Image from http://entomology.unl.edu/k12/caterpillars/hornworm/hornwormpage.html

The relationship between body surface area and morphological midgut growth of the tobacco hawkmoth, *Manduca sexta*

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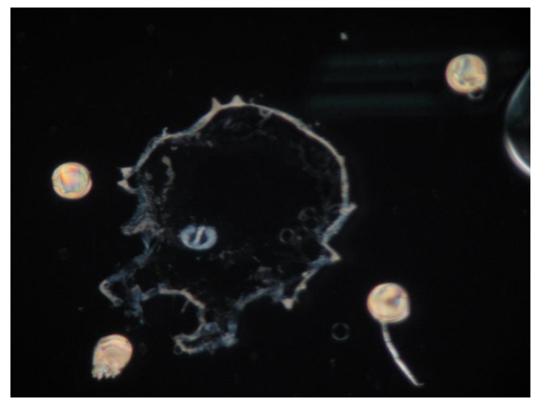

Abstract

The idea of a universal scaling relationship is one constantly being explored to help gain a better understanding of the functions of many different organisms. We have continued our work in estimating the body surface areas (BSA) of 2nd, 3rd and 4th instar larvae of the sphinx moth, *Manduca sexta*, of various body weights (BW). Three lab-reared *Manduca sexta* were embedded and sectioned using a cryostat. All transverse sections were digitized using polarization optics. The sections for two individuals were mathematically analyzed to produce a parameterization equation of their body surface areas. This information was used to construct a virtual hornworm of 2nd and 3rd instar *Manduca* and a parameterized equation for each organism was calculated. The surface area for each of these three individuals was found to be 0.303 cm^2 for 2F (2nd instar) and 0.877 cm^2 for 3AA (3rd instar.

Introduction

- An animal's size influences multiple aspects of its characteristics including its anatomy, physiology and behavior[7].
- The relationship between body size and metabolic rate is one aspect of size studied in particular, with larger organisms having a lower metabolic rate per gram weight than smaller organisms[2].
- This relationship between body weight (BW) and metabolic rate (MR) is related by the equation $MR=a(BW)^b$ with b being the experimentally determined exponent[2].
- The exponent, b, has been found to equal 0.67 according to the surface hypothesis theory(1883)[6]. However, metabolic rates across different species of animals tend to show a value of b closer to .75. This gives rise to the question regarding the existence of a universal scaling relationship between body mass and metabolic rate[1,4,9,10].
- A model organism for studying this relationship of metabolic rate and body mass in a single species is the larva of the tobacco hawkmoth, *Manduca sexta*. This larva grows exponentially, increasing in weight 10,000-fold, and passing through 5 instars in only 18 days[3] (Fig. 1).
- The ultimate goal of the project is to measure the midgut surface area, which has been found to comprise approximately 9% of the total weight of an actively feeding larva (Gillen, Itagaki pers. observation), but as a pilot study to refine our methods, we began by measuring the overall body surface area in larvae whose metabolic rates have been measured.
- Determining the scaling relationship between body weight and body surface area will help give us a better understanding of interspecific scaling relationships and through this an increased understanding of interspecific scaling relationships[5].

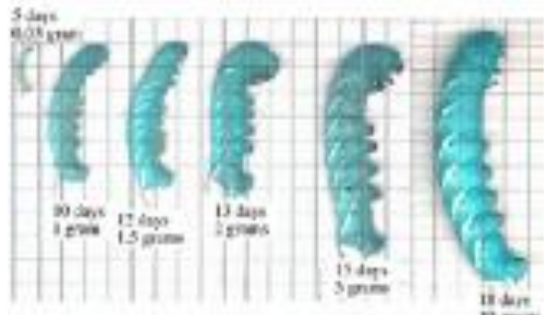

Figure 1: Growth of Manduca larvae. Image from: http://www.staff.uni-Marburg.de/~dolzer/mas_pics.html

Methods

Previous Work

Manduca sexta were reared from eggs (Carolina Biological Supply, NC) on a prepared diet. Throughout the 5 instars of the *Manduca's* growth period, larvae were starved and then fed red-dyed food to assist in the visibility of their midgut. The larvae were then frozen and embedded with Histoprep and dry ice to freeze them in a vertical position. 4 bristles from a paintbrush were embedded with each larvae to serve as a reference point between sections. A cryostat kept at -20° C was used to make transverse sections of each larva at 50µm thickness which were placed on a gel-coated slide. Each of these sections was digitized using a Zeiss Stemi 2000C stereomicroscope equipped with a Canon digital camera and polarization optics.

Image Analysis

Once these images were taken, they were imported into Image J, an NIH image program. Measurements were taken on a 2nd and 3rd instar *Manduca* between each of the 4 orientation dots and the centroid was found (Fig. 2). A line was drawn between the centroid and the upper left dot, to serve as a reference between sections. Each image was rotated so that the aforementioned reference line was located on the x-axis to serve as the 0º angle. 36 measurements were taken from the centroid to the surface of each section 10º apart.

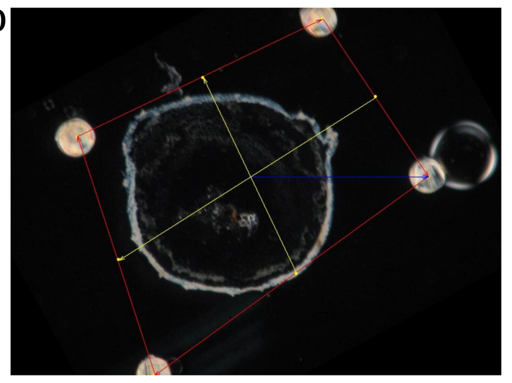

Figure 2: Section of a 3rd Instar *Manduca sexta* with orientation dots and added image analysis lines.

Mathematical Analysis

Each set of radii values was imported into MAPLE, where the complementary mathematical analysis was performed on them. Jim Boston '09 and Dr. Judy Holdener were responsible for the creation of this mathematical protocol. A parametric model of the boundary curve was taken for 14 sections in the 2nd instar and 17 sections in the 3rd instar. A second program in MAPLE was then used to create a parametric model of the *Manduca* body surface for each instar, in addition to finding the surface area. The centroid for each section was used to determine a space curve that served as the "generating curve" for the body surface.

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Results

- A 2nd, 3rd, and 4th instar *Manduca sexta* were sectioned and digitized.
- The 2nd and 3rd instar *Manduca sexta* were mathematically analyzed using NIH image and MAPLE software programs. A parameterized equation for each of the individuals was found.
- The general equation for each cross section was found to be:

$$a_0 + \sum_{m=1}^{n} \left(a_m \sin(m\,\theta) + b_m \cos(m\,\theta) \right)$$

- The parametric models of the surface area were constructed so that the data fit the sinusoidal curve of the form:

$$\langle X(u), Y(u) \rangle = \left\langle X_0 + \sum_{k=1}^{n} (a_k \sin(ku) + b_k \cos(ku)),\ Y_0 + \sum_{k=1}^{n} (c_k \sin(ku) + d_k \cos(ku)) \right\rangle$$

- This equation and the equations for the surface areas of the individual sections were used to find a full parametric virtual model of the 2nd (2F), and 3rd (3AA) *Manduca sexta* (Fig. 3).
- From these parameterized models, the surface area for each of the three organisms was calculated to be 0.303 cm^2 for the 2nd instar (2F), and 0.877 cm^2 for the 3rd instar (3AA).

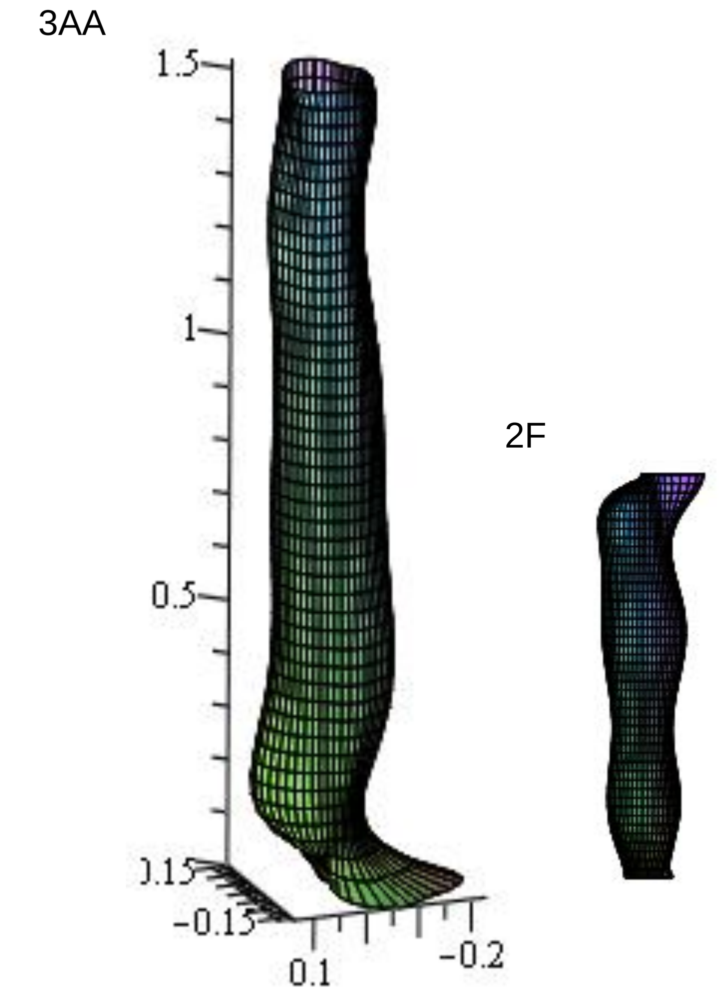

Figure 3: Fully modeled 3rd (3AA) and 2nd (2F) instar *Manduca sexta.* Measurements taken in centimeters. Models were created in MAPLE.

Discussion

This research is the continued work of what Anna Frutiger '09 had previously worked on for two years. Methods to determine the body surface area still needs to be perfected further to reduce error on these parameterizations. Despite this, however, we are hopeful that with more data, we will later be able to observe the changes in the midgut in comparison to the changes in the surface area. Although there is currently not enough data at this point in time to conclude as to whether the surface area growth of the *Manduca sexta* tobacco hornworms is allometric or isometric, it is an excellent ongoing project that we hope will provide answers to the question of whether or not a universal scaling relationship exists.