



Journal of
Entomology

ISSN 1812-5670



Academic
Journals Inc.

www.academicjournals.com

**Surface Ultrastructure of the Egg Chorion of Eri Silkworm,
Samia ricini (Donovan) (Lepidoptera: Saturniidae)**

¹Vineet Kumar, ²B.K. Kariappa, ¹A.M. Babu and ²S.B. Dandin
¹Electron Microscopy Division,
²Central Sericultural Research and Training Institute,
Srirampura, Mysore-570 008, India

Abstract: The eggshell of the eri moth, *Samia ricini* (Donovan) (Lepidoptera: Saturniidae) was investigated by scanning electron microscopy. The surface of the egg chorion of eri moth, revealed the structural elements viz., the micropylar rosette surrounding the micropyle, micropylar canals, shell imprints, aeropyles and the regional differentiation at the different poles. The oval shaped eggs were measured 820-990 (878.72±21.23) µm in length. The highly decorated chorion of *S. ricini* had a micropylar rosette of 45-51 (48.0±1.02) µm diameter with a centrally located micropylar pit of 2.08-2.31 (2.21±0.036) µm diameter. The micropylar pit consists of seven micropylar openings, each micropylar opening ranged from 0.26-0.28 (0.27±0.003) µm in diameter. Each micropylar opening opens into the seven distinct micropylar canals. The micropylar apparatus encircled by 11-14 petal-shaped primary unequal cells. These cells ranged from 12.0-12.9 (12.48±0.13) µm in length forming an asymmetrical rosette. The secondary petal-shaped cells were short in length and measured 8.4-9.1 (8.72±0.12) µm surrounded the primary petal cells. The entire surface of the chorion had a reticulate pattern of pentagonal and hexagonal cells, each boarded by 4-6 aeropyles of diameter 0.22-0.25 (0.23±0.05) µm. Further, the sunken aeropyles were also observed for the first time in second hemisphere of the egg and measured 0.22-0.24 (0.23±0.003) µm in diameter.

Key words: *Samia ricini*, ultrastructure, eggshell, micropylar rosette, aeropyles, SEM

INTRODUCTION

The insect eggs are characterized by an outer shell secreted by the follicular epithelium during the process of egg formation and provide strong and elastic mechanical protection to the developing embryo besides allowing air for respiration at the aeropylar regions. Another specialized area of the egg chorion called micropyle paves the way for the penetration of sperm (Leuckart, 1855). The morphology and architectural pattern of the egg chorion in different insects depend on the imprints of the follicular secretory cells on specialized regions such as micropyle, aeropylar, stripe and flat regions (Beament, 1948; Sakaguchi *et al.*, 1973; Kafatos *et al.*, 1977; Regier *et al.*, 1980). The structures of insect eggshells are usually quite complex and a typical insect egg capsule consists of the vitelline envelope and chorion (Kumar *et al.*, 2002a). The sculpturing of the outer part of envelope appears to be specific and is of considerable interest because of potential taxonomic applications (Roscsizewska, 1991). Further, the basic knowledge of insect eggshell structure and function, in particular of Lepidoptera, was provided by Leuckart (1855) and Korschelt (1887). Light Microscope (LM) and physiological studies of gas exchange and permeability (Beament, 1948; Wigglesworth and Beament, 1950) preceded a second phase of basic research that began with the establishment of

transmission (TEM) and Scanning Electron Microscopy (SEM). Since then, the fine structure of a large number of lepidopteran eggs has been studied with particular emphasis on surface sculpturing (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; Kumar *et al.*, 2002b; Kumar *et al.*, 2003). The high resolution and 3-dimensional clarity of the scanning electron microscope make it an invaluable tool for studying diagnostic characters of insects. Hinton (1969) used scanning electron micrographs to illustrate respiratory systems of various insect eggshells. SEM studies 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 Microscope (LM) (Arbogast *et al.*, 1980).

The surface structure of lepidopteran eggs, as revealed by scanning electron microscopy, provides reliable characters for separation of species (Arbogast *et al.*, 1989) and the external morphology of a wide variety of insect eggs has been examined using different techniques (Hinton, 1981). A taxonomy key has been formulated based on the egg chorionic characteristics and its architecture in sod webworms (Matheny and Heinrichs, 1972).

India is the only country, which produces all four kinds of silk viz., mulberry, eri, tasar and muga and the second highest country in the total production of silk, after China. The eri silk is produced by *Samia ricini* (Donovan) and is an economically important insect for sericulture point of view. Here a study has been taken on the surface ultrastructure on the egg chorion of eri silkworm, *Samia ricini* (Donovan) using scanning electron microscope. The results of the study reported here describing the surface ultrastructure of the egg chorion of *Samia ricini* (Donovan) may find use in the study of taxonomy and phylogeny of the insect.

MATERIALS AND METHODS

A laboratory culture of *Samia ricini* (Donovan) was established from adults that emerged from green pupae. Freshly emerged male and female adults were released in a cage (28×18 cm) and were provided with a 5% sucrose solution for feeding and fresh castor branches for egg layings. After mating, the freshly laid eggs were gently removed from the castor leaves using a fine tipped hair brush.

For scanning electron microscopic study, the eggs were fixed for two hours at room temperature in 2.5% glutaraldehyde prepared in 0.2 M cacodylate buffer (pH = 7.2), dehydrated in a graded alcohol-acetone series and dried in a critical point drier (EMS-850) using CO₂ as the transition fluid. The dried samples were mounted onto copper stubs and coated with gold (20 nm thickness) in a Sputter coater (EMS-550) and examined using a JEM 100 CX II electron microscope fitted with ASID 4D attachments (JEOL Ltd., Tokyo, Japan) at 20 kV.

RESULTS AND DISCUSSION

Gravid females of *Samia ricini* laid eggs on the lower surface of its food plant leaves. The eggs were found deposited upright serially arranged in 6 to 36 clutches of 317 to 510 eggs. They were attached to the leaf with their posterior poles and among each other with their lateral sides, by a sticky secretion (Fig. 1-5). The freshly laid eri eggs were slight white in colour. As the embryo developed inside the egg, the colour of the shell changed from whitish to yellowish, yellowish to ashy and finally ashy to blackish just before hatching (Fig. 2). The eri eggs were of medium size compared to mulberry or muga egg and have oval shape. Fehrenbach *et al.* (1987) have investigated the eggs of three lepidopteran moths by scanning (SEM) and transmission (TEM) electron microscopy, which revealed that the eggs of *Heliothis virescens* were standing type (upright) and ca 550 µm in diameter and ca 600 µm in length, whereas the eggs of *Spodoptera littoralis* was also standing type

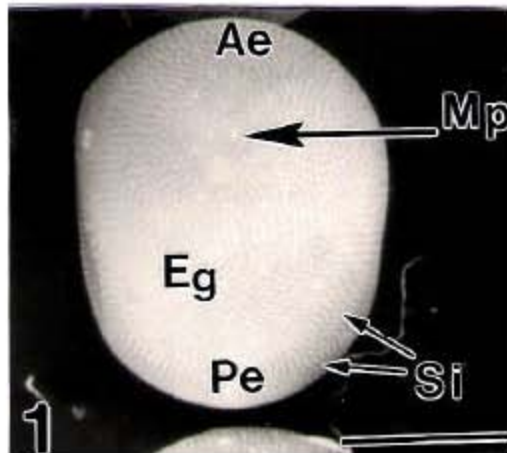


Fig. 1: A single egg (Eg) of *Samia ricini* showing anterior end (Ae), posterior (Pe) and anteriorly located micropyle (Mp) and the shell imprints (Si) on the surface. (Scale bar = 190 μ m)

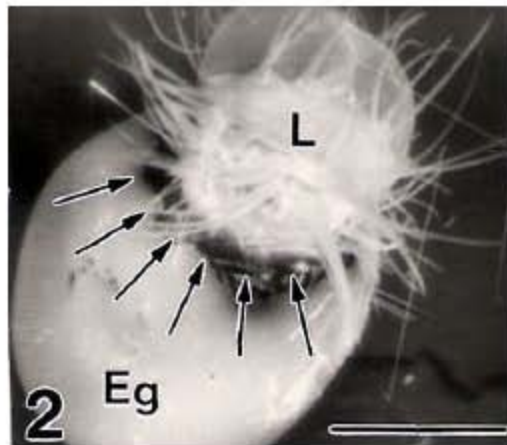


Fig. 2: A neonate larva (L) emerges out through the anterior end. Arrows indicate the anterior cut surface of the egg (Eg). (Scale bar = 120 μ m)

and almost spheroid with a diameter of 500-550 μ m and slightly less in length. However, the eggs of *Cydia pomonella* belonged to the lying (flat) type which measured ca 1350 μ m in length and ca 1050 μ m in width. Arbogast *et al.* (1989) studied the eggs of three tineid pests under scanning electron microscope and found the eggs of *Tinea pallascientella* Stainton as subcylindrical, 0.61-0.67 (0.64 \pm 0.02) mm long and 0.31-0.35 (0.33 \pm 0.01) mm in diameter at the widest point. But the eggs of *Tinea occidentella* Chambers was subcylindrical, 0.61-0.65 (0.62 \pm 0.01) mm long and 0.36-0.39 (0.38 \pm 0.01) mm in diameter at the widest point, bluntly rounded at both ends, the anterior end usually broader than the posterior. Though the eggs of *Niditinea fuscella* (L.) were also found subcylindrical their anterior end was bluntly rounded and the posterior end more acutely rounded, but occasionally ellipsoid, 0.42-0.50 (0.45 \pm 0.02) mm long with 0.26-0.30 (0.28 \pm 0.01) mm in diameter at the widest point (Arbogast *et al.*, 1989). Hinton (1981) emphasized that in most Lepidoptera, the egg is a prolate or more or less spherical or rarely hemispherical. He also revealed that the size and shape of the eggs

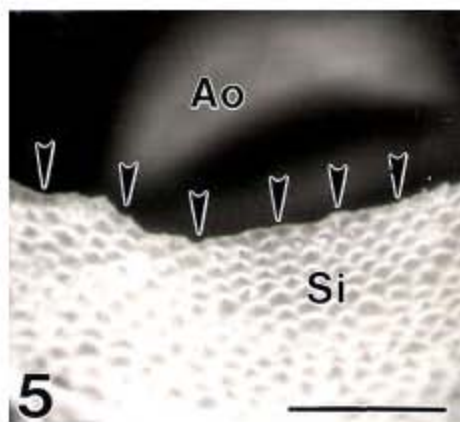
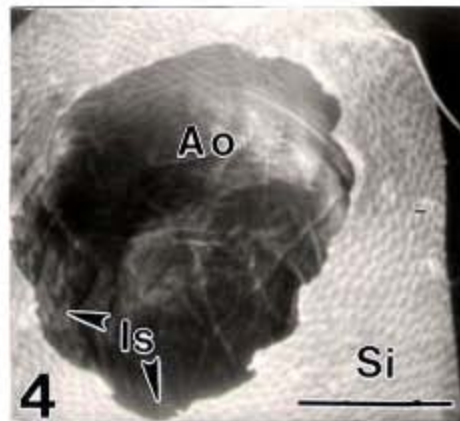
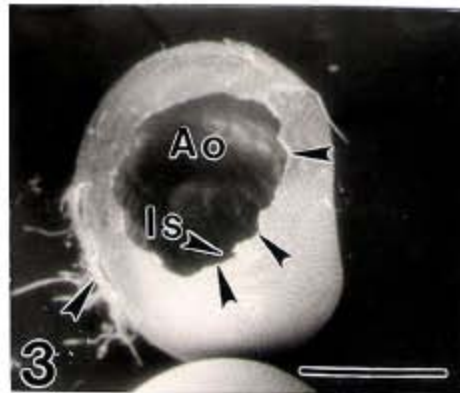


Fig. 3-5: Vacated egg shows the anterior opening (Ao), which was cut open irregularly by the hatched larvae. Arrow head shows the inner surface (Is). The detached lateral side (Left sides: single arrow head in Fig. 3) is also shown with glued substances. (Scale bar = 120 μm for Fig. 3; 60 μm for Fig. 4 and 30 μm for Fig. 5)

of certain insect like a tortricid, *Rhyaciona duplana* (Hub.) may also vary slightly according to the species of pine on which they are laid. In any insect eggshell there are three major layers viz., vitelline membrane, the endochorion and the third one is occasionally absent (Margarithis, 1985). The protection against environmental hazards during embryogenesis is one of the functions of the eggshell.

The eggs of *S. ricini* measured 820-990 (878.72 ± 21.23) μm in length. Matheny and Heinrichs (1972) have studied the egg chorion of 15 species of Lepidoptera moths and measured 0.598 ± 0.002 μm in *Thaumatoopsis edonis*, 0.425 ± 0.009 mm in *Chrysoteuchia topiaria* and 0.537 ± 0.009 μm in *Pediasia trisecta*. Recently, Kumar *et al.* (1999) observed the eggs of a lepidopteran moth, *Spilarctia obliqua* using electron microscope and measured 600-650 μm in diameter at the widest point. Further, Kumar *et al.* (2003) studied the fine structure of the egg of a lepidopterous moth, *Amata passalis* and measured the eggs 488 ± 0.595 μm in diameter under scanning electron microscope.

The eggshell of *S. ricini* has a highly decorated chorion and its micropylar apparatus is located at the anterior pole of the egg (Fig. 1, 7-16). Hinton (1981) stated that the micropyles are without exception at the anterior pole of the lepidopteran eggs. Sakaguchi *et al.* (1973) have described the arrangement of follicular imprints of the micropylar and aeropylar regions of the egg chorion of *Bombyx mori*. In recent past many workers also observed the micropylar apparatus at the anterior end in the egg chorion of lepidopterous moths (Arbogast *et al.*, 1989; Fehrenbach *et al.*, 1987; Fehrenbach, 1989; Kumar *et al.*, 1999, 2003). However, an unusual position of the micropylar structure was found on the lower surface of an egg in a lepidopterous moth *Cydia pomonella* (Fehrenbach *et al.*, 1987). A micropylar rosette consisting of micropylar openings encircled with primary petal-shaped cells, which are followed by the secondary petal-shaped cells, was observed in *S. ricini*. The micropylar rosette of *S. ricini* ranged 45-51 (48.0 ± 1.02) μm in diameter (Fig. 8). Fehrenbach *et al.* (1987) have observed a slightly bigger micropylar rosette ca 65 μm in diameter in *Heliothis virescens*, 55 μm in *Spodoptera littoralis* and about 50 μm in *Cydia pomonella*. A rosette of 11-14 petal-shaped primary cells surrounds the micropylar opening of *S. ricini* (Fig. 8-10, 13). The primary cells were again surrounded by a second row of 15-19 petal-shaped cells of unequal sizes (Fig. 8). The number of primary cells around the micropylar pit is 15-19 in *A. passalis* (Kumar *et al.*, 2003), 10-14 in *S. obliqua* (Kumar *et al.*, 1999), 5-7 in *T. pallescentella*, 5-8 in *T. occidentella* and 6-9 in *N. fuscella*

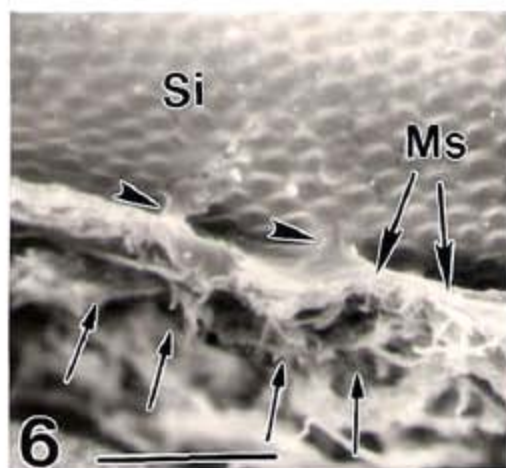


Fig. 6: The posterior end of the egg showing its surface detached from host leaves (arrows). The membranous structure (Ms) which adheres to the leaf is connected to the egg by short vertical bars (arrow heads). (Scale bar = 30 μm)

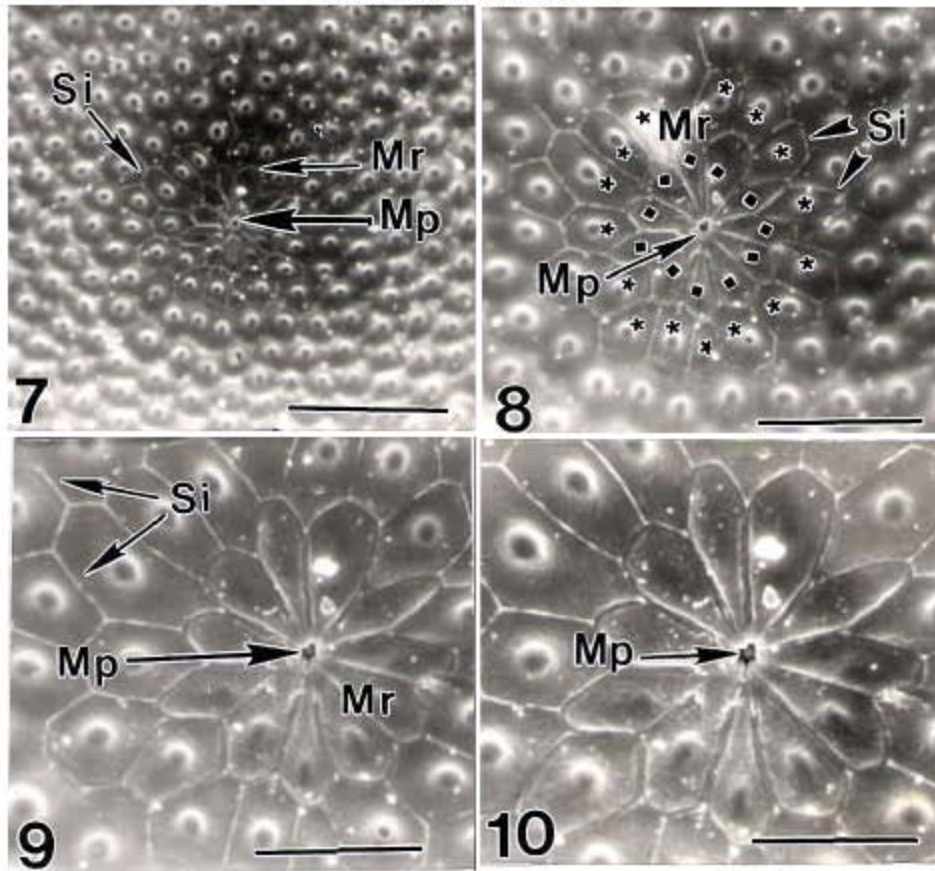


Fig. 7-10: General view of anterior egg pole revealing the rosette (Mr) of eleven primary petals (■) followed by fifteen secondary petals (*) and centrally located micropyle (Mp). Sometimes a protuberance was also observed on the rosette cells. (Scale bar = 20 μ m for Fig. 7; 12 μ m for Fig. 8; 7.5 μ m for Fig. 9 and 6 μ m for Fig. 10)

(Arbogast *et al.*, 1989). However, in some eggs of *N. fuscella* the primary cells were found to be of subequal length forming symmetrical rosettes. Felrenbach *et al.* (1987) described the fine structure of three lepidopteran moth eggs and explained that in *Heliothis virescens*, the micropylar rosette is composed of 13-15 petal-shaped primary cells with 4 or 5 micropylar canals opening into the micropylar plate, whereas in *Spodoptera littoralis* the number of the rosette petals varies between 6 and 11, 7 and 8 being the most frequent and with 3-4 micropylar openings present in the slightly depressed rosette center. In *Cydia pomonella* the micropylar rosette is composed of 8 or 9 rosette petals.

In a few eggs of *S. ricini*, the primary cells were subequal in length, arranged in a symmetrical rosette, but more often, some primary cells were longer than others and formed an asymmetrical rosette (Fig. 8 and 13). Similar asymmetrical rosettes of unequal petal-shaped cells have been reported in the eggs of six other lepidopteran moths viz., *Tinea pallenscentella*, *T. occidentella*, *Meditinea fuscella* (Arbogast *et al.*, 1989), *Spilarctia obliqua* (Kumar *et al.*, 1999) and *Amata passalis* (Kumar *et al.*, 2003).

The length of each primary rosette cells of *S. ricini* eggs ranged 12-12.9 (12.48 \pm 0.13) μ m and some of the cells were observed with a protuberance on the distal end of the cell (Fig. 9-11). The secondary petal-shaped cells were found short in length and measured 8.4-9.1 (8.72 \pm 0.12) μ m (Fig. 8 and 9).

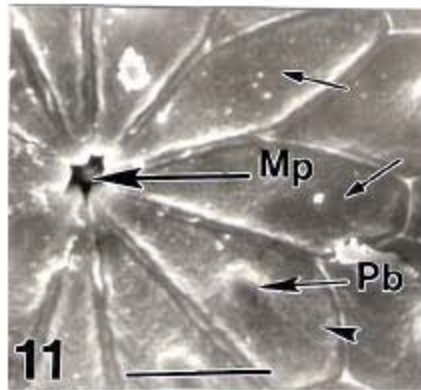


Fig. 11: Arrow head shows the petal cell with a single protuberance (Pb) while the adjacent petal cells were observed without protuberance (arrows). (Scale bar = 3 μ m)

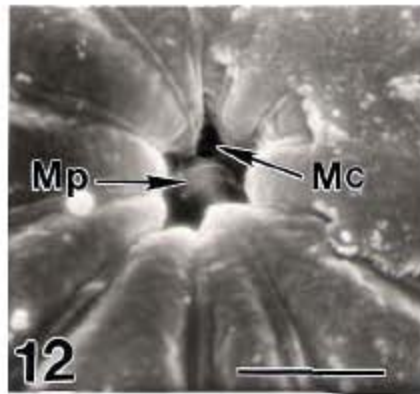


Fig. 12: Top view of egg pole showing micropylar region centrally located micropyle (Mp) and seven micropylar canals (Mc). (Scale bar = 1 μ m)

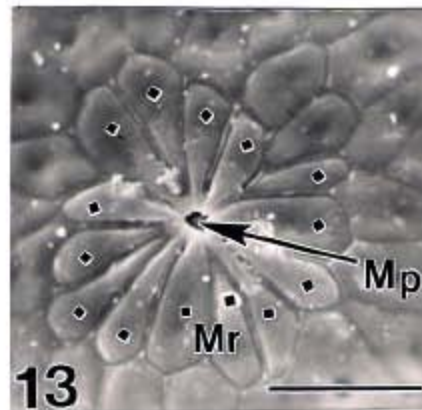


Fig. 13: A top view of the other egg shows its micropylar rosette (Mr) with fourteen petal cells (P) surrounding the micropyle (Mp). (Scale bar = 7.5 μ m)

Each micropylar pit was 2.08-2.31 (2.21 ± 0.036) μm in diameter and had seven distinct micropylar openings ranged from 0.26-0.28 (0.27 ± 0.003) μm diameters and each micropylar opening radiates down into micropylar canal in micropylar plate in the eggs of *S. ricini* (Fig. 14-16). Kumar *et al.* (1999) studied 50 eggs of *S. obliqua* under SEM and found interestingly only a single egg had three micropylar openings whereas in all other eggs the micropylar apparatus had four micropylar openings (micropylae). Hinton (1981) described that in Lepidoptera; eggs have usually four micropyles, but may have more also. In eggs of 15 species of Notodontidae studied by him, the number of micropyles varied from 4 to 20. The number sometimes varies considerably in same species, e.g., in a few shells of *Cerura vinula*, the number of micropyles varied from 16 to 20. Study of micropyles has been a favorite subject since early times. Muller (1938) gave a very good description of the 4 micropyles of the pyralid, *Plodia interpunctella* and also described how they develop. Fehrenbach (1989) studied the fine structure of eggshells of 4 primitive moths and revealed that the egg chorion of *Hepialis hecta* and *Wiseana umbracula* had a different egg surface, with two micropylar openings at the opposite sides of the longer axis of the oval micropylar plate. In *Mnesarchaea fusitella* 2-3 oval micropylar openings were seen at the base of the micropylar rim. Matheny and Heinrichs (1972) studied the egg chorion of 15 species of sod webworm moth eggs (Lepidoptera) and developed a taxonomic key for egg identification.

In *S. ricini* the rosette of two rows of petal-shaped cells at the top and anterior pole of eggs is followed by the dense ridged shell imprints which cover rest of egg surface (Fig. 7-10, 19 and 22) except at the posterior pole where the egg was attached to the substratum (Fig. 6, 7, 20 and 21). Further, it was also observed that each shell imprint bears a centrally located blunt protuberance (Fig. 8-10). Though the posterior poles of egg sometime do not have the shell imprints but the blunt protuberances were observed in that area (Fig. 17). Similar to the observation on *S. obliqua* (Kumar *et al.*, 1999), the shell imprints were mostly pentagonal or hexagonal in shape and measured 9.0-9.6 (9.24 ± 0.10) μm in diameter in *S. ricini*. Generally it was found that 4 to 6 aeropyles of the size of 0.22-0.25 (0.23 ± 0.05) μm were located on the ridges of each shell imprints, which transverse the shell down to the trabecular layer, on the anterior and middle region of the egg (Fig. 18 and 19), however, the posterior surface, which was observed with or without shell imprints, have the aeropyles in a pit (sunken aeropyles) (Fig. 20 and 21). The diameters of sunken aeropyles were measured 0.22-0.24 (0.23 ± 0.003) μm . In *S. ricini* the total number of aeropyles on a single egg is too high to be counted. Matheny and Heinrichs (1972) have investigated the egg chorion of 15 species of sod webworm moths, using scanning electron microscope and revealed a smallest aeropyle i.e., 1.46 ± 0.07 μm in *Agriphila ruricollata* whereas the largest aeropyles were 3.03 ± 0.16 μm in diameter in *Crambus lequealellus*. Fehrenbach *et al.* (1987) have reported about 50 aeropyles per egg measuring ca 1.9 μm in diameter in *Heliothis virescens*, ca 400 aeropyles per egg in *Spodoptera littoralis* measuring about 0.9 μm wide, whereas the number of aeropyles was 140 per egg and ca 0.6 μm in diameter in the eggs of *Cydia pomonella* (Fig. 23 and 24). Arbogast *et al.* (1989) reported the aeropyles ranging from 0.67-1.20 (0.94 ± 0.15) μm in *T. pallescentella*, 0.55-0.93 (0.77 ± 0.04) μm in *T. occidentella* and 0.65-1.50 (1.10 ± 0.36) μm in *Niditinea fuscilla*. Recently, Kumar *et al.* (2003) observed the aeropyles of *Amata passalis* which measured 0.36 ± 0.08 μm in diameter. However, the aeropyles were not observed on the first two rows of cells at the micropylar zone in *S. ricini*, as in the eggs of *S. obliqua* (Kumar *et al.*, 1999) and *S. littoralis* (Fehrenbach *et al.*, 1987). The panels of shell imprints adjacent to the micropylar zone in *S. ricini* have less number of aeropyles, whereas the shell imprints which are away from micropylar zone are completely boarded by aeropyles as in *S. obliqua* (Kumar *et al.*, 1999). In *T. occidentella* aeropyles are distributed over the entire surface of egg whereas in *T. pallescentella* and *N. fuscilla* they are restricted towards the anterior and posterior end of the eggs. (Arbogast *et al.*, 1989). The number of aeropyles in *S. obliqua* varies from 18 to 47 on a single shell imprint; however, some large aeropyles were also observed which may be formed by the fusion of two

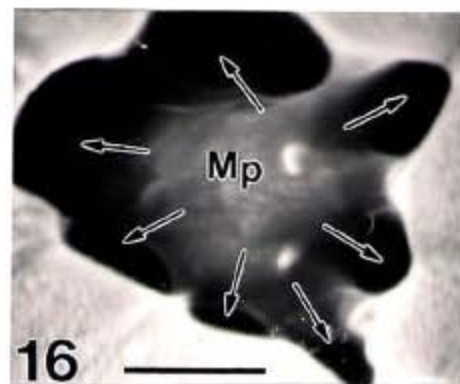
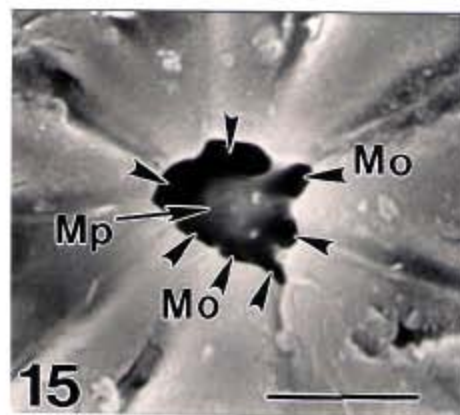
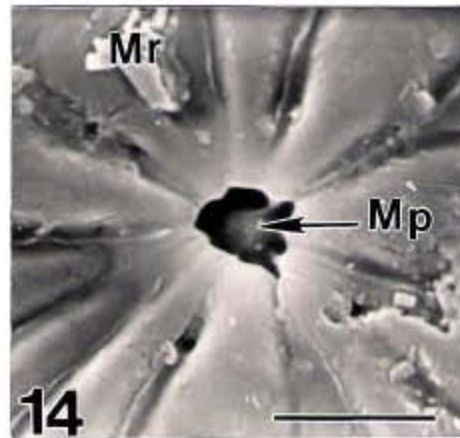


Fig. 14-16: The micropyle (Mp) reveals the distinct seven-micropylar openings (Mo), which further radiates down into the micropylar canal. (Scale bar = 2 μ m for Fig. 14; 1.2 μ m for Fig. 15 and 0.4 μ m for Fig. 16)

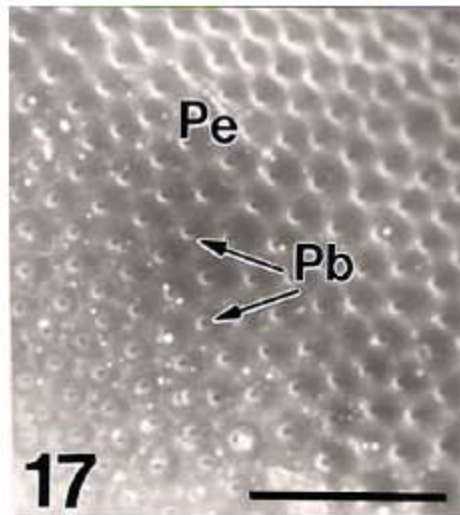


Fig. 17: Posterior end (Pe) of an egg revealing a large number of protuberances (Pb) with out definite boundary of shell imprints. (Scale bar = 30 μ m)

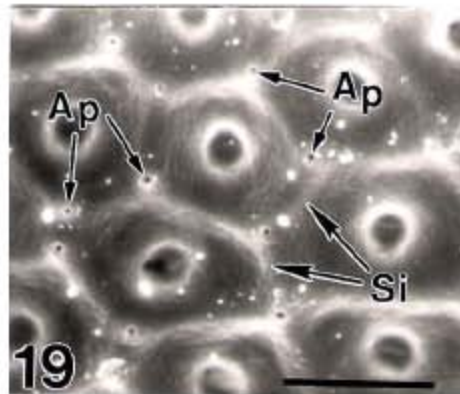
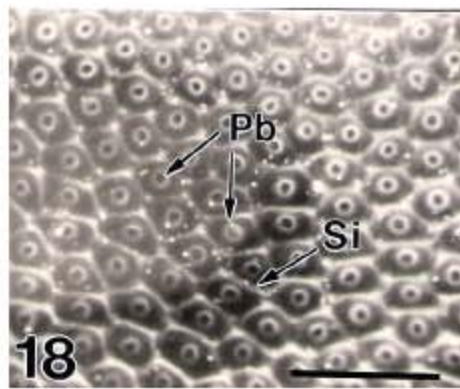


Fig. 18-19: Middle region of an egg revealing the shell imprints (Si) and centrally located protuberance (Pb). The aeropyles were observed on the shell imprints (Si). (Scale bar = 20 μ m for Fig. 18 and 6 μ m for Fig. 19)

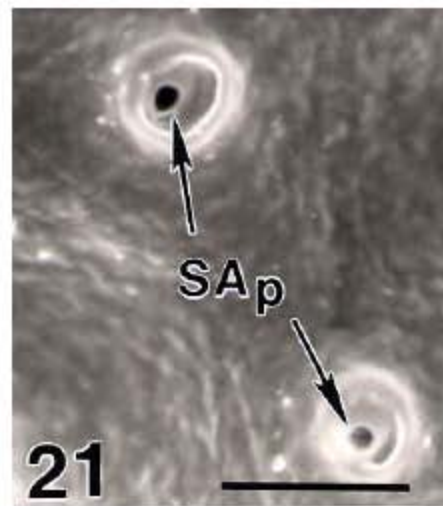
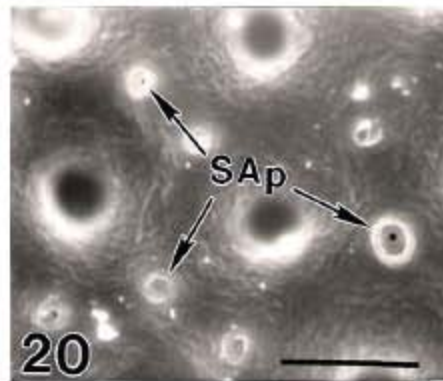


Fig. 20-21: Sunken aeropyles (SAp) were observed on the posterior region of an egg. The sunken aeropyles were not found located on the shell imprints. (Scale bar = 3 μ m for Fig. 20 and 1.2 μ m for Fig. 21)

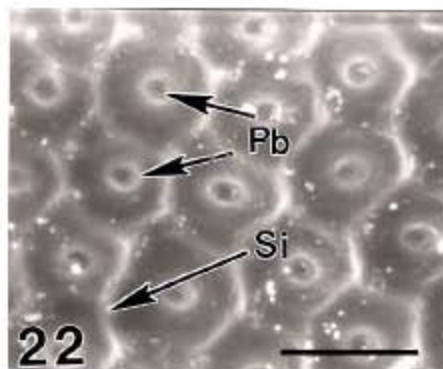


Fig. 22: Extreme posterior end of the egg shows that each protuberance (Pb) is encircled by a shell imprints (Si). (Scale bar = 7.5 μ m)

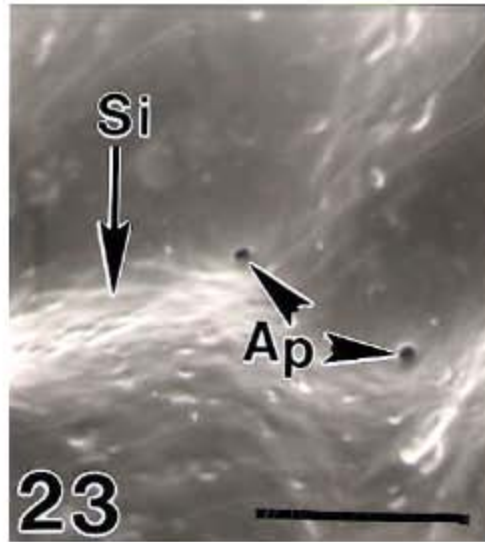


Fig. 23: Two normal aeropyles (Ap) situated on the shell imprints (Si). (Scale bar = 2 μ m)

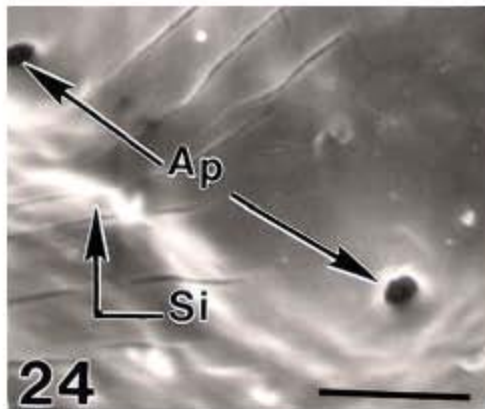


Fig. 24: Magnified view of egg chorion reveals two normal aeropyles (Ap) on shell imprints (Si). (Scale bar = 0.6 μ m)

adjacent small aeropyles (Kumar *et al.*, 1999). The main function of the aeropyles is to conduct ambient air into the trabecular layer (gas containing meshwork), which finally passes on to the oocyte. Very little is yet known about the permeability of the chorion. Tuft (1950) demonstrated that when the aeropyles of the eggs of *Rhodnius* were blocked with shellac, the egg continued to take up oxygen, though only at about a tenth of the normal rate. It may be that eggs depend not only upon oxygen entering through the aeropyles but also on that diffusing through the areas of chorion without aeropyles.

REFERENCES

- Arbogast, R.T., L.G. Le Cato and R.V. Byrd, 1980. External morphology of some eggs of stored-proodecut moths (Lepidoptera: Pyralidae, Gelechiidae, Tineidae). *Int. J. Insect Morphol. Embryol.*, 9: 165-177.

- Arbogast, R.T., J.H. Brower and R.G. Strong, 1989. External morphology of the eggs of *Tiema pallescentella*, *Tiema occidentella* Chambers and *Niditinea fuscella* (L.) (Lepidoptera: Tineidae). *Int. J. Insect Morphol. Embryol.*, 18: 321-328.
- Beament, J.W.L., 1948. The penetration of insect eggshell. I. Penetration of the chorion of *Rhodnius prolixus* Stal. *Bull. Entomol. Res.*, 39: 359-383.
- Downey, J.C. and A.C. Allyn, 1980. Eggs of Riodinidae., *J. Lepid. Soc.*, 34: 135-145.
- Downey, J.C. and A.C. Allyn, 1981. Chorionic sculpturing in eggs of Lycaenidae. Part I. *Bull. Allyn Mus.*, 61: 1-29.
- Fehrenbach, H., V. Dittrich and D. Zissler, 1987. Eggshell fine structure of three lepidopteran pests: *Cydia pomonella* (L.) (Tortricidae), *Heliothis virescens* (Fabr.) and *Spodoptera littoralis* (Boisd.) (Noctuidae). *Int. J. Insect Morphol. Embryol.*, 16: 201-219.
- Fehrenbach, H., 1989. Fine structure of the eggshell of four primitive moths: *Hepialus hecta* (L.) *Wiseana umbraculata* (Guenea) (Hepialidae), *Mnesarchaea fusilella* Walker and *M. acuta* Philp (Mnesarchaeidae) (Lepidoptera: Exoporia). *Int. J. Insect Morphol. Embryol.*, 18: 261-274.
- Hill, L., 1982. Eggs of some Tasmanian Noctuidae (Lepidoptera). *Aust. Entomol. Mag.*, 9: 49-59.
- Hinton, H.E., 1969. Respiratory systems of insect egg shells. *Ann. Rev. Ent.*, 14: 343-368.
- Hinton, H.E., 1981. *Biology of Insect Eggs*. Pergamon Press, Oxford, pp: 1125.
- Kafatos, F.C., J.C. Regier, G.D. Mazur, M.R. Nadel, H.M. Balu, W.H. Petri, R.E. Gelinas, P.B. Moore, M. Paul, A. Efstratiadis, J. Vourmakis, M.R. Goldsmith, J.B. Hunsley, N. Baker, J. Nardi and M. Koehler, 1977. The Eggshell of Insects: Differentiation-specific Proteins and the Control of Their Synthesis and Accumulation During Envelopment. In: *Results and Problems in cell differentiation*. Vol. 8. Beeman, W. (Ed.), Springer Verlag, Berlin, pp: 45-145.
- Korschelt, E., 1887. Zur Bildung der Eihüllen, der Mikropyle und Chorionanhänge bei den Insekten. *Nova Acta Ksl. Leop. Carol. Dtsch. Akad. Naturforsch*, 51: 185-252.
- Kumar, V., A.M. Babu, B.K. Kariappa, K.P. Jayaswal, R.L. Katiyar and R.K. Datta, 1999. Surface ultrastructure of the egg chorion of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). *Redia*, 82: 137-143.
- Kumar, V., M.N. Morrison, A.M. Babu and V. Thiagrajan, 2002a. Egg shell architecture of the stink bug, *Eocanthecona furcellata* (Wolff.): Ultrastructure of micropylar processes and egg burster. *Insect. Sci. Applic.*, 22: 67-73.
- Kumar, V., V. Kumar, S. Rajadurai, A.M. Babu, R.L. Katiyar, B.K. Kariappa, V. Thiagarajan and K.P. Jayasawal, 2002b. The chorionic architecture and shell structure of *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae) egg. *Russian Entomol. J.*, 11: 307-310.
- Kumar, V., S. Rajadurai, A.M. Babu and B.K. Kariappa, 2003. Eggshell fine structure of *Amata passalis* F. (Lepidoptera: Amatidae). *Insect. Sci. Applic.*, 23: 325-330.
- Leuckart, R., 1855. Ueber die micropyle und den feineren Bau der Schalenhaut bei den Insekteneiern. Zugleich ein Beitrag zur Lehre von der Befruchtung. *Arch. Anat. Physiol. Wiss. Medicin*, pp: 90-264.
- Matheny, E.L. and E.A. Heinrichs, 1972. Chorion characteristics of sod webworm eggs. *Ann. Ent. Soc. Am.*, 65: 238-246.
- Margaritis, L.H., F.C. Kafatos and W.H. Peteri, 1980. The eggshell of *Drosophila melanogaster*. I. Fine structure of the layers and regions of the wild-type eggshell. *J. Cell. Soc.*, 43: 1-35.
- Margaritis, L.H., 1985. Structure and Physiology of the Eggshell. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Kerkut, G.A. and L.I. Gilbert (Eds.), Pergamon Press, Oxford, pp: 153-230.
- Mazzini, M., 1974. Sulla fine struttura del micropila negli insetti. *Redia*, 55: 343-372.

- Muller, K., 1938. Histologische untersuchungen uber den entwicklungsbeginn bei einem kleinschmetterlung (*Plodia interpunctella*). *Z. Wiss. Zool.*, 151: 192-242.
- Reiger, J.C., G.D. Mazur and F.C. Kafatos, 1980. The silk moth chorion: Morphological and biochemical characterization of four surface regions. *Dev. Biol.*, 76: 286-304.
- Rosciszewska, E., 1991. Ultrastructural and histochemical studies of the egg capsule of *Perla marginata* (Panzer, 1799) and *Dinocras cephalotes* (Curtis, 1827) (Plecoptera: Perlidae). *Int. J. Insect Morphol. Embryol.*, 20: 189-203.
- Sakaguchi, B., H. Chikushi and H. Doira, 1973. Observations of egg shell structures controlled by gene action in *Bombyx mori*. *J. Facult. Agric. Kyushu Univ.*, 18: 53-62.
- Salkeld, E.H., 1983. A catalogue of the eggs of some Canadian Geometridae (Lepidoptera), with comments. *Mem. Entomol. Soc. Can.*, 126: 3-271.
- Salkeld, K., 1984. A catalogue of the eggs of some Canadian Noctuidae (Lepidoptera), with comments. *Mem. Entomol. Soc. Lon.*, 127: 1-167.
- Tuft, P.H., 1950. The structure of the insect eggshell in relation to the respiratory of the embryo. *J. Exp. Biol.*, 26: 327-334.
- Wigglesworth, V.B. and J.W.L. Beament, 1950. The respiratory mechanisms of some insect eggs. *Q. J. Micros. Sci.*, 91: 429-452.