Changes in midgut morphology during the growth of the tobacco hornworm, *Manduca sexta*

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Abstract

The concept of metabolic scaling has been investigated for decades as biologists worked to find a scaling exponent which links metabolic rates between organisms of different sizes. In this study, we observed the growth and change in midgut morphology in *Manduca sexta* in hopes of characterizing anatomical changes of the gut during growth. Correlations found between midgut growth and metabolic rates could indicate that the changes in the midgut affect the rate of nutrient absorption and therefore the overall metabolic rate.

Introduction

- In order for an organism to maintain constant internal conditions independent from a varying environment, it must perform many different chemical reactions that are summarized in its metabolic rate. The size of the organism performing metabolism has a large effect on its metabolic rate, which has been represented in the equation: $MR=a(BW)^b$ (e.g. Chaui-Berlinck, 2006).
- The value of *b* is calculated by graphing logBW against logMR and taking the slope of the line. Using this method, researchers have calculated *b* to have a value between 0.4 and 1.4 across a wide range of organisms (Glazier, 2005). The variation in *b* indicates that there are probably multiple factors that change the exponent between species (Darveau *et al.*, 2002), though the degree of each effect is unknown.
- *M. sexta*, the tobacco hornworm, serves as an ideal model for the study of metabolic rate and body weight due to the fact that the larvae rapidly grow 10^4-fold in weight over the course of five instars (Goodman *et al.*, 1985).
- In this study, the change in midgut morphology through the five instars of growth was documented and analyzed in hopes of better understanding how it relates to metabolic rates in animals of different sizes.

Works Cited

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Methods

Sectioning and Staining

Midgut sections from *M. sexta* individuals were fixed and embedded in paraffin blocks. The paraffin blocks were trimmed around the midgut and then sectioned at 10 uM using a rotary microtome. The sections were placed on subbed slides and then allowed to dry on a slide warmer overnight. The slides were run through 2x xylene and a hydrating ethanol series and stained using a reduced-time Hematoxylin-Eosin protocol. The slides were then dehydrated in a graded ethanol series, then in 2x xylene before being coverslipped with Permount. 585 slides made from 117 individuals were prepared and stained.

Capturing and Analyzing Images

Slides were viewed under a Nikon Optiphot II compound microscope. Three sections per portion of the midgut (anterior, middle, and posterior) were selected from each individual. The sections were photographed at 20X under brightfield conditions using a Nikon DSFi1 camera. Due to the size of the sections, multiple photographs were taken of each section and then stitched together in Adobe Photoshop9.0. Measurements were taken in ImageJ. Average goblet and columnar cell sizes were determined by averaging 30 cell lengths per section. Microvilli length and area and perimeter measurment were taken at the same magnification. Data were collected and analyzed in Microsoft Excel 2010 and Minitab16. One-Way ANOVAs were run to determine morphological differences between the different instars and GLMs were taken to determine differences between sections within and between instars.

Results and Discussion

- The perimeter of the midgut increased as a function of weight by a factor of 0.48 when transformed to a log scale (Figure 2, $r^2 = 0.87$, ANOVA p=0.00). This value indicates that the perimeter is increasing at a greater rate than by isometric growth, which would predict a slope of 0.33.
- The microvilli length changed significantly between the sections (anterior, middle and posterior) within and between instars (Figure 3, $GLM_{Section,\ Instar,\ Section*Instar}$ F=8.50, 0.41, 2.91; df=2,4,8; df_{error}=102; p=0.00, 0.80, 0.01).
- Sample size was not large in the first and fifth instars; in order to solidify results, more data need to be collected.

Figures

Figure 1. A series of composite images of anterior midgut sections from first (innermost ring) to fifth instar individuals (outermost ring).

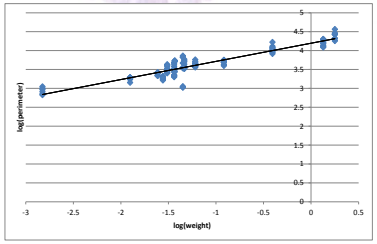

Figure 2. The relationship between the perimeter of the midgut section and the weight of the individual on a log-transformed scale (y=0.48x+4.20, $r^2 = 0.87$, ANOVA p=0.00).

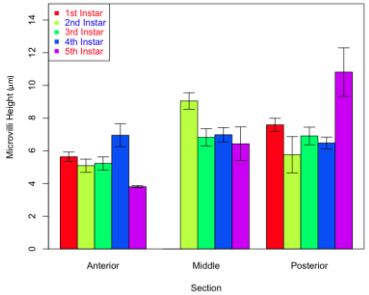

Figure 3. Microvilli lengths were dependent on the instar and section of the midgut. (Error bars= 95% CI, $GLM_{Section,\ Instar,\ Section*Instar}$ F=8.50, 0.41, 2.91; df=2,4,8; df_{error}=102; p=0.00, 0.80, 0.01)

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