

SPERMATOPHORE FORMATION AND THE MORPHOLOGY OF THE REPRODUCTIVE SYSTEM OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE)

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Li-Chen Yang and Yien-Shing Chow (1978) Spermatophore formation and the morphology of the reproductive system of the diamondback moth, *Plutella xylostella* (Lepidoptera: plutellidae). *Bull. Inst. Zool., Academia Sinica* 17(2): 109-115. The anatomy of the reproductive system of the diamondback moth, *Plutella xylostella* (L.) has been described in detail and it appears to resemble most other typical Lepidoptera with minor modifications. One significant finding is that the spermatophore is gradually formed within the bursa copulatrix of the female and is not yet completed 8 minutes after mating. A time table for spermatophore development is also presented. Once the spermatophore is formed, it can last for 96 hours, presenting itself as an assay tool to identify the mating success in the diamondback moth.

The diamondback moth, *Plutella xylostella* (L.), an important cabbage pest, attracted much attention from our entomologists recently. In attempts to control it, one biological thought is to use the disruption technique utilizing its sex pheromone^(2,3,6). Although the sex pheromone compounds of the diamondback moth have been identified and synthesized^(6,10), direct experiments for its control have not been carried out because a bioassay method was lacking to pinpoint the success of copulation between male and female adults. In other Lepidoptera, such as the almond moth *Cadra cautella* and the Indian meal moth *Plodia interpunctella*, the percentage of fertilization can be easily estimated by counting their spermatophores within the females abdominal cavity^(1,4,5). In order to develop a biological method for testing the sex pheromone of the diamondback moth in the field, additional information about its reproductive system as well as the structure and forma-

tion of the spermatophore is needed. The present paper describes our observations.

MATERIALS AND METHODS

Pupae of the diamondback moth were collected from cruciferous crops in vegetable gardens. The pupae were kept in the laboratory at 25°C and were separated by putting each pupa in individual vials to prevent mating after emergence. The vials were closed by parafilm pierced by pins for ventilation.

The male and female adults were separated by sex and held in two glass containers covered with nylon screen and fed daily with a sponge moistened with 5% honey water solution. In order to keep optimum sensitivity of the male to the female's sex pheromone, the males and females were kept in different rooms.

For observation of the reproductive system, males or females were killed and then held in place on the surface of a black paraffin dissecting plate by means of a dissecting pin through their

thorax. They were then immersed in saline solution and observed with a dissecting microscope (Olympus). A longitudinal incision was made on the dorsal side of the abdomen extending from the fourth to the last abdominal segment, so that the whole reproductive system was exposed.

In order to examine each organ in detail, the organs were carefully dissected from the abdominal cavity and placed on a glass microscope slide with a drop of saline solution, and then observed with a Nikon research microscope⁽⁴⁾.

For observation of the process of spermatophore transfer, the males were introduced to females in containers. As soon as a couple were seen copulating, they were introduced into individual vials. After various time intervals, copulation was interrupted and the females killed and dissected according to the previous method. The bursa copulatrix and other organs were dissected out and opened carefully with a micropin. They were observed with a research microscope.

The saline solution used for dissection contained:

NaCl 7 g
KCl 0.14 g
CaCl₂ 0.12 g
NaHCO₃ 0.2 g
diluted to 1000 cc with distilled water.

RESULTS AND DISCUSSIONS

The Genital Organs of the Female Diamondback Moth

When a longitudinal incision was made along the dorsal side of the abdominal segment, the large yellow ovaries were quickly exposed at the level of the fourth to sixth segment, occupying most of the abdominal cavity. Other genital organs can also be observed in the seventh and eighth segment (Fig. 1). There are two large ovaries, each consisting of four ovarioles (Fig. 2), which lead into the paired lateral oviduct, both of which join each other to form the median oviduct. The median oviduct extends posteriorly to the right of the bursa

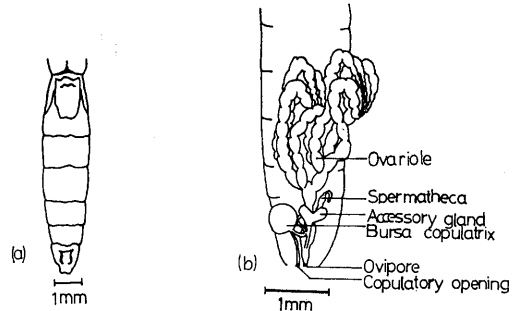


Fig. 1. (a) Ventral view of the female diamondback moth, *Plutella xylostella* (b) same, with the abdominal wall removed, showing the reproductive system.

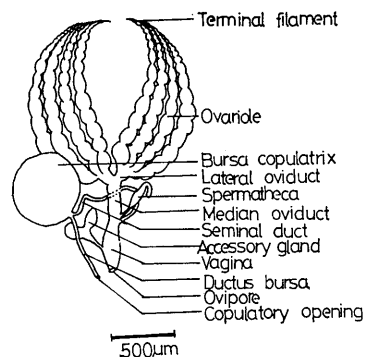


Fig. 2. The reproductive system of a female diamondback moth.

copulatrix and widens slightly to form the vagina. The vagina runs posteriorly dorsal to the rectum and meets with the rectum immediately before opening to the exterior. Hence, this represents a combination of the vaginal and anal openings.

The bursa copulatrix (Fig. 3) gives off two ducts: (1) The slightly sclerotized straight ductus bursa, which opens independently on the tergum of the eighth segment, with its distal end surrounded by a pair of sclerites, and (2) the convoluted seminal duct which runs posteriorly to enter the median oviduct.

Immediately above the seminal duct opening in the median oviduct is the spermathecal ductus which leads into the spermatheca (Fig. 2). There is a certain structure which cannot be seen very clearly that appears to

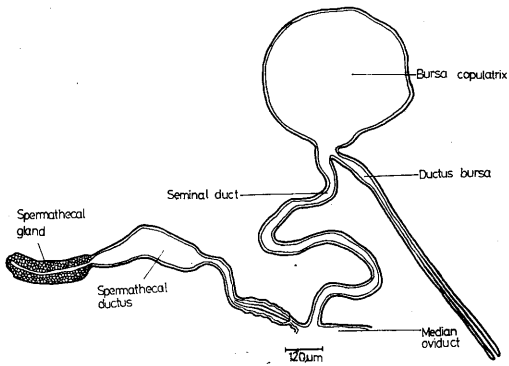


Fig. 3. The detailed structure of the bursa copulatrix and spermatheca.

extend over the two openings, so that the sperm may not accidentally spill over into the oviduct. It also appears that it may control the release of sperm into the oviduct to fertilize the eggs as the eggs pass down. At this time, the two openings appear to be separated from each other a larger distance than before the arrival of the eggs.

The spermatheca can be further subdivided into two parts (Fig. 3). (1) The spermathecal ductus, a complex short duct through which sperm can exit or enter the storage area at the same time. (2) The distally-located spermathecal gland. The spermathecal gland contains within its interior a narrow canal full of sperm which is especially active, perhaps due to a secretion from the gland.

Associated with the vagina is a slender short duct leading into a Y-shaped gland termed the accessory gland (Fig. 2).

The Genital Organs of the Male Diamondback Moth

By cutting through the mid-dorsal line of a male diamondback moth, a large yellow testis can be exposed, located between the fifth and sixth abdominal segment. There is a mass of coiled tubes situated beneath the testis at the level of the sixth to the last abdominal segment (Fig. 4).

The testis is roughly ovate in shape with its apices directed posteriorly. There are four suspensory ligaments attached on the abdominal

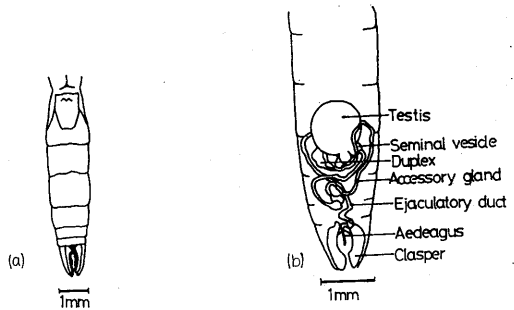


Fig. 4. (a) Ventral view of the male diamondback moth, *Plutella xylostella*. (b) same, with the abdominal wall removed, showing the reproductive system.

wall. Arising from the base of the testis is a pair of seminal vesicles (Fig. 4). This is a condition which is different from most lepidoptera⁽⁶⁾, and which was also observed by Holt and North⁽⁷⁾ in cabbage loopers, *Trichoplusia ni*. Behind the seminal vesicles the slender vas deferens continues, enlarging at its distal end and opening into a pair of sacs or duplexes (Fig. 5). A pair of long tubular accessory glands open into the tips of the duplexes, and unite with each other with their distal ends enlarged into a bulb-like process. The duplexes join basally to form the long ejaculatory duct, which is a complex tract with three valves. By observation under the microscope, the ejaculatory duct is seen to be filled with a transparent, compact liquid.

Unlike the cabbage looper⁽⁷⁾, both the seminal vesicles and duplexes of the diamondback moth are filled with sperms ever since emerging from the pupal case. Most of the sperms in the seminal vesicles are in bundles and half encased in a membrane-like structure with their tails spread out. The sperms do not become motile until they are deposited in the bursa copulatrix of a female. If a dissection of the bursa copulatrix is made in saline solution, the sperm (0.1 mm in length) can be seen wriggling about with great energy and moving forward in a spiral-like manner.

The external genital organs of the male diamondback moth consists of two distinct

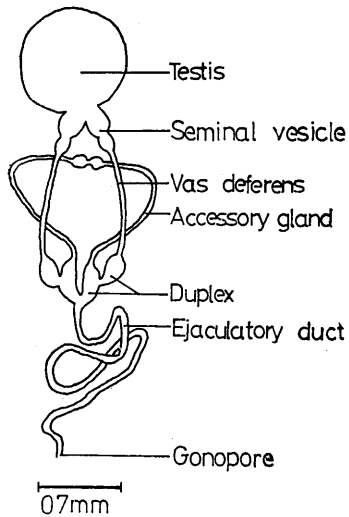


Fig. 5. The reproductive system of a male diamondback moth.

structures: phallus and harpes⁽⁹⁾.

The phallus (Fig. 6a) is somewhat differentiated into a proximal part or phallobase, and a more slender distal part or aedeagus. A pair of strong lateral processes or lateral basal lobes arise from the lateral side of the phallobase, which give attachment to muscles. Around the posterior part of the aedeagus, there is a continuously sclerotized ring termed the annulus, from which the aedeagus and anus protrude out (Fig. 6b).

A pair of strongly sclerotized claspers (Fig. 7) serve as copulatory organ and are borne on the coxopodite areas of the coxosternal plate. Two bundles of strong muscles attach to the outer walls of the two harpes.

Mating Behavior and Spermatophore Transfer

When a female diamondback moth reaches sexual maturity, it begins to release sex pheromone to attract males^(8,4,10). Under laboratory conditions, when several calling females are present in a small area of male containers, males first move their antennae, then become very excited. The stimulated males flutter their wings, and then turn their bodies around as they move backward in an attempt to contact the female from the rear. If the female is

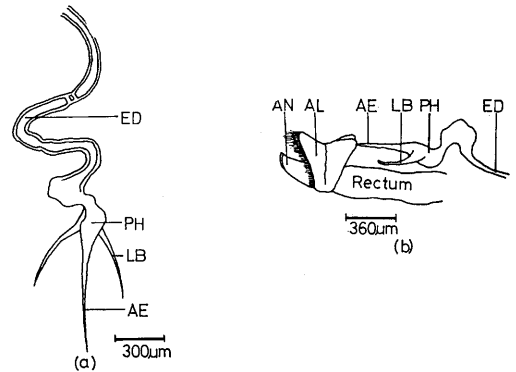


Fig. 6. The phallic organs of a diamondback moth: (a) Ventral view. (b) Lateral view showing the annulus.

ED: Ejaculatory duct, PH: Phallobase, LB: Lateral basal lobe, AE: Aedeagus, AL: Annullus, AN: Anus.

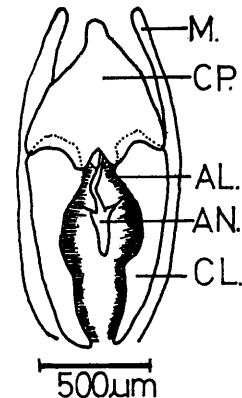


Fig. 7. The ventral view of the peripheral organs of a male diamondback moth.

M: Muscle, Cp: Coxosternal plate, AL: Annullus, AN: Anus, CL: Clasper.

receptive, she remains stationary, copulation then takes place immediately. The male protrudes his aedeagus into the ductus bursa of the female, then clasps tightly at both sides of the female's eighth abdominal segment with his clasper. Otherwise, the female will reject mating and move away. Therefore, the females not ready for mating were not forced to copulate. Homocourtships and a male attempting to copulate with another male were observed several times in the laboratory.

The bursa copulatrix of virgin females is flat and small (Fig. 8a), containing nothing but a small amount of haemolymph. A female killed about 2 minutes after the beginning of pairing shows a bursa copulatrix containing a mass of transparent viscous compact substance (Fig. 8b), which will be eventually expanded to form the sac of the spermatophore. This substance appears to be the transparent liquid which is found in the ejaculatory duct of the male before mating. A female killed about four minutes after the beginning of pairing shows a small amount of milky white seminal fluid containing highly motile sperms that have been deposited inside the transparent substance (Fig. 8c). The white seminal fluid appears to be a mixture of the half transparent mass of sperm stored in the duplex and the white secretion of the male accessory gland. The spermatophore after thirty minutes mating is spheric and surrounded by a semisolid viscous covering (Fig. 8f).

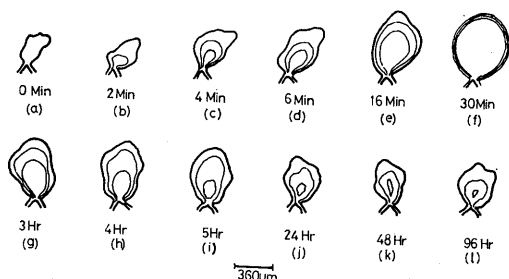


Fig. 8. The spermatophore in the bursa copulatrix in different time intervals after the initiation of copulation.

About sixteen minutes after the initiation of mating, the sperm continues to enter the bursa copulatrix through the ductus bursae concomitant with the sperm in the bursa copulatrix leaving the spermatophore, passing through the seminal duct, and migrating to the spermatheca. The migration is accomplished by the movement of the sperm itself and the muscular action of the wall of the bursa copulatrix, seminal duct, and spermatheca. The seminal fluid in the bursa copulatrix is white, but the sperm stored

in the spermatheca is half-transparent.

The whole process of copulation takes about one to two hours. A female killed immediately after completion of copulation show the spermatophore shining through the wall of the much distended bursal sac. Unlike spermatophores of most Lepidoptera mentioned by Norris⁽⁶⁾, the spermatophore of the diamondback moth is a simple spheric structure filled with seminal fluid and surrounded by a semisolid viscous covering, with an opening near the mouths of the ductus bursae and seminal duct.

Table 1 shows the number of females observed to be in certain stages of spermatophore formation as a function of time.

After completion of copulation, the spermatophore gradually becomes smaller and to some extent recedes from the wall of the bursa copulatrix. A female dissected three hours after the completion of copulation exhibits a sper-

TABLE I
The relationship between post-mating time and spermatophore formation

Time intervals after the initiation of mating	No. of pairs observed	Stage of spermatophore formation			
		1st ^a	2nd ^b	3rd ^c	4th ^d
2 min.	5	1	3	1	0
4 min.	5	1	0	4	0
6 min.	4	0	0	4	0
8 min.	4	0	0	3	1
10 min.	4	0	0	3	1
12 min.	3	0	0	3	0
14 min.	5	0	0	4	1
16 min.	4	0	0	1	3
18 min.	4	0	0	1	3
20 min.	4	0	0	0	4
22 min.	4	0	0	1	3
24 min.	3	0	0	0	3
26 min.	2	0	0	0	2

a: The bursa copulatrix is flat, containing only a small amount of haemolymph.

b: A mass of transparent viscous compact substance is entering into the bursa copulatrix.

c: The milky white seminal fluid has been deposited inside the bursa copulatrix.

d: The sperm in the bursa copulatrix is passing through the seminal duct and migrating to the spermatheca.

matophore which appears almost empty (Fig. 8g), containing only a small amount of apparently dead sperm. This small amount of dead material may disintegrate gradually (Fig. 8h, i, j, k, l). There is also a few dead sperms located outside the spermatophore in the bursa copulatrix, seminal duct, and ductus bursa.

The covering of the spermatophore in the bursa copulatrix, mentioned above, breaks into a mass of milky, shapeless fragments within four days after the introduction of the spermatophore into the female, but it is still recognizable even after the death of the female.

Since most of the sperms have transferred into the spermatheca, the spermatheca is swollen by the longitudinally arranged sperm. The amount of sperm far exceeds the number needed to fertilize the eggs. Therefore, most of the sperms remain stored in the spermatheca until the female dies. About two days after the completion of egg laying the sperm left in the spermatheca is apparently dead.

Thus, a mated female diamondback moth can be determined by a visual examination of the presence of a broken spermatophore covering shining through the wall of the bursa copulatrix, or the expanded spermatheca filled with seminal fluid.

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小菜蛾精囊包的形成與其生殖系統的解剖研究

楊麗鈺 周延鑫

本文報導小菜蛾 *Plutella xylostella* (L.) 的生殖系統，發現其組織構造和其他鱗翅目昆蟲大同小異，主要發現是其精囊包 (Spermatophore) 是在雌體之 bursa copulatrix 內漸漸形成，尤其是在雌蟲交配 8 分鐘後，精囊包尚未完全形成。一旦精囊包形成，在 96 小時之後，尚未消失，所以此物可以用做檢定小菜蛾交配成功與否之指標。