

COMPARISON OF THE HISTOLOGICAL STRUCTURE OF THE SPERMATHECA BETWEEN NORMAL AND IRRADIATED ORIENTAL FRUIT FLY, *DACUS DORSALIS*^{1,2}

SHWU-MING HWANG, WEN-YUNG LEE

*Institute of Zoology, Academia Sinica, Taipei, Taiwan,
Republic of China*

and

KAI-KUANG HO

*Department of Plant Pathology and Entomology,
National Taiwan University, Taipei, Taiwan,
Republic of China*

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Shwu-Ming Hwang, Wen-Yung Lee and Kai-Kuang Ho (1979) Comparison of the histological structure of the spermatheca between normal and irradiated oriental fruit fly, *Dacus dorsalis*. Bull. Inst. Zool., Academia Sinica 18(2): 59-70. The spermatheca of the oriental fruit fly (*Dacus dorsalis* Hendel) consists of one pair of glandular pouches which are the integumentary gland. The spermathecal cavity is a helically coiled tube. The end of the coiled tube is blind and the other end connects with the spermathecal duct. The wall of the spermathecae consist of two types of cells, the epithelial cells and the glandular cells. The former secretes the cuticular components, and the latter is surrounded by basement membrane.

The outer part of each glandular cell is packed with rough endoplasmic reticulum, secretion vesicles, mitochondria, one nucleus, and the tracheal system. The apical part of the glandular cell is filled with mass of microvilli converging on the secretory duct. Within the spermatheca, the glandular cells and the epithelial cells are tightly linked with the aid of septate desmosomes. The plasma membrane near the apical part of the epithelial cells forms many small cavities, microvilli and membrane stacks. In addition, there are also mitochondria, free ribosomes, vacuoles, and other cellular organelles in the epithelial cells.

Irradiated and normal virgin female flies show no difference in the histology of their spermathecae as determined by electron microscopy. The structure of the spermatheca is more loose in virgin females than in mated females.

The spermathecae of female insects store spermatozoa between copulation and fertilization. Structurally, they are invaginations from the integument at the posterior end of the

venter of the eighth abdominal segment, and the cavity of each of these organs is lined with cuticle continuous with that of the general body surface.

Wigglesworth⁽¹³⁾ reported that the number,

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type, and histological structure of the spermatheca varied in different insects.

In the American cockroach, *Periplaneta americana*, throughout the length of the spermatheca, the cuticular intima of each organ is lined by epithelial cells surrounded by glandular cells. Each of these cells is equipped with an intracellular duct that traverses the overlying epithelial cells as well as cuticle, then opens into the lumen. Clements and Potter⁽²⁾ stated that the epithelial cells of the spermatheca in the mosquito, *Aedes aegypti*, are surrounded by the glandular cells only in the region where the spermathecal duct enters the spermatheca. Romano⁽¹⁰⁾ found the honey bee, *Apis mellifera*, without any glandular cell in the spermatheca, but it has one pair of spermathecal glands.

This study was conducted to investigate the histological effects of a dosage of 13 Krad from Co⁶⁰ on the spermatheca of the oriental fruit fly, *Dacus dorsalis*.

MATERIALS AND METHODS

When the germ cells in the testes of the male oriental fruit flies were treated with the 13 Krad from Co⁶⁰, they were completely destroyed⁽¹²⁾. Up to the present, we still release the oriental fruit flies treated with 13 Krad Co⁶⁰ into the field to control the wild flies' population. So in this study, we want to know whether there are differences between normal flies and those treated with 13 Krad Co⁶⁰ in the structure of their spermathecae.

Normal pupae two or three days before emergence were obtained from Kwanshi Citrus Experimental Station, Taiwan Banana Research Institute. Some of them were treated with Co⁶⁰ from Industrial Technology Research Institute as irradiated flies. After emergence flies were put into individual cages (30 × 30 cm), as follows:

1. 20 normal females.
2. 20 irradiated females.
3. 20 normal females together with 20 normal males.
4. 20 normal females together with 20 irradiated males.

5. 20 irradiated females together with 20 normal males.

6. 20 irradiated females together with 20 irradiated males.

One day after mating, the females were dissected in Ringer's solution and their spermathecae used in the following studies:

(A) Light microscopical studies

(1) Whole mount method⁽⁵⁾

The spermathecae were fixed in Kahle's fluid for two hours, stained with Feulgen reagent, dehydrated in a graded series of alcohol, sealed in Canadian balsam, and then examined under the light microscope.

(2) Paraffin sectioning method⁽⁵⁾

The spermathecae were fixed in Kahle's fluid for one day, dehydrated in one to one mixture solutions of a series of alcohol and tert-butyl alcohol, and then embedded in paraffin. Thin sections of five micrometers were cut with steel knife, stained with Delafield's Hematoxylin, counterstained with Eosin, sealed in Canadian balsam, and then examined under the light microscope.

(B) Transmission electron microscopical study⁽⁹⁾

The spermathecae were pre-fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4°C, for two hours, washed with the same buffer solution with 8% sucrose, and post-fixed at 4°C in 1% osmium tetroxide in 0.1 M cacodylate buffer for one hour. After fixation, the tissue was dehydrated in a graded series of alcohol, treated with propylene oxide, and embedded in low viscosity Spurr medium. Thin sections were cut with a Sorvall Porter-Blum Mt-2 ultramicrotome using diamond knife, stained with uranyl acetate, counterstained with Reynold's lead citrate, and examined in a Hitachi 11-A type electron microscope.

RESULTS

(A) Light microscopical studies

There is a pair of oval spermathecae under the paired ovaries in the oriental fruit fly. The cells of the spermatheca are of two types, epithelial cells (EC) and glandular cells (GC) (Fig. 1a, 1b, 1c). Each spermatheca con-

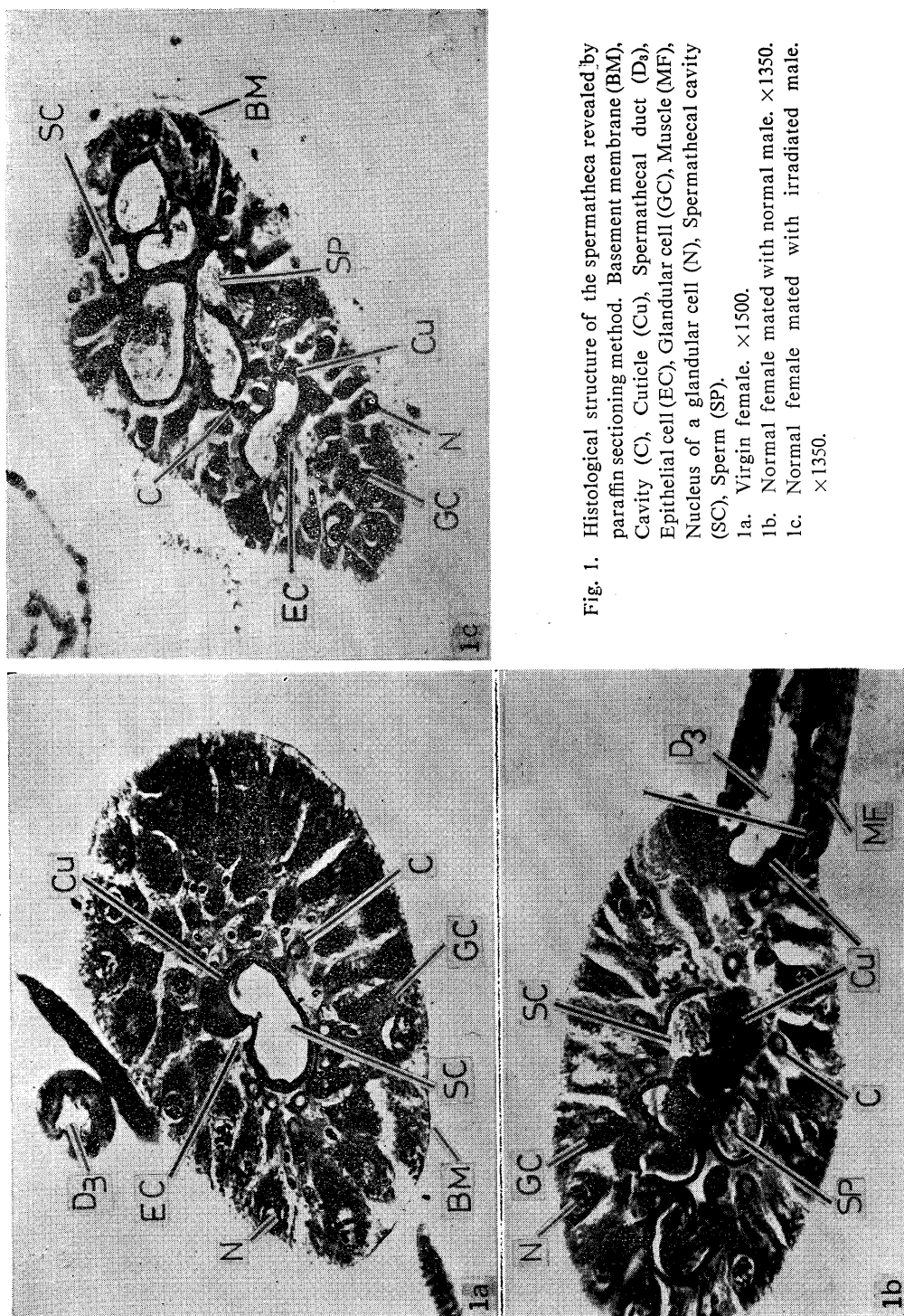


Fig. 1. Histological structure of the spermatheca revealed by paraffin sectioning method. Basement membrane (BM), Cavity (C), Cuticle (Cu), Spermathecal duct (D₃), Epithelial cell (EC), Glandular cell (GC), Muscle (MF), Nucleus of a glandular cell (N), Spermathecal cavity (SC), Sperm (SP).
 1a. Virgin female. ×1500.
 1b. Normal female mated with normal male. ×1350.
 1c. Normal female mated with irradiated male. ×1350.

sists of a spermathecal cavity (SC) which is surrounded by a layer of cuticle (Cu), thin epithelial cells, thick glandular cells, and basement membrane (BM), respectively. The spermathecal cavity (Fig. 2) forms a helically coiled tube. One end of the tube is blind and the other opens into the genital chamber by a long, slender spermathecal duct (D_3). Through

this duct sperm can enter the spermatheca. The spermathecal duct (Fig. 1b), a non-glandular epithelial tube, is surrounded by cuticle, epithelial cells, and muscle fiber (MF), respectively. An invagination cavity (C) is derived from plasma membrane near the apical region of each glandular cell. But the nucleus (N) is near the basal region.

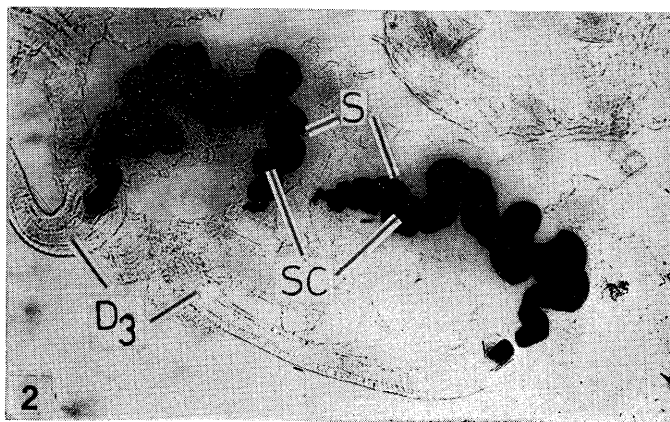


Fig. 2. This field illustrating the organization of spermathecal cavity of the spermatheca. Spermathecal duct (D_3), Spermatheca (S), Spermathecal cavity (SC). $\times 1350$.

(B) Transmission electron microscopical study

The wall of spermathecal cavity (Fig. 3) of *D. dorsalis* is surrounded by a layer of cuticle. The cuticle is covered by thin epithelial cells, thick glandular cells, and lastly basement membrane. Epithelial cells (Fig. 3) lie between the glandular cells and cuticle, thin folds of the epithelial cells extend outwards separating adjacent glandular cells. In addition, there is an epithelial duct (D_2) in each epithelial cell.

Fig. 3 also shows that each glandular cell has an outer region containing typical cytoplasmic organelles, and inner region composed of a remarkable system of microvilli (Mv) converging on the secretory duct (D_1). This duct connects with the epithelial duct with the aid of septate desmosomes (SD), then opens into the spermathecal cavity.

In Fig. 4, the plasma membrane of the apical region of each glandular cell is invaginated to form a cavity, and microvilli projecting from the surface of the cavity extend to the

secretory duct. The intima of the secretory duct is composed of loosely-packed fibrils which are called fibrillar intima (FI).

The glandular cells and the epithelial cells are tightly linked with the aid of septate desmosomes, so that the secretory ducts of the glandular cells and the epithelial ducts of the epithelial cells are brought into precise register. However, the epithelial duct (Fig. 5) penetrates the cuticle of the spermathecal cavity, therefore, the epithelial ducts can receive secretory products from the glandular cells and convey them into the main lumen.

The cuticle of the spermatheca (Fig. 6) consists of two layers: epicuticle (Ep) and procuticle. Procuticle contains exocuticle (Ex) and endocuticle (ECu), the latter forms lamellar structure.

The outer part of each glandular cell (Fig. 4) is packed with rough endoplasmic reticulum (RER), secretion vesicles (VS), and mitochondria (M). The rough endoplasmic reticulum is actively engaged in synthesis of protein⁽¹¹⁾. The

secretion vesicles containing the secretory products received from the glandular cells protude into the invagination cavity. The secretory

products enter the secretory duct easily via the loosely-packed fibrillar intima. As shown in Fig. 5 the secretory products enter the

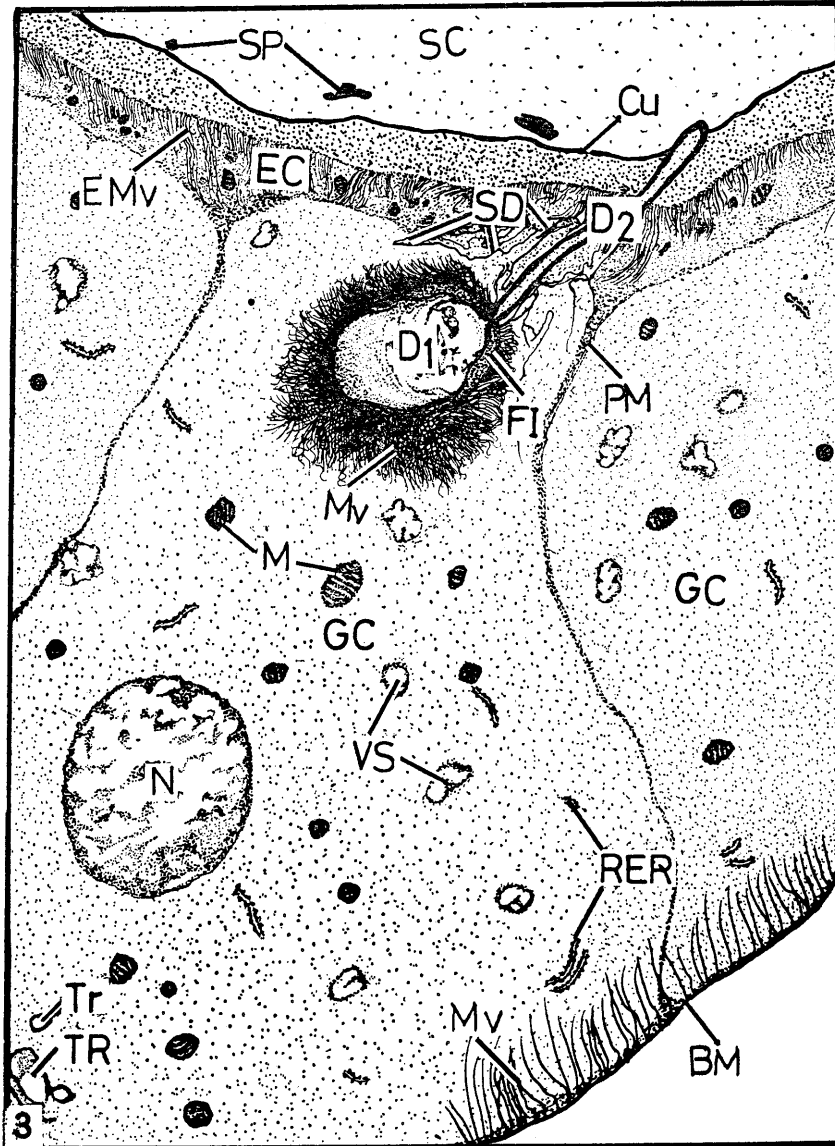


Fig. 3. A drawing of a section through part of a spermatheca of *Dacus dorsalis*. Basement membrane (BM), Cuticle (Cu), Secretory duct (D_1), Epithelial duct (D_2), Epithelial cell (EC), Microvilli of the epithelial cell (EMv), Glandular cell (GC), Fibrillar intima (FI), Microvilli (Mv), Nucleus of a glandular cell (N), Plasma membrane (PM), Rough endoplasmic reticulum (RER), Spermathecal cavity (SC), Sperm (SP), Tracheal branches (TR), Tracheole (Tr).

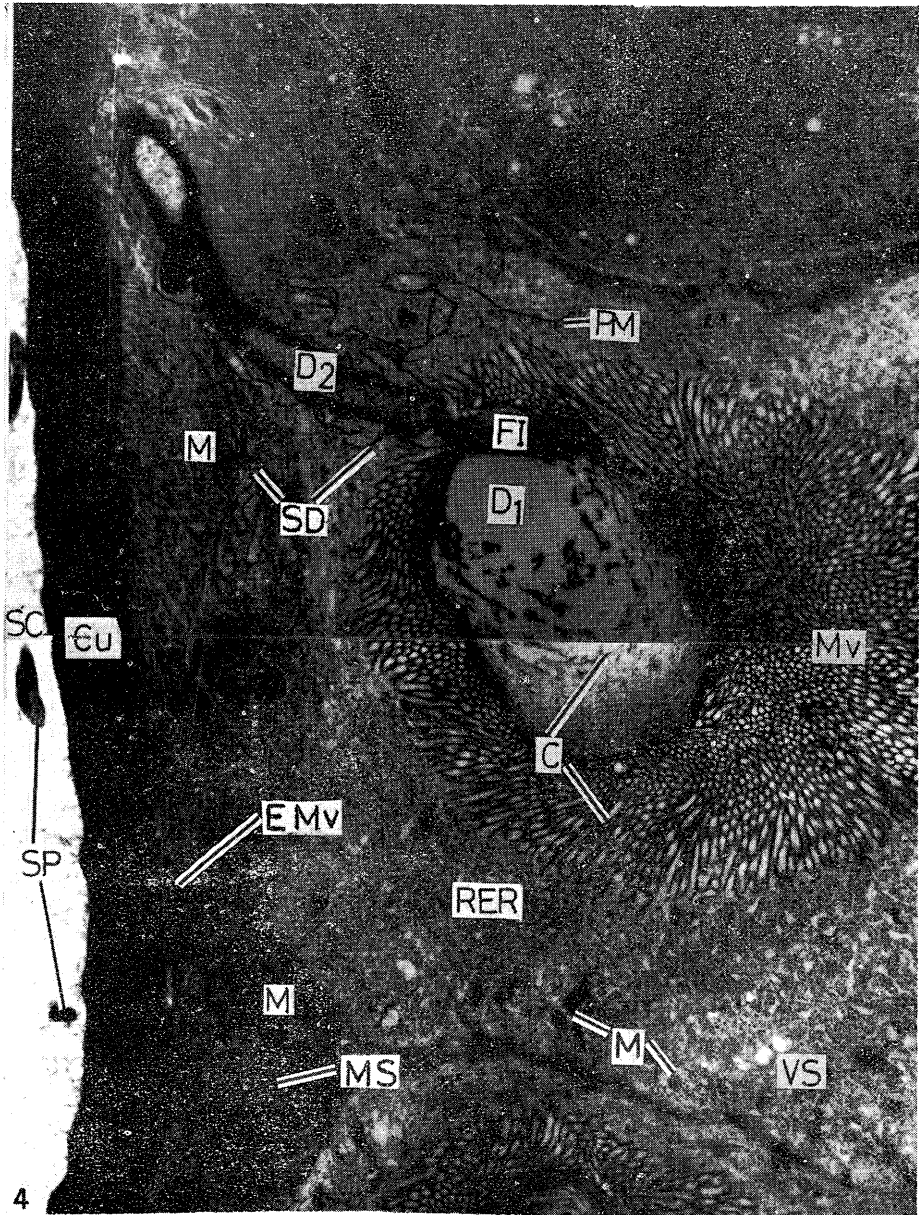


Fig. 4. A section through apical region of the spermatheca under the electron microscope in normal female mated with irradiated male. Cavity (C), Cuticle (Cu), Secretory duct (D₁), Epithelial duct (D₂), Microvilli of the epithelial cell (EMv), Fibrillar intima (FI), Mitochondria (M), Microvilli (Mv), Membrane stack (MS), Plasma membrane (PM), Rough endoplasmic reticulum (RER), Spermathecal cavity (SC), Septate desmosome (SD), Sperm (SP), Secretion vesicle (VS). $\times 13000$.

spermathecal cavity via epithelial duct. The spermathecal cavity, the epithelial duct, and the secretory duct are filled with flocculent material.

Each glandular cell is surrounded by a layer of basement membrane (Fig. 3 and Fig. 7a), a part of which invaginates to form microvilli. In

general, cell nucleus and tracheal branches (TR) are mostly located near the basal region.

The plasma membrane (PM) near the apical part of an epithelial cell (Fig. 8a) forms many small cavities, and microvilli. Plasma membranes between two epithelial cells are tightly

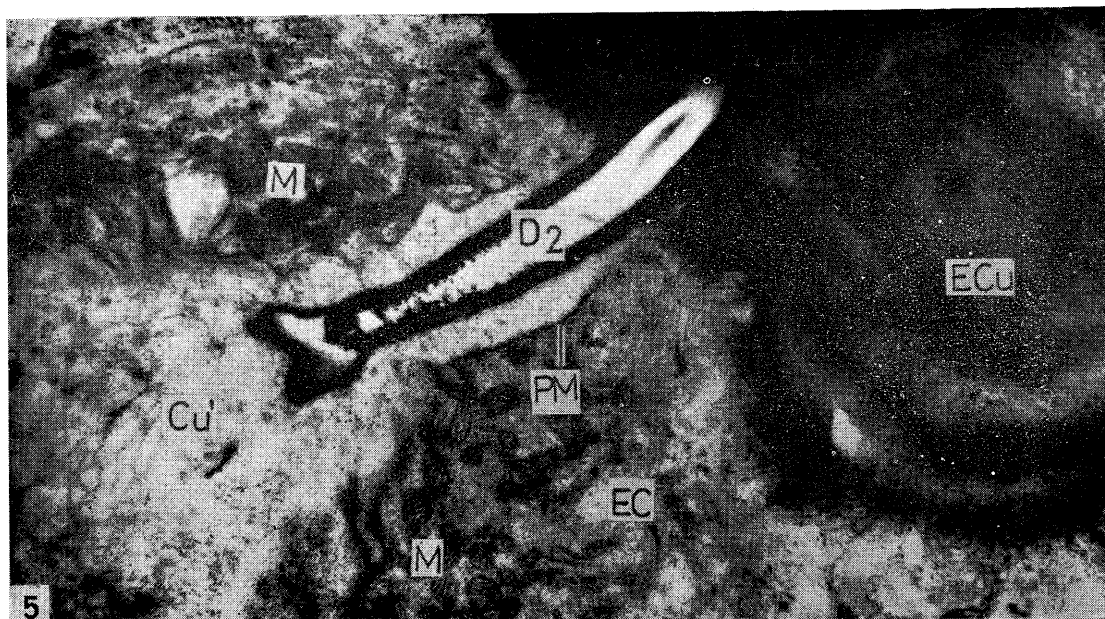


Fig. 5. Micrograph illustrating a longitudinally sectioned epithelial duct in an epithelial cell adjoining the main cuticular lining of the spermatheca. Cuticle of an epithelial duct (Cu), Epithelial duct (D₂), Epithelial cell (EC), Endocuticle (ECu), Mitochondria (M), Plasma membrane (PM). $\times 10000$.

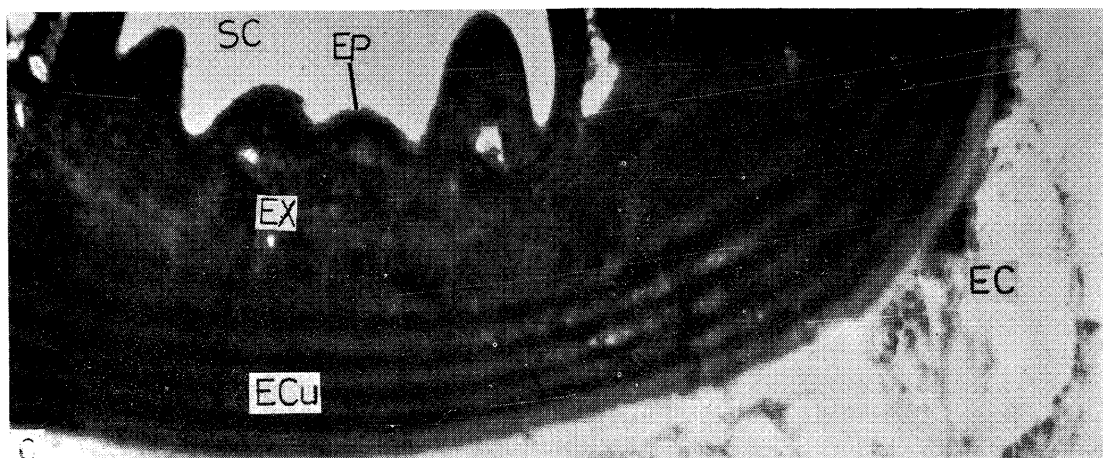


Fig. 6. Detail of the cuticular lining of the spermatheca. Epithelial cell (EC), Endocuticle (ECu), Procuticle (Ep), Exocuticle (Ex), Spermathecal cavity (SC). $\times 20000$.

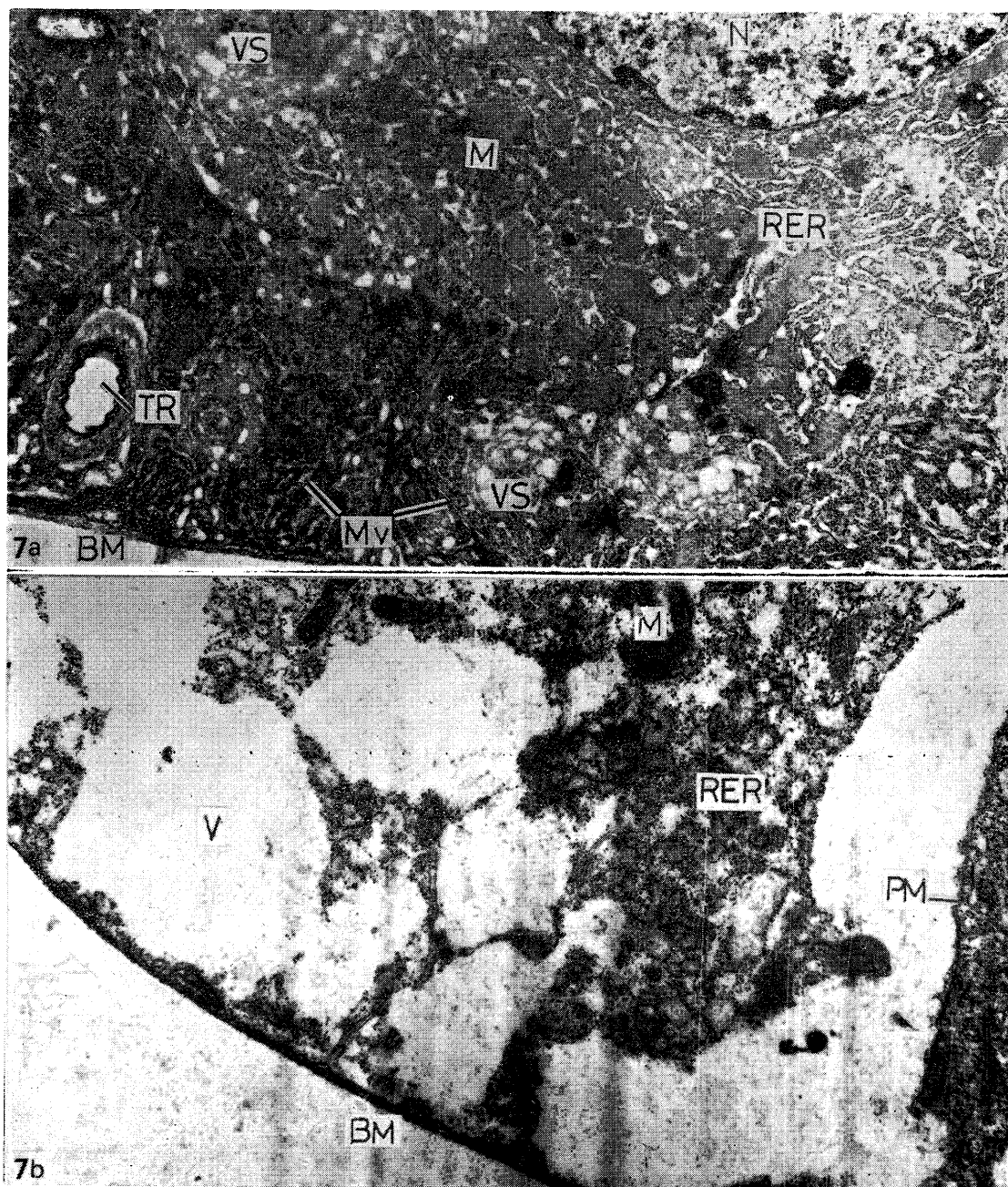


Fig. 7. A section through basal region of a glandular cell. Basement membrane (BM), Mitochondria (M), Microvilli (Mv), Nucleus of a glandular cell (N), Rough endoplasmic reticulum (RER), Tracheal branches (TR), Vacuole (V), Secretion vesicle (VS).
 7a. In mated female. $\times 13000$.
 7b. In virgin female. $\times 13000$.

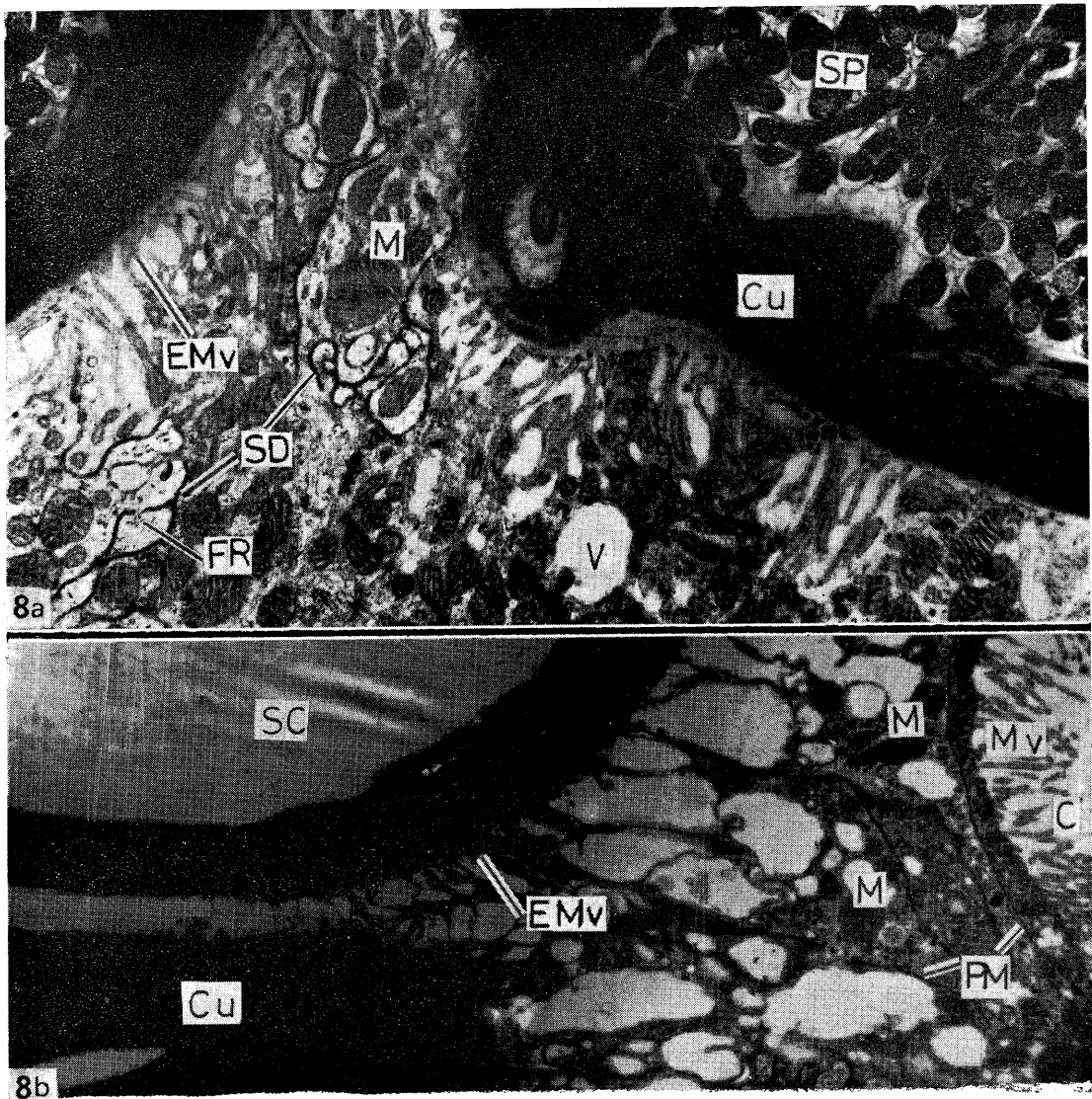


Fig. 8. A section through apical region of the spermatheca. Cavity (C), Cuticle (Cu), Microvilli of the epithelial cell (EMV), Free ribosome (FR), Mitochondria (M), Microvilli (Mv), Plasma membrane (PM), Spermathecal cavity (SC), Septate desmosome (SD), Sperm (SP), Vacuole (V).

8a. In normal female mated with normal male. $\times 10000$.

8b. In virgin female. $\times 10000$.

linked by septate desmosomes. In addition, there are also mitochondria, free ribosome (FR), and vacuoles in the epithelial cells.

(C) Comparison of the histology of the spermatheca of females in different conditions

(1) Between normal and irradiated virgin

females.

The histology of the spermatheca of the normal and irradiated virgin females shows no differences under both light microscope and electron microscope. Under EM, the apical region of the epithelial cells of the spermathecae (Fig.

8b), has many small cavities which are formed by the invagination of plasma membrane contain loose microvilli (EMV). There are secreted materials but no sperm in the spermathecal cavity. The basal region of the glandular cells (Fig. 7b) contains several large vacuoles.

(2) Normal female mated with the normal male, normal female mated with irradiated male, and normal virgin female.

Under the light microscope, the histology of the spermatheca of both virgin females and mated females shows no differences (Fig. 1a, 1b). In normal females or the irradiated females mated with the irradiated males, the spermatheca contains sparse sperm (Fig. 1c). However, in normal females mated with normal males, the spermathecal cavity contains mass of sperm bundles (Fig. 1b). This is seen more clearly under the electron microscope (Fig. 4, 8a).

In the electron microscopical study, near the apical region of the epithelial cells of the spermatheca, the cavities are smaller in the normal female mated with normal male (Fig. 8a) than in the normal virgin female (Fig. 8b). However, microvilli of these cavities are more loose in the latter than in the former. Near the basal region of the glandular cells, there are large vacuoles in the normal virgin female (Fig. 7b), however, the cells are arranged very compactly in the normal female mated with normal male (Fig. 7a).

The cell structure of apical and basal region of the spermatheca is more loose in the normal virgin female (Fig. 7b, 8b) than in the normal female mated with irradiated male (Fig. 4, 7a). In the normal female mated with the normal male, near the apical region of the epithelial cells (Fig. 8a), there are cavities which are filled with microvilli, but no membrane stacks in the middle of the epithelial cells. In the normal female mated with the irradiated male (Fig. 4), there are microvilli near the apical region of the epithelial cells, membrane stacks in the middle of the epithelial cells, but no cavities.

DISCUSSION

Wigglesworth⁽¹³⁾ reported that the histological structure of the spermatheca varied in different insects. The structure of the spermatheca of the oriental fruit fly, *D. dorsalis*, is similar to that of the American cockroach, *P. americana*, which has been described by Gupta and Smith⁽⁴⁾. The cuticle of the spermathecal cavity is lined by a layer of epithelial cells. Each epithelial cell is connected with one glandular cell. The secretory products obtained from the glandular cells are transported through secretory duct, epithelial duct, then into the lumen. The spermatheca of the mosquito, *A. aegypti*, has been described by Clements and Potter⁽²⁾. There are a few glandular cells only in the region where the spermathecal duct enters the spermatheca. In addition, there is another glandular cell which opens into the spermathecal duct by a long slender tube. However, Romano⁽¹⁰⁾ reported that the spermatheca of the honey bee, *A. mellifera*, has one pair of spermathecal gland, but no glandular cell.

Khan and Musgrave⁽⁶⁾ reported that the sperm of the Sitophilus insects are active throughout the storage period in the spermatheca, where they must obtain some energy provided by the exogenous substrate. Davey and Webster⁽³⁾ has suggested that the glandular cell of the spermatheca is one of the sources of the exogenous substrates. According to the present research, the glandular cells are active metabolic cells. On the other hand, the epithelial cells are connected between glandular cells and spermathecal cavity, therefore, the secretion of the glandular cells enters the spermathecal cavity via a series of ducts—secretory duct and epithelial duct to nourish the sperm. The secretion obtained from either glandular cells or spermathecal gland may provide nourishment for sperm within the spermathecal cavity.

The structure of microvilli formed by the invagination of the plasma membrane in the spermatheca is similar to that of the epithelial cells of the malpighian tube of the Calliphora insects. Berridge and Oschman⁽¹¹⁾ suggested

that this structure might be concerned with the balance of the permeability of water and ionic salts. Marshall⁽⁷⁾ suggested that the golgi body vesicles of the epithelial cells of the malpighian tube of the Cercopoid larvae were derived from the endoplasmic reticulum membrane. Secretion vesicles of the glandular cells of the spermatheca of the oriental fruit fly may be derived from the endoplasmic reticulum.

Gupta and Smith⁽⁴⁾ stated that the spermatheca of the insects is formed by the invagination of the body surface during the embryonic period. In the oriental fruit fly, the structure of the cuticle of the spermatheca is similar to that of the integumentary wall of the insect, its spermatheca is most likely formed by the invagination of the body wall. The main function of hypodermal cells of the integumentary wall is to secrete the cuticular components, so that the cuticle surrounding the spermathecal cavity and the cuticle of the epithelial duct are secreted by the epithelial cells. In the larval stage, the insects grow while the cuticle of integumentary wall and invagination cavity molts, but this process never occurs in the adult stage. Therefore, the region in the epithelial cells of the spermatheca becomes thin.

The present study shows that the cell structure of the spermatheca and the microvilli of the epithelial cells are arranged more loosely in the virgin females than in the mated females. We assumed that the mating behavior would stimulate the glandular cell to secrete. So there are more flocculent material in the mated females than in the virgin females. The microvilli of the epithelial cells are concerned with the reabsorption of the secretion and facilitating its passage to the spermathecal cavity. After mating, the glandular cells are active and they seem arranged tightly. The microvilli of the epithelial cells increase in number in order to reabsorb much more secretion to nourish the sperm within the spermathecal cavity. The fact that normal females mated with irradiated males have less sperm in the spermatheca than those mated with normal males, can be used to males, effectively control the oriental fruit flies'

population has been proved in another research paper (not published yet). Dosage of 3 Krad Ce^{137} damaged the ovaries of *Rhagoletis pomonella* has been suggested by Myers *et al.*⁽⁸⁾. Fine structure of the spermatheca is not different between normal and irradiated virgin females. At the same time, both spermathecal cavity are filled with flocculent material. We know that the function of the spermatheca is not damaged by irradiation. On the other hand, after the irradiated female mates, sperm can enter its spermathecal cavity, but it lays no eggs. It might be explained that the dosage of 13 Krad Co^{60} could damage the ovaries of the oriental fruit fly, but not affect on the spermathecae.

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東方果實蠅受精囊組織學之研究

黃淑明 李文蓉 何鎧光

東方果實蠅 (*Dacus dorsalis* Hendel) 具有成對的受精囊。受精囊腔呈螺旋型之管狀，一端封閉，另一端與受精囊管相連。其囊壁細胞層主由分泌表皮細胞成分之皮膜細胞及腺體細胞所構成，基部為基底膜。

每一腺體細胞外部原生質內充滿了附核糖體顆粒之內質網，分泌胞囊，粒腺體，一個核及氣管系統等；而端部內陷腔中有許多絨毛及一分泌管。皮膜細胞藉 Septate desmosome 之助與腺體細胞緊密相接，故皮膜層管道能將腺體細胞分泌物送進受精囊腔內。皮膜細胞端部原生質內陷形成許多小腔室，絨毛或重疊之膜，此外皮膜細胞內尚有粒腺體，游離之核糖體及空泡等。

在電子顯微鏡下觀察，未交尾處理及正常東方果實蠅之受精囊組織結構沒有差異；正常雌蠅交尾前受精囊細胞組織之排列較交尾後者為疏鬆。