

## A Histological and Histochemical Investigation on Oocyte Development in *Gryllus bimaculatus* (Orthoptera: Gryllidae)

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**ABSTRACT:** This study investigated the oocyte development in the ovary of two spotted black cricket, *Gryllus bimaculatus*. Oocytes mature through three developmental stages. These stages include (i) previtellogenic, (ii) vitellogenic and (iii) maturation. In previtellogenic stage, oocytes were encircled by squamous follicle cells. In vitellogenic stage, yolk granules within the oocyte were firstly observed just beneath the oolemma. As vitellogenesis proceeds, yolk granules covered the whole oocyte. In addition, small sized lipid droplets were firstly seen under the yolk granules, then spread to oocyte. This time, cubic and binucleated follicle epithelial cells encircled the oocyte. In maturation stage, oocytes were full of large yolk granules. Apart from Hematoxylin& Eosin, Periodic Acid Schiff, Bromophenol Blue and Mallory Trichrome reactions were also demonstrated. According to these three histochemical methods, yolk granules showed positive reactivity. Due to the oosorption, the resorption of oocytes, some atretic oocytes were observed. Apoptosis was also determined. This study indicated that ovary type of *Gryllus bimaculatus* is panoistic as reported in other Gryllid species.

**Key Words:** Cricket, ovary, oocyte, oosorption, reproduction, vitellogenesis

### *Gryllus bimaculatus*'ta (Orthoptera: Gryllidae) Oosit Gelişimi Üzerine Histolojik ve Histokimyasal Araştırma

**ÖZET:** Bu çalışmada iki noktalı kara çekirge, *Gryllus bimaculatus* türüne ait bireylerin ovaryumunda histolojik ve histokimyasal incelemeler yapılmıştır. Oositler üç aşamada olgunlaşır, bunlar: (i) previtellojenik, (ii) vitellojenik ve (iii) olgunluk aşamalarıdır. Previtellojenik evrede oositler yassı folikül epitel hücreleri tarafından kuşatılmaktadır. Vitellojenik evrede yolk granülleri ilk olarak oolemmannın hemen altında gözlenir. Vitellogenez ilerledikçe oositin içini doldururlar. Ayrıca küçük boyutlu lipid damlaları ilkin bu yolk granüllerinin altında bulunur ve daha sonra oositi kaplar. Bu sefer kübik ve çift nukleuslu folikül epitel hücreleri oositi kuşatır. Olgunluk evresinde oositler iri yolk granülleriyle kaplıdır. Histolojik preparatlar Hematoksilen Eosin boyaması dışında Periodic Acid Schiff, Bromophenol Blue ve Mallory Trichrome ile boyanarak incelenmiştir. Bu üç histokimyasal yöntemle yolk granülleri pozitif reaksiyon göstermiştir. Oositlerin resorpsiyonu anlamındaki oosit oosorpsiyonu sebebiyle bazı atretik oositler gözlenmiştir. Ayrıca, apoptozis belirlenmiştir. Bu çalışma *Gryllus bimaculatus* ovaryum tipinin diğer Gryllid türlerinde bildirildiği gibi panoistik tipte olduğunu göstermiştir.

**Anahtar Kelimeler:** Çekirge, ovaryum, oosit, oosorpsiyon, üreme, vitellogenez

### INTRODUCTION

Insect ovaries and the ovarioles are subdivided into two main types based on whether there are nurse cells associated with the developing egg (meroistic ovaries) or no nurse cells (panoistic ovaries) (Nation, 2015). In the panoistic ovary, oocyte itself synthesizes ribonucleic acid and protein through the action of oocyte nucleus. In the meroistic ovary, the nurse cells mostly provide ribonucleic acid together with glycogen and lipid droplets (Wigglesworth, 1974). Plenty of studies investigated the ovary structure in different insect orders, e.g. Trichoptera (Bruce and Colleen, 1995), Isoptera (Gilberto and Milvia, 1999), Dermaptera (Jun-Kong and Joon, 1999), Orthoptera (Stepanova et al., 2007), Hemiptera (Lemos et al., 2010), Diptera (Chou et al., 2012), Coleoptera (Mohamed et al, 2015). Ovaries of Orthopterans are made up of ovarioles of the panoistic type (Nation, 2015). Zeng et al (2013) reported that *Gryllus bimaculatus* ovaries, belonging to order Orthoptera, are panoistic and lack nurse cells. However, until now detailed information on the oocyte development in terms of histological and histochemical

aspects is not present in the ovary of *Gryllus bimaculatus*. From this point of view, this study aimed at the investigation of oocyte development in *G. bimaculatus*.

### MATERIAL and METHODS

#### *Cricket Culture*

*Gryllus bimaculatus*, also known as two-spotted black cricket (Lodos, 1975), specimens were cultured in laboratory conditions (temperature:  $26 \pm 2^\circ\text{C}$ ; relative humidity:  $45 \pm 5\%$ ; photoperiod: natural). They were maintained in the glass jars by feeding twice a week on lettuce and chicken grain. Cotton plugged glass tubes filled with water were put into these jars to supply the water needs of the crickets. In addition, small Petri dishes with their surface covered with moist cotton were placed in the jars to lay eggs of mating females. Upon adult emergence, these virgin female crickets were also used. Totally ten female crickets were dissected in insect physiological saline using a stereomicroscope.

### Light Microscope Analyses

After dissection, ovaries were fixed in Bouin's solution for 24 hours. Following dehydration made by graded ethanol (70%, 96% and, 100%), ovaries were put into xylol for transparency and embedded in paraffin. Tissues were serially sectioned at 5 µm thickness using a microtome (Baird&Tatlock) and stained by Harris Haematoxylin Eosin (HE) to detect general histological view. In addition, Periodic Acid Schiff (PAS) and Bromophenol Blue (BpB) were used to mark neutral mucosubstances (GAGs) and protein in the oocytes, respectively. Histological sections were also stained by Mallory's Trichrome. Sections were examined and photographed with Olympus CX 31 (Tokyo/Japan).

### RESULTS

Histologic analyses indicated that different developmental stages of oocytes were exist together in the same histological preparations. Oocytes mature through three main stages called pre-vitellogenic, vitellogenic and maturation. In Figure 1, it is seen the general view of ovary histological sections stained by different stains including Periodic Acid Schiff (Figure

1a), Bromophenol Blue (Figure 1b), Harris Hematoxylin Eosin (Figure 1c) and Mallory's Trichrome (Figure 1d).

In previtellogenic stage, the oocytes were encircled by squamous follicle epithelial cells. There was a large nucleus in this stage oocyte (Figure 2a).

In the beginning of vitellogenic stage, yolk granules firstly appeared just beneath the oolemma. In addition, small lipid droplets under these yolk granules were determined. This time, binucleated and cubic follicular epithelium encircled the oocyte (Figure 2b). As vitellogenesis proceeds, an increase in the number of yolk granules was observed. Also, larger lipid droplets than the previous stage were present. (Figure 2c). In the mature oocyte, very large yolk granules covered the whole oocyte (Figure 2d). Mature oocytes reacted with Mallory's Trichrome and Bromophenol Blue were seen in Figure 3a and 3b, respectively.

Some oocytes do not develop as mature ones. This phenomenon is known as atresia. In this process, follicle cells became hypertrophic and hyperplasic. Some of these cells gained phagocytic activity and digested the oocyte (oosorption) (Figure 4a). Vacuolization was also determined in some follicle epithelial cells. Apoptotic bodies were observed among follicle cells (Figure 4b).

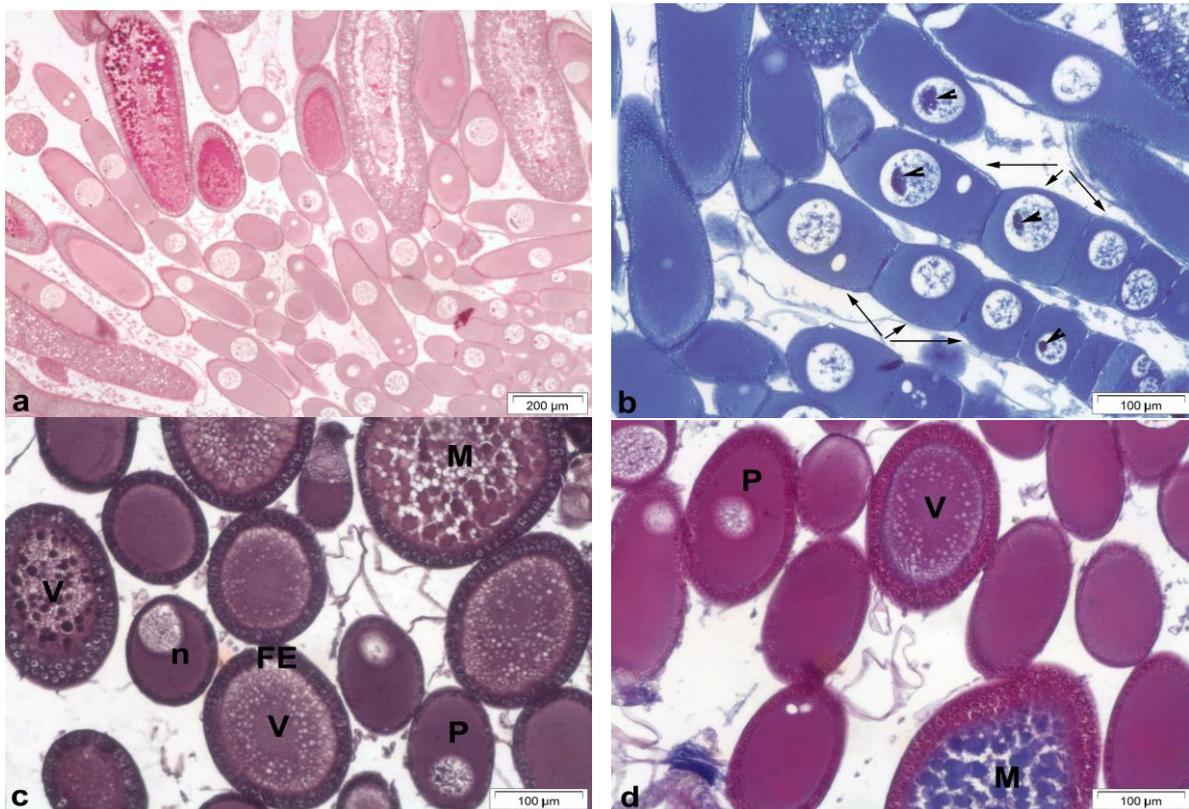


Figure 1. General view of ovary which stained different stains. **a)** Periodic Acid Schiff (PAS), Oocytes at the different stages of oogenesis, **b)** Bromophenol Blue (BpB). Note the successive previtellogenic oocytes lines (arrows) with large nucleus (n) and strongly reacted nucleolus (arrowheads) **c)** Harris Hematoxylin Eosin Stain (H&E), **d)** Mallory's Trichrome Stain (MT), Note the different oocyte developmental stages from previtellogenic (P) and vitellogenic (V) to mature (M); follicle epithelium (FE).

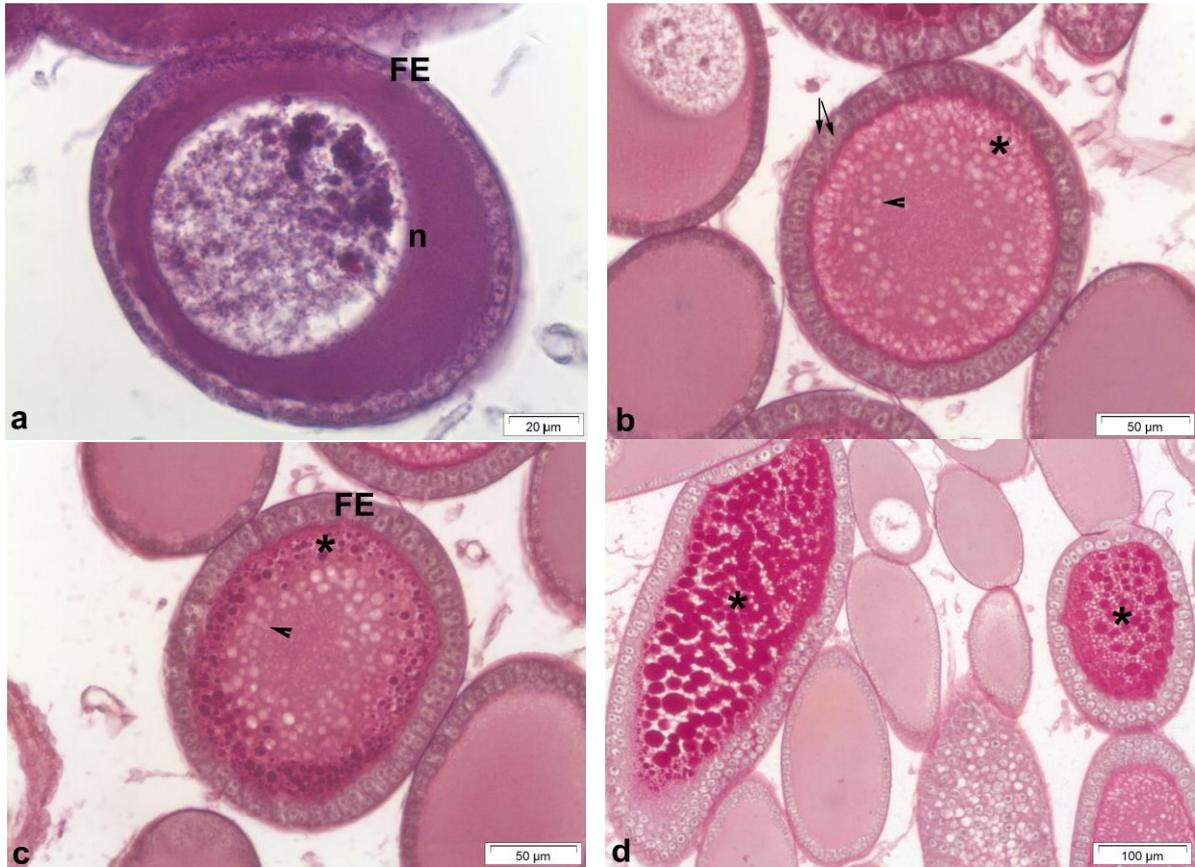


Figure 2. Stages of oocyte development, **a)** Previtellogenic stage. Note the enlarged nucleus (n) and squamous follicle epithelium (FE) surrounding oocyte (H&E), **b)** Early vitellogenic stage. Small yolk granules (asterisk) and small lipid droplets (arrowhead). Note the binucleated (arrows) and cubic follicle epithelium (PAS), **c)** Late vitellogenic stage. Large yolk granules (asterisk) and lipid droplets (arrowhead) (PAS), **d)** Maturation stage. Very large yolk granules (asterisk) covering the oocyte (PAS).

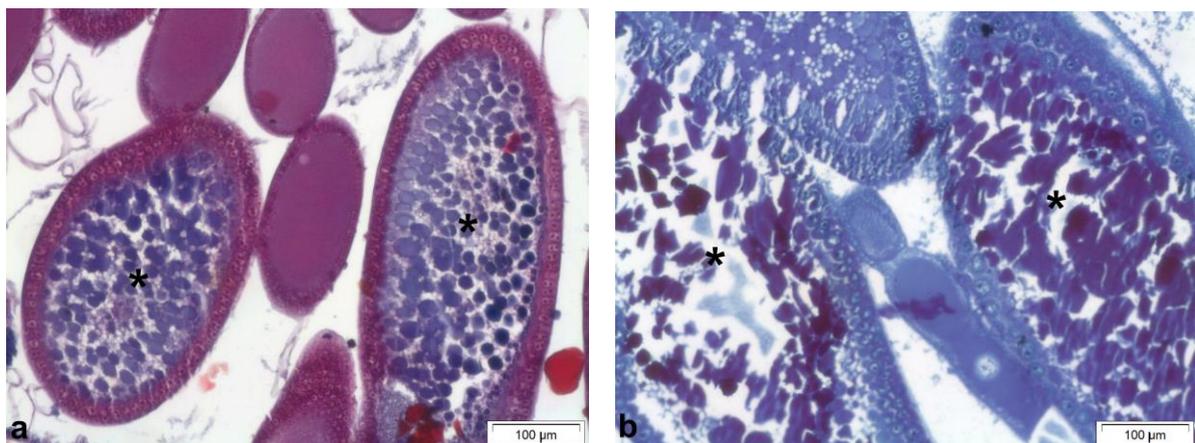


Figure 3. Maturation stage oocytes. **a)** Mallory's Trichrome (MT) stain. Yolk granules (asterisk) staining deep blue, **b)** Bromophenol Blue (BpB) stain. BpB positive materials in the yolk granules (asterisk) coloured blue-purple.

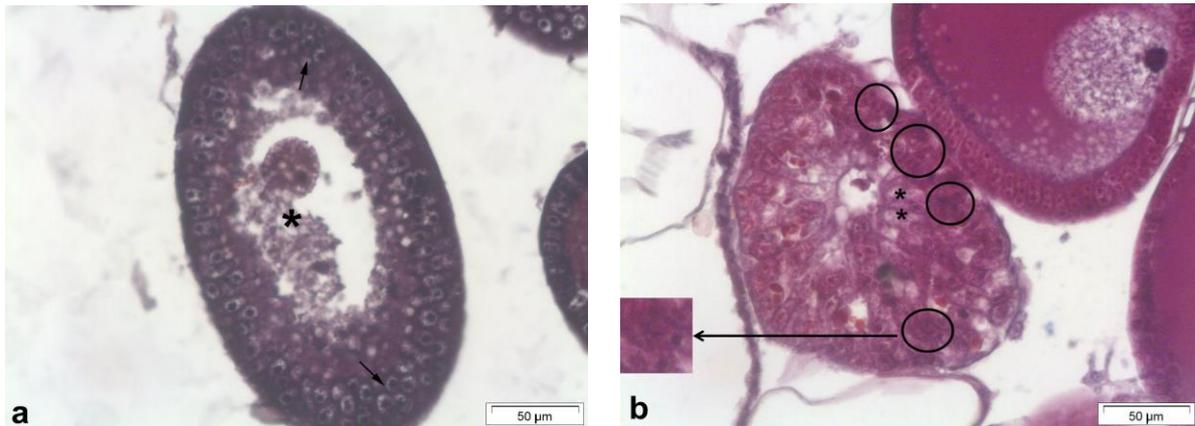


Figure 4. a) Digestion of oocyte (oosorption) (asterisk) by hypertrophic and hyperplastic follicle epithelial cells (arrows), b) Accumulation of the follicle epithelial cells after digestion of oocyte yolk. Vacuolization (asterisk) in some follicle epithelial cells and apoptotic bodies (circular).

#### DISCUSSION

In this study, histological and histochemical investigations on oocyte development in *Gryllus bimaculatus* were performed. Vitellogenesis is the period of rapid oocyte growth in which the yolk deposition occurs. During oogenesis proteins, lipids, carbohydrates and other components are stored in an organized manner. Vitellogenins that are a group of proteins synthesized outside the ovary are the main component of the yolk occurring in the vitellogenesis and stored as vitellin in oocyte (Valle 1993, Postlethwait and Giorgi, 1985; Snigirevskaya and Raikhel, 2005; Fruttero et al., 2011). During maturation, oocytes strikingly increase in size. Different developmental stages of oocytes are found in the ovary of *Gryllus bimaculatus*. Similarly, the ovary of *Rhipicephalus annulatus* is panoistic type with an asynchronous development of oocytes (Kanapadinchareveetil et al., 2015). There are well developed Balbiani bodies adjacent and on opposite sides of the germinal vesicle in the previtellogenic oocytes of the house cricket, *Acheta domesticus* (Bradley et al., 2000). Balbiani body was not determined in the oocytes of two spotted black cricket, *G. bimaculatus*.

In the oocyte of *Gryllus bimaculatus*, lipid droplets were firstly observed beneath the yolk granules which located in the periphery as in the *Lygus lineolaris* (Ma and Ramaswamy, 1990). On the other hand, lipidic granules were firstly seen at the periphery of oocyte in *Amblyomma triste* (De Oliveira et al., 2006).

In many animal species, follicular atresia is considered as a natural event during normal oogenesis (Czarniewska et al., 2014). Some oocytes undergo oosorption (Guo et al., 2011). Kotaki (2006) reported the proteolytic activity of follicle cells during oosorption. Atretic follicles were also determined in the ovary of *Gryllus bimaculatus*. Similarly, these type follicles occur as a result of oosorption. In this process,

follicle cells which became hypertrophic and hyperplastic digested the oocyte yolk. Enlarged follicle cells clearly showed phagocytic activity of these cells. Kotaki (2005) also reported that follicle cells undergo apoptosis in the late stages of oosorption. In *G. bimaculatus*, some apoptotic bodies were detected around follicle cells.

In conclusion, oocyte development is completed through previtellogenic, vitellogenic and maturation stages in *Gryllus bimaculatus* like in many insect species. Results of this study will be a basis to more comprehensive studies related to oocyte maturation process in these crickets.

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