The relationship between midgut growth and morphology and body size in the tobacco hornworm, *Manduca sexta*

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Abstract

Decades of research have focused on elucidating the association between metabolic rate and body mass. Using *Manduca sexta* as a model organism, we investigated the role of the midgut in larval metabolism; while the midgut disproportionately contributes to metabolic rate of the organism, the physiological mechanisms behind this connection are poorly understood. To determine if metabolism and body size are constrained by in-folding of the midgut, we sectioned larvae from each instar and examined the correlation between larval mass and luminal perimeter. Morphometric analyses revealed that midgut perimeter scales allometrically with body size, suggesting that in-folding may be a crucial factor in sustaining metabolism throughout the larval phase.

Introduction

- Metabolism enables an organism to maintain itself, grow, interact with its environment, and reproduce; these chemical pathways are ubiquitous, similarly operating in diverse species.
- The metabolic rate (MR) of an organism correlates with its body weight (BW) according to the proposed model:[1]

$$MR = aBW^{b}$$

- The tobacco hornworm, *Manduca sexta*, has proven invaluable for intraspecific metabolic scaling research owing to its rapid increase in BW over the course of five larval instars (10,000-fold within three weeks).[2]
- The midgut of a *M. sexta* larva comprises about 9% of its BW, while midgut metabolism encompasses 20.3% of the total metabolic output for the organism.[3]
- Since the midgut disproportionately contributes to larval metabolism, and experimental values[4,5,6] of *b* (the scaling exponent) have been greater than would be expected if the midgut scales isometrically[7] with BW, we expect that some aspect of the midgut is increasing allometrically.
- In this study we investigated the interplay between midgut in-folding and metabolic scaling.

Methods

Rearing: *M. sexta* eggs were purchased from Carolina Biological, and larvae were reared on a standard wheat germ diet at 27°C with a 14h:10h light:dark cycle.

Tissue Preparation and Microtomy: Larvae were anesthetized on ice and fixed in Bouin’s solution at 4°C. Transverse cuts were made to excise midgut regions (anterior, middle, and posterior) according to external markers. Tissues were then dehydrated in an ethanol series, cleared with xylene, and infiltrated with molten paraffin. Paraffin blocks were sectioned at 10µm using an AO Spencer Model 820 rotary microtome and put on gel-coated slides.

Histology: Slides containing sections were stained with hematoxylin and eosin according to standard protocol, dehydrated in an ethanol series, and cleared with xylene before being coverslipped with Permount.

Image Analysis: Images were captured using a Nikon DS-Fi1 camera mounted on a Nikon Optiphot-2 compound microscope at 20x magnification. Three sections per midgut region of each larva were selected for imaging, and the micrographs were stitched together with Adobe Photoshop Elements 9. Midgut perimeter measurements taken along the apical membrane were made with ImageJ 1.46, and a least-squares regression was performed on the data in Minitab 16.

Results

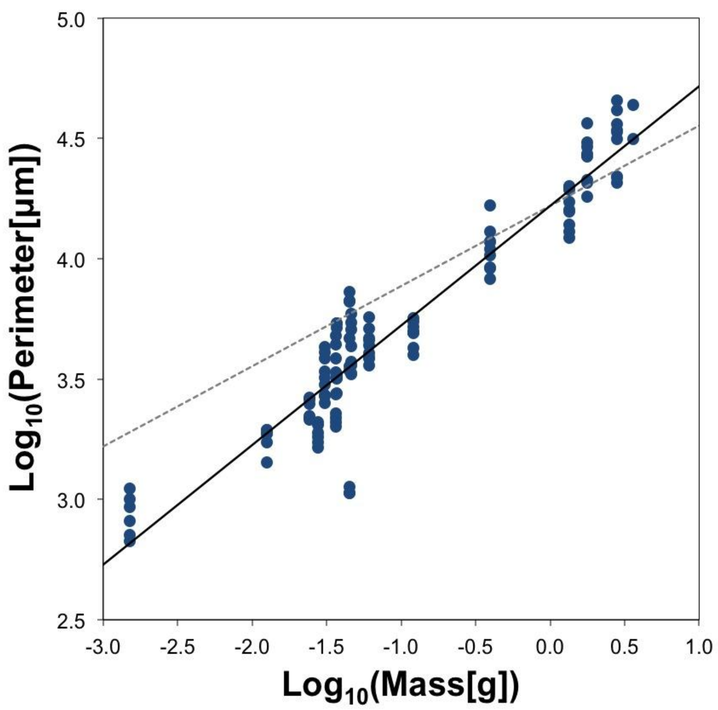

Figure 1. The relationship between midgut perimeter and larval body mass on a log-transformed scale (includes early instar data from K. Connell ‘13). The solid, black line indicates the least-squares regression line, y = 0.497x + 4.22 (n = 128, R^2 = 90.2%, ANOVA p < 0.001). The dashed, grey line indicates a regression line of slope $^1/_3$, as predicted by isometric scaling.

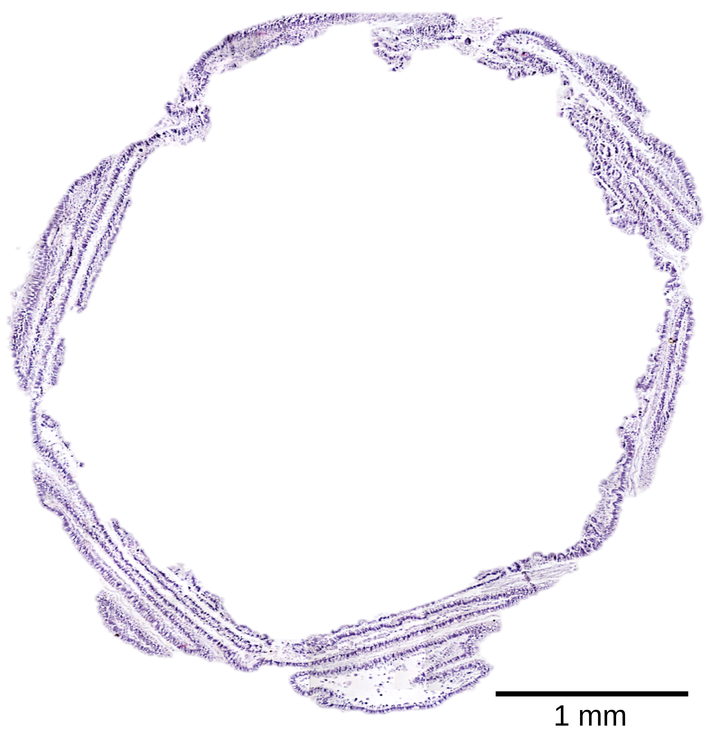

Figure 2. Composite micrograph of a 5th instar anterior midgut section showing in-folding.

Discussion

- An isometric relationship between midgut perimeter and larval body mass would yield a theoretical scaling exponent of $^1/_3$.
- However, we observed that midgut perimeter scales with larval body mass by an exponent of 0.497 (Figure 1, y = 0.497x + 4.22, n = 128, R^2 = 90.2%, ANOVA p < 0.001).
- Our findings suggest that midgut perimeter is increasing at a greater rate than predicted by isometry; this allometry is presumably the result of increased in-folding by the gut wall in later instars (Figure 2).
- Such morphological changes likely enable larger larvae to compensate for a decreased relative midgut surface area to volume ratio; in-folding facilitates diffusion by increasing surface area for nutrient exchange at a rate greater than predicted by isometry.
- While in-folding may be a factor in the metabolic scaling of *M. sexta*, changes in microvillar length and differential transporter protein densities may also influence the nutrient absorption capacity of the midgut; the interaction between these morphological variables remains unclear.

References

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