Introduction

During the 19 day pupation period of *Manduca sexta* (Tobacco hornworm) significant morphological changes occur. There is compartmentalized division of the body plan. The various adult organ systems develop, for example, an extensive tubular network ramifies to form the adult respiratory system, powerful thoracic muscles for flight, the reproductive system as well as the new adult neural network. Tracheae located in the abdomen are further modified into large sac-like structures during metamorphosis whose function is still unknown.

In this study, we used micro-computerized tomography (MCT) and conventional flow respirometry in parallel to study the effect of temperature on tissue development and to establish the relationship between the physiology and development of major organ systems during metamorphosis. MCT provided high-resolution 3-D images *in vivo* and in the same animal thereby permitting tracking of organ system development which allowed us to characterize tracheal branching in developing tissues (thoracic flight muscles). With flow respirometry, we generated a metabolic profile of the developing pupae to compare changes in respiratory needs necessary to accommodate developmental stages.

Objectives

• Utilize advanced tools for understanding organismal biology and anatomy by conducting a non-invasive study of internal and external morphogenesis during *Manduca sexta* pupal development while simultaneously studying the effects of external stimuli on developmental physiology.

• Provide a real life context for the traditional biology curriculum addressing homeostasis while cultivating scientific inquiry skills that can apply classroom lessons to real world concerns.

Materials & Methods

Temperature Treatment

A. 17°C – COLD (C)
B. 27°C – NORMAL (N)
C. 38°C – HOT (H)

*Fig. 1:* Life Cycle of *Manduca sexta*: (i) eggs, (ii) 5th instar larva, (iii) pupa & (iv) adult moth.

Conclusions

• Optimum temperature is necessary for metabolizing tissues in developing *Manduca sexta* pupae where cold temperatures delay and hot temperatures accelerate development.

• Hot temperatures show advancement in flight muscle and tracheal development compared to normal. Loss of muscle tissue on day 19 in combination with the sharp decline in respiration from days 17 to 19 may account for the failure of those pupae to emerge.

• Our data shows delay in developmental time for cold treatment compared to normal.

• This project provides an experimental perspective and application for students to illustrate the role of environmental factors at play in maintaining homeostatic systems.

Acknowledgements

Thank you to the following contributors for their support: Gilmour Lab, Cornell MCT Facility, Insect Facility at Boyce Thompson Institute, Douglas Lab for Respirometry, NSF GK-12 program, Dr. C. Schaffer, Dr. S. Archer, Kevin Dilley, Nev Singhota.