 1385-1392.

Grodzinski, W., R. Z. Klekowski & A. Duncan. 1975. Methods for ecological bioenergetics. Blackwell Scientific Publications Oxford.

Grotto, D., L. Santa Maria, S. Boeira, J. Valentini, M. Charão, A. Moro, P. Nascimento, V. Pomblum & S. Garcia (2007) Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography–visible detection. Journal of pharmaceutical and biomedical analysis, 43**,** 619-624.

Hack, M. A. (1997) The effects of mass and age on standard metabolic rate in house crickets. Physiological Entomology, 22**,** 325-331.

Hagerman, L. (1970) The oxygen consumption of Crangon vulgaris (Fabricius)(Crustacea, Natantia) in relation to salinity. Ophelia, 7**,** 283-292.

Hahn, D. A. & D. L. Denlinger (2011) Energetics of Insect Diapause. Annual Review of Entomology, 56**,** 103-121.

Hales, C. N. & S. E. Ozanne (2002) The dangerous road of catch-up growth. The Journal of Physiology, 547**,** 5-10.

Hall, M. E., J. D. Blount, S. Forbes & N. J. Royle (2010) Does oxidative stress mediate the trade-off between growth and self-maintenance in structured families? Functional Ecology, 24**,** 365-373.

Hammond, K. A. & J. Diamond (1997) Maximal sustained energy budgets in humans and animals. Nature, 386**,** 457-462.

Hayes, M., L. Jiao, T.-h. Tsao, I. King, M. Jennings & C. Hou (2014) High temperature slows down growth in tobacco hornworms (Manduca sexta larvae) under food restriction. Insect Science, In press.

Heilbronn, L. K. & E. Ravussin (2003) Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr, 78**,** 361-9.

Hermans, N., P. Cos, D. V. Berghe, A. J. Vlietinck & T. de Bruyne (2005) Method development and validation for monitoring in vivo oxidative stress: Evaluation of lipid peroxidation and fat-soluble vitamin status by HPLC in rat plasma. Journal of Chromatography B, 822**,** 33-39.

Holehan, A. M. & B. J. Merry (1986) The experimental manipulation of aging by diet. Biological Reviews, 61**,** 329-368.

Honěk, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos**,** 483-492.

Hong, Y.-L., S.-L. Yeh, C.-Y. Chang & M.-L. Hu (2000) Total plasma malondialdehyde levels in 16 Taiwanese college students determined by various thiobarbituric acid tests and an improved high-performance liquid chromatography-based method. Clinical biochemistry, 33**,** 619-625.

Hou, C. (2013) The energy trade-off between growth and longevity. Mechanisms of Ageing and Development, 134**,** 373-380.

Hou, C. (2014) Increasing energetic cost of biosynthesis during growth makes refeeding deleterious. American Naturalist, In press.

Hou, C., K. Bolt & A. Bergman (2011a) A General Life History Theory for Effects of Caloric Restriction on Health Maintenance. BMC Syst Biol, 5**,** 78-90.--- (2011b) A General Life History Theory for Effects of Caloric Restriction on Health Maintenance. BMC Syst Biol, 5**,** 78.

Hou, C., K. M. Bolt & A. Bergman (2011c) Energetic Basis of Correlation Between Catch-Up Growth, Health Maintenance, and Aging. J Gerontol A: Biol Sci Med Sci, 66A**,** 627-638.--- (2011d) A general model for ontogenetic growth under food restriction. Proceedings of the Royal Society B: Biological Sciences, 278**,** 2881-2890.

Hou, C., W. Y. Zuo, M. E. Moses, W. H. Woodruff, J. H. Brown & G. B. West (2008) Energy Uptake and Allocation During Ontogeny. Science, 322**,** 736-739.

Houthoofd, K., B. P. Braeckman & J. R. Vanfleteren. 2003. Metabolism and life span determination in C. elegans. In Advances in Cell Aging and Gerontology, ed. M. P. Mattson, 143-175. Elsevier.

Hughes, K. A. & R. M. Reynolds (2005) Evolutionary and Mechanistic Theories of Aging. Annual Review of Entomology, 50**,** 421-445.

Hulbert, A. J., D. J. Clancy, W. Mair, B. P. Braeckman, D. Gems & L. Partridge (2004) Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in Drosophila melanogaster. Experimental Gerontology, 39**,** 1137-1143.

Hulbert, A. J., R. Pamplona, R. Buffenstein & W. A. Buttemer (2007) Life and Death: Metabolic Rate, Membrane Composition, and Life Span of Animals. Physiological Reviews, 87**,** 1175-1213.

Inness, C. L. W. & N. B. Metcalfe (2008) The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish. Proceedings of the Royal Society B: Biological Sciences, 275**,** 1703-1708.

Jennings, B. J., S. E. Ozanne, M. W. Dorling & C. N. Hales (1999) Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Letters, 448**,** 4-8.

Jiao, L., M. Hayes, T.-h. Tsao, K. Koerperich, M. Jennings & C. Hou (2014) Food restriction-induced alteration of energy allocation strategy in hornworms (Manduca sexta larvae). American Naturalist, Submitted.

Karasov, W. H. & C. M. del Rio. 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press Princeton, New Jersey.

Kearney, M. R. & C. R. White (2012) Testing metabolic theories. The American Naturalist, 180**,** 546.

Kingsolver, J. G. & H. A. Woods (1997) Thermal sensitivity of growth and feeding in Manduca sexta caterpillars. Physiological and Biochemical Zoology, 70**,** 631-638.

Kitaysky, A. S. (1999) Metabolic and developmental responses of alcid chicks to experimental variation in food intake. Physiological and Biochemical Zoology, 72**,** 462-473.

Klass, M. R. (1977) Aging in the nematode Caenorhabditis elegans: major biological and environmental factors influencing life span. Mech Ageing Dev, 6**,** 413-29.

Konarzewski, M. & J. M. Starck (2000) Effects of food shortage and oversupply on energy utilization, histology, and function of the gut in nestling song thrushes (Turdus philomelos). Physiological and Biochemical Zoology, 73**,** 416-427.

Kooijman, S. 2010. Dynamic energy budget theory. Cambridge, UK: Cambridge University Press.

Kooijman, S. A. L. M. 2000. Dynamic Energy and Mass Budgets in Biological Systems Cambridge, U.K.: Cambridge Univ. Press.

Koštál, V. (2006) Eco-physiological phases of insect diapause. Journal of Insect Physiology, 52**,** 113-127.

Langleyevans, S. & D. Sculley (2006) The association between birthweight and longevity in the rat is complex and modulated by maternal protein intake during fetal life. FEBS Letters, 580**,** 4150-4153.

Lee, K. P. & C. Roh (2010) Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. Entomologia Experimentalis et Applicata, 136**,** 151-163.

Lemoine, N. & D. Burkepile (2012) Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. Ecology, 93**,** 2483–2489.

Liao, C.-Y., B. A. Rikke, T. E. Johnson, J. A. L. Gelfond, V. Diaz & J. F. Nelson (2011a) Fat maintenance is a predictor of the murine lifespan response to dietary restriction. Aging Cell**,** 629-639.--- (2011b) Fat maintenance is a predictor of the murine lifespan response to dietary restriction. Aging Cell**,** no-no.

Lighton, J. R. B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, USA.

Lin, S. J., M. Kaeberlein, A. A. Andalis, L. A. Sturtz, P. A. Defossez, V. C. Culotta, G. R. Fink & L. Guarente (2002) Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. Nature, 418**,** 344-348.

Lints, F. A. (1989) The Rate of Living Theory Revisited. Gerontology, 35**,** 36-57.

Lombard, D., K. Chua, R. Mostoslavsky, S. Franco, M. Gostissa & F. Alt (2005) DNA Repair, Genome Stability, and Aging. Cell, 120**,** 497-512.

Lucas, A., M. S. Fewtrell & T. J. Cole (1999) Fetal origins of adult disease - the hypothesis revisited. British Medical Journal, 319**,** 245-249.

Mair, W. & A. Dillin (2008) Aging and Survival: The Genetics of Life Span Extension by Dietary Restriction. Annual Review of Biochemistry, 77**,** 727-754.

Mair, W., P. Goymer, S. D. Pletcher & L. Partridge (2003) Demography of dietary restriction and death in Drosophila. Science, 301**,** 1731-1733.

Mangel, M. & S. B. Munch (2005) A life-history perspective on short- and long-term consequences of compensatory growth. Am Nat, 166**,** E155-76.

Mangel, M. & J. Stamps (2001) Trade-offs between growth and mortality and the maintenance of individual variation in growth. Evolutionary Ecology Research, 3**,** 583-593.

Marsden, I. D., R. C. Newell & M. Ahsanullah (1973) The effect of starvation on the metabolism of the shore crab, Carcinus maenas. Comparative Biochemistry and Physiology Part A: Physiology, 45**,** 195-213.

Martin, M. M. & H. M. Van't Hof (1988) The cause of reduced growth of Manduca sexta larvae on a low-water diet: Increased metabolic processing costs or nutrient limitation? Journal of Insect Physiology, 34**,** 515-525.

Masoro, E. (2005) Overview of caloric restriction and ageing. Mechanisms of Ageing and Development, 126**,** 913-922.

Mccarter, R., E. J. Masoro & B. P. Yu (1985) Does Food Restriction Retard Aging by Reducing the Metabolic-Rate. American Journal of Physiology-Endocrinology and Metabolism, 248**,** E488-E490.

McCarter, R. J. & J. R. McGee (1989) Transient Reduction of Metabolic-Rate by Food Restriction. American Journal of Physiology-Endocrinology and Metabolism, 257**,** E175-E179.

McCarter, R. J. & J. Palmer (1992) Energy-Metabolism and Aging - a Lifelong Study of Fischer-344 Rats. American Journal of Physiology-Endocrinology and Metabolism, 263**,** E448-E452.

McCay, C. M., M. F. Crowell & L. A. Maynard (1935) The effects of retarded growth upon the length of life span and upon the ultimate body size. J Nutr., 10**,** 63-79.

McCoy, M. W. & J. F. Gillooly (2008) Predicting natural mortality rates of plants and animals. Ecology Letters, 11**,** 710-716.

Merry, B. J. (1995) Effect of dietary restriction on aging – an update. Reviews in clinical gerontology, 5**,** 247.

Merry, B. J. (2002) Molecular mechanisms linking calorie restriction and longevity. Int J Biochem Cell Biol, 34**,** 1340-54.--- (2004) Oxidative stress and mitochondrial function with aging – the effects of calorie restriction. Aging Cell, 3**,** 7-12.

Metcalfe, N. B. & P. Monaghan (2001) Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution, 16**,** 254-260.

Metcalfe, N. B. & P. Monaghan (2003) Growth versus lifespan: perspectives from evolutionary ecology. Experimental Gerontology, 38**,** 935-940.

Miller, G. A., F. J. Clissold, D. Mayntz & S. J. Simpson (2009) Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. Proceedings of the Royal Society B: Biological Sciences, 276**,** 3581-3589.

Moe, B., S. Brunvoll, D. Mork, T. E. Brobakk & C. Bech (2004) Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (Phalacrocorax aristotelis). Journal of Experimental Biology, 207**,** 4067-4076.

Monaghan, P., N. B. Metcalfe & R. Torres (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecology Letters, 12**,** 75-92.

Moselhy, H. F., R. G. Reid, S. Yousef & S. P. Boyle (2013) A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC. Journal of lipid research, 54**,** 852-858.

Nagy, K. A. (2000) Energy costs of growth in neonate reptiles. Herpetological Monographs**,** 378-387.

Nagy, K. A., I. A. Girard & T. K. Brown (1999) Energetics of free-ranging mammals reptiles and birds. Annual Review of Nutrition, 19**,** 247-277.

Naim, M., J. G. Brand, M. R. Kare, N. A. Kaufmann & C. M. Kratz (1980) Effects of unpalatable diets and food restriction on feed efficiency in growing rats. Physiology & Behavior, 25**,** 609-614.

Naya, D. E. & F. Bozinovic (2006) The role of ecological interactions on the physiological flexibility of lizards. Functional Ecology, 20**,** 601-608.

Nijhout, H., G. Davidowitz & D. Roff (2006) A quantitative analysis of the mechanism that controls body size in Manduca sexta. Journal of Biology, 5**,** 16.

Nijhout, H. F. (1975) A threshold size for metamorphosis in the tobacco hornworm, Manduca sexta (L.). Biological Bulletin, 149**,** 214-225.

Nussey, D. H., J. M. Pemberton, J. G. Pilkington & J. D. Blount (2009) Life history correlates of oxidative damage in a free-living mammal population. Functional Ecology, 23**,** 809-817.

Ocak, N. & G. Erener (2005) The effects of restricted feeding and feed form on growth, carcass characteristics and days to first egg of Japanese quail (Coturnix coturnix japonica). ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES, 18**,** 1479.

Ong, K. K. L., M. L. Ahmed, P. M. Emmett, M. A. Preece & D. B. Dunger (2000) Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ, 320**,** 967-971.

Ozanne, S. E. & C. N. Hales (2004) Lifespan - Catch-up growth and obesity in male mice. Nature, 427**,** 411-412.

Partridge, L., M. Piper & W. Mair (2005) Dietary restriction in Drosophila. Mechanisms of Ageing and Development, 126**,** 938-950.

Pearl, R. 1928. The rate of living. London: University of London.

Pilz, J., I. Meineke & C. H. Gleiter (2000) Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2, 4-dinitrophenylhydrazine derivative. Journal of Chromatography B: Biomedical Sciences and Applications, 742**,** 315-325.

Promislow, D. E. L. & T. S. Haselkorn (2002) Age-specific metabolic rates and mortality rates in the genus Drosophila. Aging Cell, 1**,** 66-74.

Reynolds, S. E. & S. F. Nottingham (1985) Effects of temperature on growth and efficiency of food utilization in fifth-instar caterpillars of the tobacco hornworm, Manduca sexta. Journal of Insect Physiology, 31**,** 129-134.

Ricklefs, R. E. (2003) Is rate of ontogenetic growth constrained by resource supply or tissue growth potential? A comment on West et al's model. Functional Ecology, 17**,** 384-393.

Rikke, B. & T. Johnson (2004) Lower body temperature as a potential mechanism of life extension in homeotherms. Experimental Gerontology, 39**,** 927-930.

Roark, A. M. & K. A. Bjorndal (2009) Metabolic rate depression is induced by caloric restriction and correlates with rate of development and lifespan in a parthenogenetic insect. Experimental Gerontology, 44**,** 413-419.

Roff, D. A. 2001. Life history evolution. Sunderland, Massachusetts: Sinauer Associates.

Rollo, C. D. (2002) Growth negatively impacts the life span of mammals. Evolution & Development, 4**,** 55-61.

Rønning, B., A. S. Mortensen, B. Moe, O. Chastel, A. Arukwe & C. Bech (2009) Food restriction in young Japanese quails: effects on growth, metabolism, plasma thyroid hormones and mRNA species in the thyroid hormone signalling pathway. Journal of Experimental Biology, 212**,** 3060-3067.

Rossetto, M., G. De Leo, D. Bevacqua & F. Micheli Allometric scaling of mortality rates with body mass in abalones. Oecologia**,** 1-8.

Schiemer, F. (1982) Food dependence and energetics of freeliving nematodes. Oecologia, 54**,** 108-121.

Schoener, T. W. (1971) Theory of Feeding Strategies. Annual Review of Ecology and Systematics, 2**,** 369-404.

Sears, K. E., A. J. Kerkhoff, A. Messerman & H. Itagaki (2012) Ontogenetic Scaling of Metabolism, Growth, and Assimilation: Testing Metabolic Scaling Theory with Manduca sexta Larvae. Physiological and Biochemical Zoology, 85**,** 159-173.

Sibly, R. M. & P. Calow. 1986. Physiological Ecology of Animals: An Evolutionary Approach. Palo Alto, U.S.A.: Blackwell Scientific publications.

Sinclair, D. (2005) Toward a unified theory of caloric restriction and longevity regulation. Mechanisms of Ageing and Development, 126**,** 987-1002.

Sohal, R. S. & R. Weindruch (1996) Oxidative stress, caloric restriction, and aging. Science, 273**,** 59-63.

Speakman, J. R., D. A. Talbot, C. Selman, S. Snart, J. S. McLaren, P. Redman, E. Krol, D. M. Jackson, M. S. Johnson & M. D. Brand (2004) Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell, 3**,** 87-95.

Stearns, S. C. 1992. The evolution of life histories. Oxford, UK: Oxford University Press.

Steyermark, A. C. (2002) A high standard metabolic rate constrains juvenile growth. Zoology, 105**,** 147-151.

Stuart, J. & M. Brown (2006) Energy, quiescence and the cellular basis of animal life spans. Comp Biochem Physiol A Mol Integr Physiol 143**,** 12-23.

Timmins, W. A., K. Bellward, A. J. Stamp & S. E. Reynolds (1988) Food intake, conversion efficiency, and feeding behaviour of tobacco hornworm caterpillars given artificial diet of varying nutrient and water content. Physiological Entomology, 13**,** 303-314.

Trzcionka, M., K. W. Withers, M. Klingenspor & M. Jastroch (2008) The effects of fasting and cold exposure on metabolic rate and mitochondrial proton leak in liver and skeletal muscle of an amphibian, the cane toad Bufo marinus. Journal of Experimental Biology, 211**,** 1911-1918.

van der Meer, J. (2006) Metabolic theories in ecology. Trends in Ecology & Evolution, 21**,** 136-140.

Van Voorhies, W. A. & S. Ward (1999) Genetic and environmental conditions that increase longevity in Caenorhabditis elegans decrease metabolic rate. Proceedings of the National Academy of Sciences of the United States of America, 96**,** 11399-11403.

Verity, P. G. (1985) Grazing, respiration, excretion, and growth rates of tintinnids. Limnology and Oceanography**,** 1268-1282.

Walker, G., K. Houthoofd, J. Vanfleteren & D. Gems (2005) Dietary restriction in C. elegans: From rate-of-living effects to nutrient sensing pathways. Mechanisms of Ageing and Development, 126**,** 929-937.

Weindruch, R. & R. L. Walford. 1988. The Retardation of Aging and Disease by Dietary Restriction. Springfield, IL: Thomas.

West, G. B., J. H. Brown & B. J. Enquist (2001) A general model for ontogenetic growth. Nature, 413**,** 628-631.--- (2004) Growth models based on first principles or phenomenology? Functional Ecology, 18**,** 188-196.

Wieser, W. (1994) Cost of Growth in Cells and Organisms - General Rules and Comparative Aspects. Biological Reviews of the Cambridge Philosophical Society, 69**,** 1-33.

Withers, P. C. 1992. Comparative Animal Physiology. Fort Worth: Saunders College Pub.

Yu, B. P. (1994) How Diet Influences the Aging Process of the Rat. Proc Soc Exp Biol Med 205**,** 97-105.

Zuo, W. Y., M. E. Moses, G. B. West, C. Hou & J. H. Brown (2012) A general model for effects of temperature on ectotherm ontogenetic growth and development. Proceedings of the Royal Society B: Biological Sciences, 279**,** 1840-1846.

VITA

Lihong Jiao was born in Shijiazhuang, Hebei Province, China. In July 2009, she received her B.S. in Clinical Medicine from Hebei Medical University, Clinical Medicine College, Hebei Province, China. In May 2014, she received her M.S. degree in Biological Science from the Missouri University of Science and Technology, Rolla, Missouri, USA.