Research Ideas and Outcomes 3: e11827
doi: 10.3897/rio.3.e11827

Case Study

Know your insect: Malpighian tubules in *Trichoplusia ni* (Lepidoptera: Noctuidae)

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Reviewable v1

Received: 16 Jan 2017 | Published: 01 Feb 2017

Citation: Rivera-Vega L, Mikó I (2017) Know your insect: Malpighian tubules in *Trichoplusia ni* (Lepidoptera: Noctuidae). Research Ideas and Outcomes 3: e11827. https://doi.org/10.3897/rio.3.e11827

Abstract

Malpighian tubules are mainly known to be involved in excretion. However, recent studies have begun to look into other potential roles including detoxification, immunity, host establishment, etc. In this case study, we observed the Malpighian tubules of the cabbage looper (*Trichoplusia ni*) using confocal laser scanning microscopy. We also discuss other functions that Malpighian tubules are known for (i.e. silk-like and gall-inducing secretions) as well as the similarities between Malpighian tubules and salivary glands in endoparasitic Hymenoptera.

Keywords

cabbage looper, caterpillars, urinary bladder, uric acid

Case Study Background

The following work is the result of the "Know Your Insect" graduate course in the Department of Entomology at the Pennsylvania State University, taught during the fall semester of 2016. Briefly, the course provides the opportunity for a small group of students to discuss the morphology of the insects of interest. Regardless of which area of

entomology we are studying (e.g. chemical ecology, physiology, agroecology, pollinators, etc.), there is invariably a structure within our model insect that plays an important role in our research. In this course, each student focuses on a structure of interest and leads the class in a lecture, discussion, and live dissection. The students also have the opportunity to image their structure of interest using confocal laser scanning microscopy (CLSM). In this particular case, we focused on the Malpighian tubules of the cabbage looper (*Trichoplusia ni*).

Overview of Malpighian tubules

Malpighian tubules are part of the excretory system. They are involved in osmotic and excretory regulation [Pacheco et al. 2014]. These can be present in different numbers in different insect species ranging from 4 in Drosophila to more than 200 in locusts. During an insect's development, the need for Malpighian tubules might also increase. Insects cope with this need by enlarging the already existing Malpighian tubules through cell growth or by producing new tubules at each instar [Beyenbach et al. 2010].

If you have ever taken an entomology course and someone mentions Malpighian tubules, the first thing that will come to mind is excretion. Or say, you have never taken an entomology course but you see the term and Google it – most hits will be about their function in excretion. Yet some other functions are also being identified.

With the explosion of high throughput techniques, the transcriptome, proteome and even metabolome of Malpighian tubules from several species are being characterized [Esquivel et al. 2014, Silva et al. 2015, Dow and Davies 2006, Zhong et al. 2013]. These characterizations are consistently identifying molecules involved in detoxification as well as immunity. Given the open system in arthropods, it is not surprising that all tissues would have some innate immunity.

Malpighian tubules are also known to secret substances involved in a myriad of adaptations. For example, a common touristic destination in New Zealand are the Waitomo Glowworm Caves. The glowing that attracts so many tourists comes from *Arachnocampa luminosa* commonly known as the New Zealand glowworm. These are fungus gnats, which produce a bioluminescent substance in their Malpighian tubules [Silva et al. 2015]. The reaction and specific compounds involved in this bioluminescence are not fully understood but regardless, it makes for a beautiful spectacle.

Some Hemiptera from the superfamily Cercopoidea are known for producing a froth-like substance that resembles human spittle. This spittle is produced in the Malpighian tubules [Marshall 1966]. The spittle helps the nymphs hide from predators and parasitoids as well as functioning as protection against harsh environments (temperature, humidity, etc.).

In other leafhoppers, Malpighian tubules also produce brochosomes. These are hollow proteinaceous spheres with honey-combed walls. It's been suggested that they are used as water repellent and anti-adhesive protection against the honeydew produced by the

leafhoppers while feeding [Rakitov and Gorb 2012]. Although brochosomes can often be found on non-cycadellid insects, these findings are the results of contamination [Rakitov 2011; Fig. 1].

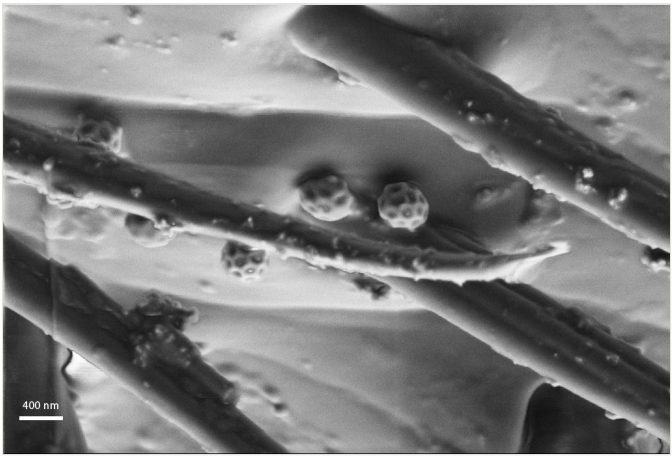

Figure 1.

SEM micrograph showing the brochosomes on the antenna of the putative parasitoids of Cicadellidae (*Trassedia luapi*, Hymenoptera, Ceraphronidae).

It has also been suggested that secretions from Malpighian tubules could be involved in host manipulation. For example, in 1914, Triggerson observed that a secretion from the Malpighian tubules in the larvae of *Dryophanta erinacei* was involved in the production of galls in its host [Triggerson 1914]. He ruled out uric crystals as the compound involved in this induction, thus indicating that Malpighian tubules contained other compounds that were inducing this response.

Methodology

Rearing: *Trichoplusa ni* caterpillars were gathered from a laboratory colony established from eggs obtained from Benzon Research, Inc (Carlisle, PA). Larvae are kept in a growth chamber at 24oC in 16:8 Light:Dark conditions. Larvae are reared pinto bean artificial diet [McEwen and Hervey 1960].

Dissections: The alimentary canal of the caterpillars were dissected out in 0.1M monobasic phosphate buffer and the Malpighian tubules along with the urinary bladders were placed in a droplet of the same buffer between two no. 1.5 coverslips with a small amount of Blue-Tack as a spacer [Mikó and Deans 2013].

Imaging: Specimens were examined with an Olympus FV10i Confocal Laser Scanning Microscope using two excitation wavelengths: 473 nm, and 559 nm. Autofluorescence was detected and assigned a pseudocolor using two channels with emission ranges of 490–590

nm (green), and 570–670 nm (red), respectively. Volume rendered micrographs and media files were created in ImageJ [Schneider et al. 2012] using maximum intensity projection.

Observations on the Malpighian tubules of Trichoplusia ni using confocal laser scanning microscopy

We observed the Malpighian tubules of the cabbage looper (*Trichoplusia ni*) using both a light microscope as well as confocal laser scanning microscope (CLSM). Under the light microscope (Fig. 2), we were able to see the regions referred by O'Donnell et al [O'Donnell and Ruiz-Sanchez 2015] as the white and yellow regions. The authors refer to the anterior region near the junction of the midgut and ileum which contains a large number of Type I cells as the yellow region and a second straight region which contains uric crystals as the white regions. These join to form an ureter which empties into the urinary bladder.

Figure 2.

Brightfield image of Malpighian tubules of *Trichoplusia ni.* Left: yellow region; Right: white region

The images of the Malpighian tubules under CLSM (Figs 3, 4, Suppl. materials 1, 2, 3) show a difference in sclerotization between the Malpighian tubules and the urinary bladder. Normally, under CLSM, soft tissue will autofluoresce under 490-590 nm wavelengths or in our case green and structures with sclerotization autofluoresce between 570-670 nm wavelengths or red in these images. Because of this, we are able to determine that there is certain level of sclerotization in the urinary bladder of the cabbage looper. The red spots observed inside the Malpighian tubules appear to be in the region associated with the ileac plexus which contains mainly Type II cells and where K+ and Na+ is reabsorbed [O'Donnell and Ruiz-Sanchez 2015]. It would be interesting to analyze these further and test whether these red areas within the Malpighian tubules always correlate with Type II cells. These are

probably the nucleus of these cells. Another option could be the uric crystal but based on where these are supposed to be found (white region), that might not be the case.

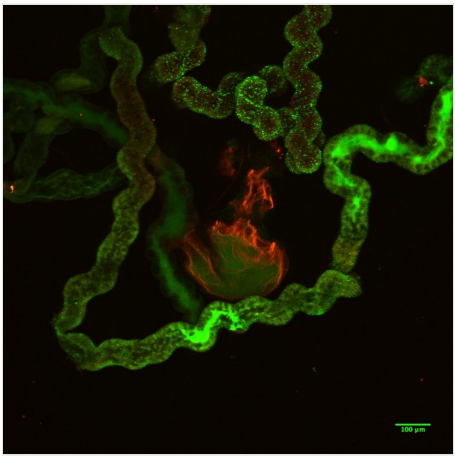

Figure 3.

CLSM volume rendered micrograph showing the Malpighian tubules and the urinary bladder of *Trichoplusia ni*.

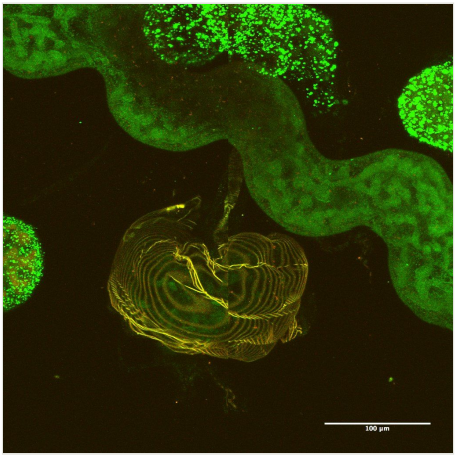

Figure 4.

CLSM volume rendered micrograph showing the Malpighian tubules and the urinary bladder of *Trichoplusia ni*.

The reasons why cuticle tends to bioluminesce at different wavelengths from soft tissue is still not fully understood. However, this case study is an example of how useful it can be to use CLSM in order to determine potential changes in the composition of certain structures and how it can lead to the development of testable hypotheses about the function of different tissues.

Relevance to ongoing research

Ongoing research by LRV focuses on understanding the complex interactions between insects and plants.

This research specifically studies the changes that occur in the saliva of cabbage looper *(Trichoplusia ni)* – a generalist pest. When an insect attempts to establish on a host, there are several challenges they need to overcome. Among these, is being able to deal with the plant defenses. Not only do plants have constitutive defenses such as physical barriers (thorns, trichomes, latex, etc.) or basal levels of secondary compounds, but upon herbivory, plants will also induce defenses such as higher levels of secondary compounds or volatiles that will attract predators and/or parasitoids [Walling 2000]. In order for these defenses to be induced, plants need to be able to recognize they are under attack. This is where insect saliva plays a role. Because it is one of the first secretions to come in contact with the feeding wound, plants could potentially be recognizing specific molecules in the saliva [Felton and Tumlinson 2008]. On the other hand, insects could also be using saliva to deposit molecules that suppress these plant defenses [Acevedo et al. 2015].

Relationship between Malpighian tubules and salivary glands

Same as with Malpighian tubules, next generation sequencing is revealing roles of saliva not previously studied such as detoxification and immunity. Malpighian tubules are also capable of secreting silk-like substances. This ‘malpighian silk’ has been observed in Neuroptera, Coleoptera and Hymenoptera [Sutherland et al. 2010]. Also, Eguileor et al. (2001) has suggested that salivary glands in the aphid parasitoid *Aphidius ervi* might be functioning as an excretory tissue.

Associations between salivary glands and MTs have been in the literature for almost 100 years. In 1938, Flanders [Flanders 1938] published an article on the potential role of Malpighian tubules in the formation of cocoon by endoparasitic chalcidoids. In this article he also reviewed previous observations of similarities between labial glands and Malpighian tubules. For example, he describes observations by Parker (1924) who showed a unique relationship in Spalangia larvae, where the the salivary glands and Malpighian tubules were joined by a protoplasmic connection.

Parasitic Hymenoptera larvae contain ileac glands which are homologous to Malpighian tubules (Fig. 5). Flanders observed that in Microterys, Metaphycus, Comperiella, and Copidosoma larvae, labial glands were attached to the extremity of a small ileac gland. He also observed that these tissues appeared identical and even hypothesized that ileac glands develop as a result of anastomosis between labial glands and proctodeum during embryonic development. To our knowledge, these relationships have not been further studied.

All this information points to several shared functions in both salivary glands and Malpighian tubules. Could it be plants are also able to detect presence of herbivory based

on secretions produced on Malpighian tubules? Or is it also possible that adaptations in Malpighian tubules are allowing generalist insects to establish on a host?

Figure 5.

Alimentary canal of the second instar larva of a cynipini inquiline (*Synergus* sp.) showing the iliac glands (Malpighian tubule analog) and salivary glands.

Acknowledgements

We thank Bipana Paudel, Kirsten Persons, Ryan Reynolds, Anne Jones, Asher Jones and Samita Limbu for their help in dissections and for useful discussions during the course of this work and Missy Hazen (Penn State Microscopy and Cytometry Facility - University Park, PA) for her help with CLSM.

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Supplementary materials

Suppl. material 1: CLSM volume rendered animated GIF showing the Malpighian tubules and the urinary bladder of Trichoplusia ni.

Authors: Loren Rivera Vega, István Mikó
Data type: animated GIF file
Filename: Loren_malphigian_60x-1(2).gif - Download file (28.19 MB)

Suppl. material 2: CLSM volume rendered animated GIF showing the Malpighian tubules and the urinary bladder of Trichoplusia ni.

Authors: Loren Rivera Vega, István Mikó
Data type: animated GIF file
Filename: suppl.mat2.gif - Download file (8.36 MB)

Suppl. material 3: CLSM volume rendered animated GIF showing the Malpighian tubules and the urinary bladder of Trichoplusia ni.

Authors: Loren Rivera Vega, István Mikó
Data type: animated GIF file
Filename: suppl.mat3.gif - Download file (8.36 MB)