LIGHT MICROSCOPIC OBSERVATIONS OF THE MESENTERO-PROCTODEAL REGIONS IN ADULT *CATOPSILLA POMONA* (FABRICIUS, 1758) (LEPIDOPTERA: PIERIDAE)

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Abstract

The aim of this study was to examine the basic histological structure of the mesentero-proctodeal regions (midgut and hindgut) of *Catopsilia pomona* (Fabricius, 1758) which were living in their natural habitat in the northern part of Thailand. All specimens were processed by the standard histological technique. Each section was cut at 6 micrometers and stained with Harris's haematoxylin and eosin. For the longitudinal sections, the results revealed that the structural wall in both the midgut and hindgut could be divided obviously into 4 layers: the mucosa, submucosa, muscularis, and serosa, respectively. The midgut was mainly shown to be 2 types of epithelial cells, based on histological localization: (i) a simple columnar epithelium and (ii) a simple cuboidal epithelium. The hindgut was classified distinctly into 2 subparts: the ilium and the rectum. The ilium was a simple squamous epithelium, whereas the rectum was a simple squamous epithelium with 5-6 rectal papillae. The results of this study showed the details of the histology of the mesentero-proctodeal regions and are considered the first such report in Thailand.

Keywords: *Catopsilia pomona*, mesentero-proctodeal regions, histology, Thailand

Introduction

The lemon emigrant *Catopsilia pomona* (Fabricius, 1758) is a common species of butterfly in Thailand, belonging to the family Pieridae (order Lepidoptera). Recently, *C. pomona has become a serious pest of several economic plants, e.g., Cassia siamea, C. fistula, C. baderiana, C. alata, C. tora, Bauhimia spp., Butea monosperma, and Sesbania grandiflora* (FAO, 2007). It is also a foliage feeder which prefers the blossoms of, and forms nurseries on, the host

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plants (Fabricius, 1775; Ek-Amnuay, 2006; Raju *et al*., 2012; Roy *et al*., 2014). From reviews of the literature, biological studies of *C. pomona* in Thailand and other countries have been relatively underreported.

Several researches of the digestive tract have been reported in both the biology and ecology of adult insects (Burgess, 1880; Bordas, 1920; Pyle, 1940; Mortimer, 1965; Chauthani and Callahan, 1967; Le Grice, 1968; Judy and Gilbert, 1970; Beals and Berberet, 1976; Gongalves, 1981). Based upon histological studies, some lepidopterans have been examined such as Diacrisia obliqua (Kabir and Ameen, 1986), *Papilio demoleus* (Goyle, 1990), and *Papilio polytes* (Gaikwad *et al*., 2011). Kabir and Ameen (1986) and Goyle (1990) described the mesentero-proctodeal regions as having been well documented with the structure particularly, due to its containing special cell types, as the absorptive function. Therefore, an investigation about the normal histology of this organ will provide basic knowledge to be applied to the nutritional physiology, as well as to the agricultural pest management. However, an investigation of the mesentero-proctodeal regions (midgut and hindgut) in *C. pomona* (Fabricius, 1758) has not been reported. In this research, we examined the *C. Pomona* mesentero-proctodeal regions of subjects inhabiting their natural habitat in the northern part of the Thailand.

Materials and Methods

Twenty-five pooled samples of adult *C. pomona* (total length about 5-7 cm) were gathered from Lampang, Phitsanulok, and Chiang Mai provinces in the northern part of Thailand during October-December 2013. The whole abdomen of each butterfly was dissected and then immediately fixed in Davidson's fixative for 36-48 hours. Each specimen was processed using standard histological techniques (Bancroft and Gamble, 2002). As to the details, the specimens were dehydrated in serially graded ethanol, cleared in xylene, and infiltrated in paraffin (m.p., 55-60°C). The paraffin block was cut at 5-6 μm with a rotary microtome and stained with Harris's haematoxylin and eosin. Afterwards, observations of the histological structure were done under a light microscope.

Results and Discussion

The digestive tract of *C. Pomona* was divided into 3 parts by considering the structure of the tissues, including the foregut, midgut, and hindgut. This study reports only on the midgut and hindgut of the *C. Pomona* in longitudinal sections, wherein the 2 walls of their regions could be divided distinctly into 4 layers: the mucosa, submucosa, muscularis, and serosa, respectively.

Histological observations of the *Catopsilia pomona* midgut

The midgut was a long and elongate tube (Figure 1) which began from the esophageal or stomodeal valve and extended to the pyloric valve. Based on the histological sections, the cardiac valve protruded into the hindgut. It consisted of 2 cell layers; the inner and outer (Figure 1A). In the outer cell layer, the epithelial cells were elongated with high simple columns and with a predominant oval nucleus and are basophilic. The cytoplasm was slightly stained as acidophilic, whereas the inner cell layer was a simple cuboidal epithelium which presented a large round or spherical nucleus along the length of the midgut. Only epithelium cells lined the basement membrane which was surrounded rarely by connective tissue (Figure 1A-1B). Then, these epitheliums gradually increase into simple columnar epitheliums. The functional structure of the cardiac valve prevents the return of food (Lacombe, 1971).

In detail, the histological structure of the midgut showed as follows (Figure 1C-1D). First, in the mucosa layer, the mucosa was raised to the lumen in longitudinal folds (100-120 μm). Moreover, it was constituted of 2 types of epithelium cells based on the histological characterization and localization. The upper longitudinal fold was lined with a simple columnar epithelium (about 15-20 μm). It presented the microvilli and was supported by a prominent basement membrane. The nuclei

of the epithelium cells were oval with a prominent nucleolus that was strongly basophilic stained. In the lower longitudinal fold, a single-layered cuboidal epithelium was observed. Its rounded or oval nucleus with a scattering of chromatin was observed. Secondly, in the submucosa layer, the layer was comprised of a slightly thin layer of loose connective tissue. Thirdly, in the muscularis layer, the layer was constituted rarely of a thin layer of smooth muscle. Fourthly, in the serosal layer, the histological structures were simple squamous epitheliums.

In addition, Malpighian tubules were observed between the hindgut and midgut (Figure 2A-B). Each Malpighian tubule showed a single layer of cuboidal epithelium. The nucleus of the tubule was oval with a scattering of chromatin. Its cytoplasm also showed the acidophilic staining. Externally, it was surrounded by loose connective tissue. This characterization was similar to research on various insects including Lepidoptera (Standlea and Yonke, 1968; Mathur, 1972), Coleoptera (Areekul, 1957), and Hymenoptera (Caetano

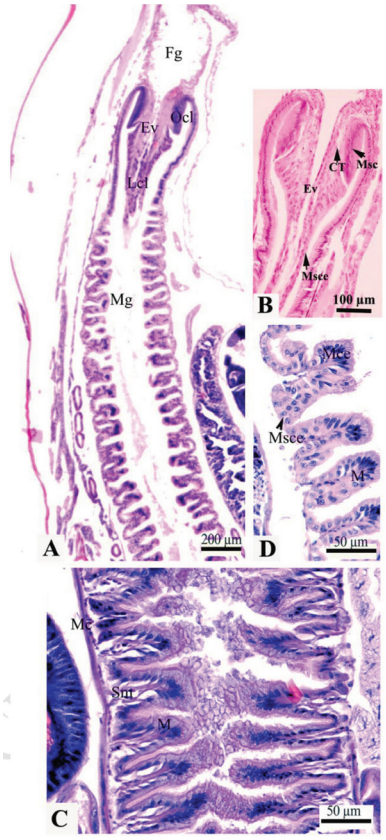

Figure 1. Micrograph of the midgut of *C. pomona* (A-D). CT = Connective tissue, Ev = Esophageal valve, Fg = Foregut, Ocl = Outer cell layer, Icl = Inner cell layer, Mg = Midgut, M = Mucosa, Sm = Submucosa, Mc = Muscularis, Msc = Simple columnar epithelium, Msce = Simple cuboidal epithelium

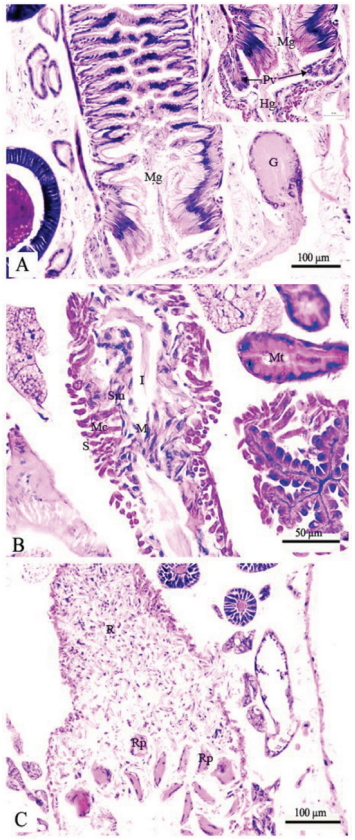

Figure 2. Micrograph of the hindgut of *C. pomona*; (A-C). G = Ganglia, Hg = Hindgut, I = Ileum, Pv = Pyloric valve, M = Mucosa, Mc = Muscularis, Mg = Midgut, Mt = Malphigian tubule, R = Rectum, Rp = Rectal papillae, S = Serosa.

and Overal, 1984; Arab and Caetano, 2002).

Histological observations of the *Catopsilia pomona* hindgut

The hindgut had different histological structures from the midgut. In the last part of the midgut, a longitudinal fold was not present but it was jointed and changed into a tall simple columnar epithelium. The histological structure between the midgut and hindgut also had a pyloric valve (Figure 2A). The function of this structure was to move food into the hindgut (Gillott, 1995).

Generally, this organ was mainly divided into 2 subparts (Figure 2B-C), the ileum and the rectum. As to detail, the ileum had a smaller lumen. Its overall histological structure was observed, and the mucosa was slightly extended into the lumen and a little branch. The epithelial cells were flat and poorly developed as a simple squamous epithelium covered with a cuticle. This finding differs from some Lepidoptera, *Papillio polytes polytes* (Gaikwad *et al*., 2011) and *P. demoleus* which are composed of single cuboidal epithelial cells (Goyle, 1990). The submucosa layer rarely consisted of a thin layer of connective tissue. The muscularis layer was externally surrounded by smooth muscle in both the outer circular and inner longitudinal muscle layers. The outer layer was the serosa showing thin connective tissue. The function of this region concerns food digestion and nutrient absorption (Irvine et al., 1988; Schumaker *et al*., 1993; Serrão and Cruz-Landim, 1996; Serrão *et al*., 2004). For the rectum, the mucosa layer extended more into the lumen with 5-6 rectal papillae. This result differs from *Micropteryx* spp. and *Hepialus* spp. with 3-4 rectal papillae (Mortimer, 1965) and the 30-35 rectal papillae in *Hyalophora cecropia* (Judy and Gilbert, 1970). Each rectal papilla was a single layer of simple squamous epithelium and it also presented an oval nucleus. The epithelium was covered with cuticle. Other layers were similar with the prior region. The function of the rectum is osmotic control (Serrão and Cruz-Landim, 1996; Serrão *et al*., 2004). In addition, Cruz-Landim (1985) reported that it also demonstrated water and nutrient absorption.

Conclusions

From this research, we can conclude that the structural wall in the mesentero-proctodeal regions could be distinctly separated into 4 layers; the mucosa, submucosa, muscularis, and serosa, respectively. The midgut was lined by 2 types of epithelial cell (simple columnar and simple cuboidal epitheliums), whereas the hindgut was lined by a simple squamous epithelium in both the ileum and the rectum. Other parts of the hindgut were similar to that described in the midgut. The information here offers a basic knowledge for further development at the immunocytochemistry and ultrastructural level.

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