The Effect of Diet on Ontogenetic Scaling of Metabolic Rate in *Manduca sexta*

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Abstract

Scientists have long understood that animal body size is an important determinant of food intake, growth, excretion and metabolic rate. A good model organism for studying these relationships is the tobacco hornworm, *Manduca sexta*. By measuring the CO_2 output, we investigated the effect of diet on the scaling of metabolism. Two different groups were reared, one on an artificial diet and one on a natural tobacco diet. The metabolic rate of the *Manduca* reared on an artificial diet was 51.5% higher than those reared on a tobacco diet. In our generated model, there is no difference between the scaling exponents found for the two diets and body weight. Thus, diet has an important influence on weight-adjusted metabolism, but does not influence the shape of the metabolic scaling relationship.

Introduction

An animal's metabolic rate can determine food intake, growth, excretion and other functions that vary with the body size of animals. Few attempts have been made to compare the metabolic scaling on different scales of observation (Glazier 2005). Here at Kenyon, this problem has been explored through a collaborative project between the biology and mathematics departments.

As the *Manduca sexta* develops, it progresses through a series of molts that characterize its larval stage (Greenlee and Harrison 2005). *Manduca* has a total of five discrete instars. The instar stages occur between the molting of the larva. The organism also grows 10,000-fold in mass and 10 times in length in about 18 days. Because of these drastic changes in mass and size, this organism is an excellent model for exploring the relation of metabolic rate to body size.

Our previous work has been dedicated to collecting data and has supplied much information on the relationships between growth, assimilation, and metabolic rate. All previous work has been done on a high nutrient, wheat germ based artificial diet. Food quality can affect the relationship between growth and metabolism. Through rearing and testing animals grown on a natural, tobacco diet, we are able to examine the changes in scaling relationship in response to food quality.

We hypothesized that growth and metabolism are affected by food quality.

Methods

Animal rearing:
We reared two cohorts of *Manduca sexta* eggs (Carolina Biological Supply) from larvae to 5th instar. Upon hatching, individuals were placed into separate containers. Half were reared on the tobacco hornworm medium bulk diet (Carolina Biological Supply)and the rest were grown on a diet composed of tobacco leaves. They grew in an incubator with 16:8 light:dark cycle at 65°F. The tobacco leaves were grown in the greenhouse and the BFEC. Each individual was weighed at the same time daily for 17 days. Food for the tobacco individuals was replenished with fresh leaves 3 times a day where food for the wheat germ diet was replaced every other day. Petri dishes were cleared of frass at the time of measurement each day. Respirometry data was collected once the animals reached second instar, or a minimum of 0.05 grams.

Data Collection:
The metabolic rate of each animal was measured approximately every other 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 30°C temperature/humidity-controlled chamber. The respirometry system was calibrated to each morning and recalibrated between each set of measurements using reference gases. After each animal had acclimated to the chamber for 15 minutes, CO_2 exchange was measured for each animal over three five minute intervals and these three measurements were averaged to obtain average values of metabolic rate, in µl CO_2 hr^{-1}. Majority of statistical analysis was produced by R statistical program.

Results

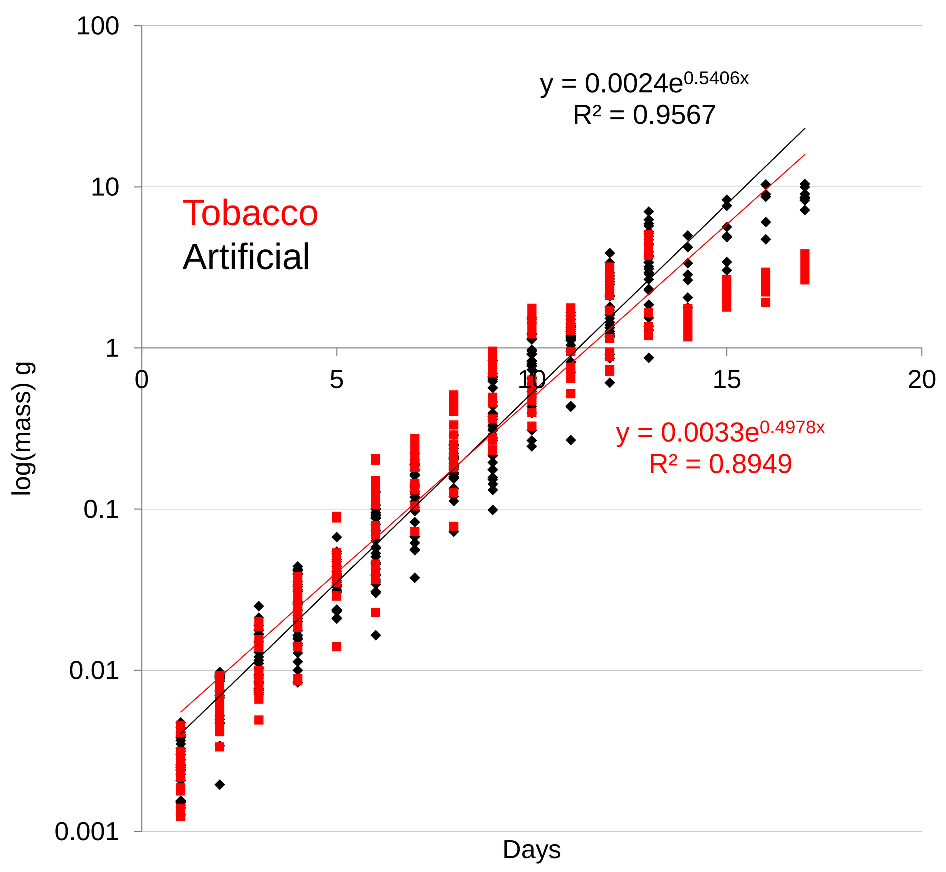
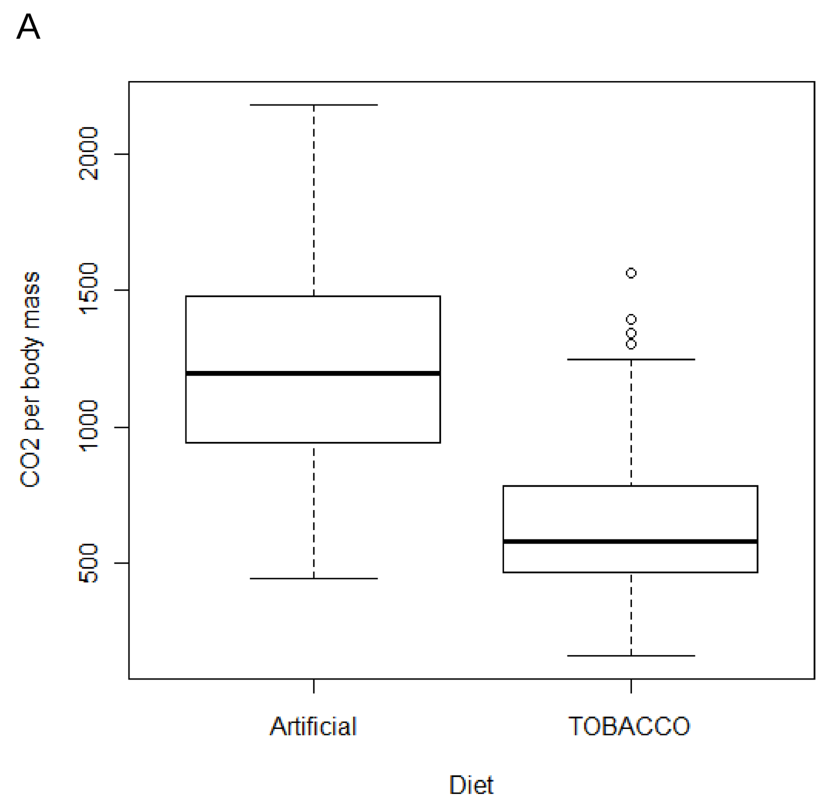


Figure 1: Growth chart of tobacco and artificially grown *M. sexta*. Because their weight grows on a logarithmic scale, data was scaled to fit. At the start of the 5th instar, tobacco trend decreases where artificial continues linearly.



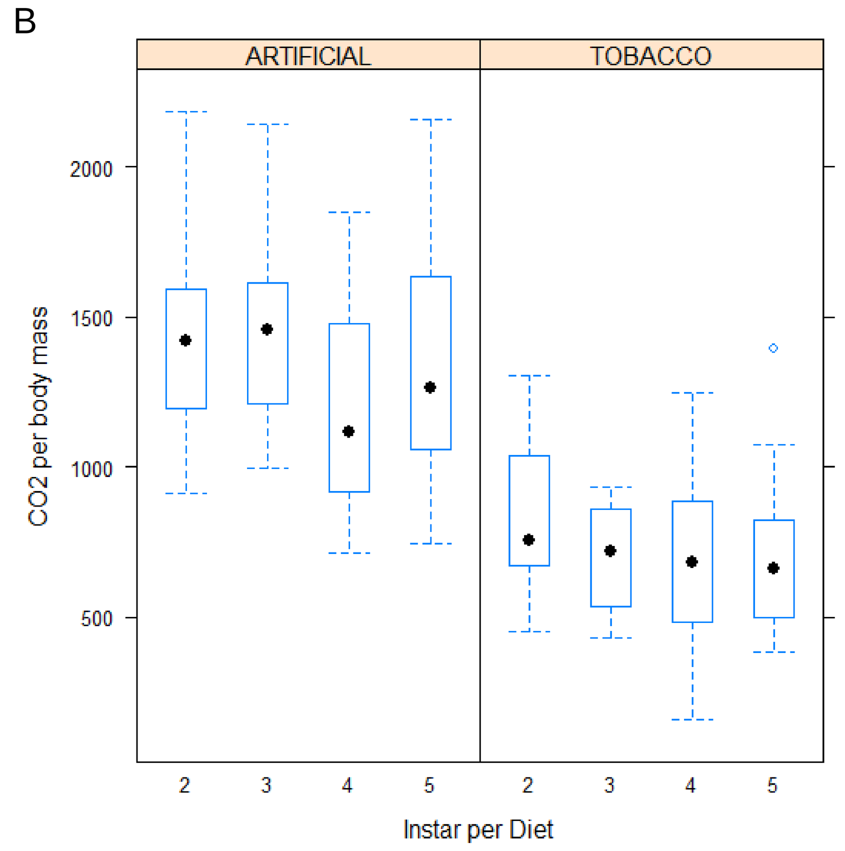


Figure 2: Box plots showing the relationship between CO_2 Exchange and the diet. Figure A shows the general significance between the relationship of CO_2 Exchange and Diet (ANOVA, F= 490.5, p= 2.2e-16). Figure B further defines Figure A by showing the significant individual effects of Instar on CO_2 per body mass (ANOVA, F= 17.27, p= 2.7e-13).

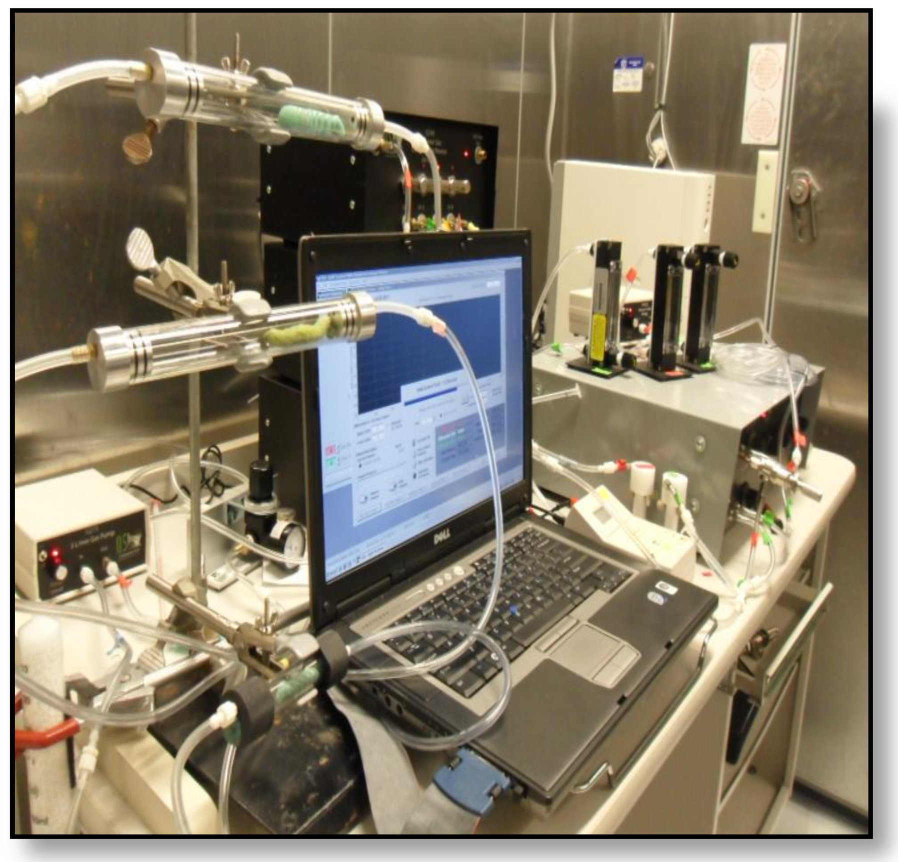


Figure 3: Qubit Systems G249 gas controller/monitor and G283 channel switcher run into a model S151 infrared gas analyzer. Data Collection of CO_2 exchange was gathered for each animal every other day.

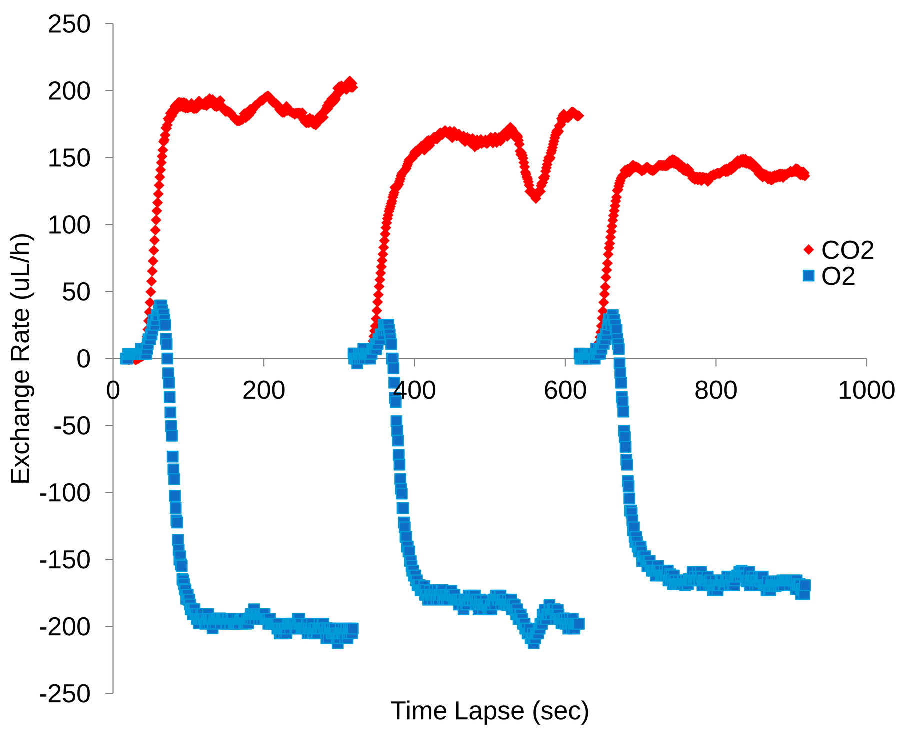


Figure 4: Trace of CO_2 and O_2 Exchange (uL/h). Output by Qubit Systems G249 gas controller/monitor and G283 channel switcher run into a model S151 infrared gas analyzer. Data was averaged to obtain metabolic rate values.

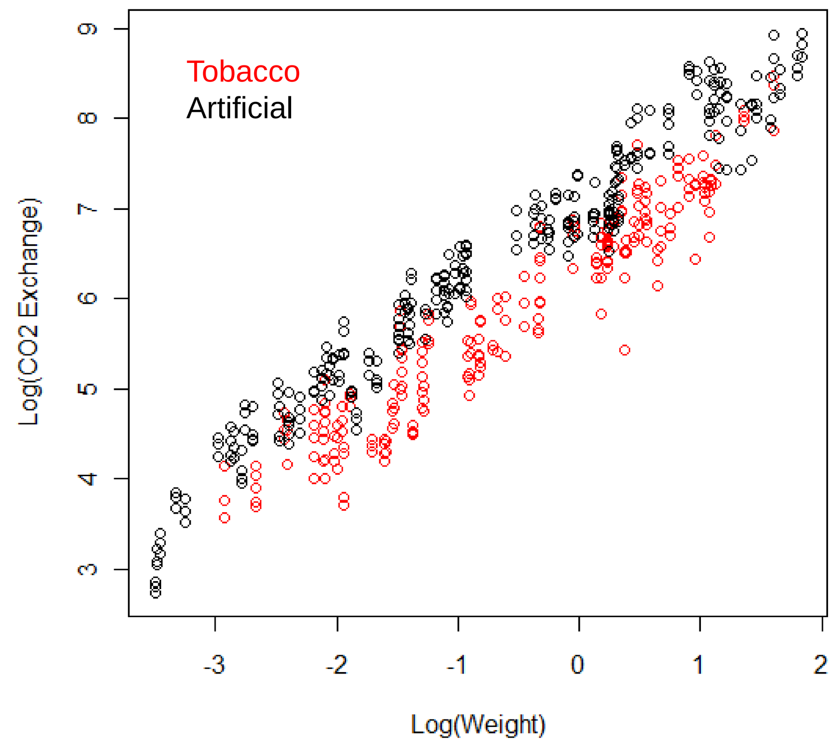


Figure 5: Scatter plot of log(CO_2) v. log(Body Mass). Respiration rate as a function of body mass. Exp. = 0.95 (Mixed-Effects Model, t= 57.2, p= 0.000).

Summary

Manduca larval metabolic scaling exponent of the tobacco diet is 0.93 which is identical to the 0.93 scaling exponent observed on the artificial diet from previous laboratory work (General Linear Model, t= 46.97, p= 2e-16).

However, we found a slightly high scaling exponent for the artificial diet alone at 0.98 (General Linear Model, t= 75.79, p= 2e-16).

Using a Mixed Effects Model, the interaction term between diet and body mass is not significant and thus has no effect on the shape of the metabolic scaling relationship (t= -1.86, p = 0.068). The common scaling exponent for both diets is 0.95. The diet affected metabolism on its own as reflected by the difference in the intercepts.

Manduca reared on the tobacco diet have lower weight-adjusted metabolism than *Manduca* on the artificial diet but the exponent of the scaling relationship does not change. In the laboratory, it is important to notice this difference because in a 'natural' environment the *Manduca* will exhibit differences because things like temperature and light exposure are not controlled. In future work we may be able to extrapolate the scaling exponents, but not necessarily the intercepts, to animals feeding on different diets.

Future Work

Looking further into the nutritional balance of the diets, is the richness and excess carbohydrates of the artificial diet affecting body content rather than just body size? To answer this, we are looking at the lipid composition of the different animals to gain better insight in to the fat storage and fitness cost in each organism and how it reflects on the growth and metabolism.

Different strains of *Manduca* can offer insight on how selection on size affects the scaling of growth and metabolism. To expand upon the data we have collected on regular stain *Manduca* and the differences between tobacco and artificial diets, we can take a look at 'mini' *Manduca* and 'mega' *Manduca* under the same project. The 'mini' and the 'mega' are 33% smaller or larger than the regular strain, respectively. When looking at the 'mini' for example, we are unsure if they are smaller due to fewer cells or just smaller cells. If the smaller size is due to smaller cells, then the surface area to volume ratio would be larger, which may allow for greater cellular metabolic rates.

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