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Arpita Shyam Roy

Insect physiology and
Biochemistry Lab Department of
Zoology Tripura University (a
Central University)
Suryamaninagar, Tripura West,
Pin799022.

Durgadas Ghosh

Insect physiology and
Biochemistry Lab Department of
Zoology Tripura University (a
Central University)
Suryamaninagar, Tripura West,
Pin799022.

Correspondence:

Arpita Shyam Roy

Insect physiology and
Biochemistry Lab Department of
Zoology Tripura University (a
Central University)
Suryamaninagar, Tripura West,
Pin799022, India

Sectioned tissues were deparaffinized with xylene and after re-hydration (100, 90, 70% and distilled water) stained with

Light microscopy revealed chorionic components in *Gesonula punctifrons* (Stal, 1861) (Orthoptera: Acrididae)

Arpita Shyam Roy and Durgadas Ghosh

Abstract

For the detection of the chorionic components and to stress out its secretion from follicle cells, living cell analysis, periodic acid-Schiff's staining and bromophenol blue staining were done. Chorion was detected as a follicular secretion secreted from matured follicle cells and contained good amount of protein and carbohydrate moiety.

Keywords: *Gesonula punctifrons*, chorion, follicle cell, PAS, Bromophenol blue.

1. Introduction

Chorion or eggshell is a follicular secretion and provides protection to the developing embryo. Follicular secretion of chorion in grasshoppers was established earlier [3]. Several investigations had been made by earlier scientists to determine the chorionic components in different insects of different orders including Orthoptera [9]. Chemical properties of eggshell were investigated in *Melanoplus differentialis* through light microscopy [1, 4]. Cuticle like property of chorion was pointed out in *Locusta* and *Melanoplus* by staining methods and light microscopy [8]. Investigation was done in maturation and development of chorion in follicle cell stage, oviduct stage and after laying stage [8]. Cuticular nature of chorion as well as its proteinaceous nature was established by earlier studies in *Drosophila* [5]. Early development and maturation of chorion was investigated in some acridids [6]. Limited information about chemical nature of the grasshoppers eggshell make premises for this study. In this study light microscopy with all its limitations has been used to give information about the chemical nature of chorion in different maturity stages and also confirms follicular origin of chorion in *Gesonula punctifrons*.

2. Materials and Methods

2.1. Egg collection

Gesonula punctifrons (paddy grasshopper) were collected from the paddy fields in and around Agartala city. Mature eggs were dissected out and collected from the mature ovarian follicle, oviduct and after laying eggs were collected just after laying of the eggs before pod formation within rearing jars. These eggs were cleaned in 100 mM Tris-HCl buffer (pH-8) with brush.

2.2. Light microscopy

The ripe ovaries were dissected out by needle and forceps and kept in Ringer's solution. For living cell analysis dissected ovaries taken in ringer's solution were directly observed under LEICA Microscope (DM 1000) and subsequently photographs were taken.

For histochemical studies the dissected ovaries were placed in 4% Formaldehyde in a watch glass overnight for fixation. After fixation ovaries were transferred to distilled water and kept overnight for complete removal of fixative. Then ovaries were transferred in graded series of alcohol (30, 50, 70 and 90%) for one hour in each grade and in 100% for overnight to dehydrate. The dehydrated tissues were cleared in xylene and then transferred in xylene-paraffin mixture (60 °C) for overnight for diffusion of xylene. After that the tissues were transferred to full paraffin for two hours at 60 °C. Then embedding was done in full paraffin and allowed the paraffin to get solid. The prepared blocks were sectioned at 5 µm thickness by using a LEICA rotary microtome (LEICA RM 2125RT).

bromophenol blue and PAS method. Stained sections were viewed under LEICA Microscope (DM 1000) and subsequently photographs were taken.

3. Result and Discussion

3.1. Living cell analysis



Fig 1: Bright field analysis of *G. punctifrons* follicle stage egg in 10X magnification. Follicle cell layer (F), Chorion (C).

In follicle stage egg of *G. punctifrons*, presence of chorion was observed under the follicular epithelium. The thickness of chorion ranged from 23.7 μm to 45.4 μm . In this insect the highest thickness was found in posterior pole but not in the terminal region, the thickness was highest at the both sides of the posterior pole (Fig. 1). Similar property of chorion deposition has been shown found in case of *Oxya* [7]. In anterior pole and other part of the egg chorion showed almost similar thickness. From these observations it appeared that in this stage, chorionic layer had just started to form and the formation and the thickness of the layer was not uniform throughout the egg covering. The pattern of chorion deposition

was also different in different part of the egg in this grasshopper.

In oviduct stage egg, fully formed chorionic layer was observed. The thickness of the chorionic layer varied from 25.1 μm to 234 μm (Fig. 2). The thickness of chorion was discontinuous throughout the egg surface. Thickness of the chorion was higher in the posterior pole and lowest in the anterior pole. With this preliminary observation and little sensitivity of the bright field microscopical technique it has been inferred that the formation of chorion might have started from posterior pole or rate of secretion of chorionic layer by follicle cells was higher in this portion.

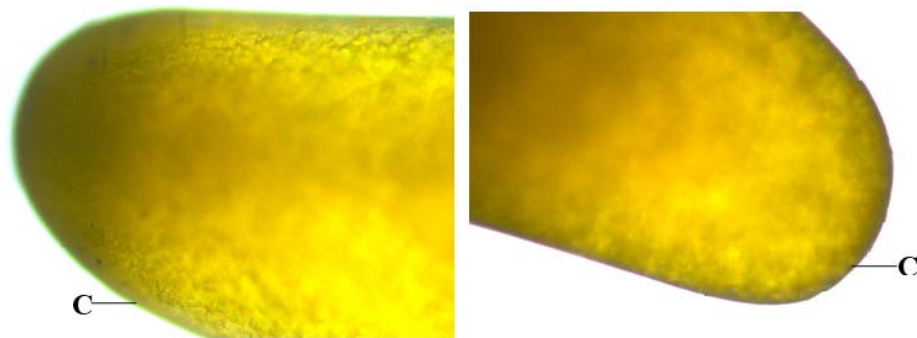


Fig 2: *G. punctifrons* oviduct stage egg in 10X magnification. Chorion (C).

3.2. Periodic Acid – Schiff's staining

In *G. punctifrons*, the immature oocyte had single follicle cell layer and it was almost PAS negative showing a faint pink colour. The thickness of the single layer of follicle cell layer was 86.5 μm to 132 μm (Fig. 3. a, b). No chorionic secretion was observed. Mature follicle cell stage had 2 to 3 layers of follicle cells. This layer had 234 μm -333 μm thickness. Single follicle cell was 89.9 μm to 250 μm tall (Fig. 3. C-f). The

chorionic secretion (C) with pink colour was observed. This result suggested that chorion started to secrete when the follicular cells had their maturity and chorion contained good amount of carbohydrate moiety which was PAS positive and suggested its histochemical nature like cuticle. Cuticle like nature of chorion was established earlier [2, 10].

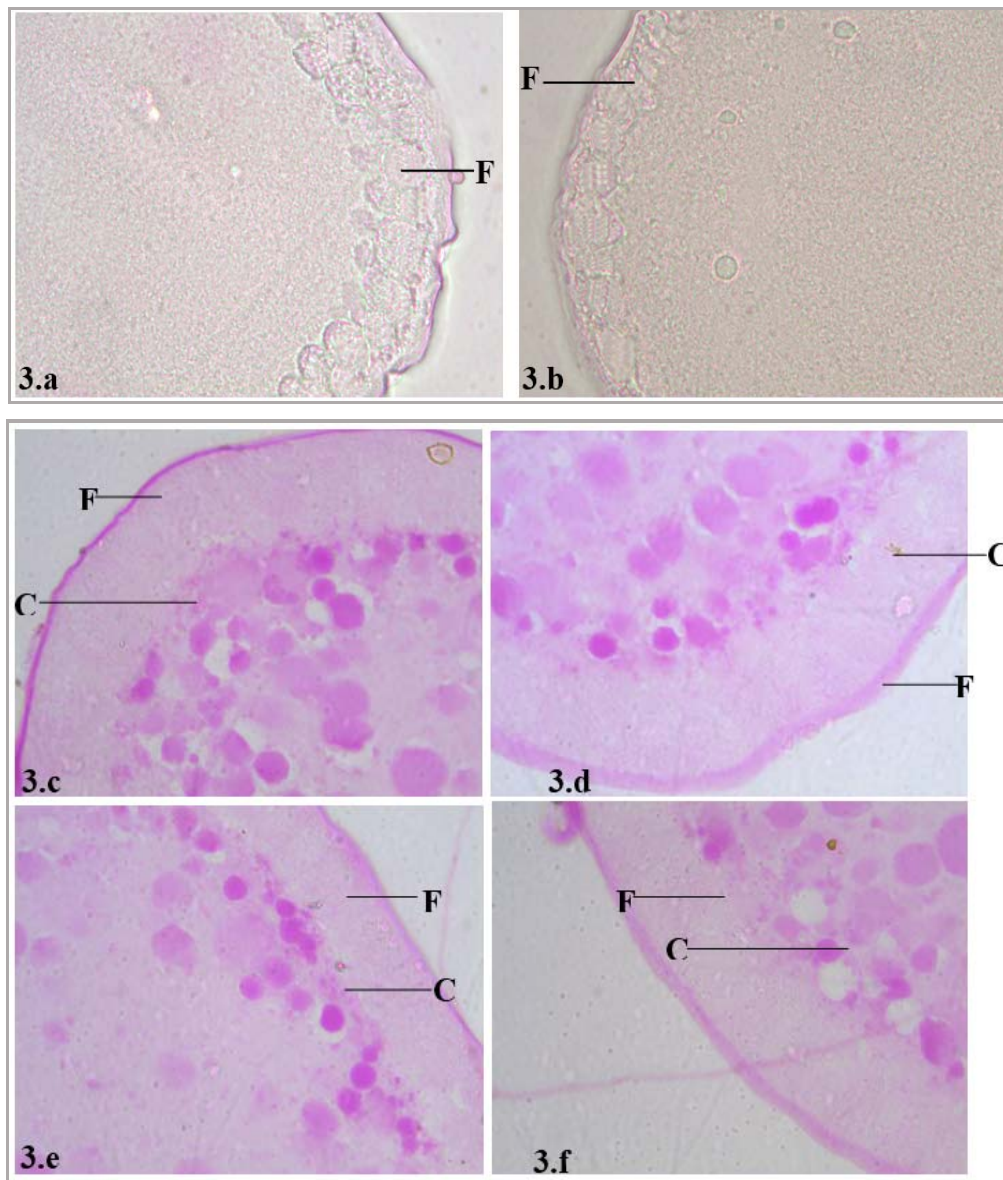


Fig 3: Photographs of PAS positive reaction of the section of the follicle stage egg of *G. punctifrons* taken in bright field 100X magnification. Follicle cell layer (F), Chorion (C).

3.3. Bromophenol blue Staining

Mercurry- bromophenol blue staining was done for observing the presence of proteinaceous compound of the eggshell of different follicle cell maturation stage. It was observed from this staining procedure that the whole section of matured egg was stained deep blue and on the other hand it was comparatively light blue for the sections of immature eggs by this method.

In immature egg of *G. punctifrons*, two layers of follicle cell were found with uniform staining with bromophenol blue and the total thickness of the layer was approximately 307 μm -353 μm . Single layer of follicle cells had 105 μm -245 μm thickness (Fig. 4.a, b). Here minimal amount of secretion of chorionic layer was found having faint blue colour. In the eggs

with higher maturity the follicle cell layer was of 3-5 layers and the thickness varied from 274 μm -464 μm . Single of follicle cell was 201 μm -296 μm in length (Fig. 4. c-f). In this stage at the periphery of follicle cell layer, deposition of chorionic secretion (C) with deep blue stain in a discontinuous manner were observed. Secretion of chorion was investigated by earlier scientist with eosin-haematoxylin staining [8]. From this observation it could be inferred that chorion started to form beneath the follicular cell layers in a discontinuous manner. With this result it was also confirmed that the chorion was proteinaceous in nature and follicular cells started to secrete chorionic layer when these were at their highest maturity state. In *Oxya hyla hyla* similar pattern of chorion secretion was found by the present workers [7].

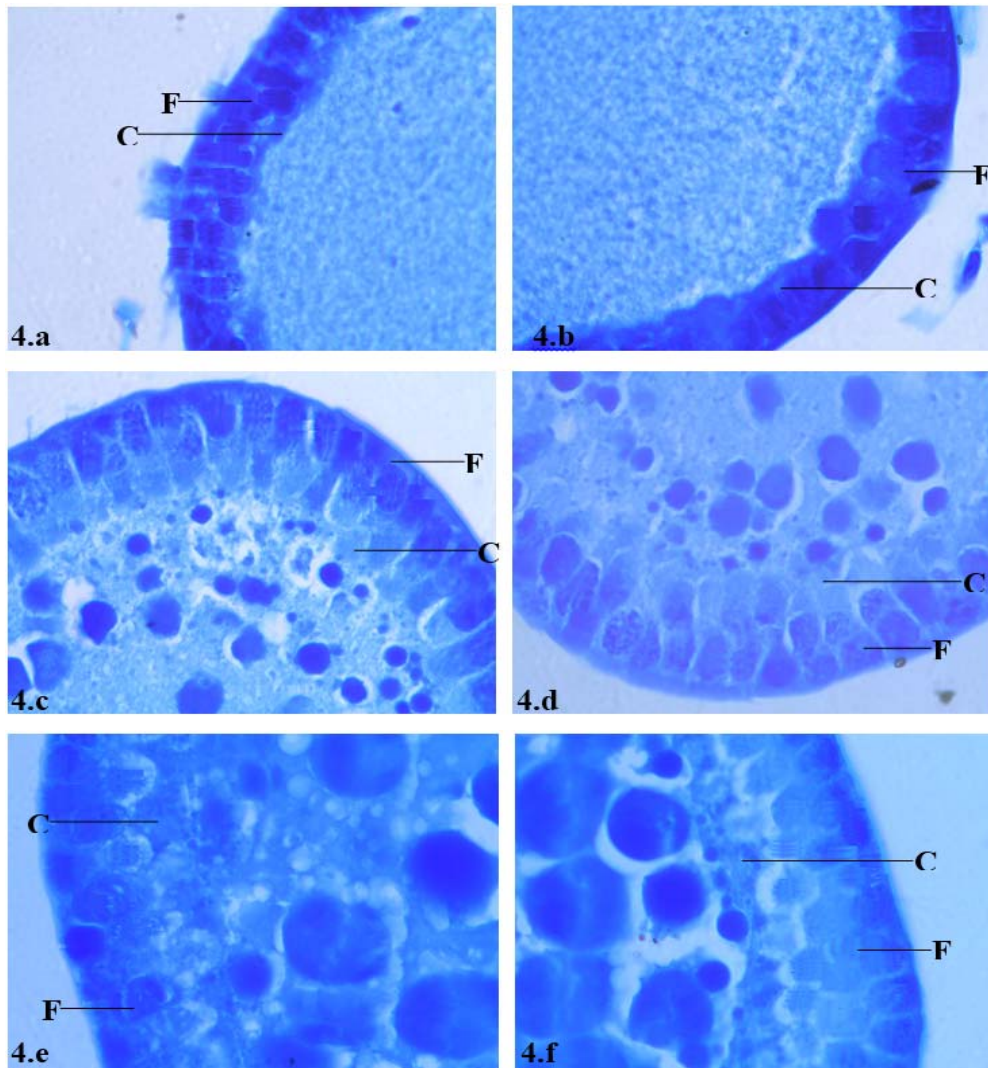


Fig 4: Photograph of Mercurry- bromophenol blue staining of the section of *G. punctifrons* taken in bright field 100X magnification. Follicle cells (F), Chorion (C).

4. Acknowledgements

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