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# Brief Note

# Ultrastructure of the Lyonet's glands in larvae of *Diatraea saccharalis* Fabricius (Lepidoptera: Pyralidae)

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**ABSTRACT:** The Lyonet's gland is found in Lepidoptera larvae, close to the excretory duct of the silk gland. The role played by this gland is still uncertain. This work aims to describe the ultrastructure of the Lyonet's gland in *Diatraea saccharalis* larvae, offering suggestions regarding its possible function. The insects were reared under laboratory-controlled conditions. The glands were conventionally prepared for transmission (TEM) and scanning (SEM) electron microscopy. SEM showed that Lyonet's glands are paired small structures located in the ventral side of the head. They are composed by clustered long cells resembling leaves. Under TEM observations, each cell is surrounded by a thin basal lamina and contains large stellate nucleus. The cytoplasm presents large and empty canaliculi with small microvilli. The basal plasma membrane forms numerous infoldings where numerous and well-developed mitochondria are concentrated. The cytoplasmic membrane system is poorly developed. Our ultrastructural results suggest that the Lyonet's gland in *D. saccharalis* larvae may be involved in the uptake of small molecules from the hemolymph; no morphological evidences of macromolecules synthesis and secretion were noticed. The detection of nerve fibers in the gland suggest a neural control for the glandular cell function.

## Introduction

The Lyonet's gland is usually found in Lepidoptera larvae close to the excretory duct of the silk gland (Waku and Sumimoto, 1974). This gland was described in the larvae of the Lepidoptera in 1760 by Lyonet (Machida, 1965); in the *Bombyx mori* it is often cited as Filippi's glands (Waku and Sumimoto, 1974; Akai, 1984). The Lyonet's gland has been considered an accessory gland of the silk gland as it communicates with the latter one (Waku and Sumimoto, 1974; Sehnal and Akai, 1990).

The role played by this gland is still uncertain. It has been postulated to be involved in the exchange of small molecules such as water and ions (Waku and Sumimoto, 1974), as well as in the secretory process of cementing substance for the silk elements (Helm, 1876, Day and Waterhouse, 1953; Wigglesworth, 1972); another hypothesis is that it secretes some lubricating substance which facilitates the extrusion of the silk from the spinning duct (Glasgow, 1936; Day and Waterhouse, 1953).

The sugarcane borer, *Diatraea saccharalis* Fabricius, is serious pest of several crops, mainly the sugarcane (Long and Hensley, 1972). This work aims to describe the ultrastructure of the Lyonet's glands in larvae

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of *D. saccharalis*, in an attempt to offer suggestions regarding its possible function.

## Material and Methods

The *D. saccharalis* larvae were maintained in the laboratory with artificial diet (Hensley and Hammond, 1968), under controlled temperature (25-27°C) and humidity (70%).

The Lyonet's glands (together with silk glands) obtained from the last instar larvae were fixed for 24 h in 2% glutaraldehyde - 4% paraformaldehyde solution buffered in 0.1M phosphate buffer (pH 7.3), post-fixed in 1% osmium tetroxide in the same buffer for 1h. The material prepared for transmission electron microscopy (TEM) was dehydrated through a graded series of acetone and embedded in Araldite. For scanning electron microscopic (SEM) observations, the specimens were dehydrated through a graded series of alcohol, critical point dried with liquid $CO_2$ and gold coated in a sputtering device.

## Results

The Lyonet's glands in *Diatraea saccharalis* larvae, as revealed by SEM analyses, are paired small structures located bilaterally in the ventral side of the head; each of them is composed by clustered long cells resembling leaves, around the posterior region of each silk gland duct (Fig. 1).

The ultrastructural observations of the Lyonet's glands in *D. saccharalis* larvae under TEM, show that each cell is surrounded by a thin basal lamina; tracheal branches penetrate deeply into the cell cytoplasm (Figs. 2-3). The basal plasma membrane forms numerous infoldings which intrude deeply into the cytoplasm; numerous, electron-dense and well-developed mitochondria are concentrated between these infoldings (Figs. 2-3). The large nucleus is stellate and extensively branched containing large nucleolus and loose chromatin (Figs. 2 and 4).

The gland cell cytoplasm presents large and empty canaliculi with small and scarce microvilli; although mitochondria may occur around the canaliculi, they are not concentrated at this cell region (Figs. 2-4). There are few rough endoplasmic cisterns, small and scarce Golgi apparatus, and the free ribosomes and microtubules are numerous; no secretory granules are detected (Figs. 2-5).

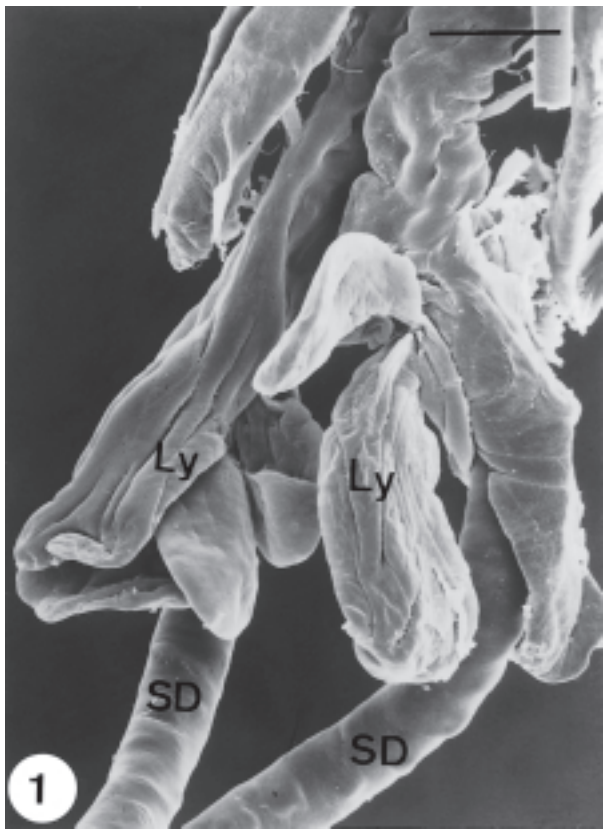
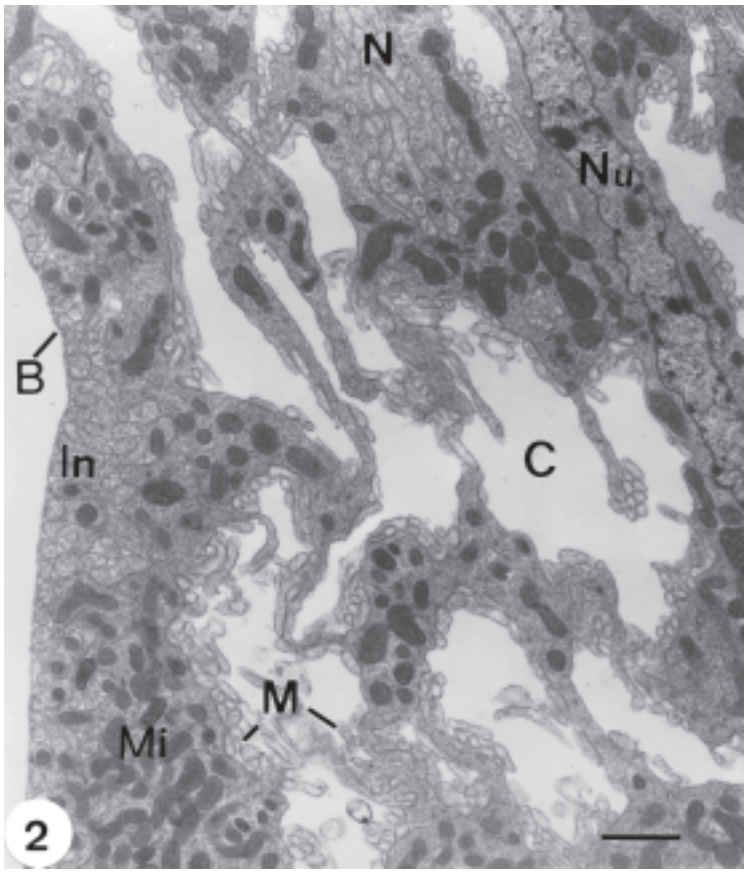



**FIGURE 1.** SEM of Lyonet's glands surface. The Lyonet's gland cells (Ly) resembling leaves are attached to the silk gland ducts (SD). Bar = 0.1mm.

**Figs. 2-5.** TEM of the Lyonet's gland cell.
**FIGURE 2.** General aspect of the gland cell with nerve fiber. Bar = 1 μm.
**FIGURE 3.** Detail of the basal cytoplasm. Bar = 0.5 μm.
**FIGURE 4.** Detail of gland cell and nerve fiber with many axons. Bar = 1 μm.
**FIGURE 5.** Detail of the nerve axon (A) with dense-cored neurosecretory vesicle (arrow). Bar = 0.25 μm.
Wide and empty canaliculi (C) with microvilli (M); mitochondria (Mi); nerve fiber (N); basal lamina (B); basal plasma membrane infoldings (In); nucleus (Nu); tracheole (T).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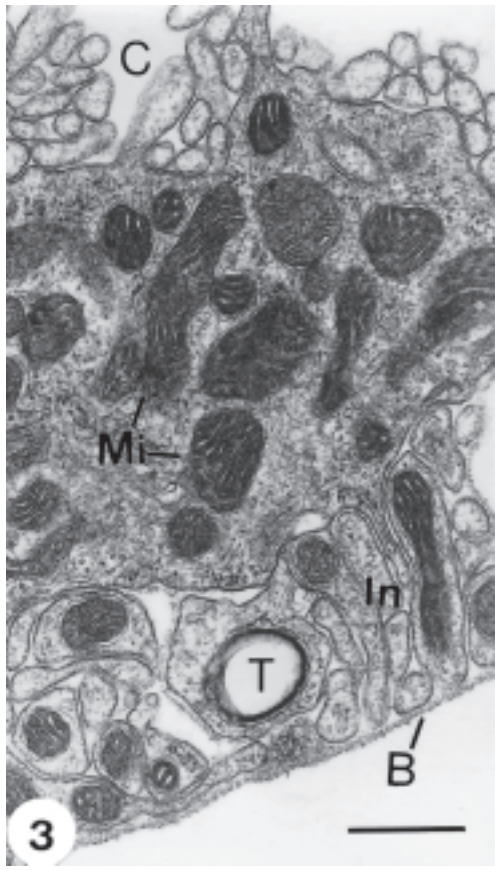
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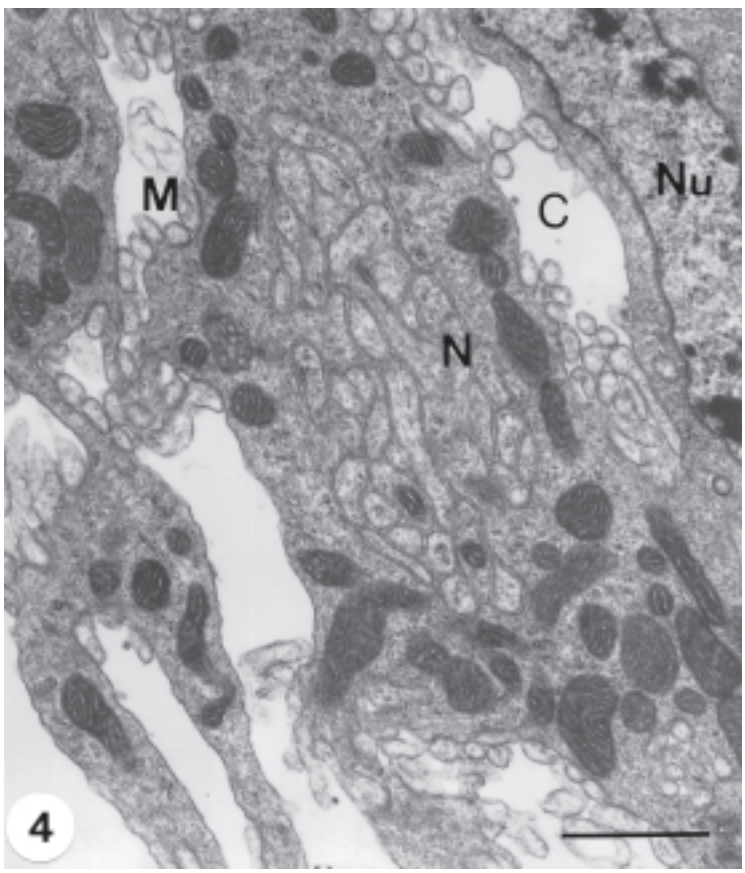
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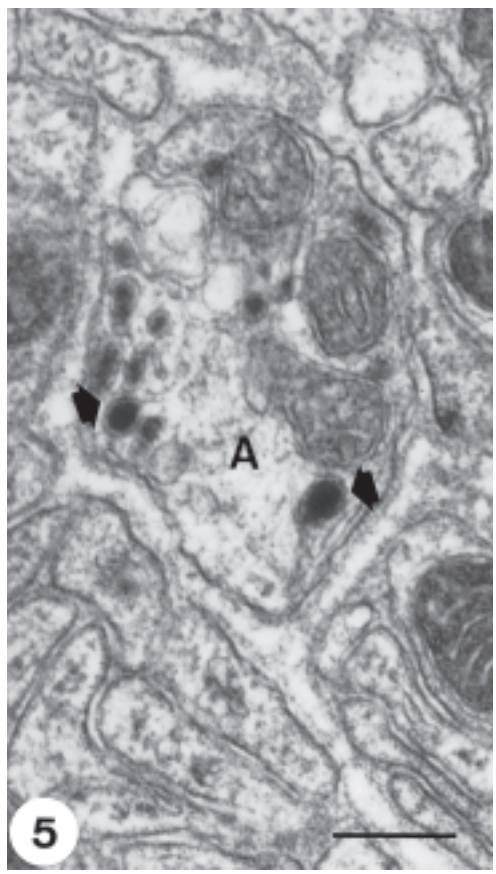
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Nerve fibers are observed between the glandular cells, being composed by groups of axons (Figs. 2 and 4); dense-cored vesicles resembling neurosecretory granules are visualized inside the fibers (Fig. 5).

## Discussion

The SEM analysis show that the Lyonet's glands in *Diatraea saccharalis* larvae are composed by clustered long cells resembling leaves, which forms paired structures located around the posterior region of each silk gland duct. There is no description in the literature of such gland under SEM, which make difficult the discussion of our observations comparing with other Lepidoptera species. However, the diagramatic representation of such gland in *Bombyx mori* (Waku and Sumimoto, 1974) and its description in *Ostrinia nubilalis* (Drecktrah *et al.,* 1966), *Spodoptera littoralis* (Sorour *et al.,* 1990) and *Spodoptera frugiperda* (Chi *et al.,* 1975), resemble the anatomical aspect of the one in *D. saccharalis,* leading us to assume that the Lyonet's gland are quite similar among the Lepidoptera.

The general ultrastructural aspects of Lyonet's gland cells in *D. saccharalis* larvae, under TEM observations are quite similar to those described for *B. mori* (Waku and Sumimoto, 1974). There are few rough endoplasmic cisterns, small and scarce Golgi apparatus, and no secretory granules are detected. The poorly developed membranous system together with the absence of secretory granules, led us to postulate that the gland cells are not involved in the macromolecules synthesis and secretion as it is well known that secretory cells may have endoplasmic reticulum and Golgi complexes (Alberts *et al.,* 1997). No morphological signs of secretory activity were detected in this gland of other Lepidoptera species (Drecktrah *et al.,* 1966; Waku and Sumimoto, 1974; Sorour *et al.,* 1990).

The cytoplasm of glandular cell presents large and empty canaliculi with small and scarce microvilli. Although mitochondria may occur around the canaliculi, they are not concentrated at this cell region. The detection of intracellular canaliculi is a remarkable morphological feature for this gland cell, being described for other studied Lepidoptera (Drecktrah *et al.,* 1966; Waku and Sumimoto, 1974; Sorour *et al.,* 1990). Waku and Sumimoto (1974), using histochemical techniques, were not able to detect any type of intracanalicular macromolecular substance in *B. mori.*

The detection of basal membrane infoldings, together with the presence of microvilli in the canaliculi, suggest that the gland cell is involved in the transfer of small molecules between the hemolymph and the cellular canaliculi. Insect cells which transfer small molecules -including water and ions- between a particular organ and hemolymph, universally share a similar architecture. However, each of them has its own characteristic ultrastructure (King and Akai, 1984). The remarkably convoluted basal plasma membranes with more or less distended spaces between them ensure a high efficiency of exchange of small molecules between the cell and the hemolymph (Oschman and Berridge, 1970). Our finding of numerous well-developed mitochondria, present particularly at the basal cytoplasm, may supply enough energy to transport the exchanged substances, as described for many other tissues. Our ultrastructural results suggest that the Lyonet's gland in *D. saccharalis* larvae may be involved in the uptake of small molecules from the hemolymph to the Lyonet's gland duct, and subsequently to the silk gland duct, which is dependent of the energy supply, supporting the hypothesis of Waku and Sumimoto (1974) about the function of such gland.

The detection of nerve fibers between the glandular cells, and dense-cored vesicles resembling neurosecretory granules inside the fibers call our attention. This is the first time that evidence of innervation of the Lyonet' s gland is presented, although other types of insect glands are known to be neuro-controlled, and the nerve fibers were mainly detected in the duct epithelial wall (Robertson, 1974; Schachtner and Bräunig, 1995; Del Bene *et al*., 1999; Victoriano and Gregório, 2002). The presence of the vesicles similar to those described for neurosecretion vesicles was also described previously for other insect glands, as in the salivary glands of both *Periplaneta americana* (Whitehead, 1970) and *Manduca sexta* (Robertson, 1974), and in the silk gland *of D. saccharalis* (Victoriano and Gregório, 2002), being suggested a functional interaction by chemical mediators between the nerve endings and the excretory duct cells. The results presented here suggest that there is also a nervous control of the functional activity of the Lyonet's gland cells in *D. saccharalis* larvae.

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