Modeling Growth and Metabolism in *Manduca sexta* Larvae: Variation Across Individuals and Instars

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ABSTRACT

Metabolism and material exchange with the environment are complex and intricately related processes that depend heavily on animal size. We developed a model of larval growth for the tobacco hornworm, *Manduca sexta*, an organism that grows 10,000-fold in approximately 18 days. Based on detailed daily measurements of food intake, frass production, carbon and nitrogen assimilation, animal mass, and metabolic rate, we document substantial physiological changes at or near times of molt. As a result, patterns of larval growth *within* each of their five instars may follow trends comparable to the ontogenetic trajectories of vertebrates. Models for growth integrating metabolic-scaling and energy uptake that have been successfully applied to mammals and birds may thus be parameterized separately for each instar when applied to larval growth. Metabolic-scaling exponents vary among instars and across individuals, and inter-individual variation may allow us to predict differences in growth among individual larvae. Our results suggest that relatively simple models relating metabolism and material exchange to growth in vertebrates can be extended to describe the complex ontogeny of insect larvae. At the same time, further studies of *M. sexta* will allow us to use controlled experiments to learn how variation in food quality, temperature, and living conditions influence the interactions between metabolic scaling, material exchange, and growth, while still considering a large range of magnitudes in animal size.

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INTRODUCTION

•Animal size has substantial effects on physiological processes like metabolism and material exchange.

•West et al. (2001) describe a model for ontogenetic growth of vertebrates based on cellular and metabolic processes. The model uses allometric scaling relationships to describe growth as the balance between the energy allocated to maintenance of existing biomass and that used to build new biomass:

$$\frac{dm}{dt} = am^{\alpha} - bm^{\beta} \qquad \text{Equation 1}$$

where a and b are constants and α and β are mass-scaling exponents. West et al. predict that α is the scaling exponent of metabolism. If this is true, we will be able to predict the value of α from measurements of larval growth.

•Due to the nature of their growth curve (see Figure 1), it is reasonable to assume that insect growth is governed mainly by the first term, which represents the creation of new biomass (Tammaru and Esperk, 2007). We test this claim by fitting this modified growth curve to *M. sexta* growth data.

METHODS

Animal Rearing: Seventeen *Manduca sexta* larvae (Carolina Biological Supply, NC) were raised from eggs to pupae in individual containers. Animal mass, food intake, frass production, carbon and nitrogen assimilation, metabolic rate, instar, and day of instar were recorded each day for each individual.

The Model: The growth model we used was modified from Equation 1, by eliminating the second term:

$$\frac{dm}{dt} = am^{\alpha} \qquad \text{Equation 2}$$

'Alpha' and 'a' values were calculated by regression fit of mass increment vs. dry mass on a log-log scale. Mass increment is an estimate of the instantaneous growth rate calculated as the difference between dry masses measured at the beginning and end of each 24 hour period.

Analysis: Due to distinct differences in growth, intake, metabolism, and other processes at times of molt, we restricted the data in the following ways:

•All Data
•Free Growth Period (FGP)—increasing mass increment, as compared to the previous day.
•Actively Feeding (AF)—increasing intake, as compared to the previous day.
•Maximum Growth Period (MGP)—highest mass increment for each instar for each animal.

FIGURES

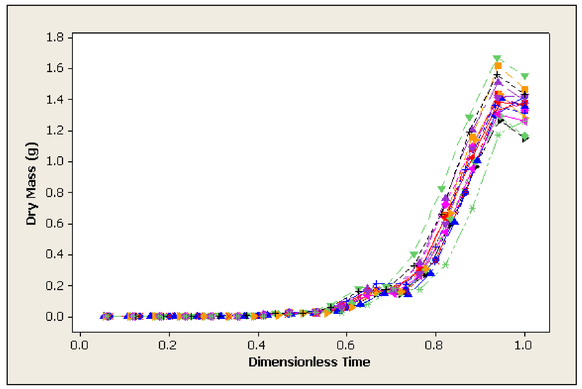
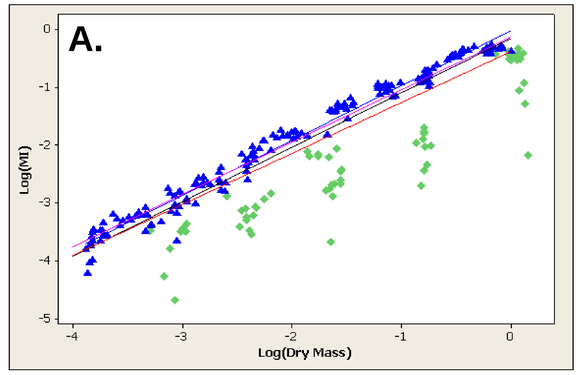


Figure 1. Growth trajectory of seventeen *M. sexta* larvae. All data is included. Dimensionless time was calculated as the ratio of days since hatching to the total number of larval days for each individual.

Data Set	Sample Size	Model		Actual	
		R^2	α	R^2	α
All	243	83.1 %	0.883	98.3 %	0.909
AF	186	92.6 %	0.942	98.5 %	0.920
FGP	178	98.0 %	0.947	98.3 %	0.928
MGP	85	98.3 %	0.905	98.1 %	0.902

Table 1. Regression statistics for each data exclusion set. Fits are shown in Figure 2. The model was fitted as mass increment vs. dry mass, while actual values were calculated from CO_2 respiration vs. dry mass. The metabolic scaling exponent (α) is the slope of each regression on a log-log scale.



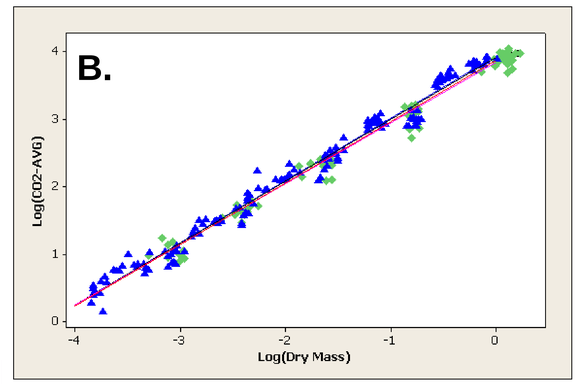


Figure 2. Regression fit of **A.)** the growth model (Equation 2 on a log-log scale) and **B.)** actual metabolic data (CO_2 respiration) over animal mass for all four data exclusion sets. All data points are shown and are colored according to free growth period (FGP) classification: blue points are included in the FGP, green points are not. Regression lines are colored as follows: red = all data, blue = FGP, black = actively feeding (AF), and pink = max growth period (MGP). See Table 1 for regression information. ANOVA p = 0.000 for each regression.

Instar	1	2	3	4	5
α	0.771	0.753	0.718	0.646	0.292
R^2	59.7 %	66.7 %	77.8 %	84.6 %	52.8 %

Table 2. Metabolic mass-scaling exponents (α) predicted from the growth model within each instar. Data was restricted to the FGP. α values were calculated from the regression of mass increment vs. dry mass on a log-log scale. ANOVA p = 0.000 for each regression.

RESULTS AND DISCUSSION

•The growth curve of *M. sexta* larvae does not follow the sigmoidal pattern observed in vertebrates. However, it resembles the early portion of this curve. We postulate that these larvae never reach the inflection point of that sigmoidal curve because their growth is arrested by hormonal signals, which prepare them for pupation. The leveling off of growth observed here could be a result of hormonal pathways rather than a manifestation of the physiological limit of growth, as in the previously modeled vertebrates.

•Of the four data exclusion sets, the FGP restriction is most suited to metabolic scaling analyses and modeling (Figure 2A). Though the MGP data set gives a slightly better fit of the model, it greatly decreases the sample size of our data (Table 1). The FGP, on the other hand, gives a very good fit, while maintaining a large sample size. This method of data restriction ensures that the points that are included in analyses represent days when larval growth was not impeded by molting and is especially important when comparing insect growth to that of vertebrates, which do not have comparable molt cycles.

•The model predicts metabolic mass-scaling exponents (α) that are close to those calculated directly from metabolic measurements (Figure 2B, Table 1). The variation in α observed among individuals and instars, however, was not correlated with variation predicted from the growth model.

•Hou et al. (2008) propose a model of energy uptake and assimilation based on the growth model of West et al. In the future, we will be able to apply this modified growth curve to parameterization of the Hou et al. model for insect larvae.

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