

Case Study

Know your insect: The structural backgrounds of regurgitation, a case study on *Manduca sexta* and *Heliothis virescens* (Lepidoptera: Sphingidae, Noctuidae)

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Abstract

Background

Insect herbivores often regurgitate on wounded sites while feeding on plants. Their regurgitant contains different kinds of elicitors that could trigger different plant responses. While feeding on the same host plant, *Heliothis virescens* and *Manduca sexta* caterpillars deposit different amount of regurgitant on the damage site (Peiffer and Felton 2009). According to literature data the foregut of regurgitating caterpillars is longer and more strongly muscled than that of not or rarely regurgitating caterpillars (Grant 2006).

New information

We compared the gross morphology of fore and midgut of the rarely regurgitating caterpillar of *Manduca sexta* with the more often regurgitating caterpillar of *Heliothis virescens*. The foregut of the rarely regurgitating caterpillar is longer than that of the regurgitating caterpillar, which contradicts the literature data.

Keywords

regurgitation, herbivory, elicitor molecules

Overview of regurgitation in insects

Insect herbivores have evolved a myriad of strategies to suppress plant anti-herbivore defensive responses. One of the herbivore strategies receiving most attention by researchers is the use of herbivore elicitors to suppress induced plant defense mechanisms. While feeding on plants, insect herbivores regurgitate on wounded sites (Peiffer and Felton 2009, Vadassery et al. 2012). Insect regurgitant contains different kinds of elicitors. These elicitors can be small molecules like fatty acid conjugates (FAC), inceptin, calliferins and/or larger molecules like the specific enzyme glucose oxidase (Alborn et al. 2003, Alborn et al. 1997, Fürstenberg-Hägg et al. 2013). Plants can recognize these elicitors to distinguish between mechanical injury and herbivore damage (Fürstenberg-Hägg et al. 2013). Although chemical characteristics and functions of elicitors have been studied in depth, little attention is given to mechanism of deposition of these elicitors through the process of regurgitation.

Herbivores deposit different amounts of regurgitant on damaged sites of host plant (Peiffer and Felton 2009). This difference in amount of regurgitation depends on herbivore behavior rather than nutritional quality of the host plant (Vadassery et al. 2012). On the basis of regurgitation behavior, herbivores can be divided into 3 groups; primary regurgitators regurgitate actively during feeding and respond immediately to attack, non-regurgitators neither regurgitate during feeding nor during attack, and secondary regurgitators are intermediate in between primary regurgitators and non-regurgitators (Grant 2006). Insect regurgitation behavior is directly associated with gut morphology and motor patterns of foregut. A primary regurgitator possesses a larger crop than a secondary- or non-regurgitator (Grant 2006).

Methodology

Heliothis virescens eggs purchased from Benzon Research (Carlisle, PA) were used to start laboratory colonies at Penn State University (State College, PA). *Manduca sexta* eggs were obtained from the Stephenson Lab, Penn state. Newly hatched larvae were reared with a commercial artificial diet and kept in a growth chamber maintained at 25°C and 16:8-h Light:Dark condition. Early 4th instar *H. virescens* larvae and early 3rd instar *M. sexta* larvae were used in this study.

For studies with a light microscope, the larvae were dissected in a Petri dish filled with 0.1M monobasic phosphate buffer. The fat body and the tracheal system were removed carefully without damaging the alimentary canal. The cleaned up alimentary canal was analyzed and imaged under a light microscope.

For imaging with confocal laser scanning microscope (CLSM), foregut was isolated and fixed in between two coverslips spaced with a small amount of Blue-Tack (Mikó and Deans 2013). A small drop of 0.1M monobasic phosphate buffer was added to the specimen to prevent desiccation. The specimens were imaged with an Olympus FV10i Confocal Laser Scanning Microscope. Volume rendered micrographs and media files were visualized using ImageJ (Schneider et al. 2012).

All the works described in this paper were done as a part of “Know your insect- ENT 597”, a course offered by the Entomology Department at The Pennsylvania State University during the fall semester 2016. During this course, every student was assigned to give a mini-lecture on the morphological structure of an organ/a system of the insect they are working on followed by dissection, imaging, and discussion of the finding in the light of previous literature.

Result and discussion

Alimentary canals of two lab reared herbivore species, *M. sexta* and *H. virescens*, were dissected under a light microscope and imaged using a confocal laser-scanning microscope (CLSM). Under the light microscope, we were able to differentiate three segments of gut (foregut, midgut and hind gut). The foregut to midgut ratio is obviously higher in *Manduca sexta* (Fig. 2) than in *Heliothis virescens* (Fig. 1). The foregut is well muscled in both species. We were not able to find any differences in the junction between the fore and midgut of the two species (Figs 3, 4, 5).

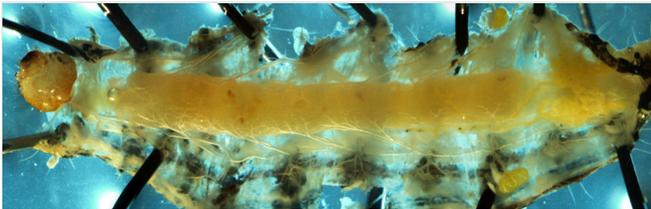


Figure 1.

Alimentary canal of early fourth instar larvae of *Heliothis virescens* imaged by Light microscope



Figure 2.

Alimentary canal of early third instar larvae of *Manduca sexta* imaged by Light microscope

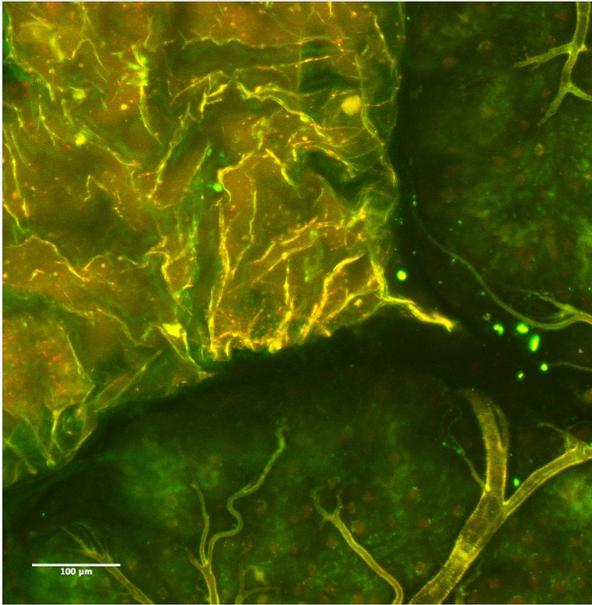


Figure 3.

CLSM volume rendered micrograph showing the junction of foregut and midgut of *Heliothis virescens* larva

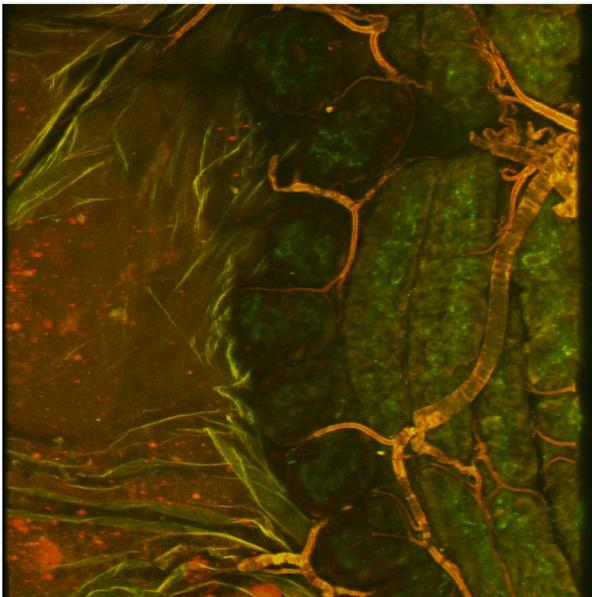


Figure 4.

CLSM volume rendered micrograph showing midgut epithelium of *Manduca sexta* at the junction of foregut and midgut

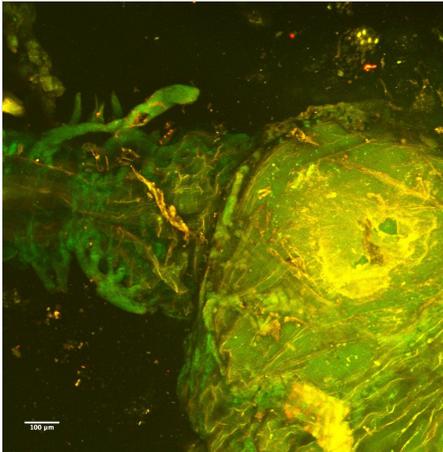


Figure 5.

CLSM volume rendered micrograph showing the junction of foregut and midgut of *Manduca sexta* larva

While feeding on the same host plant, *H. virescens* larvae deposit higher amounts of regurgitant than *M. sexta* larvae (Peiffer and Felton 2009). Although Grant 2006 claimed that insect regurgitant behavior is directly associated with foregut to midgut ratio, we observed higher foregut to midgut ratio in *M. sexta* than that in *H. virescens* (Figs 1, 2). Further investigation is needed to elucidate the mechanism of regurgitation. One of the possible mechanisms is voluntary movement of foregut muscle.

Two types of rhythmic movements occur in the foregut region of *M. sexta* larvae. Peristalsis movements push food materials towards the mid gut while constriction along the esophagus region retains food inside the crop (Miles and Booker 1994). It is likely that synchronization of these two foregut-movements prevents regurgitation.

Relevance to ongoing research

The senior author studies plant defense mechanisms against herbivores, mainly focusing on herbivore specificity of induced volatile production. After feeding damage by two different herbivores of the same feeding guild, *Nicotiana benthamiana* plant produce significantly different amounts of volatiles. Feeding damage by *Heliothis virescens* (generalist herbivore) produces 2–3 times as much volatiles as feeding damage by *Manduca sexta* (specialist herbivore). This variation is due to the presence of different elicitors in the insects' regurgitant. *M. sexta* regurgitant contains volicitin, glutamine fatty acid conjugate and glutamic acid conjugates, whereas *H. virescens* regurgitant contains the former two elicitors only (Alborn et al. 2003, Yoshinaga et al. 2014). While feeding on the same host plant, these two herbivores deposit different amounts of regurgitant on the damage site (Peiffer and Felton 2009). So, it is likely that this difference in regurgitation

behavior is also causing variation in volatile production. However, no study has determined how variation in the amount of regurgitant deposition on a wound site affect induced volatile production by plant.

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