Lyonet's gland of the tomato fruitworm, *Helicoverpa zea* (Lepidoptera: Noctuidae)

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ABSTRACT

The Lyonet's gland is a widespread accessory labial gland in Lepidoptera. Albeit its function is ambiguous, the Lyonet's gland arguably plays an important role in silk production. Our knowledge on the Lyonet's gland in heliothine species is limited; it is apparently missing from *H. armigera* and *Heliothis virescence*, whereas reduced in size in *H. zea*. Using confocal microscopy and brightfield imaging, we show that the Lyonet's gland in *Helicoverpa zea* is present and the size is relatively enlarged relative to other lepidopterans.

INTRODUCTION

The Lyonet's gland is an accessory gland located at the proximal region of the silk gland Waku and Sumimoto (1974); Sehnal and Akai (1990). The gland is also referred to as "Fillipi's gland" (Waku and Sumimoto, 1974) and was first described in 1760 Machida (1965); Waku and Sumimoto (1974); Akai (1984). A gland with a similar location is also recorded from larvae of the other amphiesmenopteran order, Trichoptera (Cianficconi et al., 1992; Allegret and Denis, 1963; Glasgow, 1936). The homology of these trichopteran glands with the lepidopteran Lyonet's gland, however, is questionable, as these glands are absent from non-dytrisian lepidopteran lineages (Vegliante, 2005; Victoriano and Gregório, 2004).

Several functions are being suggested for the secretion of Lyonet's gland. Numerous authors Day and Waterhouse (1953); Helm (1876); Wigglesworth (1972) suggested that the gland produces a cementing substance, while others hypothesized that the secretion serves as a lubricant and helps in the extrusion of silk Day and Waterhouse (1953); Glasgow (1936).

Although an ablation study shows that removal of Lyonet's gland does not impact silk quality in *Bombyx mori* (Machida, 1965), based on more recent studies on the gland ultrastructure (Waku and Sumimoto, 1974) and transcriptome (Wang et al., 2016) suggests that the Lyonet's gland has an important role in transporting small molecules to the labial gland duct. Importance of the Lyonet's gland in silk production is also supported by the fact that these glands are missing from some taxa that don't produce silk, *e.g.*, *Manduca sexta* (Leslie and Robertson, 1973).

Many heliothine moths (Lepidoptera: Noctuidae) are major insect pests in several crops worldwide, and disruption of their silk production could have potential as a management strategy. While there are several studies on main silk glands Akai et al. (2003)Sorensen et al. (2006)Corbet (1971)Li et al. (2010) de la Paz Celorio-Mancera et al. (2011) , limited information is about their Lyonet's gland. Sorensen et al. (2006) and Chi et al. (1975) did not find the Lyonet's gland in *Helicoverpa armigera*, *H. zea* and *Heliothis virescence*. This finding is surprising, as these taxa all produce silk. Only a single, superficial illustration of a putative Lyonet's gland from MacGown and Sikorowski (1982) suggests its presence in heliothines where it is described as a small, bi-lobed dilution of the proximal region of the silk gland.

The aim of the present study was to investigate whether the Lyonet's gland is present in *H. zea* using dissection, bright field and confocal laser scanning microscopy.

The current work is the result of the "Know Your Insect" 2017 fall graduate course of the Entomology Department at the Pennsylvania State University (Miko, I, 2017).

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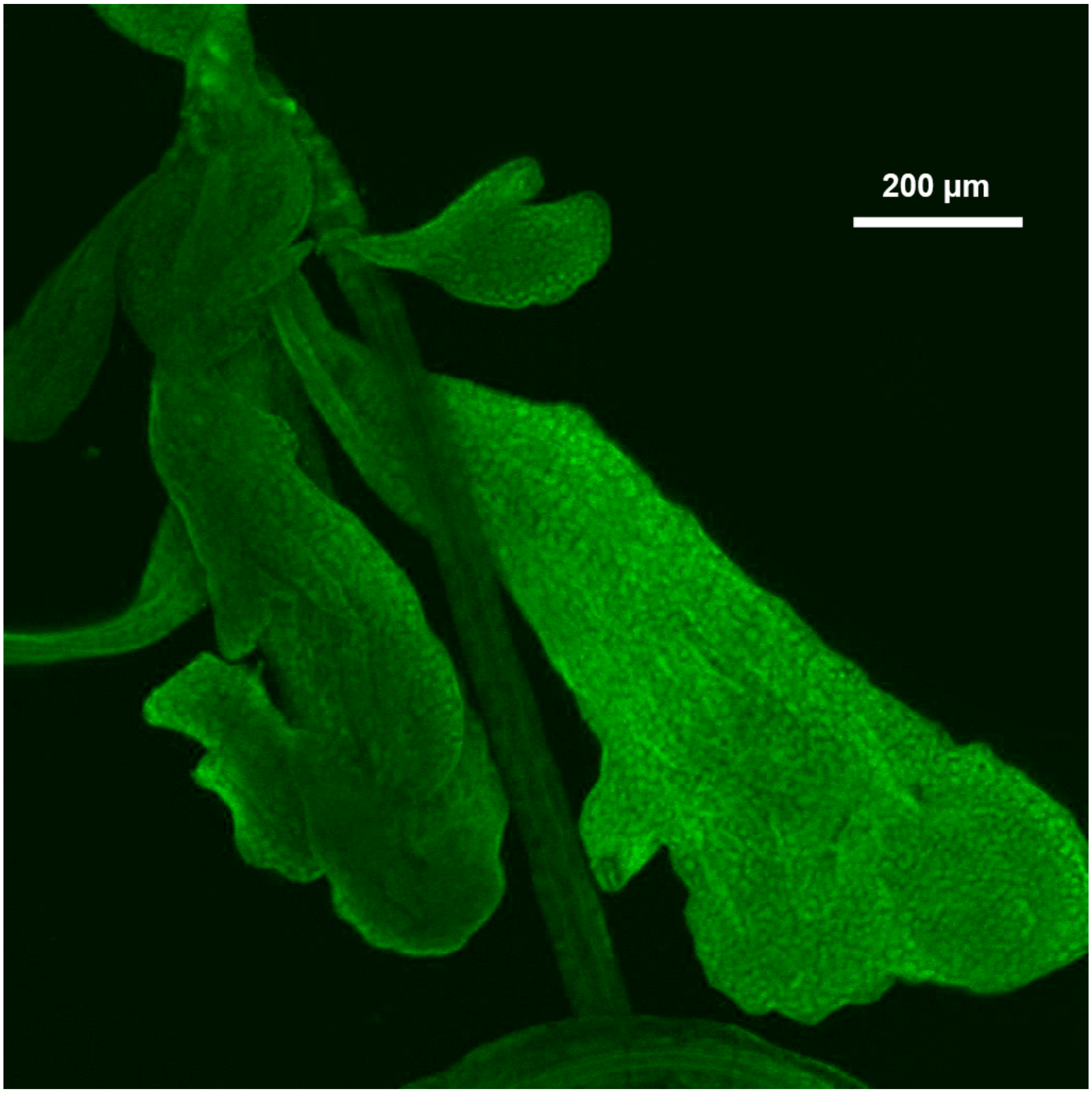

Figure 1. CLSM volume rendered image showing the Lyonet's gland of *Helicoverpa zea*.

MATERIALS AND METHODS

5th instar larvae of *Helicoverpa zea* were dissected in 0.1 M phosphate buffer (pH 7.4). Lyonet's gland were fixed in 2.5% glutar-aldehyde in 0.1 M phosphate buffer, and 5% sucrose for 24 hours on room temperature, washed in phosphate buffer, transfered and imaged in glycerol on concavity microscope slides. The glands were imaged with an Olympus BX41 compound microscope equipped with a Cannon EOS 70D SLR digital camera and with an Olympus FV10i Confocal Laser Microscope using two excitation wavelengths: 473 nm, and 559 nm. Autofluorescence was detected using three channels with emission ranges of 490–590 nm (green), and 570–670 nm (red), respectively. Volume rendered micrographs and media files were generated with ImageJ (Schneider et al., 2012) using maximum intensity projection.

RESULTS AND DISCUSSION

The Lyonet's glands in *Helicoverpa zea* larvae are branched from the proximal region of the silk glands (Figs 1, 2. Their lumen is substantially smaller than the silk gland lumen (Lgl, sgl: Fig. 3). The Lyonet's gland of *Helicoverpa zea* is composed of multiple elongate lobes of 30–500 micrometers each, whose surfaces are scattered with less fluorescing wavy areas. We were not able to differentiate cell borders on the lobes even on higher magnifications (Fig. 4). The structure of the gland is different from

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Figure 2. Bright field micrograph showing the Lyonet's gland of *Helicoverpa zea*.

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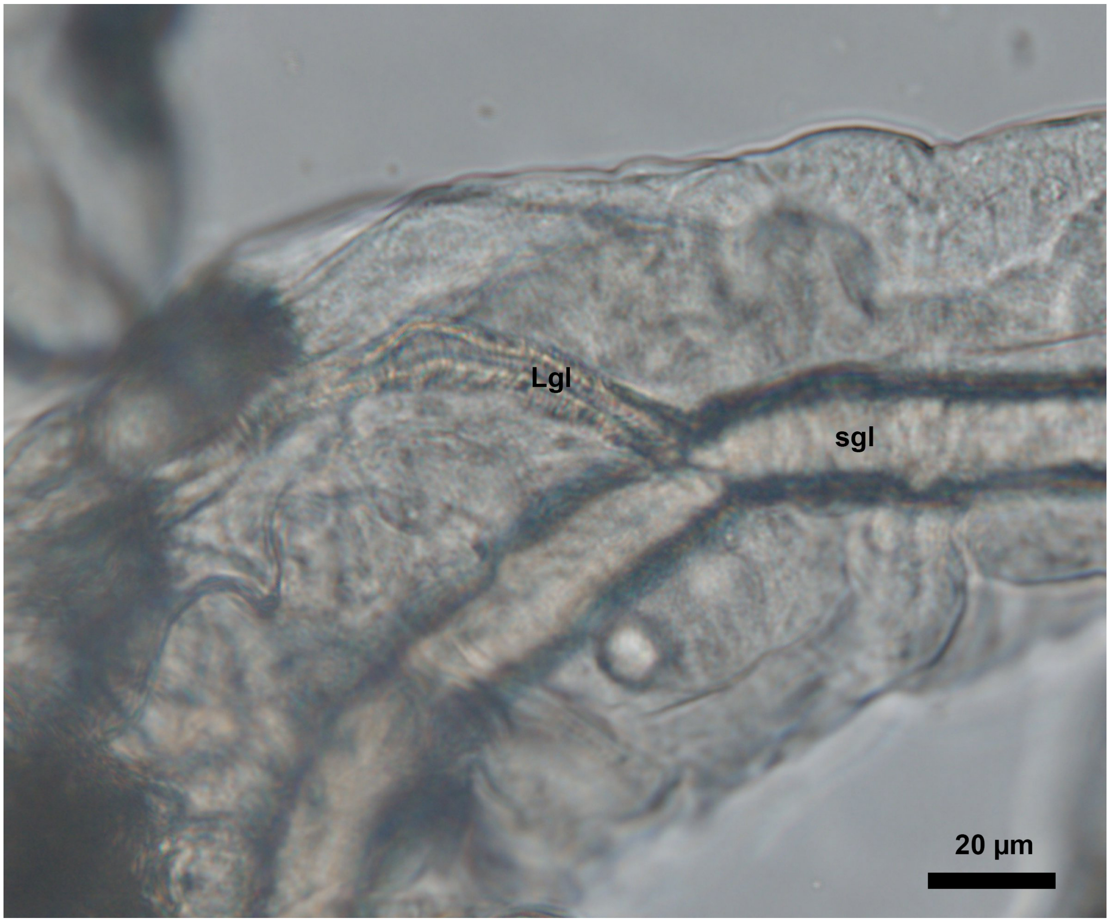

Figure 3. Bright field micrograph showing the branching point of the Lyonet's gland and main silk gland of *Helicoverpa zhea*.

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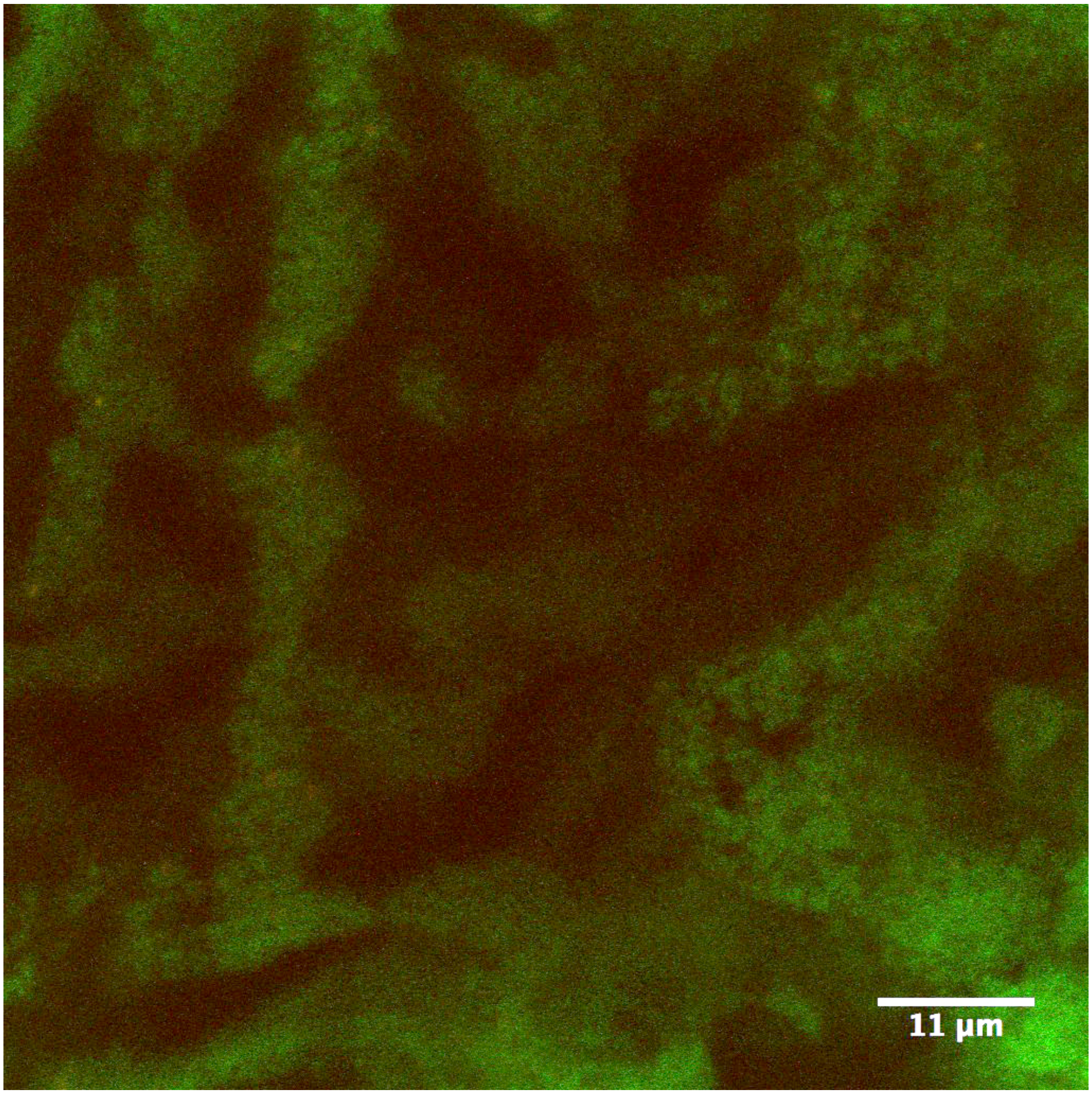

Figure 4. CLSM volume rendered image showing granules on the surface of Lynoett's gland of *Helicoverpa zea*.

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what was illustrated by Macgown (1981), as it is much larger relative to the size of the main salivary gland and is composed of multiple, leaf-like lobes.

Helm (Helm, 1876) classified the Lyonet's glands into three types based on their gross morphology. The first and second types have proximal canal-like components while in the third type, the leaf-like glandular lobes arises from the main silk gland without any gland canal. The Lyonet's gland of *Helicoverpa armigera*, similarly to that of most other noctuids Vegliante (2005) belongs to the third type, as the lobes of the gland arises directly from the silk gland (Figs 1, 2.

The glandular lobes in lepidopteran Lyonet's glands are composed of single cells (Waku and Sumimoto, 1974; Helm, 1876; Patra et al., 2012). These cells are much larger than normal epidermal cells and, based on their polyploid nuclei, might be the result of the fusion of multiple cells during their development (Waku and Sumimoto, 1974). The largest cells (lobes) of the Lyonet's gland are 140 micrometer in *Antheraea mylitta* (Patra et al., 2012) and around 600 micrometer in *Bombyx mori* (Waku and Sumimoto, 1974). The cell (lobe) size in *Helicoverpa armigera* varies between 200 and 1200 micrometer. Unlike in other studied lepidopterans, the glandular cells are lobate in *H. armigera*.

The Lyonet's gland wasn't reported in numerous heliothine species, including *Helicoverpa zea* Sorensen et al. (2006); Chi et al. (1975). Delicacy of the Lyonet's glands might be the explanation for these reports and it is also possible that specimen used by MacGown et al. (1982) was partially destroyed and the illustration was mostly based on the proximal portion of the gland.

We also experienced difficulties to keep the Lyonet's gland attached to the main salivary gland during our dissections, but were always able to easily regain pieces of the gland.

Based on the size and accessibility of the Lyonet's gland we think that it is possible to perform transcriptomic and secretomic studies in *Helicoverpa zea*. The relative size of this gland in H. zea, which is 2–3 times larger than the same gland in *Bombyx mori* suggests that this gland plays an important role in the biology of *H. zea*. Examination of the gross morphology of other heliothines would be also reasonable, as authors who reported the absence of the Lyonet's gland from *H. zea* also reported that this gland is absent from *H. armigera* and *Heliothis virescence* (Sorensen et al., 2006; Chi et al., 1975).

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