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The extremely low energy cost of biosynthesis in holometabolous insect larvae

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ABSTRACT

The metabolic cost of growth, which quantifies the amount of energy required to synthesize a unit of biomass, is an important component of an animal's ontogenetic energy budget. Here we investigated this quantity as well as other energy budget variables of the larvae of a holometabolous insect species, *Vanessa cardui* (painted lady). We found that the high growth rate of this caterpillar cannot be explained by its metabolic rate and the percentage of the metabolic energy allocated to growth; the key to understanding its fast growth is the extremely low cost of growth, 336 Joules/gram of dry mass.

The metabolic cost of growth in caterpillars is 15–65 times lower than that of the endothermic and ectothermic species investigated in previous studies. Our results suggest that the low cost cannot be attributed to its body composition, diet composition, or body size. To explain the "cheap price" of growth in caterpillars, we assumed that a high metabolic cost for biosynthesis resulted in a high "quality" of cells, which have fewer errors during biosynthesis and higher resistance to stressors. Considering the life history of the caterpillars, i.e., tissue disintegration during metamorphosis and a short developmental period and lifespan, we hypothesized that an energy budget that allocates a large amount of energy to biosynthesizing high quality cells would be selected against in this species. As a preliminary test of this hypothesis, we estimated the metabolic cost of growth in larvae of *Manduca sexta* (tobacco hornworm) and nymphs of *Blatta lateralis* (Turkestan cockroach). The preliminary data supported our hypothesis.

1. Introduction

The importance of the metabolic cost of growth, defined as the amount of metabolic energy required to synthesize one unit of biomass (Brody, 1945, Wieser, 1994, Hou et al., 2008, Moses et al., 2008), has been highlighted since the era of Rubner (Rubner, 1908) and Brody (Brody, 1945). It has been typically estimated using the energy budget model (Wieser, 1994, West et al., 2001):

$$B = E_m G + B_{M,A} \quad (1)$$

where B and $B_{M,A}$ are the metabolic rate and the rate of energy allocated to maintenance and activity respectively, both in unit of energy/time; G is the growth rate (biomass gain/time); and E_m is the metabolic cost of growth. Equation (1) suggests that for a given metabolic rate, growth rate depends on (1) the amount of energy allocated to growth; and (2) the value of E_m. For a given fraction of B allocated to growth, a smaller E_m (cheaper growth) would lead to a higher G (faster growth).

The metabolic cost of growth, E_m, also called the "indirect cost of growth," is equivalent to the efficiency of production and related to the economic profit in animal husbandry and fishery, and it has been heavily studied in agricultural and aquacultural animals (e.g., (Brody, 1945, Rattray et al., 1974, Webster et al., 1976, Lupatsch et al., 2003), but see (Bayne, 1999, Peterson et al., 1999)). Less effort has been made to investigate the values of E_m in insects, despite its significance in physiology and ecology (Brody, 1945, Ricklefs, 1974, Vleck and Vleck, 1987, Wieser, 1994, Konarzewski, 1995, Peterson et al., 1999, Kooijman, 2010). Moreover, some of the existing studies on insects suffer from a conceptual flaw (e.g., (Booth and Kiddell, 2007, Sears et al., 2012). The authors of those studies simplified the energy budget model, Eq.1, as $B = E_m G$, assuming the energy allocated to maintenance and activity is negligible in developing insects, and therefore overestimated the value of E_m of insects.

The main purpose of the current study was to apply Eq. (1) to investigate the ontogenetic energy allocation strategy and the E_m-value of a holometabolous insect larva, the caterpillar of *Vanessa cardui* (painted lady). Unlike previous studies on insect energy budget, we do not treat

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the maintenance and activity costs as negligible. Instead, we follow the previous models (Jobling, 1985, Peterson et al., 1999), and assume that the term of $B_{M,A}$ scales with body mass M as $B_{M,A} = aM^b$. Equation (1) thus becomes

$$B = E_m G + aM^b \quad (2)$$

With the empirical inputs of B, G, and M, the nonlinear regression of Eq.2 yields the values of the parameters, E_m, a, and b (Peterson et al., 1999). This model assumes that E_m is a constant over ontogeny and is independent of growth rate, G, and maintenance term, $B_{M,A}$. Thus, the values of the parameters obtained from the nonlinear regression of Eq. (2) can be considered the average over the period of measurements.

Painted ladies complete larval growth in ~2–3 weeks. Equation (2) indicates that this fast growth may stem from the concerted effects of three factors, namely high metabolic rate (B), high allocation of energy to growth (the ratio of E_mG and B), and cheap cost of biosynthesis (E_m). To put the values of B, E_mG/B, and E_m obtained from caterpillars, high or low, into perspective, we used the nymph of a hemimetabolous species, *Blatta lateralis* (Turkestan cockroach), as a reference. The body mass range of cockroach nymphs during development is similar to that of painted lady caterpillars, but it takes 100 ~ 200 days for the cockroach to finish the nymphal stage (Kim and Rust, 2013). Based on Eq. (2), we predicted that the great difference in the growth rates of these two species may not simply come from the difference between their metabolic rates; additionally, the allocation ratio and the indirect cost of biosynthesis may both attribute to the difference. We also attempted to explain the extreme low E_m-value found in the holometabolous species (painted lady caterpillars). As a preliminary test of the hypothesis, we use Eq. (2) to estimate the value of E_m in another holometabolous insect larva, the tobacco hornworm (*Manduca sexta*), in addition to the nymph of Turkestan cockroach.

2. Materials and methods

2.1. Animal rear, food supplies, and growth rate

Sixty five painted lady caterpillars, 73 cockroach nymphs, and 40 tobacco hornworms were reared at 25 ± 1 °C on a long day cycle (17 h light: 7 h dark). Painted lady caterpillars were fed *ad libitum* with sucrose and protein-based diet (Carolina Biological Supply, NC. 80% moisture; per unit of dry food has 13–15% of protein content and negligible amount of lipid content). Cockroaches were supplied with Wardley Pond Pellets (Hartz Mountain Corp., Secaucus, NJ; the protein and lipid contents of the dry mass are 33% and 5.5%, respectively). Water supply was unlimited. Tobacco hornworms were fed a wheat germ-based diet (tobacco hornworm medium bulk diet, Carolina Biological supply, NC).

The body mass of each painted lady caterpillar was measured to the nearest 0.1 mg using a digital microbalance (Perkin-Elmer AD6) at approximately the same time every day. Thirty five caterpillars were measured during the 4th instar, and 30 were measured during the 5th instar (the final instar). It is statistically convenient to compare the energy budget during growth between the caterpillars and cockroaches within a similar body size range. Thus, the body masses of 13 cockroaches were measured twice during the 2nd instar with a one-week interval, and 60 were measured from the 3rd to the 5th instar twice or three times with an interval of two weeks. About 1/3 of the individuals became adults before the third measurements. The measurements on tobacco hornworms were performed during the entire 5th instar (the final instar).

2.2. Respirometry

The same method described in our previous publication was used to measure the metabolic rate of painted lady caterpillars, tobacco hornworms, and cockroach nymphs (Hayes et al., 2015). For painted lady caterpillars and tobacco hornworms, our experiences suggest that their CO_2 production does not fluctuate greatly, probably because they eat constantly and their activity level is low. Thus, we performed respirometry on each animal for 7–10 min time interval every day. The sample from such a short period is sufficient to represent the average. The rates of CO_2 production, $\dot{V}_{CO_2}$, of each animal was measured using Sable System International (Las Vegas, U.S.A.) CA-10 CO_2 analyzer (incurrent flow-through respirometry). During the trials, temperature was controlled at 25 °C using a PELT5 temperature controller (SSI) that housed the respirometry chambers. Respirometry chambers for all species were 60-cc syringe barrels fitted with rubber stoppers and connected to inlet and outlet tubing. CO_2 production rate, $\dot{V}_{CO_2}$, in unit of ml/min, was calculated as $\dot{V}_{CO_2} = FR \times [CO_2]/100$, where FR is the flow rate, set at 50 ml/min, and $[CO_2]$ is the concentration of CO_2 in the respirometry chamber (Lighton, 2008). Our previous unpublished data suggest that $\dot{V}_{CO_2}$ of cockroaches varies considerably at different time of the day, and therefore the results from a short measurement period, e.g., 7 ~ 10 min, are not sufficient to represent the metabolic rate of cockroaches. Thus, we performed respirometry on each cockroach for a 24-hour period. The method is the same as described above, except that the air flow rate was set at 25 ml/min due to the low metabolic rate of cockroaches. During the respirometry, all three species had access to food and water. Metabolic rate (in unit of energy/time) was converted from CO_2 production rate as $4.98 \times \dot{V}_{CO_2} \times 4.18$, where the factor 4.98 converts the emission rate of CO_2 (in unit of ml/min) to metabolic rate (calorie/min) (Blaxter, 1989), and the factor 4.18 converts calories to joules.

2.3. Body composition assays

Since the study of energy budget is performed on painted lady caterpillars and cockroaches, we only assayed the body composition of these two species. Sixteen painted lady caterpillars and 25 cockroaches with different body sizes, which were not included for respiromitry, were sacrificed and oven-dried at 65 °C for 72 h to determine the dry mass/wet mass ratio. Our previous unpublished data showed that roughly 5% of the dry mass of painted lady caterpillars is comprised of the food in the midgut. To accurately estimate the body composition, we rinsed the midgut with distilled water and removed the gut content before oven-drying.

Following Foray et al's modified protein and lipid content assays (Foray et al., 2012), the protein and total lipid contents in both species were measured in 96-well microplates. The insects were dried and crushed into an aqueous lysis buffer to extract and quantify their protein content using the Coomassie-Bradford assay. Then a chloroform:methanol (1:2 v/v) solution was added to reach the following proportions of water, chloroform and methanol (2:5:10 v/v/v) allowing the extraction of total lipids. Lastly, the extraction solution is further assayed with van Handel's methods (Van Handel, 1985, 1988). The experiments were carried out on the various insects. Each set of insects was considered to have similar levels of nutrients, with each of the samples treated individually as well as being randomly assigned to groups. Homogenized samples were split into ten groups of 15 mg.

2.4. Protein content determination

Each sample was placed in a 2-mL Eppendorf tube, 180 μL of aqueous lysis buffer solution [100 mM KH_2PO_4, 1 mM dithiothreitol (DTT), and 1 mM ethylenediaminetetraacetic acid (EDTA)] was added, and the tube was then vortexed. Each homogenate was subjected to low-spin centrifugation (150 g at 4 °C for 10 mins), which allowed gentle sedimentation of cell debris that would otherwise alter the clarity of the sample. In accordance with the manufacturer's instructions, duplication

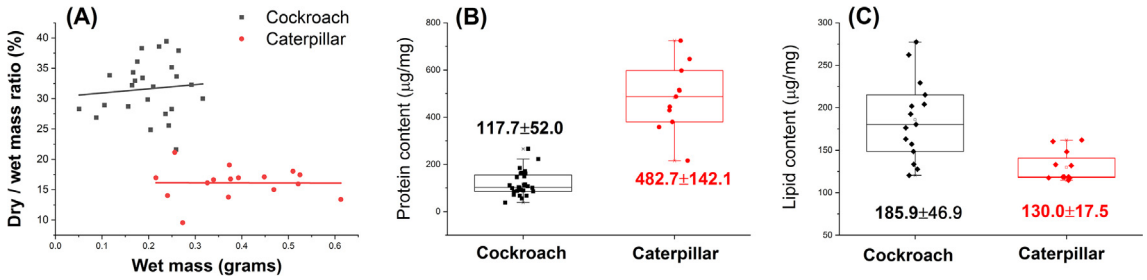

Fig. 1. Body composition of cockroaches and painted lady caterpillars. (A) The ratio of dry and wet mass; (B) Protein contents of dry mass; (C) Lipid contents of dry mass.

of 5 μL of each supernatant was transferred into a 96-well microplate, together with 250 μL of Coomassie (Bradford) micro-assay reagent (23200, Thermo Fisher Scientific, Rockford, IL). Samples were incubated at room temperature for at least 15 min (the protein-dye complex is stable up to 50 mins). Protein concentration was determined spectrophotometrically at 595 nm (FLUOstar Omega, Cary, NC) using a dilution-series of fetal bovine serum albumin dissolved into the same buffer as the samples. Before reading, the microplates were gently shaken (3 secs at 10 Hz) to disrupt protein-dye aggregates.

2.5. Lipid content determination

To replace the taken supernatant, 10 μL of the aqueous lysis buffer was added to the homogenate followed by 20 μL of 20% sodium sulfate (Na_2SO_4, 191444, MP Biomedicals LLC, Solon, OH) to dissolve all the carbohydrates and to reach the final solution of 0.2 ml of 2% Na_2SO_4 (van Handel, 1965; van Handel & Day, 1988). This solution was then mixed with 1500 uL of 1:2 v/v chloroform–methanol solution to solubilize the total lipids (Van Handel, 1965, Van Handel, 1988). After vigorous vortexing, each sample was centrifuged (200 g at 4C for 15 mins) to remove the glycogen from the supernatant. The total amount of lipids in each sample was determined in accordance with the vallinin assay procedure (Van Handel, 1985) using triolein (122327, MP Biomedicals LLC, Solon, OH) as the standard. Vanillin reagent was prepared by mixing vanillin (121335, Acros Organics, Pittsburgh, PA) with ortho-phosphoric acid 68%, reaching a final concentration of 1.2 g L^{-1}. For the assay, 100 μL of the supernatant was transferred into a glass-coated microplate well (60180P304, ThermoScientific, St. Peters, MO) and heated at 80°C until the solvent was completely evaporated. After removing the plate from the bead bath, the plate sat at room temperature for 2 min before 10 uL of 98% sulphuric acid was added to each well. Then the microplate was again incubated at 80 °C for 5 mins. After removing the plate from the bead bath, the microplate was cooled on ice for 10 min prior to 190 μL of vanillin reagent was added to each well. The plate was homogenized using glass rods, incubated at room temperature for 15 min and its absorbance was measured spectrophotometrically at 525 nm.

2.6. Data analysis

We measured the metabolic rate and body mass of each painted lady caterpillar and tobacco hornworm multiple times with a one-day interval between measurements, and those of each cockroach twice with a 1- or 2-week interval between measurements. The growth rate of the animals were calculated as the difference of dry body mass between two successive measurements. In Eq. (2), $B = E_m G + aM^b$, each growth rate (G) over a particular time interval (one day for painted lady caterpillars and tobacco hornworms, and one or two weeks for cockroaches) corresponds to a certain body mass (M) and a certain metabolic rate (B). We took the average values of dry body mass and metabolic rate at two successive measurements, and then treated the average values as the corresponding values for a particular time interval for the regression.

Since dry mass/wet mass ratios are independent on body size in painted lady caterpillars and cockroaches, we used the empirically derived dry/wet mass ratios to estimate the dry mass of each animal on the days of measurements. For tobacco hornworms, the food content in the midgut makes up to as much as ~20% of their body mass. This large portion of mass does not cost energy to synthesis, and therefore needs to be removed in the calculation of the E_m-value of tobacco hornworms. We sacrificed 44 individuals in the 5th instar, which were not included in the respirometry, removed the food content in midgut, and measured the dry mass. We then regressed the dry mass onto the wet mass before scarification, and obtained a linear correlation between the dry and wet mass. We use this linear correlation to estimate the dry masses and growth rates of the living individuals involved in the respirometry and the estimate of E_m-value. The data analysis and calculation methods are the same as employed for painted lady caterpillars and cockroaches.

The time scale in this study is daily, instead of hourly or weekly. Thus, for convenience, the units of metabolic rate and growth rate are presented as Joules/Day and Grams/Day. The nonlinear regression was performed on IBM SPSS Statistics 23.

3. Results

3.1. Body composition of painted lady caterpillars, cockroach nymphs, and tobacco hornworms

The dry mass/wet mass ratios of painted lady caterpillar and cockroach nymph are independent on body mass ($P = 0.65$ and 0.98 for cockroach and caterpillar respectively, Fig. 1A), and the average values of the ratios are 31.6 ± 4.6% ($N = 25$) and 16.1 ± 2.6% ($N = 16$) for cockroach and caterpillar, respectively.

The protein content of cockroach and caterpillar dry mass are 117.7 ± 52.0 μg/mg ($N = 28$) and 482.7 ± 142.1 μg/mg ($N = 11$), respectively (Fig. 1B). The lipid content of cockroach and caterpillar dry mass are 185.9 ± 46.9 μg/mg ($N = 15$) and 130.0 ± 17.5 μg/mg ($N = 12$), respectively (Fig. 1C).

The regression of dry mass without gut content onto wet mass of living tobacco hornworms gives Dry mass =0.1132 × Wet mass − 0.0061 ($N = 44$, $R^2 = 0.981$, Fig. 2). This relation is used to infer the dry mass and growth rate in the tobacco hornworms under respirometry.

4. Growth rate and metabolic rate of three species

The dry body mass increase of the cockroaches averages at 2.47 ± 1.89% per day. The linear regression of growth rate (daily dry body mass gain) on dry body mass yields $G = 0.0130M$ ($R^2 = 0.67$, $P < 0.001$, $N = 117$, Fig. 3A). The average dry mass increase of the painted lady caterpillars is 51.3 ± 31.1% per day. The linear regression shows that $G = 0.354M$ ($R^2 = 0.65$, $P < 0.001$, $N = 154$, Fig. 3A). For tobacco hornworms, the average growth is 35.6 ± 13.1% per day, and the linear regression of growth rate is $G = 0.302M$ ($R^2 = 0.86$, $P < 0.001$, $N = 117$, Fig. 3A).

The metabolic rates (Joules/day) scale with dry mass as

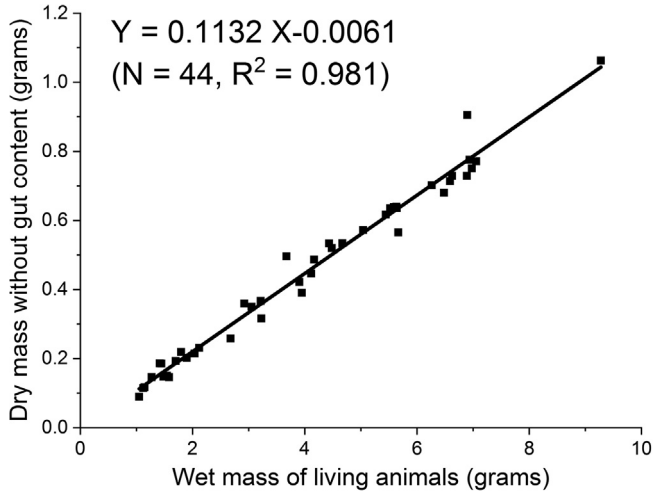

Fig. 2. The linear correlation between dry mass without gut content and wet mass of living tobacco hornworms.

$B = 1101.9M^{1.068}$ ($R^2 = 0.77$, P < 0.001, N = 117, Fig. 3B) for cockroaches, $B = 2976.5M^{0.794}$ ($R^2 = 0.85$, P < 0.001, N = 154, Fig. 3B) for painted lady caterpillars, and $B = 2531.3M^{0.592}$ ($R^2 = 0.56$, P < 0.001, N = 117, Fig. 3B) for tobacco hornworms.

4.1. Estimates of E_m-values in three species

Nonlinear fitting of Eq. (2), $B = E_mG + aM^b$, with the empirical data of metabolic rate (B), growth rate (G), and dry body mass (M) yields $B = 6905.4G + 1261.0M^{1.165}$ for cockroach nymphs (N = 117; $R^2 = 0.80$), $B = 335.6G + 2982.4M^{0.804}$for painted lady caterpillars (N = 154; $R^2 = 0.87$), and $B = 1303.9G + 2182.4M^{0.586}$ for tobacco hornworms (N = 116; $R^2 = 0.58$). The results of the regressions suggest that the values of the metabolic cost of growth, E_m, are 6905.4, 335.6, and 1303.9 Joules/gdbm for cockroach nymphs, painted lady caterpillars, and tobacco hornworms respectively.

4.2. The energy allocation strategies of cockroach nymphs and painted lady caterpillars

We use the fitted functions, $B(M)$, $G(M)$, and $B_{M,A}(M)$ (listed in Table 1), to investigate the energy budget of painted lady caterpillars and cockroach nymphs. The dry body masses of these two species during development are in a similar range, varying from 0.00258 to 0.119 g in caterpillars and from 0.00158 to 0.153 g in cockroaches. This allows us to use cockroach nymph as the reference to study the causes of the fast growth in caterpillars. Tobacco hornworms, on the other hand, have a much larger body mass range (Fig. 2). Since the allometric scaling powers of B and $B_{M,A}$ are different in these three species, the energy budget can only be compared between the species with similar body mass range. Thus, tobacco hornworms are excluded in the analysis below. We will primarily focus on cockroaches and painted lady caterpillars in this pilot study.

Using the fitted values of E_m and the empirical data of G and B, we estimate the percentage of total metabolic energy that is allocated to biosynthesis by calculating the ratio E_mG/B. On average cockroaches allocate 17.9 ± 14.4% of the metabolic energy to biosynthesis, and the allocation in caterpillars is 3.1 ± 2.5%. In cockroaches, the allocation percentage declines with body mass as $0.009M^{-0.24}$ (N = 117, $R^2 = 0.13$, P < 0.001, Fig. 4A), whereas in painted lady caterpillars the allocation percentage is roughly a constant independent on body mass (N = 154, $R^2 = 0.007$, P = 0.196, Fig. 4A).

The metabolic rate (B), growth rate (G), and the rate of energy allocated to maintenance and activity ($B_{M,A}$) vary with dry body mass (M). The cost of growth (E_m) in cockroach nymphs is about 20-fold (=6905.4/335.6) that of painted lady caterpillars. The growth rate (G, dry body mass gain/day) of painted lady caterpillars is 27-fold that of cockroach nymphs ($0.354M/0.0130M$). The energy spent on maintenance and activity in painted lady caterpillars is 7.06 ± 3.06-fold that of cockroach nymphs ($B_{M,A} = 2982.4M^{0.804}/B_{M,A} = 1261.0M^{1.165}$, M varies from 0.002 to 0.15 g. Fig. 4B). The metabolic rate of painted lady caterpillars is 6.1 ± 1.87-fold that of cockroach nymphs ($B = 2976.5M^{0.794}/B = 1101.9M^{1.068}$ with the body mass range 0.002–0.15 g. Fig. 4B).

5. Discussion

5.1. Comparing this study to a study based on the dynamic energy budget model

Employing the Dynamic Energy Budget (DEB) model, Llandres et al. (2015) conducted a study on an endoparasitic wasp, *Venturia canescens* parasitoid (hymenoptera: Ichneumonidae). It was the first study that investigated the complete energy budget of a holometabolous insect during its entire lifecycle. Here we make two points to compare our study to Llandres et al. (2015).

First, it is unclear how Llandres et al obtained the E_m-value of the wasp listed in their Table 4, which is 21210 J/cm^3, equivalent to roughly 21,210 Joules/gram of wet mass. It seems that the authors obtained this value from other species, or made a theoretical assumption about it. In contrast, one of the major goals of our study is to empirically measure this value, which showed a great difference between painted lady caterpillars and previously studied species.

Second, the scope of Llandres et al.'s study is much broacher than ours. Their study includes a wide suite of ecophysiological variables

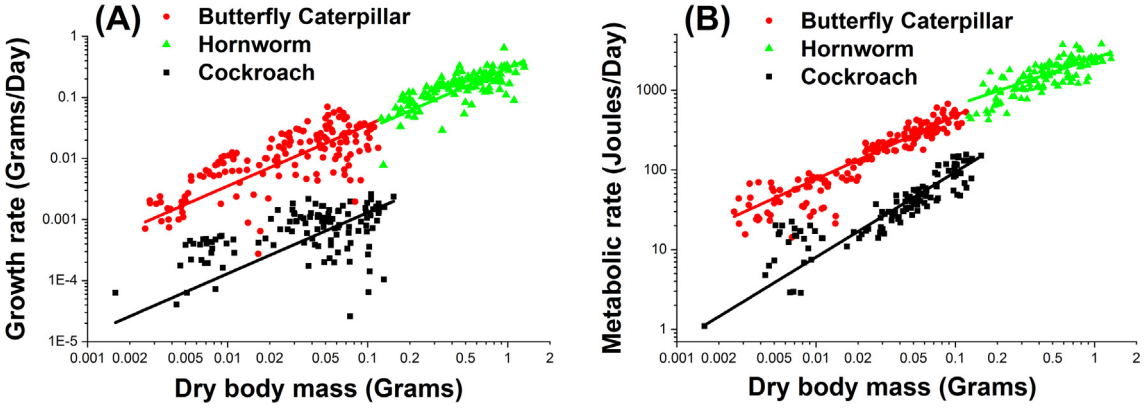

Fig. 3. Growth rate (A) and Metabolic rate (B) of painted lady caterpillars, cockroach nymphs, and tobacco hornworms.

Table 1
Comparison of the energy budget parameters of painted lady caterpillar and cockroach.

	E_m (Joules/gdbm)	G (grams/day)	$B_{M,A}$ (Joules/day)	B (Joules/day)
Cockroach	6905.4	$G = 0.0130M$	$B_{M,A} = 1261.0M^{1.165}$	$B = 1101.9M^{1.068}$
Caterpillar	335.6	$G = 0.354M$	$B_{M,A} = 2982.4M^{0.804}$	$B = 2976.5M^{0.794}$
Ratio of caterpillar to cockroach	0.049	27.2	7.06 ± 3.06 (M varies from 0.002 to 0.15 g)	6.1 ± 1.87 (M varies from 0.002 to 0.15 g)

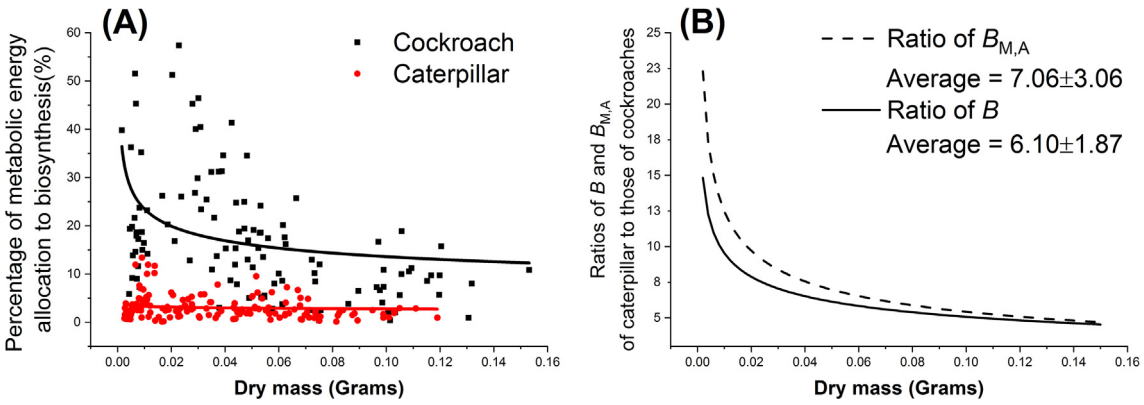

Fig. 4. Comparison of energy allocation in cockroaches and painted lady caterpillars. (A) Percentage of metabolic energy allocated to biosynthesis as functions of body mass; (B) The ratios of metabolic rate (B) and rate of energy allocated to maintenance and activity ($B_{M,A}$) between painted lady caterpillars and cockroaches.

during the entire lifecycle, ranging from food intake, embryo development, number of eggs, silk production, aging, lifespan, and so on. They performed a complete analysis on one species. In contrast, we only focus on two life history traits during larval stage, biosynthesis and maintenance, and our focus is to highlight the extremely low biosynthetic cost in painted lady larvae.

5.2. Energy budgets of cockroach nymphs and painted lady caterpillars

Two surprising findings of our study stand out, and are associated with each other. First, counterintuitively, the fast-growing painted lady caterpillar was found to allocate less of its metabolic energy to biosynthesis (3.1%) compared to the relatively slow-growing cockroach nymphs (17.9%). However, the painted lady caterpillars' growth rate (dry body mass gain per day) is about 27-fold that of the cockroach nymphs. In the Introduction, we explained that physiologically, the relatively high growth rate may be associated with a higher metabolic rate, a higher energy allocation to growth, a cheaper growth cost, or a combination of these factors. The metabolic rate of the painted lady caterpillars is roughly 6.1-fold that of cockroach nymphs, and the fraction of energy allocated to biosynthesis in painted lady caterpillars is 3.1%/17.9% ≈ 1/6-fold that of cockroach nymphs. Thus, the total metabolic energy allocated to biosynthesis are roughly the same in both species ($6.1 \times 1/6 = 1.06$), which means the combination of high metabolic rate and low fraction of energy allocation to biosynthesis in painted lady caterpillars cannot explain its high growth rate. This highlights the second major finding of this study: the metabolic cost of growth is about 21 times lower in painted lady caterpillars (335.6 Joules/gdbm) than in cockroach nymphs (6905.4 Joules/gdbm). Together with the 1.06-fold greater energy allocation to growth, the 21-fold cheaper cost of growth in painted lady caterpillars almost explains the 27-fold greater growth rate.

5.3. The extreme low cost of growth of painted lady caterpillars and tobacco hornworms

As an important component of animal energy budget, the metabolic cost of growth, E_m, has been estimated in multiple species. In the seminal review (Wieser, 1994), Wieser concluded that the "consensus value" of E_m is about 7.2 Kilojoules/gram of dry body mass (KJ/gdbm) for a wide range of organisms, assuming one gram of dry tissue contains 22 Kilojoules of combustion energy. This value means that it takes one unit of metabolic energy to deposit three (~7.2:22) units of combustion energy in dry body tissue, which agrees with the value estimated by Ricklefs (1974). In Table 2, we list the values of E_m in multiple endothermic and ectothermic species collected from the studies that employed the energy budget model, Eq. (2). Several previous studies are excluded in Table 2 for two reasons. First, some studies did not include the energy allocation to maintenance and activity, $B_{M,A}$ (e.g., (Booth and Kiddell 2007, Sears et al., 2012)), and therefore overestimated the cost of growth; Second, instead of using the empirical values, some

Table 2
Previously reported values of the metabolic cost of growth, E_m. The values from Wieser 1994 were converted based on the assumption that 1 μmol oxygen consumption is equivalent to 0.45 Joules of energy. For trout, bream, bass, and grouper, the authors reported the amount of digestible energy from food that is required to deposit one gram of dry body mass. This value, denoted as γ here, is related to, but different than, E_m, because it includes the combustion energy content in the dry body mass. Thus, E_m was estimated as γ – 22 KJ/gdbm, assuming the combustion energy of one gram of dry tissue is 22 KJ (Wieser 1994).

Species	E_m (KJ/gdbm)	References
cattle and sheep	22	(Blaxter, 1989; Peterson et al., 1999)
horse	13.8	(Blaxter, 1989; Peterson et al., 1999)
dog and pig	8.6	(Blaxter, 1989; Peterson et al., 1999)
man, rat, chicken	6.4	(Blaxter, 1989; Peterson et al., 1999)
catfish	5.0	(Wieser, 1994)
pollock	5.1	(Wieser, 1994)
sole	6.0	(Wieser, 1994)
plaice and flounder	6.3	(Wieser, 1994)
mullet	7.4	(Wieser, 1994)
rainbow trout	9.9–14.0	(Azevedo et al., 1998; Rodehutscord and Pfeffer, 1999)
sea bream	11.8	(Lupatsch et al., 2003)
sea bass	10.4	(Lupatsch et al., 2003)
White grouper	9.9	(Lupatsch et al., 2003)
toad	7.4	(Jorgensen, 1988)
mussel	6.9	(Hawkins et al., 1989)
Garter snake	6.8–8.5	(Peterson et al., 1999)

studies assumed an allometric scaling law for total metabolic rate as $B = B_0M^{0.75}$ with a fixed value of the scaling power, 0.75, and a constant normalization coefficient B_0 for a given species (e.g., (Moses et al., 2008)). Using the theoretical form of metabolic rate in the energy budget model leads to inaccurate estimates of the values of E_m.

The values of E_m from the species listed in Table 2 range from 5.0 to 22.0 KJ/gdbm with an average of 9.16 ± 4.30 KJ/gdbm, close to Wieser's "consensus value". The E_m of cockroach nymphs obtained in this study, 6.90 KJ/gdbm, is bracketed in the range in Table 2, and similar to most of the ectothermic species. However, the value obtained for the painted lady caterpillars, 0.354 KJ/gdbm, is 27 times lower than the average, and 15 times lower than that of catfish, which has the lowest value in Table 2, 5.0 KJ/gdbm. This is similar for tobacco hornworms. Its E_m-value, 1.30 KJ/gdbm, is 7 and 4 times lower than the average and lowest values listed in Table 2, respectively. Thus, our result raises an interesting and important question: why do the painted lady caterpillar and tobacco hornworm have such low metabolic costs of growth?

It is generally believed (Millward et al., 1976, Parry, 1983, Sibly and Calow, 1986, Aoyagi et al., 1988, Jorgensen, 1988, Blaxter, 1989, Wieser, 1994, Peterson et al., 1999, Hou et al., 2008, Moses et al., 2008, Hou, 2014) that the value of E_m can be attributed to three types of physiological and ecological processes. First, a major component of E_m is the cost of assembling, including the energy cost of forming peptide bonds, RNA transcription, mitosis, lipid synthesis, etc.; second, growing animals require energy to digest and transport absorbed nutrients to the sites of biosynthesis. The energy requirements for these processes attribute to E_m; and third, the cost of growth includes the energy spent on foraging.

The last two components of E_m indicate that the value of E_m may depend on body mass. Peterson et al. (1999) considered two hypothetical animals with different body sizes, which gain the same amount of bio-tissue per unit time, i.e., same growth rate. In addition to the physiological costs of assembling, the cost of growth must include "the minimum energetic effort required to forage for…… the necessary amount of food to supply the amino acids and other raw materials" (Peterson et al., 1999). For the same growth rate, the minimum food required can be obtained from the same spatial distribution. However, foraging over the same time and space would cost more energy for the animal with a larger body size. Thus, the larger animal would have to pay more energy for the same amount of body-mass increase, i.e., a larger E_m. Moreover, once food is absorbed, a larger body size requires more energy to transport the nutrients because of the larger spatial distance (Moses et al., 2008). Thus, the extremely low value of E_m in painted lady caterpillars may stem from their small body size, 0.0026–0.12 g (dry mass). However, we noticed that several species in Table 2 had a similar body size, e.g., < 0.12 g for walleye pollock and < 0.01 g for mussel. The cockroach nymphs in this study also have similar body size range (Fig. 2), yet a 20-fold greater E_m. Thus, the small body size of the painted lady caterpillars, and the consequences of the energy cost of foraging and transporting nutrients, may not be the major contributing factors to the extremely low E_m in this species. The body size cannot explain the low E_m-value in tobacco hornworms either, because it had a much larger range of body mass (Fig. 2).

Numerous studies have suggested that for a given diet composition and ration, synthesizing protein costs more energy than synthesizing lipid (Morowitz, 1978). For example, in three fish species, it takes 0.79 to 0.90 KJ of metabolic energy to deposit one KJ of protein, but only 0.10 to 0.31 KJ to deposit one KJ of lipid (Lupatsch et al., 2003). Moreover, the various biochemical transformations of diet ingredients to body tissue have different efficiencies. Millward et al. (1976) estimated that the efficiency of transforming fat to fat is 0.99, i.e., 99% of the energy from dietary fat can be deposited as fat, and only 1% of the energy in the fat is lost during the process. The efficiency of transforming protein into fat is 0.31, and 0.15 for both transforming protein into protein and carbohydrate into fat. Thus, a species with a low protein and high fat content in its bio-tissue and a high protein content in its diet may have a low E_m value. However, this is not the case in this study. Painted lady caterpillars have a higher protein fraction in their bio-tissue than cockroach nymphs (Fig. 1B) and a lower lipid fraction than cockroaches (Fig. 1C). The caterpillar diet also has lower protein content than the cockroach diet.

We did not measure the fiber level in these species—another body component that requires metabolic energy to be synthesized. One of the most important components of fiber is chitin, which consists of polysaccharides and form the major constituent in the exoskeleton of insects. Previous studies on several insect species that are relevant to this study show that the chitin levels, expressed as mg/gram of the dry body mass, are 122 in adult American cockroaches (Kim et al., 2017), 67 in silkworm larvae, 82 in cricket nymphs, 56 in giant mealworm larvae, and 38 in waxworm larvae, respectively (Finke, 2007). Besides chitin, fiber also contains a considerable amount of amino acid (Finke, 2007). So, a complete estimate of metabolic cost of biosynthesis should not only include chitin, but also the fiber level. Finkle's work on aid detergent fiber (ADF) and neutral detergent fiber (NDF) (Finke, 2007) show that the sum of ADF and NDF is 13%, 24%, 16%, and 14% of the whole body dry mass of silkworm larvae, cricket nymphs, giant mealworm larvae, and waxworm larvae, respectively.

Thus, both the chitin and fiber (ADF and NDF) levels in silkworm caterpillars, which are likely similar to the painted lady caterpillars, is only two-fold or less compared to the adult cockroaches and cricket nymphs. It is unclear how much the metabolic energy cost of synthesizing fiber or chitin is. But, even if it is so large that the cost of synthesizing protein and lipid can be ignored when estimating the whole body biosynthetic cost, the two-fold difference in fiber and chitin levels cannot explain the 20-fold difference in E_m observed in this study.

5.4. The "Cost-Quality" hypothesis

Here we propose a hypothesis, attempting to explain the extremely low metabolic cost of growth in the painted lady caterpillars. We assume that a higher energy cost of synthesizing results in a higher cellular homeostasis. Considering the same organ in two insect species, e.g., midgut or muscle, our assumption indicates that the organ in the species that spends more energy synthesizing it has a better biomolecular "quality", including fewer errors in protein and DNA sequences, higher resistances to stresses, and slower cellular senescence. This assumption is yet to be tested, but there exists evidence that indirectly supports it. For example, amino acid composition is associated with protein stability (Argos et al., 1979, Ponnuswamy et al., 1982), and the syntheses of different amino acids have different energy costs (Akashi and Gojobori, 2002, Swire, 2007, Kafri et al., 2016). It has been suggested that the differentiation in the energy cost of amino acid synthesis is one of the selection pressures for protein evolution (Swire, 2007, Kepp and Dasmeh, 2014). Perhaps what is more important is the process of proofreading during protein synthesis, which is energetically costly (Blomberg, 1983, Jakubowski, 1994), and is closely related to the protein fidelity (Cochella and Green, 2005).

Among all the species in Table 2 and in this study, the painted lady caterpillar and the tobacco hornworm are the only holometabolous (complete metamorphous) species. During the metamorphosis of such species, only a few groups of imaginal discs and the tracheal system remain and grow (Madhavan and Schneiderman, 1977, Aldaz and Escudero, 2010, Lowe et al., 2013). A large portion of body tissues accumulated during the larval stage are disintegrated and remodeled (Locke, 1981, Lockshin, 1981), because most of them serve as an energy storage for future reproduction, instead of functional body structures. Moreover, the larvae of many holometabolous insects need to reach a critical weight for successful pupation (D'Amico et al., 2001, Davidowitz et al., 2003), and insects' fecundity is positively correlated to body size (Honěk, 1993). Thus, selection favors a high growth rate. Painted lady caterpillars need to finish their larval development in a

short period of ~2 weeks, and they have a relatively short adult lifespan of 2–3 weeks. Considering tissue disintegration during metamorphosis, the pressure of fast larval growth, and the short adult lifespan, we hypothesize that a high cost of synthesizing high quality tissues would be a "waste", and therefore is selected against in such a species. This is because not only a high cost would result in a slow growth, but also most of the high quality tissues would be disintegrated during metamorphosis—such a species needs to quickly accumulate a large amount of "low quality" mass that serves as the energy reservoir for future reproduction. Our hypothesis suggests that, compared to cockroaches and the other animals in Table 2, painted lady caterpillars may spend less energy on proofreading during protein and DNA synthesis, and perhaps also possess an amino acid composition that is more energetically efficient (Akashi and Gojobori, 2002), but less stable. Taking this strategy, the caterpillars achieve cheap and fast growth at the cost of a relatively poor cell quality. The cockroach, in contrast, spends 100–200 days on nymphal development, and the adult lifespan is longer than a year (Kim and Rust, 2013). Thus, from a life history perspective, the cockroach is comparable to small rodents, and therefore is found to have a relatively high E_m value. We need to distinguish the "buildings" (cells, tissues, organs, etc.) and the "building blocks" (biochemical units, such as amino acid). What is recycled during metamorphosis are the building blocks, not the buildings, and the biosynthetic cost lies in assembling the building blocks, not in building blocks themselves. Our hypothesis suggests that the buildings are "cheap" to make in caterpillars, but the building blocks, the units of the assembling, are the same in caterpillars and cockroaches.

Although we do not have the body composition data from tobacco hornworms, its E_m-value can be used to support this hypothesis as an incomplete and pilot test. Similar to painted lady caterpillars, tobacco hornworms have a short larval stage (~4 weeks), they need to reach a critical weight for pupation, they breaks down most of their tissues during pupation, and they have a short adult lifespan (Davidowitz et al., 2003). Our result show that the E_m-value of tobacco hornworm is 1303 Joules/gdbm, 7 and 4 times lower than the average and lowest values listed in Table 2, respectively. It is worth pointing out that Sears et al. (2012) obtained a similar value (1197 Joules/gdbm), using an overly simplified energy budget equation. As explained in the Introduction, the correct energy budget equation is $B = B_{M,A} + E_mG$ (Eq. (1)). The simpler version employed in Sears et al. (2012), $B = E_mG$, omits the maintenance/activity term, $B_{M,A}$. Assuming that all the metabolic energy was allocated to biosynthesis, the values of E_m obtained Sears et al was overestimated. The reason that their value is lower than ours is because in tobacco hornworms a considerable portion of the measured body mass comes from the food in their midgut. When estimating the value of E_m, Sears et al. (2012) did not remove the midgut content from the analysis, whereas this mass does *not* require any energy to synthesize. Note, both of ours and Sears et al's tobacco hornworm cohorts were measured alive, and both cohorts had food in the guts. So, both Sears et al's and our results on metabolic rate include the contribution from the gut microbiota and peritrophic membranes. The difference in the data analysis between these two studies is that we employed another cohort to obtain a relationship between living wet mass and dry mass without food content (Fig. 2), and we used this relationship to exclude the gut food weight from the daily body weight gain when estimating E_m. Needless to say, counting food as biotissue in data analysis underestimates the E_m-value. Overall, using the simplified energy budget equation overestimated the value, but failing to remove the food mass underestimated the value, so the combination of these two leads to a similar value of E_m to ours.

We need to emphasize that the quantity E_m is conceptually and practically different than the combustion energy. E_m is the "indirect cost of growth", which quantifies the amount of energy to synthesize one unit of biotissue. It is included in the metabolic energy, and dissipates as heat. Combustion energy is the energy content of the biomass, equivalent to energy allocated to production. It is stored in the body, and usually measured with bomb calorimeter. It does not dissipate as heat, and is not included in the metabolic energy. Thus, the same unit of dry mass in different species may have similar combustion energy, but very different E_m, for the reasons we hypothesized.

We call for two lines of future research to test our hypothesis. First, estimates of E_m using the correct energy allocation model and comparisons need to be performed on more holometabolous and hemimetabolous insect species. Second, the quality of the same type of cells from these species, such as the amino acid composition, the molecular damage on protein and DNA, the vulnerability to external insults, and cellular senescence, needs to be compared.

6. Authors statement

C.H. conceptualized the idea; C.H., N.F., N.G., K.H., and Y.-W.H. designed the experiments; N.F., K.H., and M.F. performed respirometry; N.G., H.N., and C.P performed body composition assays; C.H., N.F., N.G., K.H., and Y.-W.H. analyzed the data; C.H., N.G., and N.F. wrote the first draft of the manuscript, and K.H., and Y.-W.H. contributed to revisions.

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