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【Research report】

利用掃描電子顯微鏡研究東方果蠅 (Dacus dorsalis Hendel) 性費洛蒙腺體之發育【研究報告】

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Abstract

摘要

利用掃描電子顯微鏡方法研究東方果實蠅 (Dacus dorsalis Hendel)，從蛹期第六天至成蟲期第30天性費洛蒙腺體 (Sex pheromone producing gland) 發育的經過。用斷裂方法藉掃描電子顯微鏡觀察研究該腺體內部構造之變化和發育。 性費洛蒙腺體的分泌囊 (Secretory sac) 於蛹期第六天時才開始形成。囊體的大小隨蟲齡增長而長大。囊內真皮細胞 (Epithelial cells) 的形狀由骰子形逐漸發育為柱狀形，依蟲齡，其真皮細胞之成長至衰老退化可分為五期來描述和討論。

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STUDIES ON THE DEVELOPMENT OF SEX PHEROMONE PRODUCING GLAND OF THE MALE ORIENTAL FRUIT FLY, *DACUS DORSALIS* HENDEL WITH THE SCANNING ELECTRON MICROSCOPY

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ABSTRACT

The development of the sex pheromone producing gland in the male of oriental fruit fly, *Dacus dorsalis* was studied by scanning electron microscopy. The fracturing technique was used for the internal morphological studies of the gland. The secretory sac of the pheromone gland in the fly has been found firstly on the sixth day old male pupa. The size of the secretory sac increases and epithelial cells of the sac develop from the cuboidal to the columnar shape while the flies grow up. The morphology of the epithelial cells can be divided into five stages from the growth to the degeneration of the cells, is described.

INTRODUCTION

The production and storage of the sex pheromone in the gland associated with the rectum was firstly described for the male *Dacus tryoni* (Froggatt) by Fletcher (1968), and the male *D. oleae* Gmel. by Economopoulos *et al.* (1971). These glands have been hypothesized as the sex pheromone glands for the males of *D. dorsalis*, *D. oleae* and *D. cucurbitae* by Schultz and Boush (1971). The anatomy and histology of the gland have been studied for *D. tryoni* by Fletcher (1969) and *D. oleae* by Economopoulos *et al.* (1971) with the light microscopy. The ultrastructure of the gland had been observed for *D. oleae* by De Marzo *et al.* (1978) and *D. dorsalis* by Lee *et al.* (unpublished data) with the transmission electron microscopy. Published studies on the changes of the sex pheromone gland in the flies are rather rare (Fletcher, 1969). In view of this, an investigation of the development of the sex pheromone gland in the male *D. dorsalis* with scanning electron microscopy was taken.

MATERIALS AND METHODS

The oriental fruit flies, *Dacus dorsalis*, have been reared in the insectarium of Institute of Zoology, Academia Sinica for over 30 generations. However the wild flies collected from the infested fruits in the field were introduced into the laboratory colony every year, in order to add new genes into the colony. The flies were fed with the mixture of sugar and protein hydrolyzate (5:1), and water. The larvae were fed with the artificial diet which the formula was instructed by Chiu (1977). The whole colony was kept at normal laboratory and light/dark conditions.

A tiny structure of the secretory sac could be found since the 6th day old pupa. The insects, therefore, were examined from the 6th day to the 30th day male adult flies. The recta of these insects were cut off from their digestive tracts, and subsequently fixed in cold 2.5% glutaraldehyde with 0.1 M acacodylate buffer of pH 7.2 at 6 C for 2 hours. Specimens were washed in the same buffer twice, and then dehydrated with a serial concentrations of acetone from 50% to 100%, and treated in the Critical Point Dryer. Tissue were sticked on stubs and coated in IB-2 Ion Coater with a gold membrane, then observed under a scanning electron microscope type Hitachi S-450 operated at 15 KV. The secretory sac of each rectum was fractured on the stub by a knife and moved inside up on the stub with a tiny needle. Specimens were coated again with a gold membrane and observed under the scanning electron microscope.

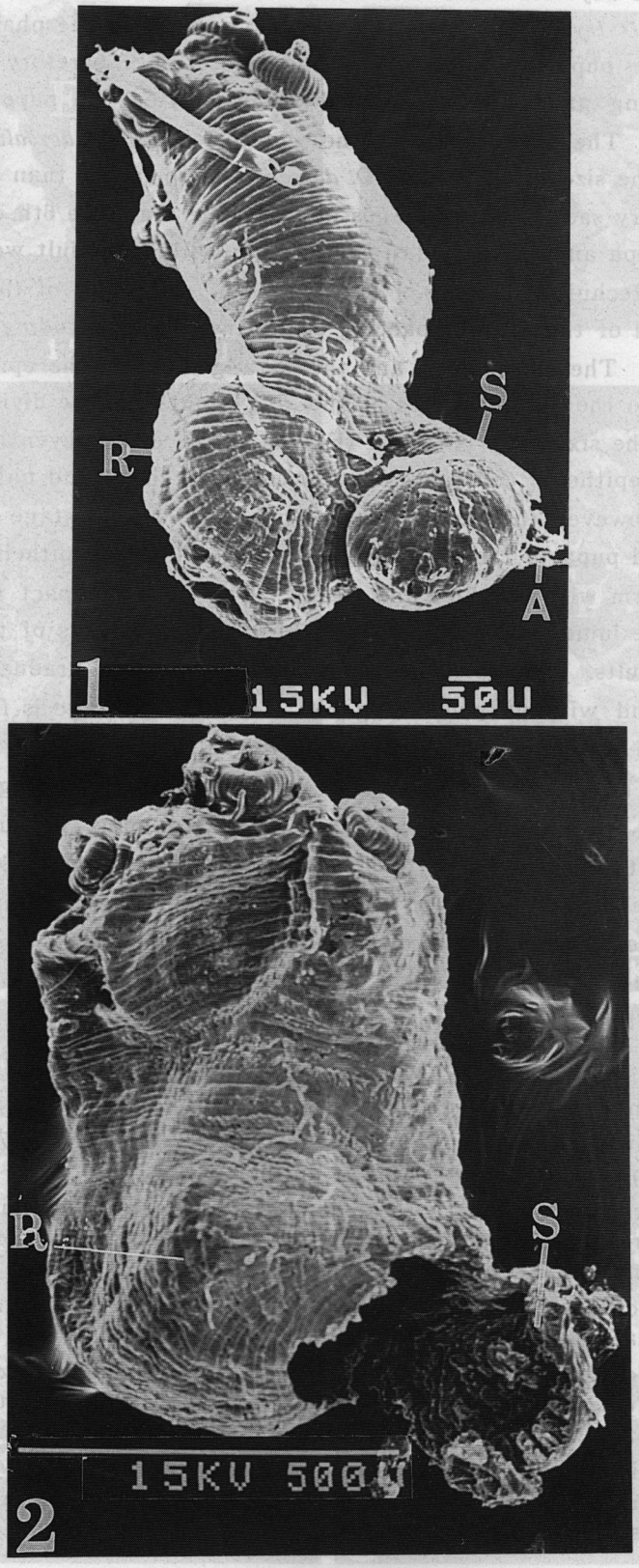
RESULTS AND DISCUSSIONS

The pheromone producing gland of the male oriental fruit fly, *Dacus dorsalis* locates at the posterior portion of the rectum near the anal tube (A) as a small bulbous shape termed as the secretory sac (Fig. 1, S). The left side of the anterior portion to the secretory sac is an envaginated structure as the reservoir (R). While pheromone was secreted from the epithelial cells of the secretory sac, the reservoir was enlarged (Lee and Chang, 1987). These structures of the rectum have also been found in the males of *D. tryoni* (Fletcher, 1968), *D. olear* (Economopoulos *et al.*, 1961), *D. cucurbitae* and *D. dorsalis* (Kobayashi *et al.*, 1978).

The small bulbous shaped secretory sac initiates to appear at the posterior portion of the rectum on the sixth day old pupa. The size of the sac is approximately 70 μ long and 100 μ wide. The sac is getting larger following the insect age. The day before eclosion, the size of the sac becomes 170 μ long and 170 μ

Fig. 1. The external morphology of the rectum of the male oriental fruit fly, *Dacus dorsalis*. A: anal tube, R: reservoir, S: secretory sac.

Fig. 2. The scanning electron micrograph shows the secretory sac (S) cut by fracturing technique. R: reservoir.


S
R
A
1
15KV
50U
R
S
15KV 500U
2

wide. In the 10 days old adult flies the size of sac increases to 200 μ long and 340 μ wide. In *D. tryoni*, the secretory sac first appears in the pharate adult male 2 days after the pupul-adult apolysis. The size of the secretory sac is approximately 130 μ long and 100 μ wide on the 3 days after the pupal-adult apolysis (Fletcher, 1969). The development of the secretory sac of *D. dorsalis* is earlier than *D. tryoni*, and the size of the sac of *D. dorsalis* is also larger than *D. tryoni*.

The secretory sac in different ages of the male flies, the 6th day old pupa to 10th day old pupa and the first day adult to the 30th day adult were observed by the fracturing technique (Fig. 2). The epithelial cells (Ep) of the secretory sac line on the wall of the sac, compacting each other and protruding into the lumen (L) of the sac. The morphology and the arrangement of the epithelial cells are changing due to the different ages of the five stages can be divided: The first stage is from the sixth day old pupa (Fig. 3) to the early seventh day old pupa (Fig. 4). The epithelial cells appear soft, cuboidal shape and not well developed at the stage, however, they are well arranged. The second stage is from the late seventh day old pupa to the first day adult (Fig. 5). The epithelial cells become an irregular form with wrinkles and peaks. The cells compact each other and project into the lumen of the sac. The third stage consists of the third to the seventh day adults. The epithelial cells increase the size gradually, and appear to be flatten and without packs (Fig. 6). The fourth stage is from the 9th or 10th day to the 20th day adults which are sexually maturated. Their epithelial cells appear to be a columnar shape and well arranged protruding into the lumen of the sac (Fig. 7). In the fourth stage, there are many granules attached on the surface of the cells. The fifth stage is from 20 days old to 30 days old adult males while the sexual activation is decreased or completed. During this stage, the epithelial cells degenerated and some cells become slender in shape.

According to the morphological changes, the epithelial cells of the secretory sac from the first stage to the third stage may be involved as the stage of

Fig. 3. The epithelial cells (Ep) of the secretory sac on the 6th day old pupa, represent the first stage of epithelial cells.

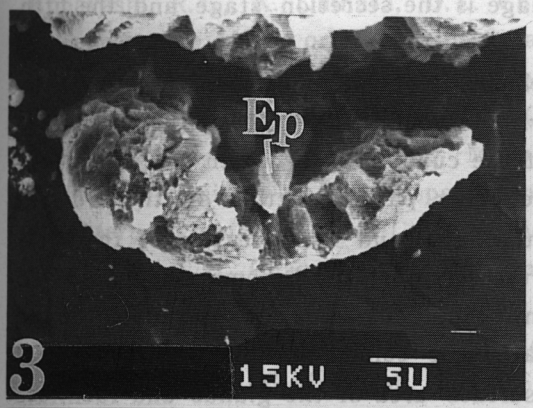
Fig. 4. The epithelial cells (Ep) of the secretory sac on the early 7th day old pupa, also represent the first stage of epithelial cells.

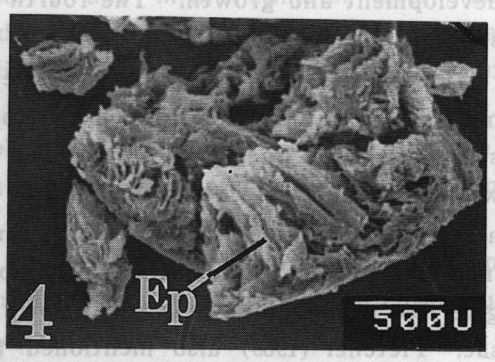
Fig. 5. The secretory sac on the first day old adult male. The epithelial cells (Ep) show irregular arrangement and with the sharped peaks and wrinkles, represented the second stage of epithelial cells.

Fig. 6. The micrograph of the secretory sac on the fifth day old adult male. The epithelial cells (Ep) are large and flattened.

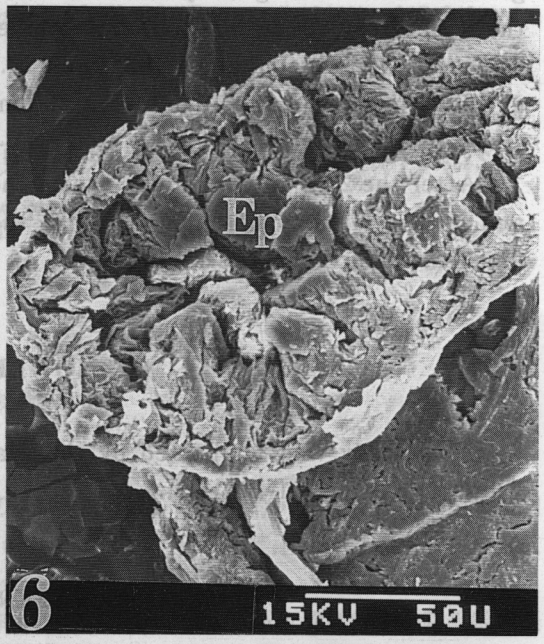
Fig. 7. The micrograph of the secretory sac the 10th day old adult male during the sexual maturation, represented the fourth stage of epithelial cells. The epithelial cells (Ep) are in columnar shape.

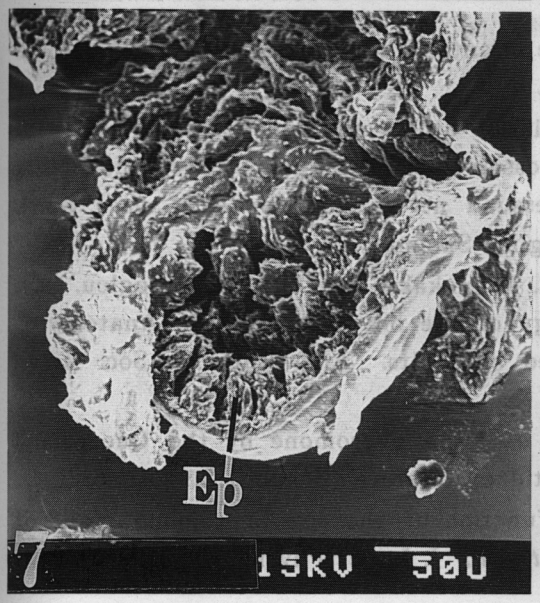
Fig. 8. The micrograph of the secretory sac in the 25th day old adult male, represented the fifth stage of epithelial cells. The epithelial cells (Ep) degenerated. L: lumen of the secretory sac.

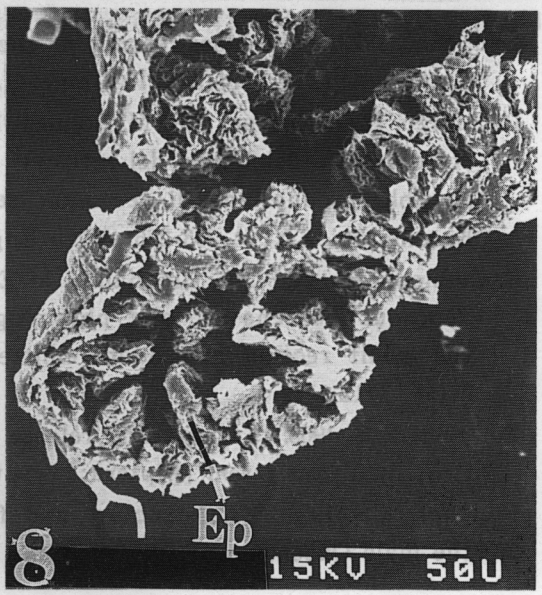

Ep
3
15KV 5U


Ep
4
500U


Ep
5
15KV 50U


Ep
6
15KV 50U


Ep
7
15KV 50U


Ep
8
15KV 50U

development and growth. The fourth stage is the secretion stage and the fifth stage is the degeneration stage.

Fletcher (1969) has studied the development of the pheromone gland in the male *D. tryoni* with the light microscopy. He found that the epithelium of the secretory sac is lined by a single layer of the cuboidal cells which are continuous with and similar to the epithelium of the other part of rectum on the 3rd day after the pupal-adult apolysis. Between the day 3 and 4, the rectum of the adult increases in size. The epithelial cells of the secretory sac become attenuated and give rise to columnar cells and thrown into numerous folds into the lumen of the sac. Fletcher (1969) also mentioned that these columnar cells are the typical glanular cells and greatly increase the surface area of the glands and facilitate their secretory function. The developmental processes of the secretory sac in *D. dorsalis* is almost similar to that of in *D. tryoni*. The shape of the epithelial cells develops from the cuboidal to the columnar, as the flies develop from the first stage to the fourth stage.

After the 20th day old adult, the epithelial cells of the secretory sac degenerated and loosen. It may be explained that the secretory function of the pheromone is finished. Therefore, the surface area of the epithelial cells greatly decreases.

ACKNOWLEDGEMENT

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利用掃描電子顯微鏡研究東方果實蠅 (*Dacus dorsalis* Hendel) 性費洛蒙腺體之發育

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