Scanning Electron Microscope (SEM) Studies on the Egg Ultrastructure of Some Wild Silk Moths Collected from Meghalaya, North-East India

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Abstract: ***Scanning electron microscopy of the eggs of nine species of wild silkmoths belonging to the family Saturniidae has been studied. These species include: Samiaricini (Boisduval, 1854), Samiacanningi (Hutton, 1860), Actiasselene (Hubner, 1807), Antheraeaassamensis (Helfer, 1837) A. royleiMoore, 1859, Caligula simla (Westwood, 1847) C. thibeta Westwood, 1853, Leopakatinka Westwood, 1848 and Rhodinianewara Moore, 1872. These eggs of the moths exhibit different colours and shapes and the study revealed the different patterns of structural elements occurring in these silkmoths. The surface of the chorion had a reticulate pattern of pentagonal and hexagonal cells which also differs among the species studied. The present investigation shows oval main cells, aeropyles and micropylar rosette throughout the egg surface.***

Keywords: wild silkmoths, Saturniidae, Scanning electron microscope, chorion

1. Introduction

Insect eggs show a characteristic feature by the presence of an outer shell. This outer shell is secreted by the follicular epithelium during the process of egg formation (Kumar et al 2007). The structure of insect egg shell are quite complex and consists of the vitelline envelop and chorion (Kumar et al 2002). The insect eggs perform functions like allowing air for respiration and also provide strong and elastic mechanical protection to the developing embryo. Another specialised area of the egg chorion called the micropyle is involved in the penetration of sperm (Leuckart, 1855). The aeropyles are present in the form of aeropylar crowns in the area of egg shell edge zone reported in Saturniidsilkmoths. mylitta (Barsagade et al 2009).

The morphology of the egg chorion has been studied by a number of workers (Beament, 1948, Sakaguchi et al 1973, Kafatos et al 1977, Rieger at al 1980). The morphology in different insects depend on the imprints of the follicular secretory cells on specialised regions such as micropyle, aeropylar, stripe and flat regions The structure of shells of the insect eggs are usually quite complex and a typical insect egg capsule was found to consist of the vitelline envelope and chorion (Kumar et al., 2002). The sculpturing of the outer part of the envelope appears to be specific and is of considerable interest because of potential taxonomic applications (Roscizzewska 1991). Further the basic knowledge of insect egg shell and function in particular species of Lepidoptera was provided by Leuckart 1855 and Korschelt 1887. Light Microscopy (LM) and physiological studies of gas exchange and permeability (Beament, 1948), (Wigglesworth and Beamet 1950) preceded a second phase of basic research that began with the establishment of transmission (TEM) and Scanning Electron Microscopy (SEM). The fine structure of a large number of Lepidopteran eggs has been studied by various workers (Matheny and Heinrichs 1972, Mazzini 1974, Downey and Allyn 1980, 1981, Hill 1982, Salkeld 1983, 1984, Fehrenbach et al 1987, Arbogast et al., 1989, Kumar et al., 1999, 2002b, 2003)

Hinton 1969 used scanning electron micrographs to illustrate respiratory systems of various insect eggshells. SEM studies of the egg has revealed the egg surface to be regionally differentiated (Kafatos et al 1977, Margaritis et al 1980) also allowing a better distinction between closely related species than by Light Microscopy (LM) (Arbogast et al 1980).

Studies on the Scanning Electron Microscopy of the surface structure of Lepidopteran eggs provide reliable characteristic features for the separation of different species of Lepidopterans (Arbogast *et al.,* 1989). Matheny and Heinrichs, 1972 studied the external morphology of a wide variety of insect eggs based on the egg chorionic characteristics

2. Materials and Methods

The adult stages of the wild silkmoths were allowed to mate under laboratory conditions. The female moths which then laid the eggs were kept in a separate chamber. The eggs were then collected with the help of a brush, washed thoroughly with distilled water and dried with blotting paper and air.

Processing of Eggs for SEM:
Eggs were fixed in neutral buffer formalin solution for 4-5 days at 40^0 C. It was then dried and secured in brass stubs (10mm dia.X30mm high) and was gold coated in a JFC - 1100 (Jeol) ion sputter (Wooley and Vossbrinck 1977).

The coated samples were examined in JSM-6360 (Jeol) scanning electron microscope at an accelerating voltage of 20kV in the secondary electron emission mode

3. Results

The eggs of *Antheraeahelferi*, *Rhodinianewara*, *Criculatrifenestrata* are flat, ellipsoidal or oval and bilaterally symmetrical along the antero posterior axis. At the time of deposition, the eggs looked either dark brown or brown but on washing slight changes of colour was observed. The shape of the concavity varies from round as seen in the eggs of *Caligula simla*, *Actiasselene* and *Rhodinianewara* to oval as seen in *Caligula thibeta*, *Antheraearoylei*, *Loepakatinka* to elongated as seen in the wild silk moth *Samiacanningi*. Aeropyles can be distinct or not distinct but in some species like *Loepakatinka* and *Rhodinianewara* the aeropyles are not connected. The micropylar pit is visible only in two species of wild silk moth studied i.e the *Actiasselene* and *Antheraeaassamensis* whereas in the case of *Samiacanningi*the micropylar pit is degenerating. In other species, the micropylar pit is not visible during the study.

The whole egg is covered by follicular imprints which are different in different regions such as aeropylar and micropylar region. The scanning electron microscopic examinations of the chorion shows that the follicular imprints are characterised by main cell, aeropyle and intercellular space. The micropyle is at the anterior polar region. It has been observed that the micropylar rosette consists of micropylar openings encircled with unequal petal- shaped cells, followed by a row of secondary petal shaped cells. The rosette of petal shaped cells is followed by the polygonal shell imprints which lack aeropyles and intercellular space (Choudhury and Devi 2013). There is no intercellular space and aeropyles between the petal cells.

Ultrastructural characteristics of the eggs of some wild silk moths are described below:

1) *Actiasselene*: Egg oval, with the micropylar area large located at the sides of the egg attached to the substrate, sculpture is less expressed on micropylar area. It is represented by 6-7 rows of polygonal cells. Micropylar rosette with 10-16 cells. Central portion of the rosette is occupied by deep depression. Large, very distinct aeropyles are present at the cell edges junctions. Fig. 1(a).
2) *Antheraearoylei*: The shape of the concavity is oval with continuous demarcating lines which outline the concavity. In the present study, the aeropyles are not distinct and the micropylar pit is also not clearly visible. Fig. 1(b).
3) *Antheraeaassamensis:*The micropylar structures are at the anterior pole of the oval shaped eggs opposite to its side of distal pole. The central cavity is encircled by a primary rosette of 16-18 cell imprints. The primary petal shaped cell imprints are followed by secondary and tertiary rows of cell imprints. Except the central cavity and micropylar rosette, the entire surface of the chorion is marked by a circular shell imprints which are boarded by aeropyles. Each cell imprint has 4-6 aeropyles. Fig.1(c).
4) *Rhodinianewara*: The shape of the concavity is round. Walls concave, columnar cells narrow. Aeropyles clearly expressed at walls and not connected. Central portion of rosette like a small round depression. Fig.1(d).
5) *Samiacanningi:* Egg marked by concave cells. Aeropyles small, cells arranged by regular radial lines. The quantity of lines increases to egg base. Micropylar area not clearly expressed, and the micropylar pit is degenerating. Fig.1(e).
6) *Samiaricini:* The egg shell of Samiaricini has highly decorated chorion and the micropylar apparatus is located at the anterior pole of the egg. A rosette of few petal shaped primary cells surrounds the micropylar opening. The primary cells were again surrounded by a row of petal shaped cells of unequal size. Some primary cells are longer than the others. Fig. 1(f).
7) *Caligula simla*: Egg oval, with the micropylar area located at the sides of the egg attached to the substrate. The aeropyles are small and distinct. Fig. 1(g).
8) *Caligula thibeta*: Aeropyles are very small, poorly expressed. Micropylar area not clearly expressed. Chorion weakly pebbled everywhere, pebbles more distinct in area of cell walls. Fig. 1(h).

4. Discussion

SEM studies of the eggs showed that the surface of the egg chorion revealed structural elements viz., the micropylar rosette surrounding the micropyle, micropylar canals, shell imprints, aeropyles and regional differentiation at the different poles. The micropylar apparatus is at the anterior pole of the egg, it is encircled by 11-14 petal shaped primary unequal cells which formed an assymmetrical rosette. The secondary petal shaped cells were short in length surrounded by the primary petal cells. The entire surface of the chorion has a reticulate pattern of pentagonal and hexagonal cells each boarded by 4-6 aeropyles. Aeropyles consists of a large number of oval or round spaces or concavities surrounded by a network pattern mostly hexagonal unit. These network patterns are polygons, which are imprints of follicular epithelial cells that secrete the chorion proteins (Palash*et al.*, 2013). Tazima (1978) reported that the colour variation of egg shell is due to serosa pigment. The variations in egg size and weight were also revealed by Nath (2009) and Zamal (2012). The present investigation shows oval main cells, aeropyles and micropylar rosette throughout the egg surface. The aeropyles are the main root through which oxygen exchange takes place between outside and inside the egg.

Each polygon has aeropyle at each ridge so that there are six aeropyles in a hexagonal structure, five in pentagonal, seven in heptagonal and so on.The variability in the size of aeropyles during egg development is difficult to distinguish functionally. However, as they mainly serve for gaseous exchange between the egg and the outside environment, it may be that gradual increase in size at least up to the required day demands more oxygen uptake by the developing larva (Renthlei et al 2010)
The chorion of lepidopteran insects has spatially and morphologically differentiated surface regions. Renthlei et al (2010) in their studies on the chorion of *Samiaricini*observed that this species lack the aeropyle crown region which is also observed in the present study.

The polygonal shell imprints away from the micropylar region in the case of *Antheraeaassamensis* and change their shape to round. They are completely boarded by aeropyles as also seen in the studies of the chorion of *Antheraeaassamensis* (Choudhury and Devi 2013).

Leather et al., (1993) described that several species exhibit a large variability in the colour present within the population which contribute to the morphological and physiological changes. It is also possible that the host plant may determine the colour and it is a cryptic mechanism determined by the host plant to avoid predators.

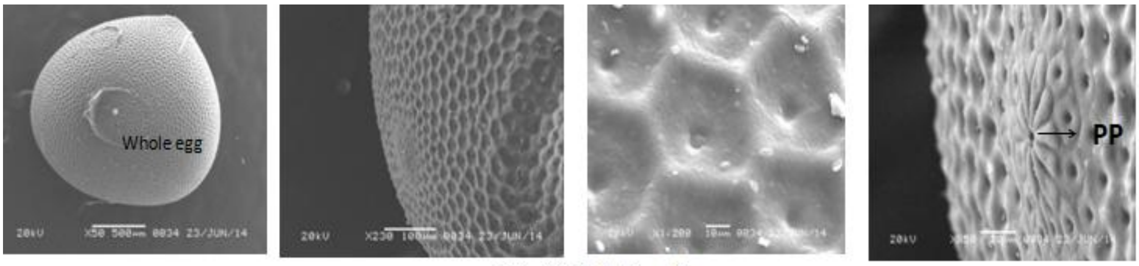

Fig. 1(a) *Actiasselene*

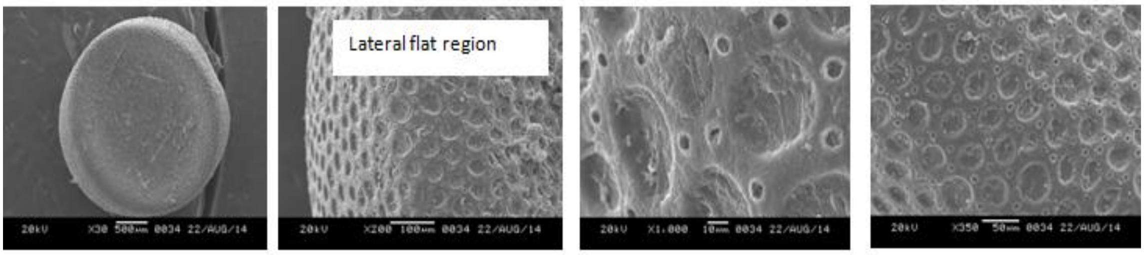

Fig. 1(b) *Antheraearoylei*

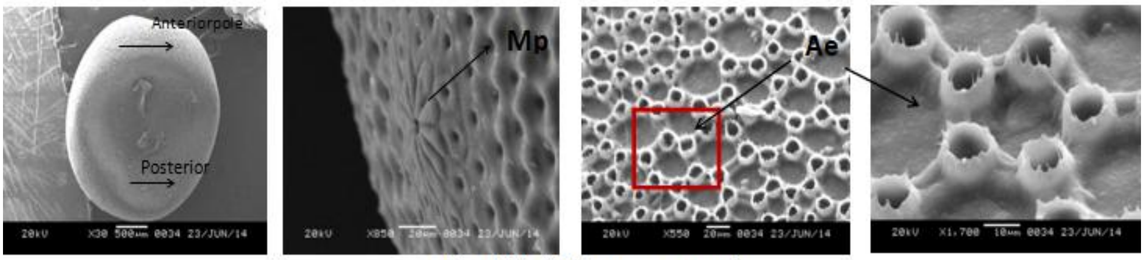

Fig. 1(c) *Antheraeaassamensis*

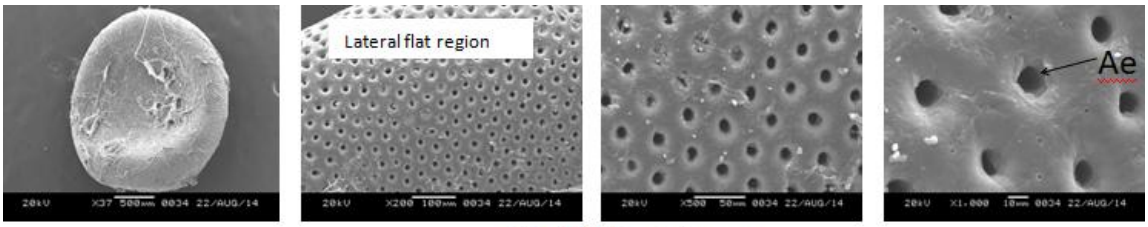

Fig. 1(d) *Rhodinianewara*

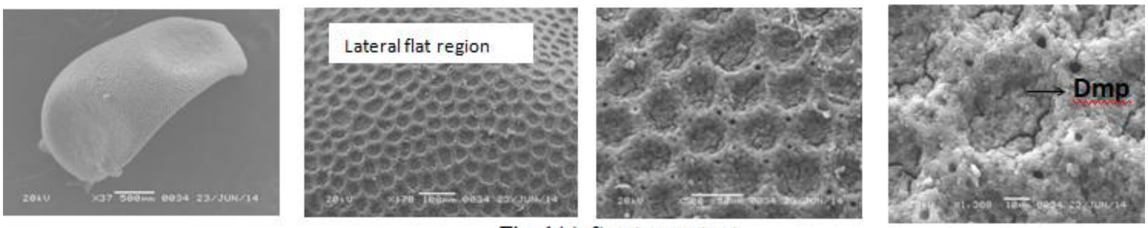

Fig. 1(e) *Samiacanningi*

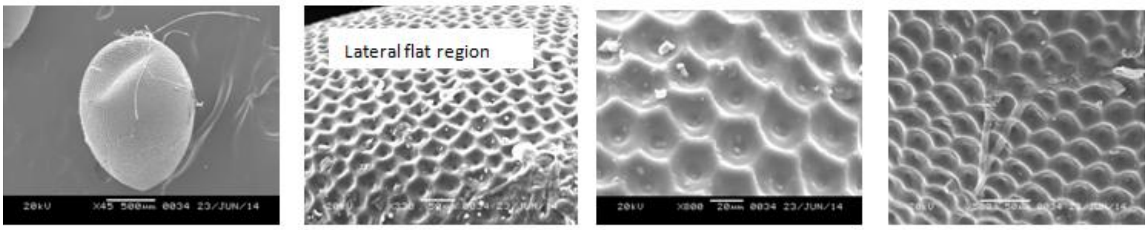

Fig. 1(f) *Samiaricini*

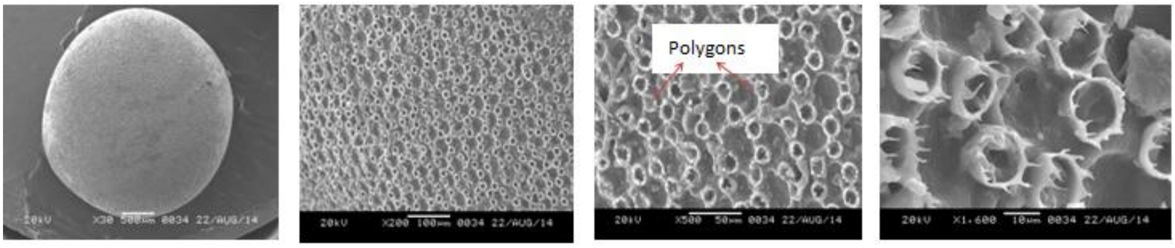

Fig. 1(g) *Caligula simla*

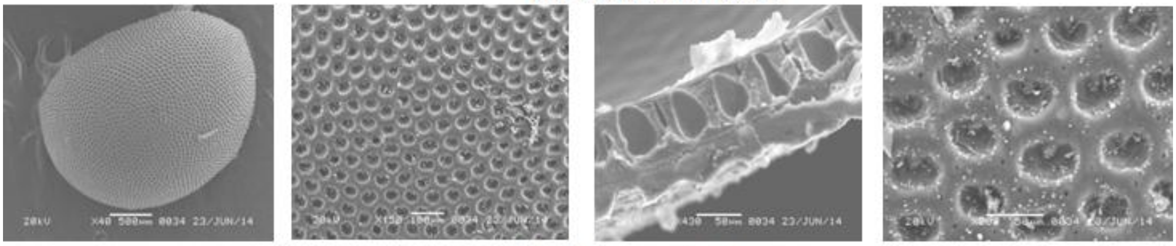

Fig. 1(h) *Caligula thibeta*

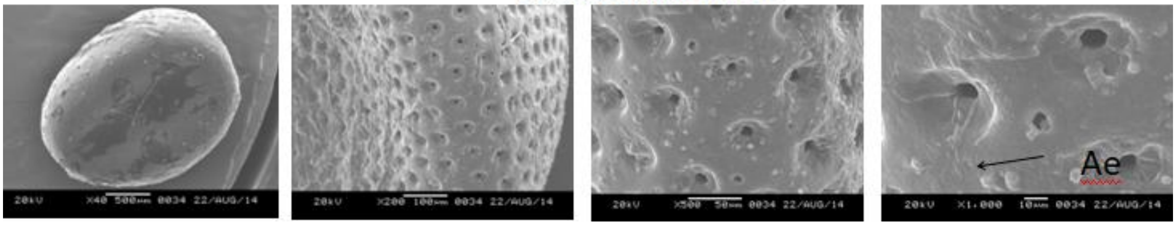

Figure 1: SEM of the eggs of wild silk moths. Ae: Aeropyles, Mp: Micropylar pit, PPC: Primary petal shaped Cells, Dmp: Degenerating micropylar pit, Pb: Protuberances without definite boundary of shell

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