Effect of strain and diet on *Manduca sexta* midgut mass

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Abstract

We investigated masses of the midgut and its contents in tobacco hornworm *Manduca sexta* reared on a tobacco leaf diet compared to those reared on an artificial diet. The midguts of larvae reared on tobacco were 27.4% lighter than those reared on artificial food. In contrast, midgut contents were 27.8% heavier in the larvae reared on tobacco compared to those reared on artificial food. We also compared two strains of *Manduca sexta*, mini and mega, which are smaller and larger, respectively, than control strains. The scaling exponents of midgut mass for mini, mega, and regular stains were 0.97, 0.93, and 0.85, respectively. The scaling exponents of midgut content mass were 1.23, 1.00, and 1.27, respectively. Finally, we studied the relationship between midgut region and body mass of the larvae. Scaling exponents of anterior and posterior midgut were 0.92 and 0.85, above the 0.67 value predicted for isometric scaling, while the exponent for middle midgut was 0.71. Dry masses showed the same pattern.

Introduction

Manduca sexta larvae are a useful model for evaluating effects of body size on physiology because they grow approximately 10,000 fold in body mass over approximately 18 days (Goodman et al.,1985). Overall body structure and behavior are mainly unchanged across instars. On the other hand, relative midgut surface area probably decreases substantially in later instars in comparison to growth rate, body mass and metabolic rate. Estimates of midgut surface area based on measurements of cell size and number show that surface area increases by only 256 fold while growth rate increases by 717 fold (Baldwin and Hakim, 1991; Gilbellato and Chamberlin, 1994; Goodman et al., 1985).

We compared two strains of *Manduca sexta*, mini and mega, to control strains. The mini and mega strains were artificially selected to be 30% smaller and larger, respectively, than the original strain. We expected to see the midgut scale differently with each strain.

We studied the relationship between midgut region and body mass of the larvae. The midgut is composed of three cell types: columnar cells, goblet cells and small regenerative cells. The fine structure of goblet and columnar cells changes along the length of the midgut, allowing the midgut to be divided into structurally and perhaps functionally distinct anterior, middle and posterior regions (Cioff, 1979). We predicted that the anterior and posterior regions would have higher weights (in relation to body mass) than the middle region.

Methods

Animals

Manduca sexta were obtained from Carolina Biological Supply Co. and raised at 27 degrees Celsius. Larvae were raised on 2" x 2" x 0.5" wire supports in plastic containers with air holes. Larvae were individually caged and staged daily starting in the 2nd instar.

Diets

Animals in the diet experiment were either reared on an artificial wheat germ diet (supplied by the Carolina Biological Supply Co.) or on a tobacco-leaf diet. Artificial food was replaced daily while tobacco leaves were replenished two or three times daily. Frass was removed at each feeding. Tobacco plants were grown in the greenhouse and at the Brown Family Environmental Center at Kenyon College.

Scaling of midgut tissue and contents

Body mass of 3rd, 4th, and 5th instar larvae was measured and the larvae were anesthetized on ice. Gut contents, including the peritrophic membrane, and the entire gut tissue, including the foregut, midgut, and hindgut, were carefully dissected, placed on pre-weighed aluminum pans, and their masses measured. The remainder of the body was also placed on a pre-weighed pan.

Scaling of midgut tissue sections

The above procedure was applied once again and microscopic observations were used to distinguish the sections of the midgut (anterior, middle, and posterior), especially in 3rd instars.

References

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Results

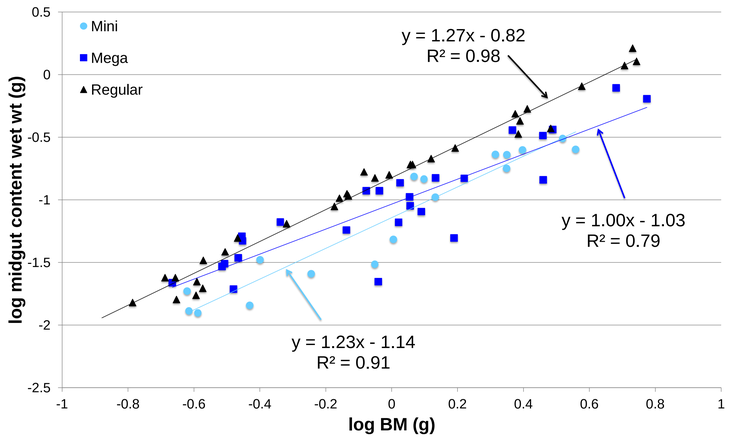

Figure 1. The relationship between midgut content wet weight and the body mass of 3rd through 5th instar in Mini, Mega and regular strains of *Manduca sexta*. Least squares regression lines are shown.

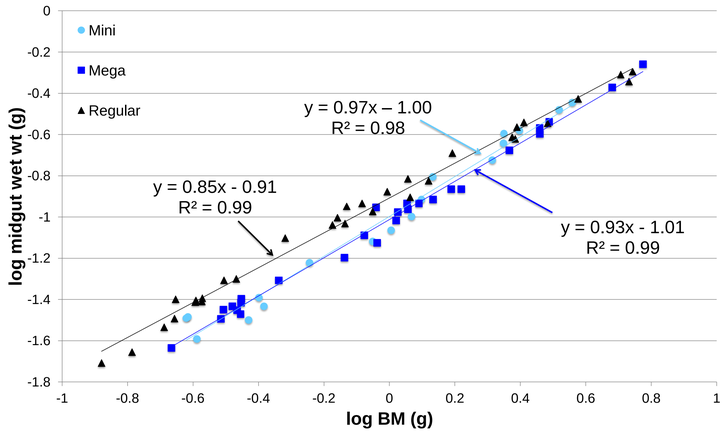

Figure 2. The relationship between midgut wet weight and the body mass of 3rd though 5th instar in Mini, Mega and regular strains of *Manduca sexta*. Least squares regression lines are shown.

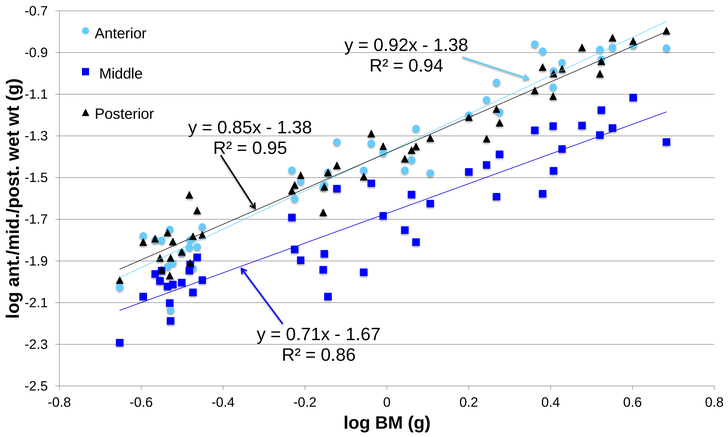

Figure 3. The relationship between anterior, middle and posterior midgut wet weights and body mass of 3rd through 5th instars of *Manduca sexta*. Least squares regression lines are shown.

Results

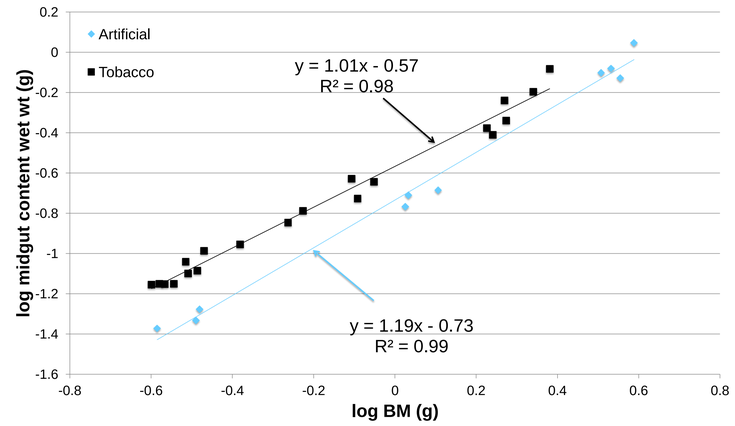

Figure 4. The relationship between midgut content wet weight and body mass in 3rd through 5th instar of *Manduca sexta* reared on artificial food and tobacco leaves. Least squares regression lines are shown.

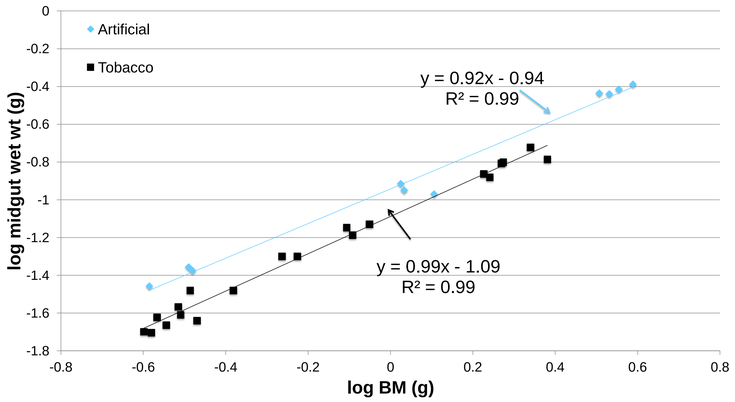

Figure 5. The relationship between midgut wet weight and body mass in 3rd through 5th instar of *Manduca sexta* reared on artificial food and tobacco leaves. Least squares regression lines are shown.

Summary

•The scaling exponents were different between the midgut content mass and the midgut mass in animals of all three strains eating artificial diet. Midgut contents scaled with exponents above 1, while midgut tissue scaled with exponents below 1.

•In contrast, scaling exponents for midgut tissue and content were similar in animals reared on the tobacco diet. Midgut contents scaled with an exponent 1.01 and midgut tissue scaled with an exponent of 0.99.

•Difference between scaling of midgut content and tissue mass between animals reared on natural and artificial diets suggests that food quality is an important determinant of scaling.

•All regions of the midgut scaled with exponents lower than one, with the middle region forming a smaller portion of the midgut as animals grew.

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