Ontogenetic Scaling of Metabolism, Growth, and Assimilation: Testing Metabolic Scaling Theory with *Manduca sexta* Larvae

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ABSTRACT

Metabolism, growth, and the assimilation of energy and materials are essential processes that are intricately related and depend heavily on animal size. However, models that relate the ontogenetic scaling of energy assimilation and metabolism to growth rely on assumptions that have yet to be rigorously tested. Based on detailed daily measurements of metabolism, growth, and assimilation in tobacco hornworms, *Manduca sexta*, we provide a first experimental test of the core assumptions of a metabolic scaling model of ontogenetic growth. Metabolic scaling parameters changed over development, in violation of the model assumptions. At the same time, the scaling of growth rate matches that of metabolic rate, with similar scaling exponents both across and within developmental instars. Rates of assimilation were much higher than expected during the first two instars and did not match the patterns of scaling of growth and metabolism, which suggests high costs of biosynthesis early in development. The rapid increase in size and discrete instars observed in larval insect development provide an ideal system for understanding how patterns of growth and metabolism emerge from fundamental cellular processes and the exchange of materials and energy between an organism and its environment.

Introduction

All animals consume food to gain energy and materials from the environment. This finite supply of energy and matter must be allocated to maintenance, growth, reproduction, movement, and their attendant metabolic costs. Both metabolism and material exchange with the environment are complex and intricately related processes that depend heavily on animal size (Peters 1983; Schmidt-Nielsen 1984). However, although assimilation, metabolism, and growth are clearly linked through the constraints of mass and energy balance (Kleiber 1961; Scriber and Slansky 1981; Kooijman 1993; West et al. 2001), biologists still lack an agreed-upon theory of ontogenetic growth.

A Scaling Model of Ontogenetic Growth

Recently, West et al. (2001) proposed a model of ontogenetic growth (West et al. 2001; Hou et al. 2008; Moses et al. 2008), based on fundamental cellular and biological principles, beginning with the law of conservation of energy. They assumed that the total metabolic rate (typically measured as O_2 consumption or CO_2 production and then converted to W) of an organism (B) is simply the sum of the energy devoted to growth (i.e., the synthesis of new biomass) plus the energy devoted to maintaining existing biomass:

$$B = E_{\mathrm{m}}\frac{dm}{dt} + B_{\mathrm{m}}m, \tag{1}$$

where m is animal mass, E_{m} is the energy required to synthesize a unit of biomass (e.g., in J g^{-1}), and B_{m} is the metabolic rate required to maintain a unit of biomass (e.g., in W g^{-1}). Rearranging to solve for the growth rate and taking into account the fact that metabolic rate scales as a power law of animal mass, $B = b_0 m^{\alpha}$, West et al. (2001) then derived a function describing growth rate in terms of animal mass:

$$\frac{dm}{dt} = am^{\alpha} - bm, \tag{2}$$

where $a = b_0/E_{\mathrm{m}}$ and $b = B_{\mathrm{m}}/E_{\mathrm{m}}$ and b_0 is a taxon-specific constant. Based on an equilibrium between growth and assimilation, Hou et al. (2008) arrived at a model of assimilation rate with a similar form.

Although it differs significantly in its derivation, this model shares the same form as several previous growth models (von Bertalanffy 1957; Reiss 1989; Ricklefs 2003). As with these other models, one of the main strengths of this approach is that it links patterns of assimilation and growth directly to the allometric scaling of metabolic rate. Growth is modeled as the energetic balance between the cost of maintaining existing biomass (bm) and the energetic capacity to synthesize new biomass (am^{α}). At the same time, several core assumptions of the ontogenetic growth and assimilation model have never been

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tested. First, it has been assumed that α is constant over ontogeny. Second, it has been assumed that the exponents governing growth and assimilation rates are identical to the scaling exponent of the metabolic rate allometry. Finally, more recent extensions of the model to describe assimilation rate (Hou et al. 2008) have implicitly assumed that the assimilation efficiency is constant over development. Here, we use the lepidopteran tobacco hornworm, *Manduca sexta*, to evaluate these assumptions by measuring growth, assimilation, and metabolism on the same animals over their entire larval developmental period.

Insect larvae generally grow nearly exponentially (von Bertalanffy 1957; Nylin 1992; D'Amico et al. 2001), which effectively simplifies the model above by ignoring the metabolic costs of maintenance, changing the model of growth rate from a parabolic to a monotonically increasing function of body size,

$$\frac{dm}{dt} = am^{\alpha}. \tag{3}$$

Truly exponential growth requires the further assumption that $\alpha = 1$. However, based on growth data from nine species of caterpillar, Tammaru and Esperk (2007) argue that while growth rate does appear to increase throughout ontogeny, it tends to increase at a decreasing rate, consistent with a value of $\alpha < 1$. This pattern of monotonically increasing growth rates, as modeled by equation (3), is to be expected as long as the larvae are well below the asymptotic mass predicted from the balance of assimilatory capacity and maintenance metabolism (i.e., $am^{\alpha} \gg bm$; West et al. 2001; Tammaru and Esperk 2007; Moses et al. 2008). Such monotonically increasing growth is consistent both with empirical growth curves (Tammaru and Esperk 2007) and with the fact that the cessation of larval growth and the transition to pupation results from a complex but well-documented set of hormonal triggers, rather than from energetic limitation per se (Sehnal and Meyer 1968; Nijhout 1975; Davidowitz and Nijhout 2004). Indeed, both classic and recent investigations of the hormonal control of larval development have demonstrated that altering hormonal titers can sometimes lead to additional larval instars and caterpillar gigantism (Sehnal and Meyer 1968; Reynolds et al. 2009). Thus, caterpillars may indeed be well below their asymptotic size, as assumed by this simplified model of larval growth.

In the original derivation of the ontogenetic growth model, West et al. (2001) made the additional, more specific assumption that $\alpha = 3/4$, based on the observation that many interspecific metabolic scaling analyses indicate that the 3/4 exponent is typical for most animals (Kleiber 1961; Peters 1983; Schmidt-Nielsen 1984), a pattern that they had earlier sought to explain based on the fractal-like structure of exchange surfaces and resource supply networks (West et al. 1997). Initial analyses of the model provided reasonable fits to patterns of growth and assimilation observed over a wide variety of taxa, suggesting broad generality of the underlying theory (West et al. 2001; Hou et al. 2008; Moses et al. 2008). However, comparisons with alternative models (which differ principally in the assumed value of α) have demonstrated that it is not possible to distinguish among them based simply on fitted growth trajectories (Banavar et al. 2002; West et al. 2002; Ricklefs 2003). At present, there is little theoretical or empirical agreement about "the" value of the metabolic mass-scaling exponent (White and Seymour 2003; Savage et al. 2004; Glazier 2005; Moses et al. 2008; Kolokotrones et al. 2010; Isaac and Carbone 2010; DeLong et al. 2010). Both alternatives to (Banavar et al. 1999; Kozlowski et al. 2003; Glazier 2005) and extensions of (Savage et al. 2008; Banavar et al. 2010; Kolokotrones et al. 2010) the network model predict a variety of different metabolic scaling exponent values, with varying degrees of support from the data. However, it is important to note that the mathematical logic of the ontogenetic growth model does not depend on the network model and that growth trajectories are determined by both the energetic demands of growing tissues and the capacity of the organism to meet those demands (Banavar et al. 2002; Ricklefs 2003; West et al. 2004; Moses et al. 2008). Moreover, despite the largely theoretical controversy, we know of no studies that have tested the assumptions of the ontogenetic growth model based on direct measurements of metabolic rate in growing organisms.

Ontogenetic scaling of metabolic rate is a distinctly different phenomenon from interspecific (or phylogenetic) scaling (Wieser 1984), and the values of ontogenetic scaling exponents are considerably more variable than those reported for interspecific relationships (Glazier 2005). Although the distribution of intraspecific exponents shows limited variation in the range of 2/3 to 1, especially when observed over large ranges of body size (Moses et al. 2008), systematic variation in metabolic scaling across taxa may be very important for explaining interspecific differences in ontogenetic growth (von Bertalanffy 1957). In larval *M. sexta*, metabolic rate has been found to scale with exponents ranging from 0.77 (Alleyne et al. 1997) to 0.98 across the entire larval period (Greenlee and Harrison 2005).

A recent comprehensive review has shown that both the scaling exponent (α) and the scaling coefficient (b_0) vary over ontogeny in a variety of taxa (Glazier 2005), which can greatly complicate estimates of intraspecific scaling relationships. For example, larval tiger puffer fishes (*Takifugu rubripes*) exhibit approximately isometric scaling of metabolic rate (i.e., $\alpha = 1$) over their entire developmental period (Yagi et al. 2010), as do many aquatic invertebrates and larval fish (Glazier 2006). However, in the puffer fish, more careful analyses demonstrate that three separate increases in metabolic coefficient correspond to four distinct developmental stages associated with the development of morphological antipredatory defenses against cannibalistic conspecifics (Yagi et al. 2010). Within each developmental stage, the scaling exponent is constant ($\alpha = 0.80$) but substantially shallower than that observed over all stages (Yagi et al. 2010).

In this study, we ask whether the allometry of metabolic rate is constant over *Manduca* ontogeny and whether the scaling of assimilation and growth rates are similar to the scaling of metabolic rate, as predicted by West et al.'s (2001) ontogenetic growth model. Further, we examine whether the efficiencies of growth and assimilation are constant over development. If the

above simplified growth model is correct, based on West et al.'s (2001) derivation, we can make the additional prediction that the scaling coefficients for metabolic and growth rate scaling should yield a realistic estimate of the energetic costs of biomass synthesis; that is, $E_m = b_0/a \sim 1$–7 kJ g^{-1} (Calow 1977; Wieser 1994; Moses et al. 2008).

Manduca serves as an excellent model organism for investigating scaling relationships because it grows almost 10,000-fold in mass in less than 3 wk. Additionally, *Manduca* larvae have five discrete developmental stages (instars). Between instars, larvae go through a molt period, during which most of their cell proliferation occurs (Baldwin and Hakim 1991). These discrete developmental stages provide a natural basis for asking whether the parameters of scaling relationships and the efficiencies of growth and assimilation change over ontogeny. As far as we know, this is the first study to directly address the scaling of metabolism, assimilation, and growth on the same animals, a key step in testing the underlying assumptions of West et al.'s (2001) model of ontogenetic growth.

Methods

Animal Rearing

In the summer of 2009, we reared two cohorts of *Manduca sexta* larvae from eggs (Carolina Biological Supply) to adults at a constant temperature of 27°C and "long-day" (16L : 8D) photoperiod. Individuals were placed in separate containers upon hatching. All larvae were fed a wheat germ–based laboratory diet (tobacco hornworm medium bulk diet, Carolina Biological Supply) ad lib. but in controlled amounts to facilitate accurate measures of ingestion and assimilation rates (see below). To facilitate the collection of frass, caterpillars and food were elevated above the floor of the containers on a small wire mesh frame constructed of hardware cloth. Data were analyzed only for larvae that survived to the wandering stage and successfully entered pupation, yielding a total of 253 animal-days of observation on 15 different animals.

Growth, Metabolism, and Assimilation Measurements

Animal mass, instar, and day of instar were recorded for each larva at approximately the same time each day. Early-instar animals (<10 mg) were weighed on a digital microbalance (Perkin-Elmer AD6) while larger animals were weighed on a Mettler-Toledo XS-204. Values of animal mass were converted to dry mass based on a preliminary study that measured the water content of *Manduca* larvae. We measured the wet and dry masses of three larvae at each day of each instar, providing a wide range of animal sizes and accounting for all stages of the larval cycle, and found that dry animal mass was best described by a quadratic regression on log-transformed weights ($\log(\text{dry mass}) = -0.9 + 1.05 \times \log(\text{wet mass}) + 0.037 \times (\log(\text{wet mass}))^2$, $r^2 = 99.9\%$, both terms $P < 0.001$). Growth rate (g d^{-1}) was then measured as the dry mass increment from one day to the next.

The metabolic rate of each larva was also measured at approximately the same time every day using a four-channel respirometry system (Qubit Systems G249 gas controller/monitor and G283 channel switcher run into a model S151 infrared gas analyzer) in a 27°C temperature-controlled chamber. The respirometry system was calibrated (both zero and span) each morning and recalibrated between each set of measurements using standardized reference gases. After acclimation to the chamber for 25 min, CO_2 exchange was measured for each animal over three 5-min intervals, and these three measurements were averaged to obtain average values of metabolic rate (in μL CO_2 h^{-1}).

To avoid stress responses, animals had food available right up to their introduction to the respirometry chambers. Although the animals were not actively feeding, which can increase insect metabolic rates substantially (Gouveia et al. 2000), they were still processing and digesting food during the metabolic measurements, as evidenced by the frequent production of frass while in the chamber. Thus, our measured respiration rates are "resting" rates and likely underestimate the average metabolic rate of free-ranging animals.

We used the quantitative nutritional approach (Scriber and Slansky 1981) to calculate daily food assimilation. Each day, at the time of animal weighing, we replenished food supplied and collected all frass. Animals were provided with a serving of food roughly three times the mass of maximum daily consumption for an animal that size, as estimated from preliminary experiments. During the experiment, no animals ran out of food. We controlled for water loss in food samples by measuring control food samples of approximately the same size and shape as the food given to our experimental larvae and under the same conditions each day. These control samples were weighed at three different times: at the beginning and end of a 24-h incubation period and also after being oven-dried. Based on the control food samples, food mass lost due to evaporation during the 24-h incubation period was accounted for by estimating the equivalent wet food remaining ($\log(\text{wet food}) = 0.08 + 0.87 \times \log(\text{incubated food}) + 0.066 \times (\log(\text{incubated food}))^2$, $r^2 = 100\%$, both terms $P < 0.001$). Daily ingestion rates were then estimated as the difference between the food provided and the food remaining, converted to dry mass based on 80% water content of the food (based on 33 food samples, SD = 0.3%). Frass was collected at the time of feeding and weighing each day and was oven-dried to obtain mass measurements of dry frass production.

Assimilation was calculated daily by subtracting the dry mass of frass produced from the dry mass of food ingested (both in g d^{-1}) over each 24-h period. Within each instar we also examined the distribution of two different measures of efficiency within each instar. Daily assimilation efficiency (AE) was calculated by dividing the rate of assimilation by the rate of food intake. Gross growth efficiency (GGE) was also calculated for each 24-h period as the ratio of dry mass increment to dry food intake.

We use a dry mass basis because the underlying growth model is derived in terms of mass. Alternatively, assimilation and gross growth efficiencies could be calculated on an energy basis by

multiplying dry mass terms times the energy content of food, frass, and animal tissue samples. However, while energy-based efficiencies of insect larvae tend to be approximately 3%–5% higher than dry mass efficiencies, the differences show no systematic ontogenetic trend in lepidopteran larvae (Slansky 1985), so our test of the assumption of constant efficiency should not be sensitive to the material basis of our estimates.

Data Analysis

We assume that rates of metabolism, growth, and assimilation all scale as allometric power laws of the form

$$R = cM^d, \tag{4}$$

where R is the measured rates of interest, M is measured animal body weight (g, dry mass), and c and d are fitted parameters. For analysis, we linearize the relationship by log transformation and assume that errors are lognormally distributed, giving us

$$\log R = \log c + d\log M + \varepsilon_{\mathrm{R}}, \tag{5}$$

where $\varepsilon_{\mathrm{R}} \sim N(0, \sigma_{\mathrm{R}}^2)$ are the normally distributed error terms. After log transformation, the scaling exponent (d) corresponds to the slope term of the linear model, while the coefficient (c) is estimated by the intercept term. Log transformation was used not only to normalize residual variation but also because ontogenetic growth is a fundamentally multiplicative process and spans, in this case, several orders of magnitude, making a multiplicative error model more appropriate (Kerkhoff and Enquist 2009). Based on the general model in equation (5), we used linear mixed-effects models (Pinheiro and Bates 2000; Bolker et al. 2009) to compare the scaling of growth rate and assimilation rate to the scaling of metabolic rate. Because both the slope and the intercept of the model may change over development, we treat instar as a fixed effect. We treat individual as a random effect to account for the fact that we were making multiple measurements on the same animals repeatedly.

We analyzed the scaling of metabolic, growth, and assimilation rates in two steps. First, examining each rate separately, we tested the hypothesis that scaling is constant over ontogeny using an analogue to ANCOVA. In this case, our full statistical model is

$$\begin{aligned}\log R = {} & \beta_0 + \beta_1 \log M + \beta_2 \mathrm{instar} \\ & + \beta_3 \log M \times \mathrm{instar} + \varepsilon_{\mathrm{L}} + \varepsilon_{\mathrm{R}},\end{aligned} \tag{6}$$

where the β terms are fitted coefficients and the error terms correspond to residual variation within (ε_{L}) and between (ε_{R}) individual larvae. A significant $\log M \times$ instar interaction term (i.e., $\beta_3 \neq 0$, $P < 0.05$) was taken to indicate separate slopes, that is, a developmental shift in the scaling exponent. If the interaction was not significant, we then assessed the significance of instar terms (β_2) in a simplified common-slope model (i.e., $\beta_3 = 0$) to assess developmental differences in intercepts (scaling coefficients). For comparison, we also present analyses for data pooled across all instars but still treating individuals as a random effect to control for repeated measures; that is, $\beta_2 = \beta_3 = 0$ in equation (6).

Second, to test whether the scaling of assimilation and growth rates match the scaling of metabolic rate, we made pairwise comparisons, again using a mixed-effects analogue to ANCOVA. In particular, we combined data for the rates and created an indicator variable (I_{R}) describing which rate was measured (i.e., either growth vs. metabolic or assimilation vs. metabolic). We then expanded our model to test for significant $\log M \times I_{\mathrm{R}}$ interactions:

$$\begin{aligned}\log R = {} & \beta_0 + \beta_1 \log M + \beta_2 \mathrm{instar} + \beta_3 \log M \times \mathrm{instar} \\ & + \beta_4 I_{\mathrm{R}} + \beta_5 \log M \times I_{\mathrm{R}} + \beta_6 \mathrm{instar} \times I_{\mathrm{R}} \\ & + \beta_7 \log M \times \mathrm{instar} \times I_{\mathrm{R}} + \varepsilon_{\mathrm{L}} + \varepsilon_{\mathrm{R}}.\end{aligned} \tag{7}$$

Here, significant β_5 and/or β_7 terms would indicate differences in the scaling exponent between the two measured rates. Conversely, we expect to see differences in β_4 simply because the rates differ in their units and their magnitude.

We fitted the mixed-effect models using restricted maximum likelihood (REML) in the "nlme" and "lme4" packages in R, version 2.10.1 (R Development Core Team 2011). We assessed the significance of the fixed effects and their interactions based on Wald F-tests (Pinheiro and Bates 2000; Bolker et al. 2009) and calculated 95% confidence intervals for all fixed-effect parameter estimates using a normal approximation to the distribution of REML estimators (Pinheiro and Bates 2000). For the estimates of the scaling coefficients, we present back-transformed values, so that the numbers correspond to the original scale of measurement and conform to the power law form of equation (4). Note that since the linear model provides the best unbiased parameter estimate for the log-transformed data, this back-transformation process yields asymmetrical confidence intervals for the intercept terms.

Larvae undergo substantial physiological and behavioral changes at or near times of molt (Chamberlin et al. 1997) that warrant the restriction of our data set for analysis. For example, larvae decrease their food intake, frass production, and growth rate considerably as they approach times of molt. We thus restricted our analysis to the "free growth period," days on which animals grew more than they had on the previous day (Esperk and Tammaru 2004). A brief comparison between analyses of the free growth period and the entire data set are provided in the appendix.

Results

Variation in Scaling over Ontogeny

Over their entire developmental period, larvae grew nearly exponentially, with little interindividual variation and an abrupt cessation of growth at the end of the larval period, when the larvae stop eating and start "wandering" in preparation for pupation (fig. 1). The scaling exponent (α) from the power law growth model, estimated across the entire larval period, was 0.94 (fig. 2; table 1; df = 1, 142, $P < 0.0001$). This estimate

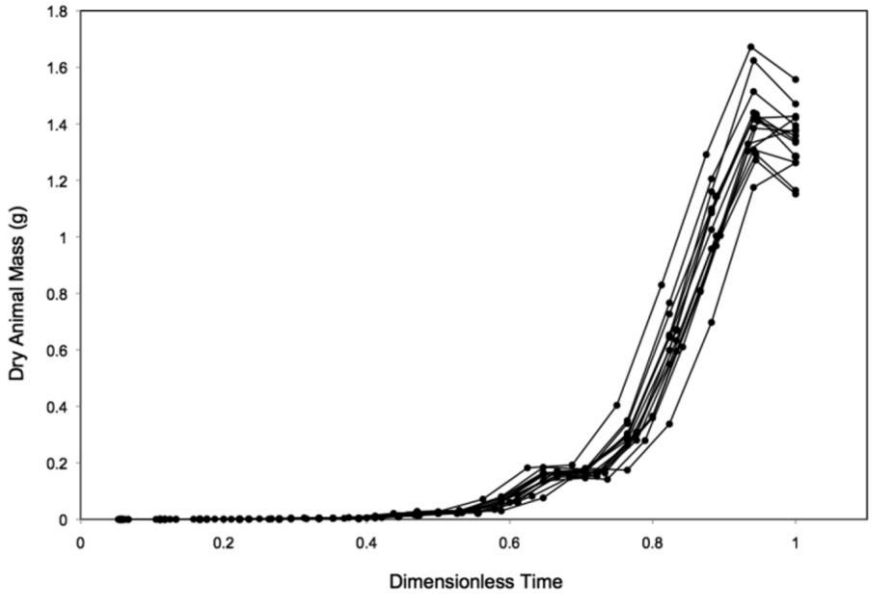

Figure 1. Growth curves of 15 *Manduca* larvae over ontogeny, from the day of hatching to pupation. Dimensionless time was calculated for each measurement by dividing the day on which the measurement was taken by the total number of larval days for that animal. Animal mass was recorded at approximately the same time every day.

of α was nearly identical to the metabolic scaling exponent estimated from the respirometry data (0.93; fig. 3; table 1; $df = 1,135$, $P < 0.0001$).

Examined in isolation, instar had no effect on the scaling exponent of metabolic rate ($\log M \times$ instar, $F = 0.99$, $df = 4,127$, $P = 0.46$), but the scaling coefficients did vary (instar, $F = 19.47$, $df = 131$, $P < 0.0001$). Metabolism scaled as the 0.65 power of body mass (95% confidence interval [CI] = 0.58–0.72) within instars, but the scaling coefficient increased 50%–75% over the first three instars and then jumped more than 120% during the last molt (fig. 3; table 1). Similarly, the scaling of growth rate was dependent on instar, but in this case both the scaling exponent and coefficient varied across instars (fig. 3; table 1; $\log M \times$ instar, $F = 3.45$, $df = 4,134$, $P = 0.01$). The growth scaling exponent varied modestly around a value somewhat higher than the measured metabolic scaling exponent (0.75–0.74) across the first two instars and then decreased during the third and fourth instars (0.70 and 0.61, respectively). During the fifth instar, the growth scaling exponent decreased further to 0.27 (fig. 2; table 2). As with the metabolic data, the scaling coefficient of growth rate also increased in each successive instar, showing a pattern of ~50% increase through the first two molts, with 17% and 10% increases in the final two molts.

While assimilation did scale with caterpillar mass, there were clear differences among instars (fig. 4*A*; table 2; $\log M \times$ instar, $F = 12.52$, $df = 4,100$, $P < 0.0001$). The scaling of assimilation was approximately flat during the first two instars (95% CIs include 0; table 2) and then increased for the final three instars (fig. 4*A*; table 2). Further analyses of the components of assimilation (intake and frass production rates) demonstrate that, like assimilation, food intake scaling was practically flat over the first two instars and then increased ($\log M \times$ instar, $F = 4.898$, $df = 4,121$, $P = 0.01$) but never matched the scaling of growth and metabolism. In contrast, the scaling of frass production shows a more modest variation in scaling exponents, although they did vary significantly between instars ($\log M \times$ instar, $F = 2.99$, $df = 4,107$, $P = 0.022$).

Comparison of Scaling among Rates

When compared directly, the scaling exponent for growth rate was statistically indistinguishable from the scaling exponent of metabolic rate. This result applies both across all instars (common value = 0.94, $\log M \times I_R$, $F = 1.29$, $df = 1,291$, $P = 0.26$) and when the scaling exponent was allowed to vary over development ($\log M \times I_R$, $F = 2.26$, $df = 1,275$, $P = 0.13$). The common metabolic growth scaling exponent did vary across instars (figs. 2, 3; $\log M \times$ instar, $F = 2.70$, $df = 4,280$, $P < 0.03$) and was generally intermediate between the values estimated for the two scaling relationships independently (table 3). As in the analyses of the two rates separately, the scaling coefficient was found to shift at each molt, but while the growth rate scaling coefficients increased with each instar, the metabolic rate scaling coefficients tended to decrease with each molt, with the exception of the last molt (table 3). In contrast, the scaling of assimilation rate consistently differed from that of metabolic rate ($\log M \times I_R$, $F = 195.34$, $df = 4,241$, $P < 0.0001$). Moreover, the differences in scaling them-

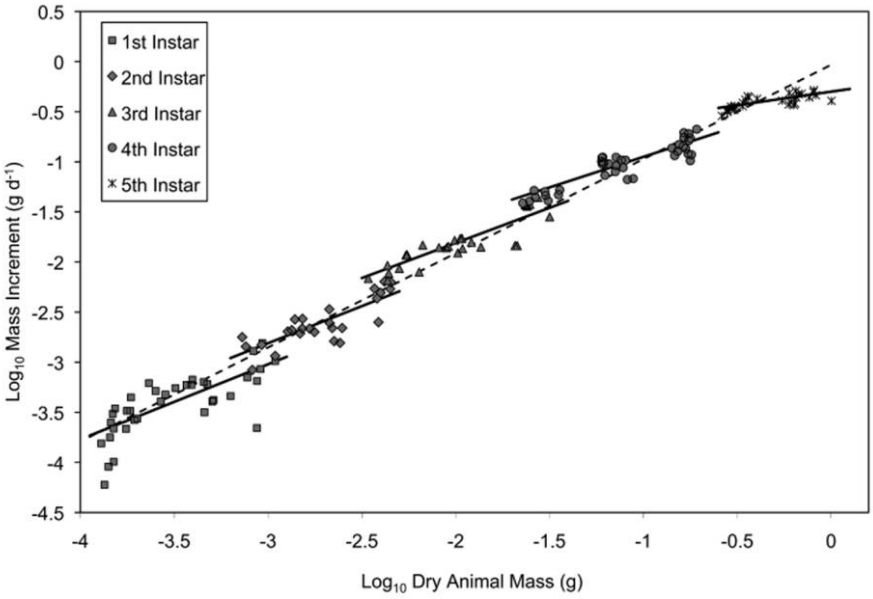

Figure 2. Scaling of growth rate by instar in *Manduca sexta*. Dashed line is fitted over all five instars. Solid lines represent separate exponents and coefficients for each instar fitted by the linear mixed-effects model. See table 1 for model parameters.

selves varied developmentally, as evidenced by the three-way interaction between mass, instar, and rate ($\log M \times$ instar $\times$ I_R, $F = 12.53$, df $= 4, 241$, $P < 0.0001$).

Assimilation and Growth Efficiencies

Early in development, food intake greatly exceeded frass production, but they converged over ontogeny. Thus, larvae retained nearly 90% of the food they took during the first instar, but their AE decreased as they grew, converging on about 70% in the final three instars (fig. 5*A*; $F = 14.6$, df $= 4, 105$, $P < 0.0001$). Ignoring a single outlying observation with the lowest food intake value from a first-instar animal (which led to an unrealistic efficiency value >5), GGE generally increased over the first three instars, leveling off at around 40% (fig. 5*B*; $F = 34.1$, df $= 4, 125$, $P < 0.0001$).

To calculate the energy required to generate a unit of biomass (i.e., $E_m = b_0/a$, we converted the metabolic coefficient (b_0) from microliters of CO_2 per gram to the α per hour to joules per gram to the α per day, using a respiratory quotient of 0.877 (Alleyne et al. 1997) and a respiratory yield of 20.1 J (mL O_2)$^{-1}$ (Peters 1983) and then divided through by the corresponding growth scaling coefficient (a). Based on the common slope scaling relationship for metabolic and growth rates (which keeps the units constant) and averaged over the five instars, $E_m \approx 2{,}160$ J g^{-1}. Within instars, the estimates ranged from a high of 5,410 J g^{-1} in the first instar to a low of 960 J g^{-1} in the fourth instar (fig. 5). Ignoring variation in the scaling exponent and coefficients across instars yields an estimate of $E_m \approx 3{,}980$ J g^{-1}.

Discussion

Metabolic Scaling Parameters Change over Ontogeny

Although the precise value of the metabolic scaling exponent was not the primary focus of this study, it is interesting to note

Table 1: Mass-scaling exponents and coefficients for metabolic and growth rates by instar

	Metabolic rate (μL CO_2 h^{-1})		Growth rate (g d^{-1})	
Instar	Scaling exponents	Scaling coefficients	Scaling exponents	Scaling coefficients
1		906 (517–1,588)	.753 (.619–.888)	.169 (.057–.503)
2		1,408 (1,170–6,960)	.741 (.507–.975)	.257 (.051–1.297)
3		2,497 (1,899–3,286)	.705 (.498–.915)	.385 (.103–1.434)
4		3,727 (2,503–5,551)	.616 (.432–.800)	.451 (.145–1.406)
5	.647 (.578–.716)	8,527 (5,080–14,314)	.272 (.000–.544)	.496 (.164–1.500)
All	.928 (.908–.948)	8,680 (7,850–9,580)	.945 (.924–.966)	.928 (.822–1.046)

Note. Results are for linear mixed-effects models with individual treated as a random effect to control for repeated measurements on individuals. Error is 95% confidence interval.

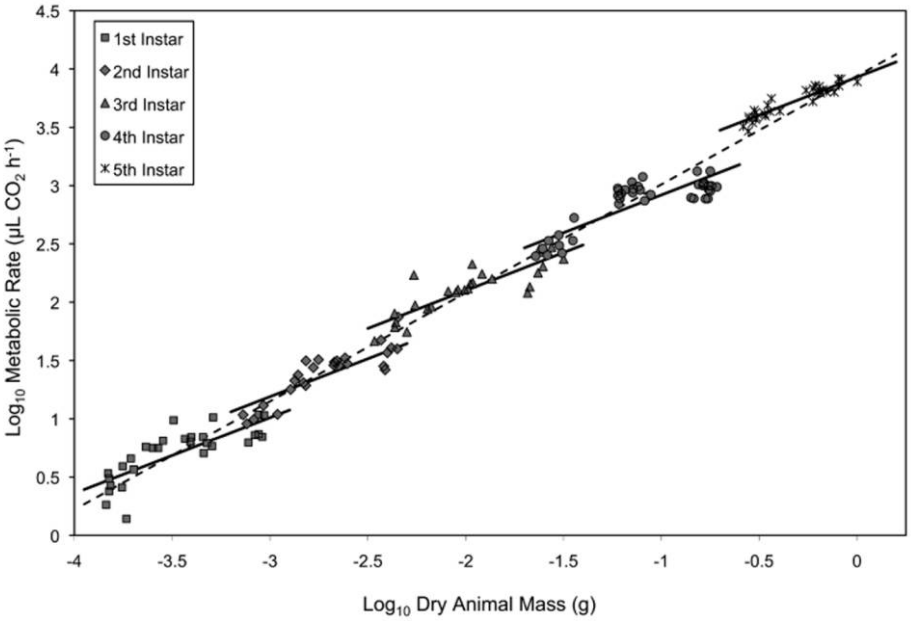

Figure 3. Scaling of metabolic rate by instar in *Manduca sexta*. Dashed line is fitted over all five instars. Solid lines represent the common exponent and separate coefficients for each instar fitted by the linear mixed-effects model. See table 1 for model parameters.

that the metabolic scaling exponent observed here across instars was close to that reported in other studies of *Manduca* larvae (e.g., 0.98; Greenlee and Harrison 2005). This value is steeper than the 0.76 exponent reported for the most comprehensive recent interspecific analysis of insects (Chown et al. 2007), a discrepancy that has been observed previously in other ontogenetic scaling analyses of insects and other invertebrates (Glazier 2006; Blossman-Myer and Burggren 2010).

After controlling for sequential increases in the scaling coefficient (b_0) between instars, we found that the metabolic scaling exponent conformed more closely to the interspecific pattern and was in fact very close to the value of 2/3 expected from the argument of geometric similarity and changing surface-area-to-volume ratios (Rubner 1883; Glazier 2005). Similar sequential shifts in scaling coefficient over development have been observed in larval tiger puffer fish (Yagi et al. 2010). Interestingly, these ontogenetic shifts lead to a near-linear scaling of metabolism over the entire larval period for the fish (Yagi et al. 2010), as observed in our study of *Manduca*. Thus, we speculate that such ontogenetic shifts may play an unrecognized role in the linear scaling of metabolism that has been widely observed in invertebrates (Glazier 2006).

Differences between scaling exponents across versus within instars may be related to the particulars of the growth process. Based on histological studies of the *Manduca* gut epithelium (Baldwin and Hakim 1991), it appears that during each instar, larvae grow primarily through cell expansion rather than cell division. Thus, the scaling of metabolism observed within instars ($\alpha = 0.65$) may represent the limitation of metabolism by a decreasing surface-area-to-volume ratio of cells (Kozlowski et al. 2003; Chown et al. 2007; Savage et al. 2007) if the cells are retaining the same shape during expansion. During molt, not only do new cells proliferate from stem cells but also existing cells shrink, such that epithelial cells are approximately the same size at the beginning of each instar, despite an estimated 200-fold increase in cell number and epithelial surface area from first to fifth instar (Baldwin and Hakim 1991). Now by itself this increase in exchange surface area cannot account for the roughly 900-fold increase in metabolism observed over larval development, but if we include the roughly ninefold increase in metabolic scaling coefficient (from first to fifth instar; table 1), the new estimate ($9 \times 200 = 1{,}800$-fold) is more than enough. However, this begs the question of what could cause increases in the metabolic scaling coefficient across the molting cycle.

The observed increases in scaling coefficient may be linked to gas-exchange limitations. In larval insects, the diameters of major tracheae and spiracles are generally fixed during an instar, and the tracheal system can increase in volume only by tracheole sprouting (Beitel and Krasnow 2000). In *Manduca*, mass-specific tracheal system conductance decreases almost 50% on average as animals grow within each of the first four instars (Greenlee and Harrison 2005), suggesting that as animals grow, the delivery capacity of the tracheal system may not be able to keep up with their expanding oxygen demands. Thus, the increase in metabolic intensity observed across instars may result from the restoration of oxygen supplies when the major tracheae and spiracles are replaced at molt.

As animals grow within an instar, metabolic rate may thus become supply limited both by the gas exchange capacity of

Table 2: Mass-scaling exponents and coefficients (with 95% confidence intervals) for rates of assimilation, food intake, and frass production by instar and across all instars

	Assimilation		Food intake		Frass production	
Instar	Scaling exponents	Scaling coefficients	Scaling exponents	Scaling coefficients	Scaling exponents	Scaling coefficients
1	−.316 (−.648 to .015)	3.82×10^{-4} (2.67×10^{-5} to 5.47×10^{-3})	−.102 (−.462 to .259)	.0021 (1.11×10^{-4} to .039)	.632 (.371–.893)	.0532 (.0064–.441)
2	.157 (−.373 to .687)	.0333 (8.29×10^{-4} to 1.341)	.516 (−.016 to 1.047)	.272 (.060–12.40)	.741 (.278–1.205)	.250 (.0104–6.01)
3	1.172 (.740–1.604)	6.073 (.319–115.6)	.986 (.499–1.473)	3.82 (.141–103.3)	.593 (.226–.960)	.175 (.0157–1.97)
4	.555 (.162–.948)	.681 (.0450–10.32)	.486 (.041–.930)	.767 (.038–15.6)	.198 (−.127 to .524)	.090 (.0102–.789)
5	.562 (.046–1.078)	1.069 (.0731–15.64)	.531 (−.074 to 1.137)	1.49 (.077–29.0)	.452 (.0034–.901)	.428 (.0506–3.62)
Across all instars	.696 (.655–.737)	.984 (.821–1.180)	.737 (.697–.777)	1.45 (1.20–1.76)	.889 (.853–.925)	.551 (.465–.653)

Note. Coefficients are back-transformed to the original units (all g dry matter d^{-1}).

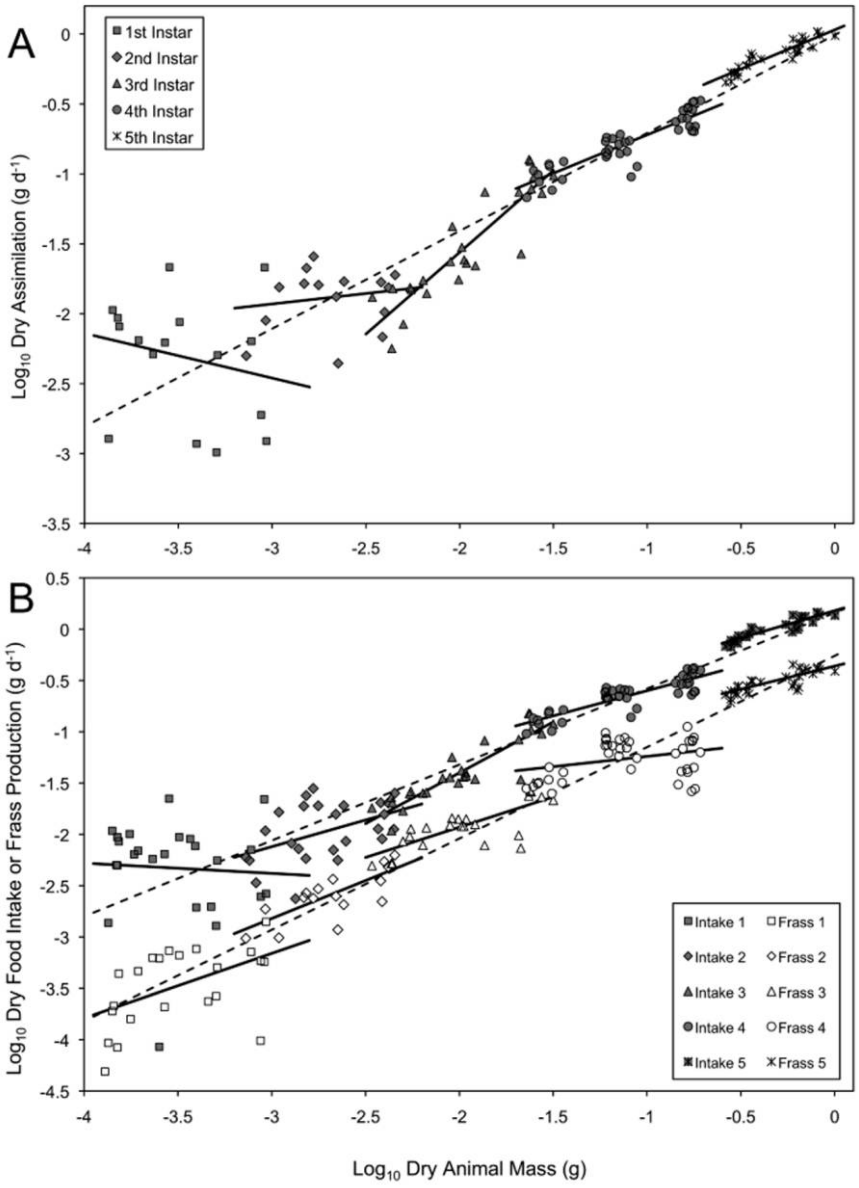

Figure 4. Scaling of the rates of (*A*) assimilation and (*B*) food intake and frass production in *Manduca sexta*. Dashed lines are fitted over all five instars. Solid lines represent the separate exponents and coefficients for each instar fitted by the linear mixed-effects models. See table 2 for model parameters.

the tracheal system and by the surface-area-to-volume ratio of cells. Alternatively, sublinear (exponent <1) scaling may be explained by decreased metabolic demand over development. Such a decrease is consistent with the decrease in cytochrome oxidase activity, a measure of mitochondrial density, observed in silkworms (*Bombyx mori*) across instars (Blossman-Myer and Burggren 2010). However, based on citrate synthase activity in the midgut, mitochondrial densities and aerobic capacities do not appear to change over development in *Manduca* (Gibellato and Chamberlin 1994; Blossman-Myer and Burggren 2010). Interestingly, silkworms also showed an opposite pattern of metabolic shifts: near-linear metabolic scaling within instars led to an exponent of 0.82 over the entire larval period (Blossman-Myer and Burggren 2010), which suggests that different patterns of metabolic scaling may be present in supply- versus demand-driven systems.

Growth and Metabolism Share Common Scaling Properties

The simplified growth model (eq. [3]) predicts that, over ontogeny, rates of assimilation, growth, and metabolism will all scale similarly. In partial accordance with this prediction, the

Table 3: Common slope mass-scaling exponents and coefficients for metabolic and growth rates by instar

	Metabolic rate (μL CO_2 h^{-1})		Growth rate (g d^{-1})
Instar	Scaling coefficients	Scaling exponents	Scaling coefficients
1	1,599 (1,400–1,826)	.717 (.615–.818)	.125 (.055–.285)
2	1,262 (907–1,757)	.764 (.597–.931)	.294 (.091–.947)
3	1,064 (766–1,478)	.660 (.510–.812)	.314 (.119–.828)
4	967 (708–1,321)	.594 (.460–.728)	.425 (.181–1.000)
5	1,636 (1,184–2,260)	.478 (.285–.671)	.578 (.250–1.334)
All	8,713 (7,518–10,097)	.945 (.925–.965)	.926 (.831–1.033)

Note. Results are for a common slope linear mixed-effects model with individual treated as a random effect to control for repeated measurements on individuals. Error is 95% confidence interval.

scaling exponent of growth rate was indistinguishable from that of metabolic rate in freely growing larvae, both within and across instars. Thus, even though the scaling of metabolism was not constant over development, the scaling of growth and metabolism appear to be closely coupled as predicted by West et al.'s model. However, during the fifth instar, even as the animals gained nearly 90% of their final weight (fig. 1), the scaling of growth rate decreased substantially, likely in preparation for pupation (Davidowitz and Nijhout 2004).

While the scaling exponents for metabolic and growth rates were indistinguishable over ontogeny, they did not match the scaling of assimilation rate, as predicted based on the assumed balance between assimilation and growth (West et al. 2001; Hou et al. 2008; Moses et al. 2008). Thus, the assumption of constant assimilation efficiency over ontogeny is not realistic for *Manduca* larvae. In particular, food intake and assimilation rates during the first two instars were both highly variable and sometimes substantially higher than would be expected by a matched scaling of assimilation, metabolism, and growth. In contrast, the scaling of frass production over ontogeny followed a pattern similar to that of metabolism and growth over ontogeny.

The substantial residual variation observed in the assimilation data likely has a technical explanation in part, but changes in the scaling exponents may still be biologically meaningful. The observed variability in food intake rates could result in part from the difficulty of precisely measuring the very low rates of food intake exhibited by small larvae, the episodic nature of larval feeding behavior, and variation in gut passage time across instars. Alternatively, when the animal is small, the surface-area-to-volume ratio of the food inside the gut is higher (because the gut is smaller), which may allow the animal to assimilate a higher fraction of its ingested food. Because frass production lags behind ingestion, our restriction of data to the free growth period may not have eliminated all frass production measurements that were affected by molt periods, contributing to the variability of the data and parameter estimates reported here. Despite this noise in the data, our results support the overall notion that excretion rates generally scale similarly to metabolic rates (Peters 1983), as has recently been shown for nitrogen excretion across multiple caterpillar species (Meehan and Lindroth 2007).

Assimilation and Growth Efficiencies and the Energetics of Biosynthesis

As mentioned above, our results do not support the assumption that assimilation and growth efficiencies are constant over development, a result also found in studies of embryo growth (Rombough 2011). Early in development, very high AE values are associated with extremely low GGEs. Moreover, the high first-instar AE values are primarily driven by unusually high size-specific intake rates (fig. 4). These trends in AE and GGE across instars are commonly observed in insect larvae, and our efficiency estimates are consistent with other studies of lepidopteran larvae (Scriber and Slansky 1981; Slansky 1985). However, West et al.'s growth model provides a novel explanation for the pattern: changes in the costs of biosynthesis (E_m). Even though E_m was estimated without reference to ingestion or frass production, it follows a very similar pattern of decline and stabilization over ontogeny (fig. 5), and the range of values (960–5,400 J g^{-1}) is in general agreement with previous theoretical and empirical estimates (Calow 1977; Wieser 1984; Moses et al. 2008; Rombough 2011). Thus, we suggest that high AE (and perhaps low GGE) are due to the higher energetic costs of biosynthesis early in development.

Changes in E_m may be associated with differences in tissue composition (Calow 1977; Wieser 1994), and our results suggest that early instars may face a trade-off between tissue quantity (reduced GGE) and tissue quality (increased E_m). Early in development, larvae may have to invest disproportionately in tissues rich in the molecular machinery of metabolism and biosynthesis (e.g., mitochondria, ribosomes, and endomembrane systems), which have high nutrient overhead, particularly the nitrogen and phosphorus necessary for the construction of proteins and RNA (Sterner and Elser 2002). This early investment in high-quality tissues may have important ramifications later in life. For example, *Manduca sexta* larvae reared on low-

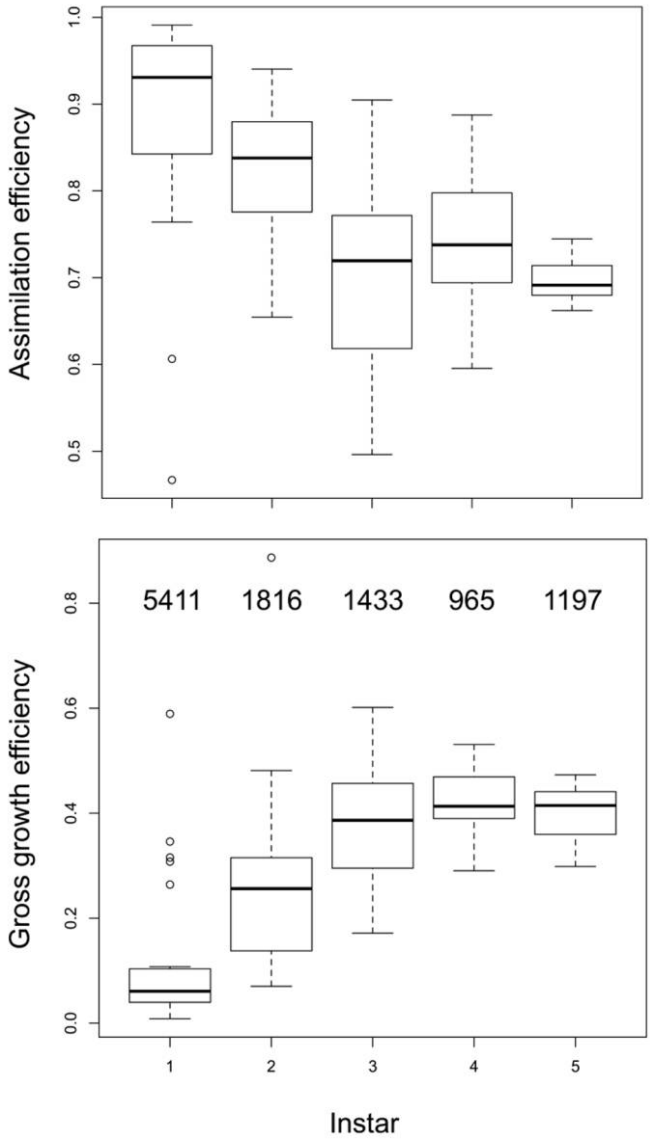

Figure 5. Distributions of (*A*) assimilation efficiency and (*B*) gross growth efficiency for each instar of *Manduca sexta*. Numbers are estimates of the energetic cost of biosynthesis (E_m; J g^{-1}), based on metabolic and growth parameters (see text).

protein diets early in life exhibit reduced fifth-instar growth rates and assimilation efficiencies, even when they are switched to a high-protein diet (Woods 1999).

Reduced values of E_m, AE, and GGE in the later instars may represent increased proportional investments in the synthesis of less costly and less metabolically active fatty tissues (Scriber and Slansky 1981; Ojeda-Avila et al. 2003). At the same time, the stabilization of assimilation and growth efficiencies leads to a closer match between the scaling of assimilation, metabolic, and growth rates in the later instars, as assumed by the growth model. Thus, we arrive at a hypothesis that deviations from the assumptions of the growth model early in larval development are caused by the costs associated with a sort of "capital investment" in the machinery of metabolism and biosynthesis. More direct measures of changes in tissue composition (e.g., protein and ribosomal RNA) and energy content over development, and particularly early in development, will be helpful in addressing this hypothesis. Moreover, because this molecular machinery has varied elemental composition, biological stoichiometry may play an important role in models of biological scaling and ontogenetic growth (Raubenheimer and Simpson 2004; Gillooly et al. 2005; Allen and Gillooly 2009).

Conclusions

Once it is simplified to reflect the near-exponential nature of larval insect growth (Tammaru and Esperk 2007) and gener-

alized to admit variation in the metabolic scaling exponent (Moses et al. 2008), West et al.'s (2001) growth model appears to provide a useful and biologically relevant, if somewhat simplified, description of larval growth, metabolism, and development. Still, it is clear from our metabolic, growth, and assimilation measurements that some of the core model assumptions (e.g., constant metabolic scaling parameters and constant assimilation and growth efficiencies) do not hold through all stages of larval development. At the same time, these violations of the model's assumptions provide an opportunity for understanding how variation in assimilation and growth efficiency may be related to changes in the underlying costs of biosynthesis early in development, and calculation of these costs is based on the derivation of the model.

The discrete developmental stages and wide size range of insect larvae such as *Manduca* provide a unique experimental model for the study of metabolic scaling, growth, and energetics. More specifically, our results highlight the potential importance of the discrete changes in the morphology, structure, and activity of exchange surfaces (midgut) and networks (tracheae) at times of molt and the predominance of cell expansion rather than proliferation during the instar. Further study of these changes will help us to understand how patterns of organismal growth emerge from fundamental biological processes at the cellular and tissue level, as mediated by the exchange of materials and energy between the organism and its environment.

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APPENDIX

While the metabolic scaling exponent did not change appreciably in response to restricting the data (fig. A1), the substantial decreases in growth rate observed at molt affected the scaling of growth rate much more strongly (fig. A2). Because free growth period restriction eliminated measurements recorded at or near times of molt while maintaining a relatively large data set, all analyses are reported only for the free growth period data.

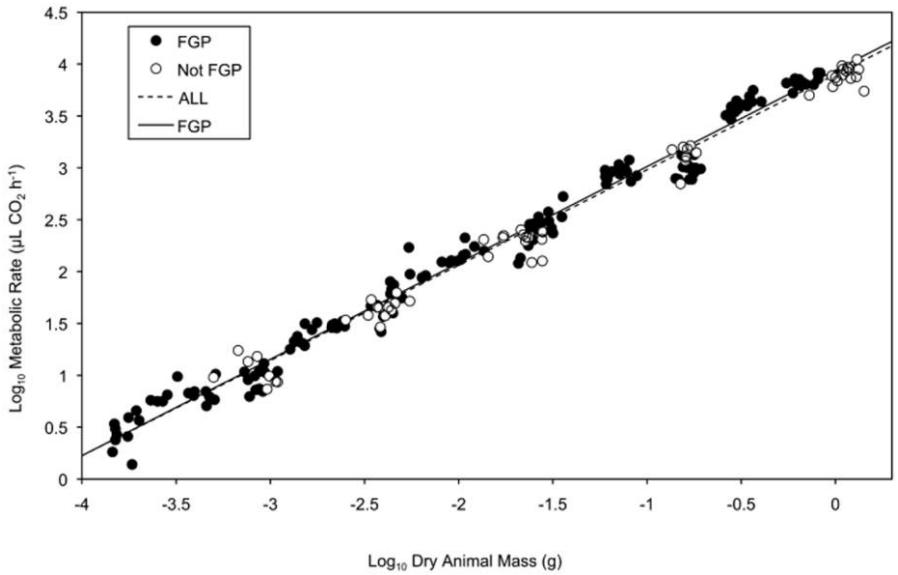

Figure A1. Scaling of metabolic rate during ontogeny for 15 *Manduca* larvae shown with regression for all data and limited to data from the free growth period (FGP).

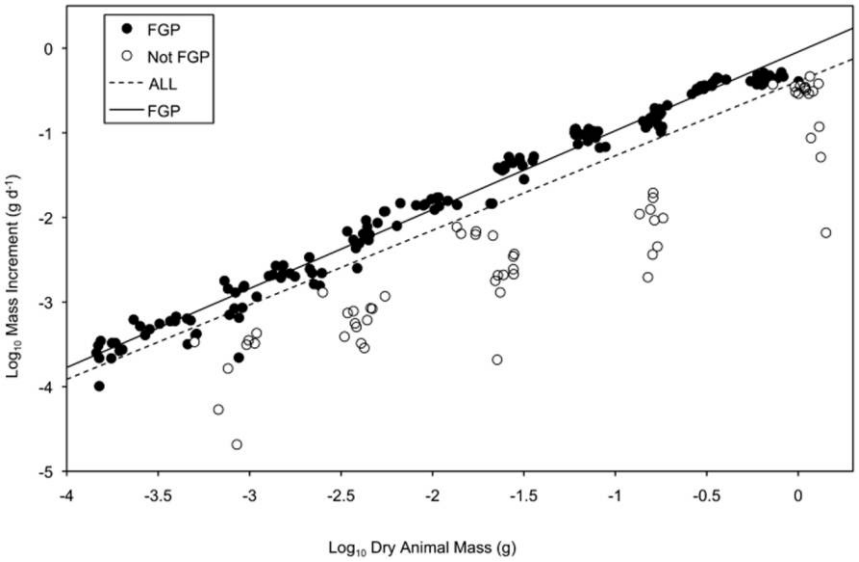

Figure A2. Scaling of growth rate over ontogeny for 15 *Manduca* larvae shown with regression for all data and limited to data from the free growth period (FGP).

Literature Cited

Allen A.P. and J.F. Gillooly. 2009. Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. Ecol Lett 12: 369–384.

Alleyne M., M.A. Chappell, D.B. Gelman, and N.E. Beckage. 1997. Effects of parasitism by the braconid wasp *Cotesia congregata* on metabolic rate in host larvae of the tobacco hornworm, *Manduca sexta*. J Insect Physiol 43: 143–154.

Baldwin K.M. and R.S. Hakim. 1991. Growth and differentiation of the larval midgut epithelium during molting in the moth, *Manduca sexta*. Tissue Cell 23:411–422.

Banavar J.R., J. Damuth, A. Maritan, and A. Rinaldo. 2002. Ontogenetic growth: modelling universality and scaling. Nature 420:626.

Banavar J.R., A. Maritan, and A. Rinaldo. 1999. Size and form in efficient transportation networks. Nature 399: 130–132.

Beitel G.J. and M.A. Krasnow. 2000. Genetic control of epithelial tube size in the *Drosophila* tracheal system. Development 127:3271–3282.

Blossman-Myer B.L. and W.W. Burggren. 2010. Metabolic allometry during development and metamorphosis of the silkworm *Bombyx mori*: analyses, patterns, and mechanisms. Physiol Biochem Zool 83:215–231.

Bolker B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and J.S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135.

Calow P. 1977. Conversion efficiencies in heterotrophic organisms. Biol Rev 52:385–409.

Chamberlin M.E., C.M. Gibellato, R.J. Noecker, and E.J. Dankoski. 1997. Changes in midgut active ion transport and metabolism during larval-larval molting in the tobacco hornworm (*Manduca sexta*). J Exp Biol 200:643–648.

Chown S.L., E. Marais, J.S. Terblanche, C.J. Klok, J.R.B. Lighton, and T.M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Funct Ecol 21:282–290.

D'Amico L.J., G. Davidowitz, and H.F. Nijhout. 2001. The developmental and physiological basis of body size evolution in an insect. Proc R Soc B 268:1589–1593.

Davidowitz G. and H.F. Nijhout. 2004. The physiological basis of reaction norms: the interaction among growth rate, the duration of growth and body size. Integr Comp Biol 44:443–449.

DeLong J.P., J.G. Okie, M.E. Moses, R.M. Sibly, and J.H. Brown. 2010. Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life. Proc Natl Acad Sci USA 107:12941–12945.

Esperk T. and T. Tammaru. 2004. Does the "investment principle" model explain moulting strategies in lepidopteran larvae? Physiol Entomol 29:56–66.

Gibellato C.M. and M.E. Chamberlin. 1994. Midgut metabolism in different instars of the tobacco hornworm (*Manduca sexta*). J Exp Zool 270:405–409.

Gillooly J.F., A.P. Allen, J.H. Brown, J.J. Elser, C. Martínez del Rio, V.M. Savage, G.B. West, et al. 2005. The metabolic basis of whole-organism RNA and phosphorus content. Proc Natl Acad Sci USA 102:11923–11927.

Glazier D.S. 2005. Beyond the "3/4-power law": variation in the intra- and interspecific scaling of metabolic rate in animals. Biol Rev 80:611–662.

———. 2006. The 3/4-power law is not universal: evolution of isometric, ontogenetic metabolic scaling in pelagic animals. BioScience 56:325–332.

Gouveia S.M., S.J. Simpson, D. Raubenheimer, and F.P. Zanotto. 2000. Patterns of respiration in *Locusta migratoria* nymphs when feeding. Physiol Entomol 25:88–93.

Greenlee K.J. and J.F. Harrison. 2005. Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta*. J Exp Biol 208:1385–1392.

Hou C., W.Y. Zuo, M.E. Moses, W.H. Woodruff, J.H. Brown, and G.B. West. 2008. Energy uptake and allocation during ontogeny. Science 322:736–739.

Isaac N.J.B. and C. Carbone. 2010. Why are metabolic scaling exponents so controversial? quantifying variance and testing hypotheses. Ecol Lett 13:728–735.

Kerkhoff A.J. and B.J. Enquist. 2009. Multiplicative by nature: why logarithmic transformation is necessary in allometry. J Theor Biol 257:519–521.

Kleiber M. 1961. The fire of life: an introduction to animal energetics. Wiley, New York.

Kolokotrones T., V. Savage, E.J. Deeds, and W. Fontana. 2010. Curvature in metabolic scaling. Nature 464:753–756.

Kooijman S.A.L.M. 1993. Dynamic energy budgets in biological systems. Cambridge University Press, Cambridge.

Kozlowski J., M. Konarzewski, and A.T. Gawelczyk. 2003. Cell size as a link between noncoding DNA and metabolic rate scaling. Proc Natl Acad Sci USA 100:14080–14085.

Meehan T.D. and R.L. Lindroth. 2007. Modeling nitrogen flux by larval insect herbivores from a temperate hardwood forest. Oecologia 153:833–843.

Moses M.E., C. Hou, W.H. Woodruff, G.B. West, J.C. Nekola, W.Y. Zuo, and J.H. Brown. 2008. Revisiting a model of ontogenetic growth: estimating model parameters from theory and data. Am Nat 171:632–645.

Nijhout H.F. 1975. Threshold size for metamorphosis in tobacco hornworm, *Manduca sexta* (L). Biol Bull 149:214–225.

Nylin S. 1992. Seasonal plasticity in life-history traits: growth and development in *Polygonia c. album* (Lepidoptera, Nymphalidae). Biol J Linn Soc 47:301–323.

Ojeda-Avila T., H.A. Woods, and R.A. Raguso. 2003. Effects of dietary variation on growth, composition, and maturation of *Manduca sexta* (Sphingidae: Lepidoptera). J Insect Physiol 49:293–306.

Peters R.H. 1983. The ecological implications of body size. Cambridge Studies in Ecology. Cambridge University Press, Cambridge.

Pinheiro J.C. and D.M. Bates. 2000. Mixed effects models in S and S-plus: statistics and computing. Springer, New York.

Raubenheimer D. and S.J. Simpson. 2004. Organismal stoichiometry: quantifying non-independence among food components. Ecology 85:1203–1216.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Reiss M.J. 1989. The allometry of growth and reproduction. Cambridge University Press, Cambridge.

Reynolds S.E., A.M. Brown, R.K. Seth, L.M. Riddiford, and K. Hiruma. 2009. Induction of supernumerary larval moulting in the tobacco hornworm *Manduca sexta*: interaction of bisacylhydrazine ecdysteroid agonists with endogenous juvenile hormone. Physiol Entomol 34:30–38.

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. Funct Ecol 17:384–393.

Rombough P. 2011. The energetics of embryonic growth. Respir Physiol Neurobiol 178:22–29.

Rubner M. 1883. Über den einfluss der körpergrösse auf stoff- und kraftwechsel. Z Biol 19:536–562.

Savage V.M., A.P. Allen, J.H. Brown, J.F. Gillooly, A.B. Herman, W.H. Woodruff, and G.B. West. 2007. Scaling of number, size, and metabolic rate of cells with body size in mammals. Proc Natl Acad Sci USA 104:4718–4723.

Savage V.M., E.J. Deeds, and W. Fontana. 2008. Sizing up allometric scaling theory. PLoS Comput Biol 4:e1000171.

Savage V.M., J.F. Gillooly, W.H. Woodruff, G.B. West, A.P. Allen, B.J. Enquist, and J.H. Brown. 2004. The predominance of quarter-power scaling in biology. Funct Ecol 18:257–282.

Schmidt-Nielsen K. 1984. Scaling: why is animal size so important? Cambridge University Press, Cambridge.

Scriber J.M. and F. Slansky. 1981. The nutritional ecology of immature insects. Annu Rev Entomol 26:183–211.

Sehnal F. and A.S. Meyer. 1968. Larval-pupal transformation: control by juvenile hormone. Science 159:981–984.

Slansky F. 1985. Food utilization by insects: interpretation of observed differences between dry-weight and energy efficiencies. Entomol Exp Appl 39:47–60.

Sterner R.W. and J.J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ.

Tammaru T. and T. Esperk. 2007. Growth allometry of immature insects: larvae do not grow exponentially. Funct Ecol 21:1099–1105.

von Bertalanffy L. 1957. Quantitative laws in metabolism and growth. Q Rev Biol 32:217–231.

West G.B., J.H. Brown, and B.J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. Science 276:122–126.

———. 2001. A general model for ontogenetic growth. Nature 413:628–631.

———. 2004. Growth models based on first principles or phenomenology? Funct Ecol 18:188–196.

West G.B., B.J. Enquist, and J.H. Brown. 2002. Ontogenetic growth—modelling universality and scaling: reply. Nature 420:626–627.

White C.R. and R.S. Seymour. 2003. Mammalian basal metabolic rate is proportional to body mass$^{(2/3)}$. Proc Natl Acad Sci USA 100:4046–4049.

Wieser W. 1984. A distinction must be made between the ontogeny and the phylogeny of metabolism in order to understand the mass exponent of energy-metabolism. Respir Physiol 55:1–9.

———. 1994. Cost of growth in cells and organisms: general rules and comparative aspects. Biol Rev 68:1–33.

Woods H.A. 1999. Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. Physiol Biochem Zool 72:445–454.

Yagi M., T. Kanda, T. Takeda, A. Ishimatsu, and S. Oikawa. 2010. Ontogenetic phase shifts in metabolism: links to development and anti-predator adaptation. Proc R Soc B 277: 2793–2801.