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# 交配后扶桑绵粉蚧雌成虫卵巢结构及 其他相关结构的变化

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摘要:利用显微光镜和超微电镜技术观察明确了扶桑绵粉蚧雌成虫卵巢及相关结构的变化,结果表明该虫的内生 殖系统含1对卵巢、输卵管、储精囊和1对附腺。每个卵巢有数以百计的端滋式卵巢小管。卵巢小管缺末端纤丝, 包含滋养部分和卵黄部分。储精囊表面被丰富的肌原纤维包裹,未经交配的雌虫体内储精囊在显微镜下呈透明状 圆形,电镜观察囊内只含液态物质;交配后,储精囊不再维持规则的球状,囊内出现精细胞等物质;精细胞呈典 型的 "9+2"结构。在初孵化的雌性成虫体内,卵巢小管内的滋养细胞部分中间为营养核,以此联接滋养细胞和 卵细胞。卵黄部分包含1个卵细胞。雌性成虫只有通过交配,卵巢内的胚胎才可得以顺利发育;若未经交配,卵巢 内的卵细胞将出现发达的内质网结构,标志着细胞将降解而被母体重吸收。

关键词:扶桑绵粉蚧;雌成虫;生殖系统;结构

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# Structure of ovaries and changes in the reproductive components of female *Phenacoccus solenopsis* adults after mating

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**Abstracts**: Structure of ovaries and changes in reproductive components of female *Phenacoccus solenopsis* were studied using light microscopy and transmission electron microscopy. Reproductive system of female *P. solenopsis* was composed of a pair of ovaries , a common oviduct , a spermatheca and two pairs of accessory glands. Each ovary was composed of approximately hundreds of telotrophic ovarioles. The ovariole was devoid of terminal filaments , and was subdivided into an apical tropharium and a vitellarium. Spermatheca was surrounded by a network of myofibril. Before mating , the spermatheca was round and translucent in the microscopy observation; nothing except liquid substance was observed under TEM. Once mated , the round shape could not be supported , and the sac was full – filled with substances proposed from the males involving with sperms. Sperms of *P. solenopsis* had a typical characteristic "9 + 2" structure. In the newly emerged female adults , the center of the tropharium was occupied by a trophic core , through which trophocytes and oocytes were connected. The vitellarium contained one oocyte. Only if the females were mated , sperms in the sac of the spermatheca , and embryogenesis in the ovariole scould be observed; otherwise , endoplasmic reticulum in the inner layer of the ovariole developed well , which was proposed to

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absorb the substance in the degenerated oocytes.

Key words: Phenacoccus solenopsis; amphigenesis; spermathecae; reproductive system

*Phenacoccus solenopsis* (Hemiptera: Coccoidea: Pseudococcidae) has recently been one of the most focused coccids since it broke out in Pakistan and India (Dhawan *et al.*, 2007; Prasad *et al.*, 2012; Kumar *et al.*, 2014). *P. solenopsis* is known to the world as a cotton pest, but is recorded from more than 100 host plants in 27 families (Abbas *et al.*, 2010). It is an important pest threatening agriculture and horticulture in its zoogeographic regions (Wang *et al.*, 2010).

Due to its economic importance , the biology of P. solenopsis has been studied (Vennila et al., 2010; Zhu et al., 2011). Same as other members of the superfamily Coccoidea, P. solenopsis has distinct sexual dimorphism (Huang et al., 2013). Eggs are laid singly and hatch within 1 - 3 h into first instar crawlers. The mobile crawler even since the first instar has behavioral and morphological adaptions for dispersal (Zhu et al., 2010). From the late first instar stage onwards, sexual dimorphism is exhibited by showing distinct morphological differences between females and males. Females undergo hemimetabolous metamorphosis: the eggs hatch into crawlers; nymphal characteristics has been remained from the 1<sup>st</sup> moult till the adult emergency, only growing in size into a globular, sedentary stage. The males undergo complete metamorphosis: the eggs hatch into the 1<sup>st</sup> instar crawlers; the second instar crawlers spin a white , silky cocoon inside which crawlers enter into pupal stage; and finally develop into white-winged adults. The adult male is a weak flier with short-lived surviving for 3 -5 days. The numerical paucity in visual displaying of adult males, comparing to females and crawlers in a population, lead to the conclusions that P. solenopsis is parthenogenetic or facultative parthenogenetic (Vennila et al., 2010).

Facultative parthenogenesis is typically amphimictic; but known in Hymenoptera and Hemiptera , unmated females may produce some viable offspring by thelytoky (Normark , 2003). Conflicting comments concerning whether a mealybug species is facultative parthenogenetic often occur. Mealybug species, such as *Planococcus citri*, *Formicococcus njalensis*, *Ferrisia virgata* (three above mealybugs in Padi 1997), and *Planococcus vovae* (Francardi & Covassi, 1992), are firstly reported to be facultative parthenogenetic, but their reproductive modes are later doubted based on observation of no progeny produced by unmated females (da Saliva, 2010).

Several authors state *P. solenopsis* comprises both sexual and parthenogenetic lineages (Vennila *et al.*, 2010; Sahito *et al.*, 2010). However, some researchers insiste *P. solenopsis* should be obligate sexual reproduced (Aheer *et al.*, 2009; Prasad *et al.*, 2012). Our previous studies reveale that female of *P. solenopsis* lays eggs only after mating, and its oviposition behavior is dynamic at the level of egg load, responding to variation in ovarian development which is highly correlated to female's copulation age (Huang *et al.*, 2013). To reinforce and confirm *P. solenopsis* should be of gamogenesis, structure of ovaries and changes of the reproductive tracts after mating were investigated, focusing on the structural alteration of spermatheca.

## **1** Materials and methods

## 1.1 Insects

Solenopsis mealybugs, *P. solenopsis*, were originally collected from *Hibiscus syriacus* L. in Hangzhou, China, and reared on cotton, *Gossypium hirsutum* L., in an incubator at  $27^{\circ}$ C ± 1 °C and 65% -75% relative humidity (RH) under a photoperiod of 12:12 (L:D) h.

Mealybugs for tests were single-reared as described in our previous study (Huang *et al.*, 2013). Two days after the female adult emerged, a male adult was introduced; a > 5 sec copulation was considered as a successful mating.

## 1.2 Microscopy observations

For light microscopy observation , pairs of ovaries were dissected from female mealybugs at nymphal

(early and late  $2^{nd}$  and  $3^{rd}$  instar nymph) and adult (unmated and mated adults) phases. P. solenopsis were rinsed in 75% ethanol for 5-8 sec to remove waxes over the body surface, dried at room condition, and then used for dissection in 0.1 M phosphate buffer (pH7.4). Dissected ovaries or spermatheca were observed under a Nikon SMZ 1500 microscope (www. nikon. com) equipped with a Nikon digital sight DS-L1 camera or an Olympus BX 51 microscope (www.olympus - global.com) equipped with a QImaging Micropublisher 5.0 RTV camera ( www. qimaging. com) .

## **1.3** Ultrastructure observations

Female reproductive system were dissected in 0.1 M phosphate buffer (PBS, pH7.4) and immediately fixed in 2.5% glutaraldehyde at 4°C for 24 h, rinsed in PBS, and then postfixed in 1% osmium tetroxide. After dehydration in a graded series of ethanols and acetone, the material was embedded in epoxy resin Epox 812 (Fullam Inc., Latham, N. Y. , USA). Target scenes involved with oocytes or spermatheca were positioned through checking in semithin sections (0.7 mm thick), which stained with 1%methylene blue in 1% borax. The positioned part was then cut into ultrathin sections (90 nm thick) by a Reichert-Jung Ultracut E microtome ( Leica, German), and examined in a JEM 100 SX EM at 60 kV (Nec, Japan).

#### 2 **Results**

## 2.1 Gross architecture of the female reproductive

The female reproductive system of P. solenopsis consisted of a pair of ovaries, a spermatheca and a pair of accessory glands (Fig. 1). Ovaries were individually connected to a pair of lateral oviducts, which joined to form a median oviduct opening posteriorly into a genital chamber. Opening from the chamber was the spermatheca for storing sperm through copulation, and a pair of accessory glands. There was no apparent expansion part of the oviduct, which commonly termed as calyx. Each ovary had hundreds of ovarioles (Fig. 2).



Fig. 1 Schematic representation of female reproductive system of Phenacoccus solenopsis (  $Bar = 100 \mu m$ )



Fig. 2 Schematic representation of the ovary of mated females of Phenacoccus solenopsis

## 2.2 Spermatheca

Spermatheca inserted at the anterior end of the median oviduct (Fig. 1). A network of myofibril surrounded on its surface (Fig. 3). Before mating , the spermatheca was round and translucent in the microscopy observation; nothing except liquid substance was observed under TEM (Fig. 4). Ultrastructural observations showed that spermatheca sac wall was composed by columnar epithelial cells (Fig. 5A). The epithelial cells contained abundant mitochondria and some endoplasmic reticulum, but Golgi apparatus (Fig. 5A, C). Once mated, the round shape could not be supported , and the sac was full filled with substances proposed from the males involving with sperms (Fig. 5). Sperms of P. solenopsis had a typical characteristic "9+2" structure, two central singlet microtubules were encircled by nine outer doublet microtubules (Fig. 5B).

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Fig. 3 Light microscopy of spermatheca with numerous myofibril surrounded ( Bar = 100  $\mu$ m) and transmission electron microscopy of the cross section of the myofibril ( Bar = 0.1  $\mu$ m)



Fig. 4 Ultrastructure of spermatheca in unmated female adult of *Phenacoccus solenopsis* (Bar = 5  $\mu$ m)



Fig. 5 Ultrastructure of spermatheca in mated female adult of *Phenacoccus solenopsis* 

A , spermatheca involving with sperms , Bar = 1  $\mu m;~B$  , enlarged view of details in the dashed box in A , Bar = 0.2  $\mu m;~C$ , reservoir wall of spermatheca , Bar = 2  $\mu m.$ Lu , lumen; RER , rough endoplasmic reticulum; Mt , mitochondria.

## 2.3 Development of the ovary

In newly emerged adult , the cystocytes on the oviduct protruded from the ovary surface into the body cavity forming ovarioles. Ovariole was teardrop–shaped , and terminal filaments were absent; vitellaria ( tip of the teardrop) and tropharia ( transparent and spherical with a diameter of 15 – 25  $\mu m$ ) could be distinguished ( Fig. 6A) . In the vitellaria , pre-oocyte was surrounded by follicle cells ( Fig. 7A) , and in the tropharia , several trocytes were clustered ( Fig. 7B) .



Fig. 6 Ovaries of *Phenacoccus solenopsis* at 1 , 5 , 10 days after adult emerged ( $Bar = 200 \mu m$ )

As a result of cystocyte differentiation , the oocytes expanded ( ellipse-shaped with a major axis of

 $30-50~\mu m$  and a minor axis of  $25-40~\mu m$ ) with trophocytes (spherical with a diameter of  $20-45~\mu m$ ) attached at the apex in the following 5 days (Fig. 6B). At this stage , center of the tropharium was occupied by a cell-free region termed the trophic core (Fig. 8) , through that trophocytes and oocytes were connected.



Fig. 7 Ultrastructure of cystocyte in the ovaries of newly-emerged *Phenacoccus solenopsis*A, pre-stage of oocyte surrounding by follicle cells; B, prestage of trophocyte (Bar = 2 μm)



Fig. 8 Trophic core (  $Bar = 1 \mu m$ ) in the center of the tropharium (  $Bar = 5 \mu m$ )

Ten days post-emergence, oocytes expanded drastically with a major axis of  $180 - 220 \ \mu\text{m}$  and a minor axis of  $100 - 120 \ \mu\text{m}$ ; while trophocytes was still in a spherical shape with a diameter of about 30  $\mu$ m (white arrows in Fig. 6C). At this point of time, if mating succeeded previously, oocytes

continued to expand and trophocytes atrophied; otherwise, the oocytes would atrophy. In the following time under the former situation (i. e. in the mated females), embryo began to develop, and oocytes coexisted with embryo in every stage (Fig. 9). Once an egg was ovulated, an empty sac could be observed; and the sac gradually contracted to a small dense plug attached to the oviduct (Fig. 9).



Fig. 9 Inner reproductive system of *Phenacoccus solenopsis* females who have laid eggs (Bar = 200 μm)

## 2.4 Development of the oocyte/embryo

In mated females, oocytes arised and expanded drastically in 3-8 days after adult emerged (Fig. 10A -B). The developing oocytes nuclei were spherical, enclose decondensed chromatin and single nucleoli. The oocyte developing in the vitellarium was encompassed by a single layered follicular epithelium; and the cytoplasm was filled with ribosomes, mitochondria and endosymbionts (Fig. 11). Near the collapse of the trophocytes, an egg underwent subdivision (Fig. 10B). In the following 2 days, an obvious serosal membrane could be observed under light microscopy, and then gastrulation proceeded (Fig. 11C). Gastrulation duration lasted about 1 -2 days and was followed by segmentation (Fig. 10D), which was complete after another 1 - 2 days. When abdomen segments (arrow1 in Fig. 10E) and legs (arrow3 in Fig. 10F) were completely formed, compound eyes in red color were shown (Fig. 10F).

In umated females , oocytes in the ovariole degenerated. Endoplasmic reticulum in the inner layer

of the ovariole developed well to absorb the substance stored in the oocytes ( Fig. 12) .



Fig. 10 Phenacocccus solenopsis oocytes and embryo ( $Bar = 50 \mu m$ )



Fig. 11 Ultrastructure of Phenacocccus solenopsis oocytes with a layer of follicle cells. ( Bar = 2  $\mu m)$ 



Fig. 12 Ultrastructure of ovariole in unmated female *Phenacocccus solenopsis* (Bar =  $10 \mu m$ )

## 3 Discussion

Nucleic acids and ribonucleoproteins are known as the two major classes of stored compounds that support the embryogenesis (Berry, 1985). If these compounds are synthesized by trophocytes that connect to the oocytes, the ovary is classified as meroistic; if trophocytes were absent, the ovary is of phanoistic type. In meroistic ovaries, two subtypes, polytrophic and telotrophic, are divided according to location of trophocytes. In polytrophic ovaries, the trophocytes are included within the follicle; while in telotrophic ovaries, the trophocytes are attached to the oocytes by a long cellular process at the distal end (Blum, 1985). Judged by Blum's principle of classification, ovary of P. solenopsis is of the telotrophic ovaries, which is suggested as one of the fundamental ovary characteristics in scale insects (Szklarzewicz, 1998a).

Previous researches reported that in some studied scale insects, for example, Nipaecoccus nipae (Szklarzewicz, 1998a), Cryptococcus (Szklarzewicz, 1998a), Newsteadia floccose (Szklarzewicz, 1998b) and Orthezia urticae (Szklarzewicz, 1998b), at the beginning of the ovariole differentiation ( usually in third instar larva), female gonads were usually composed of two spindle shaped ovaries, which were surrounded by peritoneal sheath and filled with cluster of germ cells (cystocytes). Cluster was formed in a rosette, which was regarded as ovariole anlage; cystocytes of the anlage then protruded into the body cavity to form ovariole. However, female gonads of P. solenopsis were not in a spindle shape due to the lack of the peritoneal sheath, but arranged in an irregular coarse strip shape.

Before vitellogenesis, fully developed ovaries of female *P. solenopsis* are similar, both in structure and functioning, to those of other studied Sternorrhyncha insects, for example, *Newsteadia floccose* (Szklarzewicz, 1998a), *Orthezia urticae* (Szklarzewicz, 1998b), and aphids (Tionnaire *et al.*, 2008). As known in aptery adult aphids, occurrences of oogenesis and/or embryogenesis between in asexual and sexual females are different, in which the key point is whether syngamy occurs (Miura et al., 2003). For the components in the reproductive tract system, the sexual female ovaries additionally possess spermathecae and accessory glands (Tionnaire et al., 2008). It is demonstrated that during the formation of the germarium of the future sexual female aphids, the future oocytes remain blocked in metaphase I (Blackman, 1976); while in unmated P. solenopsis, oocytes development blocked in stage II (Huang et al. , 2013). Embryogenesis in both of them occurred until the fertilization of the fully grown oocyte. It is suggested whether the following choriogenesis ( as the beginning of embryogenesis) in P. solenopsis occur after vitellogenesis is closely associated with the contents in the spermatheca. Thus, the existence of spermatheca could be regards as a mark and/or a guarantee for the sexual-reproduction type in P. solenopsis.

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