Physiological changes in the midgut during the growth of the larval Tobacco Hornworm, *Manduca sexta*

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Abstract

Metabolic scaling has been investigated for decades as biologists searched for a link between body mass and metabolic rate by determining the scaling exponent. In this study, we investigated *Manduca sexta* as our model organism; observing their changes in midgut morphology in various stages of growth. By measuring the increase in luminal perimeter in relation to body mass, we were able to determine that there is an increase in midgut surface area through infolding as body mass increases. This allometric scaling of surface area to volume of the midgut could be responsible for increased nutrient absorption and therefore increase total metabolic rate.

Introduction

- Every organism must perform many chemical processes in order to maintain internal homeostasis despite changing external conditions. The energy required for these processes are obtained through its ability to extract nutrients during digestion.
- An organism's metabolic rate (MR) is largely dependent on its body weight (BW) (Gilooly, 2001) and the correlation can be defined by the equation:

  $$MR=a(BW)^b$$

  where b is the exponent of metabolic scaling (Banavar *et. al.*, 2002). B can be calculated by determining the slope between log(BW) and log(MR).
- Manduca are an ideal organism as they increase in size 10^4-fold over 5 instars in about 3 weeks (Goodman et. al., 1985). By using only one species, the effects of differences in morphology, habitat, and life strategy are eliminated and the the effect of body weight on metabolic rate is isolated.
- *M. sexta* midgut comprises 9% of the organism's total BW yet it is responsible for 20.3% of the organism's total metabolic output (Kerkhoff *et. al.*, 2008), indicating that some aspect of the midgut is increasing allometrically.
- In this study, changes in midgut morphology through all 5 instars of growth were documented and analyzed.

Acknowledgements

I would like to thank Professor Itagaki for his guidance throughout this process as well as K. Connell '13 and A. Sanderlin '15 for their preliminary research and instruction on midgut morphology. This project was sponsored by the Kenyon Summer Science Program and the Kenyon Biology Department, and The National Science Foundation Interdisciplinary Training for Undergraduates in Biological and Mathematical Sciences program (UBM Grant #0827208).

Methods

Sectioning and Staining

Midgut from *Manduca sexta* individuals were dissected, fixed and embedded in paraffin blocks. Each block was then trimmed around the midgut sample and sectioned at 10uM using an American Optical rotary microtrome. Each section was then placed on a subbed slide and allowed to dry on a slide warmer for 8+ hours. Slides were then run through 2x xylene and hydrating ethanol series before staining using a standard Hematoxylin-Eosin reduced time staining protocol and then dehydrated in a graded ethanol series (30%, 50%, 70%, 95%, 2x100%) and then 2x xylene before being coverslipped with Permount.

Capturing and Analyzing Images

Slides were viewed under brightfield conditions on a Nikon Optishot II compound microscope. The three best sections from each portion of midgut (anterior, middle and posterior) were photographed using a Nikon Digital Analysis System and saved as .jpg files. For larger instars, images were stitched together using Adobe Photoshop CS6. Measurements were taken with ImageJ. The perimeter measurement was taken by tracing the boundary between the base of the microvilli and the midgut lining. Data were collected and analyzed in Microsoft Excel 2010 and Minitab16. A regression analysis was done to determine the metabolic scaling exponent.

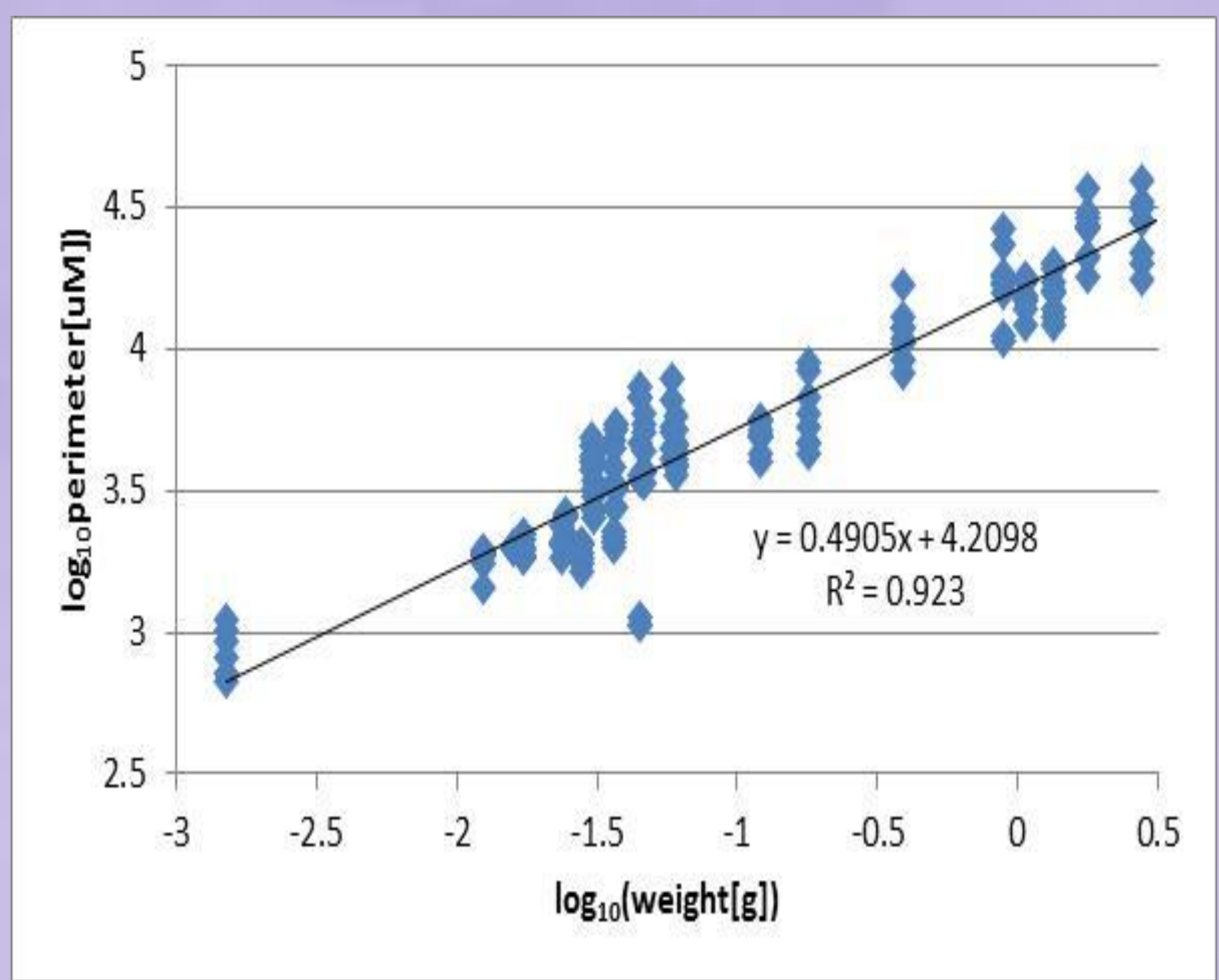

Figure 1. The relationship between the body weight of the individual and the midgut perimeter of the cross-section on a logarithmic scale (y=0.4905x+4.2098, r^2=0.923, ANOVA p=0.000)

Results and Discussion

- An isometric relationship between larval body weight and midgut perimeter would yield a theoretical metabolic scaling exponent of 0.33.
- We observed a metabolic scaling exponent of 0.4905 (Figure 1. y=0.4905x+4.2098, r^2=0.923, ANOVA p=0.000).
- This suggests that the midgut perimeter is increasing at a faster rate than the caterpillar's body mass. This allometry is caused by increased infolding of the midgut in larger instars (Figure 2.)
- The increase in midgut surface area ultimately allows the increase nutrient absorption to enable larger larvae to accommodate a generally decreasing surface area to volume ratio.
- In addition to increased infolding, other factors may influence the nutrient absorption of *M. sexta* larvae. Changes in microvillar length and density of transport proteins in the epithelia membrane of the midgut could also influence nutrient absorption rates although the relationship between these mechanisms is unclear.

Figure 2. A series of composite images of anterior midgut sections from first (innermost ring) to fifth instar individuals (outermost ring). By K. Connell, '13.

Works Cited

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