Metabolism, trachea, and mitochondria: What makes a tobacco hornworm molt?

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Insect larval growth is characterized by a growth trajectory periodically interrupted by molts, during which the larva sheds and replaces its cuticle. In the tobacco hornworm (*Manduca sexta*), the molting process is initiated when larvae attain the critical weight. Although this phenomenon is well documented, the physiological event that initiates the molting process is not known.

Figure 1. A fifth instar *Manduca sexta* larva.

I hypothesize that, during the intermolt period (instar), the tracheal system is fixed in size, which limits the rate of oxygen supply. Metabolic demand for oxygen increases with body size, so oxygen supply could become inadequate beyond a certain size. Lack of oxygen might therefore trigger the molt, during which the old tracheal system is shed and replaced with a new, larger one. The physiological basis of critical weight could be the threshold of oxygen below which cells become hypoxic.

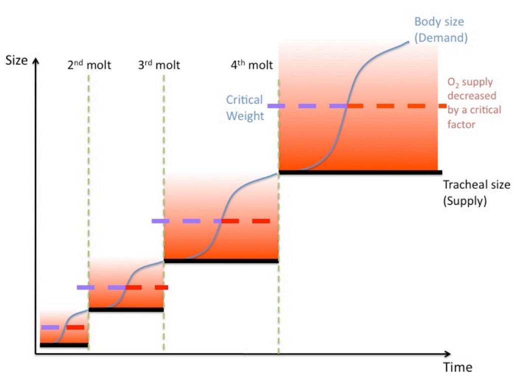

Figure 2. Graphic of the hypoxia hypothesis.

I tested this hypothesis by rearing larvae in low oxygen environments. Lower oxygen concentrations should reduce the critical weight because oxygen delivery should be limiting at smaller body size. I found that larvae reared in hypoxic conditions do indeed have lower critical weights, and attain approximately *half* the size of the equivalent normoxic controls.

I then created a mathematical model in the form of a differential equation to simulate growth curves of caterpillars reared in normoxic conditions. In my model the growth rate dM/dt is given by

$$dM / dt = a \cdot M^{1-b \cdot M / R_{\max}}$$

where M is the larva's mass; a is the rate at which oxygen and food consumption is converted into an increase in mass; b is the demand for oxygen per unit mass; and R_{max} is the maximum rate of oxygen supply for a given instar. At the beginning of the instar, demand is low, so the exponent approaches one: Mass grows exponentially. As the larva increases in mass, demand increases, and the exponent decreases toward zero. This reproduces the observed decrease in growth rate as the larva progresses through each instar. The growth equation is supplemented by the condition that, when the ratio of demand ($b \cdot M$) becomes excessive relative to supply (R_{max}), the molt is triggered and the larva proceeds to the next instar.

Based on respirometry measurements from Greenlee and Harrison (2005), I was able to quantitatively predict the magnitude of the decrease of the critical weight for larvae reared in hypoxic conditions.

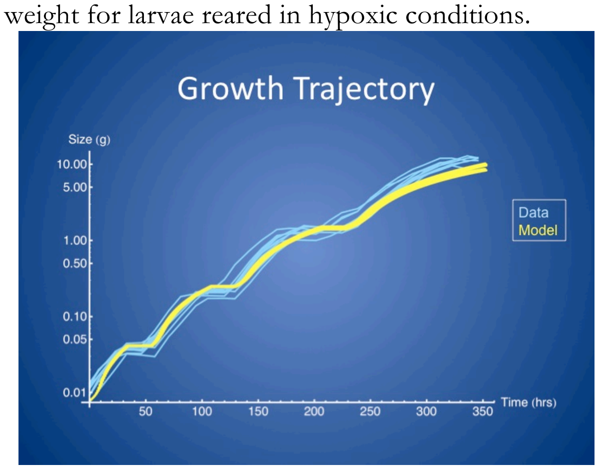

Figure 3. Measured (light blue) and simulated (yellow) growth curves.

These measurements and model will help us understand how larvae translate hypoxia-induced physiological changes into a size-sensing mechanism. Understanding the normal regulation of growth and size could help us better understand diseases, such as cancer, where these processes go awry.