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Morphological Characteristics of Reproductive System of the Codling Moth *Cydia Pomonella*

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Abstract

The codling moth *Cydia pomonella* is one of key pests in fruit orchards. The strong reproductive ability is one of the main reasons for serious damage by the codling moths. In this study, the morphological characteristics of the male and female adult reproductive systems are dissected and described. The two testicular follicles are closely connected to each other like a single organ and enveloped in the lavender adventitia. The corpus bursae have a pair of horn-shaped signa instead of *cysticerci*. The aedeagus has 7 *cornuti*, among which five are close to the end and two are in the middle. During the process of sexual maturation, we observed that an antheridium is formed in the section of ductus seminalis between corpus bursae and ostiumbursae. The study of the reproductive systems, especially the female systems could provide evidence for prevention and forecasting on the codling moth population, and male characteristics are helpful in its identification.

Keywords: Lepidoptera, Codling moth, Cydia pomonella, Reproductive system, Morphology

Introduction

The codling moth *Cydia pomonella L.* (Lepidoptera, Tortricidae) was originally found in the southeast regions of Europe and then spread to the apple tree *Malus pumila* Miller around the world. In China, it was first discovered in Korla city, Xinjiang province in 1953 [1,2]. It shows a remarkable ability to adapt to a variety of climatic and trophic conditions [3], even those in North America, South American, and South Africa. The codling moth is one of the main quarantine insect pests of fruit trees in the world (Jin 1997) and a key pest of the plant family Rosaceae. It causes a great damage to pome fruits (apple, pear and quince); [4] and occasionally apricots [2,5]. It has been also found in walnut orchards of the tree family Juglandaceae. Recently, it was reported to harm members of plant family *Magnoliaceae* [6].

The strong reproductive ability is one of the main reasons for their ability to cause serious damages [2]. Under laboratory conditions, each female adult can lay 12 to 345 eggs [7-10]. The eggs were always laid in fruits, as well as on leaves and twigs of trees, rarely on petioles or stems because of their oviposition behaviours [5]. Therefore, the egg mortality is low (4.7-6.9%) in integrated control orchards due to unviability to nature enemies and other animals [5], causing serious fruit dropping and rotting by the larvae feeding on fruits [11], (Du et al. 2012). The morphological characteristics of reproductive systems are of significance to predict the emergence period and population trend, and to determine the optimum timing for pest control and to identify species [12-16], especially in the cases of Lepidoptera pests, such as *Grapholita molesta* [17], Palumbina spp. [12], *Spodoptera exigua* [12]. Besides, insect genitalia are often species-specific, and their structures are often more divergent among closely related species than other structures such as legs, antennae, and eyes [18], which make genitalia more useful in distinguishing closely related species [18].

Allman (1930) and Wiesmann (1935) [19, 20] described the gross morphology of the external and internal reproductive systems of C. pomonella females. Williams (1941) compared the structure of the codling moth spermatophore with those of other Tortricoidea species. Ferro and Akre (1975) [4] illustrated both female and male reproductive systems of the codling moth *Laspeyresia pomonella* [4]. Since then, mostly studies have focused on their chemical control. There are very few reports about the reproductive characters of this species. Until 2018, the process of egg maturation and classification of ovary development in the codling moth were described and reported [21]. In the present study, the C. pomonella reproductive systems from a Chinese codling moth population in Gansu Province were dissected. The morphology of each parts was described for the first time in detail. The study aims to provide morphological information for identification and support for the prevention and prediction of the codling moth.

Methods and Materials

Codling moth colony

The codling moth *Cydia pomonella* was collected from an abandoned apple orchard in Wuwei City, Gansu Province of China. The larvae were reared in fruits, the emerged adults were reared with 10% honey water in Laboratory under the condition of 25±1°C temperature, 75±5% relative humidity and a photoperiod of 16h light: 8h dark.

Reproductive system dissection and observation

The 1-2 d old female and male adults were used. The inner organ was prepared by putting the abdomen in Bouin's fluid for 30 minutes in a centrifuge tube, and then kept in Ringer's saline for dissection. The genitalia were treated by boiling with 10%NaOH for 5-10 min to remove all soft tissue, and then kept in 75% alcohol ready for observation.

The reproductive system was observed under Zeiss SteREO Discovery V12 stereoscopic microscope. The photographs were taken with Carl Zeiss CCD AxioCam MRc 5 digital cameras. All images were processed by using Adobe Photoshop CS6.

Results and Analysis

Male internal reproductive organs

The male internal reproductive organ consisted of a testis (Figure1: A-1), a pair of seminal vesicles (Figure1: A-2), a pair of vas deferens (Figure1: A-3), a ductus ejaculatorious duplex (Figure1: A-4) and a ductus ejaculatorious simplex (Figure1: A-5), a pair of accessory glands (Figure1: A-6). The testis is the main sperm productive structure and formed by two testicular follicles enveloped in the lavender adventitia. The seminal vesicles are constricted in the middle and swollen at apex. The vas deferens are the access to ductus ejaculatorious duplex for sperms. The ductus ejaculatorious simplex can be divided into four sections: the first section is transparent, thick and short; the second section is translucent, as thick as the first but longer; the third section is transparent and the longest one; the fourth section is narrowed at the base and then distorted at the connecting section with the aedeagus.

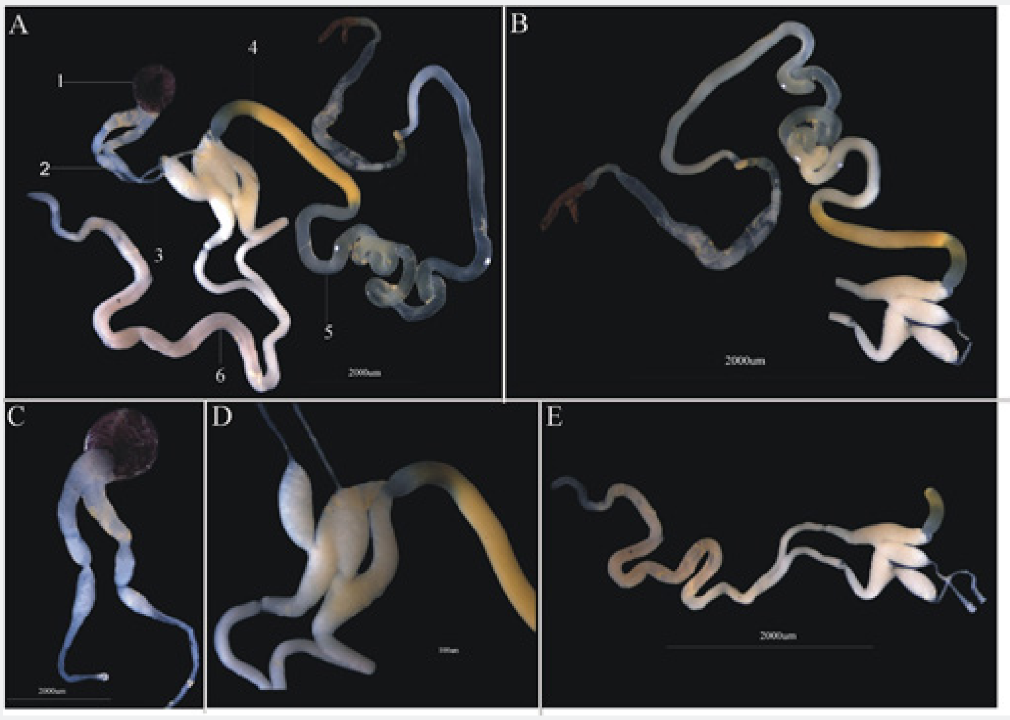

Figure 1: Male internal reproductive system of Cydia pomonella (L.). A. male internal reproductive organ in dorsal view; B. enlargement of ductus ejaculatorious simplex (A-5); C. enlargement of testis and seminal vesicles (A-1 and A-2); D. enlargement of ductus ejaculatorious duplex (A-4); E. enlargement of accessory glands (A-6). 1. testis; 2. seminal vesicle; 3. vas deferens; 4. ductus ejaculatorious duplex; 5. ductus ejaculatorious simplex; 6. accessory gland.

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Male external reproductive organs

The male genitalia mainly consist of a pair of valvae (Figure 2A-6 and B), a pair of aedeagus (Figure 2A-3, D and E) and other accessory structures. The valvae are ear shaped. The end of the valvae (Figure 2: A-6) is round, densely covered with thin and irregular hairs and finger like ventral process (Figure 2: A-7). The uncus (Figure 2: A-5) is short. The aedeagus is cecum-like with strong sclerotization and seven cornuti dorsally. Five of the cornute are close to the apex and the other two are in the middle (Figure 2: E). The juxta (Figure 2: F) is V-shaped and membranous.

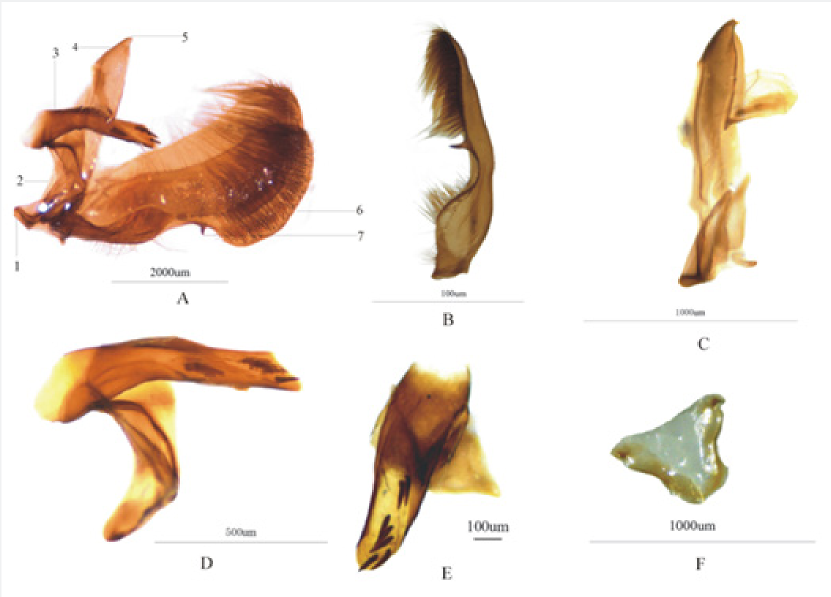

Figure 2: Male external reproductive organs of *Cydia pomonella* (L.). A. male genitalia in lateral view; B. left valva in lateral view (A-6); C. a ring in lateral view; D. aedeagus in lateral view (A-3); E. aedeagus in dorsal view (A-3); F. juxta in ventral view. 1. saccus; 2. vinculum; 3. aedeagus; 4. tegumen; 5. uncus; 6. valva; 7. vental process.

Female internal reproductive organ

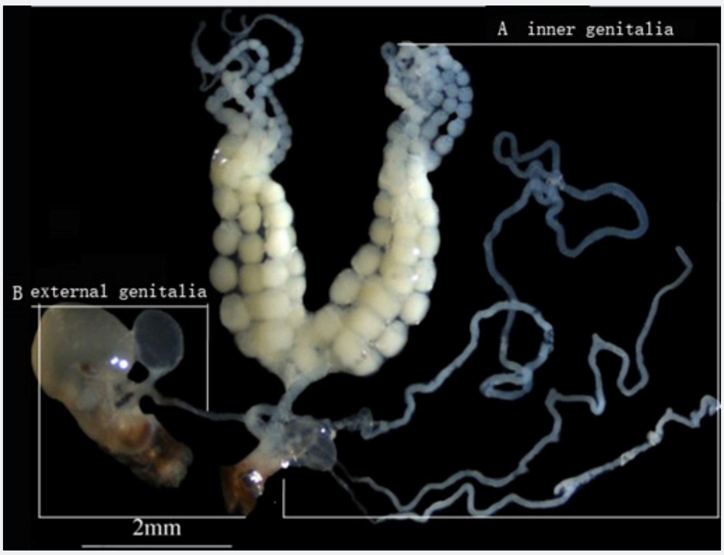

Figure 3: Female reproductive system of Cydia pomonella (L.). A. Female internal reproductive organs; B. Female external reproductive organs.

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The female internal reproductive organ (Figure 3A) consists of a pair of ovaries (Figure 4: A-2), a pair of lateral oviducts (Figure 4: A-4), a common oviduct (Figure 4: A-5), a bursa of accessory glands (Figure 4: A-8), accessory glands (Figure 4: A-9), a spermathecal (Figure 4: A-10) and a spermathecal gland (Figure 4: A-11). The ovaries are symmetrical, and each ovary has four polytrophic ovarioles. The ovarioles twin together through tracheoles, merge at the base, then incorporate into lateral oviducts. The common oviduct is thin and straight initially, becomes widen and short afterward. The ovarioles (Figure 4: A-3) are divided into four parts: terminal filament (Figure 4: A-1), germarium, vitellarium and pedicel. The two accessory glands are connected with the bursae of accessory glands.

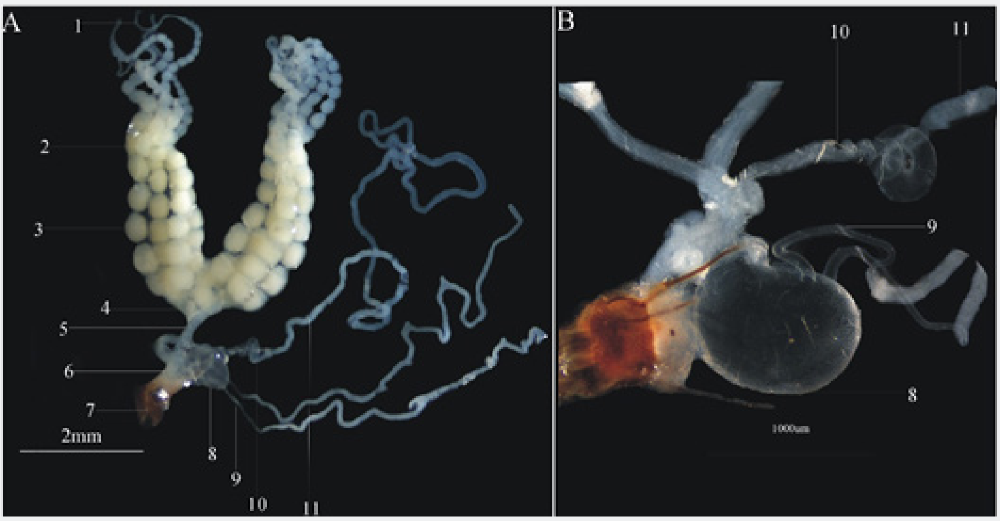

Figure 4: Female internal reproductive organs of Cydia pomonella (L.). A. female internal reproductive organs in dorsal view; B. enlargement of bursae of accessory glands. 1. terminal filament; 2. ovary; 3. ovariole; 4. lateral oviduct; 5. common oviduct; 6. genital chamber; 7. oviporus; 8. bursae of accessory glands; 9. accessory gland; 10. spermathecal chamber; 11. spermathecal gland.

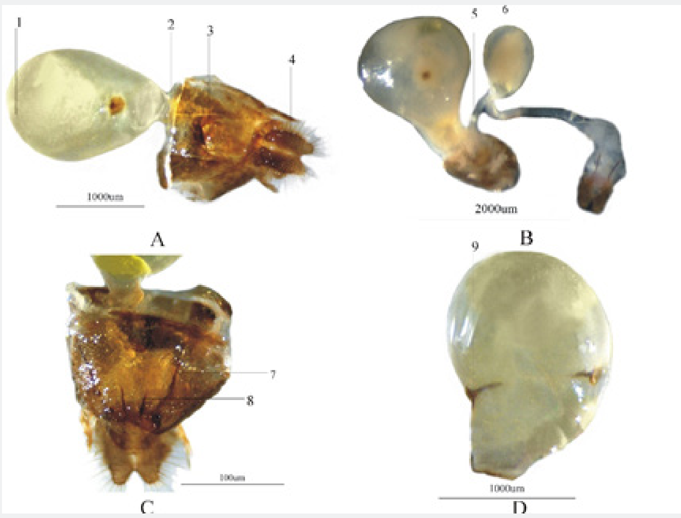

Figure 5: Female genitalia of Cydia pomonella (L.). A. female genitalia in ventral view; B. enlargement of ductus seminalis and antheridium; C. enlargement of base in dorsal view; D. enlargement of corpus bursae and signa in ventral view. 1. corpus bursae; 2. ductus bursae; 3. ostium bursae; 4. papillae anales; 5. ductus seminalis; 6. antheridium; 7. apophyses anteriores; 8. apophyses posteriores; 9. signum.

Female external reproductive organs

The female external reproductive organs (Figure 3B) consist of a corpus bursa (Figure 5: A-1), a ductus bursa (Figure5: A-2), papillae anales (Figure5: A-4), a ductus seminalis (Figure5: B-5), antheridium (Figure5: B-6) and accessory structure, apophyses anteriores (Figure5: C-7), apophyses posteriores (Figure5: C-8), and two horn-shaped signa (Figure5: D-9). The ductus bursae (Figure 5: A-2) is short, the ostium bursae (Figure5: A-3) is flat

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and broad. The length of apophyses anteriores is longer than that of apophyses posteriores. Both anus and oviporus have an opening between the papillae anales. The antheridium (Figure5: B-6) is oval and milky and located in the section of the ductus seminalis.

Disscussion

The morphological differences of insect reproductive systems mainly lie on the shape and number of ovary and testis [18, 22], the presence of antheridium, the shape of corpus bursae and signum. In our study of the codling moth, the two testicular follicles are closely connected to each other like a single organ and enveloped in the lavender adventitia, which is similar to those of most species in Lepidoptera [23-26], but inconsistent with the structure of *Heliothis zea* [27,28] and *Papilio xuthus* [29]. Moreover, the corpus bursae of the codling moth have a pair of horn-shaped signa instead of cysticerci, but the corpus bursae have cysticerci in *Pyrausta nubilalis, Catopsilia Pomona* and *Colias fieldii* [25, 26, 30]. In addition, some species such as Lipographis and Etielloides have no signa [31,32]. Wiesmann (1935) [20] illustrated the accessory gland reservoir being bifurcate, but no reservoirs were bifurcate in our study and nor in Ferro's sample [4].

In addition, the aedeagus of the codling moth has 7 cornuti, among which five are close to the end and two are in the middle. But Ferro (1975) [4] described 6 large cornuti (spines) that radiate from the endophallus and evert from the aedeagus into the female cervix bursae during copulation. It is probably due to different geographical populations or may be because of intraspecific variations. Moreover, in the process of sexual maturation, we observed that an antheridium is formed in the section of ductus seminalis between corpus bursae and ostiumbursae. Originally, the antheridium is small and transparent, then it becomes large, milky color, and finally turn into large, yellowish color. This feature can also be found in those species in the genus Eurema but missing in other genera of subfamily Coliadinae [26]. In the present study, we have clarified the structural characteristics of both internal and external reproductive organs of the codling moths of a Chinese population. We will conduct the spermatogenesis in the near future in order to study futher the reproductive isolation mechanism and reproductive biology of the codling moth, and to provide more guidance for integrated control them [33-36].

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Notes

All authors declare no conflicts of interest.

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