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Short Note

Surface Morphology of Egg Chorion of the Uzi Fly, *Exorista bombycis* (Louis), (Diptera: Tachinidae) — an Endoparasite of the Silkworm, *Bombyx mori* Linn.

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Sunil K. Tewari, Vineet Kumar, Arvind K. Awasthi and Rajat K. Datta (1995) Surface morphology of egg chorion of the Uzi fly, *Exorista bombycis* (Louis), (Diptera: Tachinidae) — an endoparasite of the silkworm, *Bombyx mori* Linn. *Zoological Studies* **34**(1): 62-66. Scanning electron microscope observations on the egg chorion of the Uzi fly *Exorista bombycis* (Louis) (Diptera: Tachinidae) were carried out to obtain further understanding of the probable function and development of chorionic structures of eggs. The study reveals morphologically and physiologically important structures viz. aeropyles, micropyles, a circular lid with a well defined hatching line, a chorionic respiratory network, etc. The egg of *E. bombycis* is considered indehiscent, i.e., the egg chorion is not modified for hatching and the larva emerges from the ventral egg surface. However, a lid surrounded by a well defined hatching line is present in *E. bombycis*, but is generally found in dehiscent Tachinid eggs and is used for hatching. Further, the type of chorionic respiratory systems in the family Tachinidae are discussed in detail.

Key words: Eggshell, Chorionic respiratory system, Hatching line, Uzi fly, Scanning electron microscope.

For some time the insect eggshell and chorion and its associated follicular epithelium, considered at the molecular and morphological levels have provided a model system for areas of research, such as the control of gene expression in differentiating cells, organisation and evolution of gene families, morphology of supra-molecular structure, formulating strategies of insect pest control, and impact of biopesticides, etc. The eggshells of insects have been studied extensively and categorized from different points of view by a number of workers (Regier and Kafatos 1985, Margaritis 1985, Hinton 1981, Kafatos et al. 1977).

The sericulture industry in India has been under threat from various diseases of the silkworm and from insect pests. For two decades a considerable loss has been reported due to infestation of the Uzi fly, *Exorista bombycis* (Louis), an endoparasite of the mulberry silkworm, *Bombyx mori*. The Uzi fly, *Exorista bombycis* (= *Tricholyga bombycis* Beck = *Exorista sorbillans* Wiedemann = *Tricholyga sorbillans* Wiedemann = *Tachina sorbillans* Wiedemann) is a member of the subfamily Goniinae, the family Tachinidae and the order Diptera (Crosskey 1976, Sabrosky and Reardon 1976, Kumar 1987). The family Tachinidae is second to the Hymenoptera in number of parasitic species and is used for biological control of other insects. The serious threat to the silk industry by the Uzi fly has led scientists to try to understand the biology of this pest in order to develop control measures. Attempts include a study of the general biology of Tachinids by Dupuis (1963) and Thompson (1963 1968), correlation of growth rate and development under certain environmental conditions, ovicidal effect of organic acids, and the chemosterilant effect of benzoylphenyle urea by Kumar (1987), a life history by Ayuzawa et al. (1972), Datta and Mukharjee (1978), a review on taxonomy, economic status, general biology and management by Siddappaji and ChannaBasavanna (1990) and a study of the taxonomic status and ovarian developmental stages by Manjunatha and Puttaraju (1992a, b). Further, Manjunatha and Puttaraju (1993) reported briefly on the microscopic structure and function of the egg of *E. bombycis*. There remains a lack of information on the surface topography of the chorion at the ultrastructural level. This is important for developing integrated eradication strategies.

Materials and Methods—The colony of *E. bombycis* was maintained in the laboratory and gravid female flies of required age were utilized in the present studies. Fifth instar larvae of the silkworm, *B. mori* and a female fly were placed along with sugar cubes in a conical flask (opening closed by a muslin cloth). The unhatched and mature Uzi eggs were collected from silkworm larvae after three hours of infestation and the empty eggshells were collected after 48 hours of infestation. The mature Uzi eggs and empty eggshells were gently removed and fixed for scanning electron microscopy as follows.

Two separate batches of eggs were washed thoroughly in bleaching powder and 0.5% KOH to remove debris and

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mucilaginous material. The eggs were then fixed in 2.5% glutaraldehyde (in 0.2 M cacodylate buffer, pH 7.2) for one hour, washed in cacodylate buffer, followed by double distilled water, dehydrated in a graded series of ethanol, dried in a critical point dryer (EMS 850), mounted onto copper stubs by using double sided tape and coated by gold (300 nm thickness) in a sputter coater (EMS 550). Stubs with different angles were scanned under the electron microscope (JEOL 100 CX II-ASID 4D) at 20 kV.

Results—Although the *E. bombycis* lay their eggs on the entire larval body, they are preferably deposited at the sides of the larvae of *B. mori*. The eggs are dehiscent, creamy white at the time of deposition but later changing to a grey whitish colour. The dorsal portion of the chorion is thick and opaque, whereas the ventral surface is flat with slight depressions on the inside. The dorsal surface of the egg has a well defined chorionic respiratory system. The eggs carry a quantity of mucilaginous material at the time of deposition which helps them to adhere to the body of the host. The egg is ovoid; measuring 260-320 μm in length and 90-140 μm in width, tapered anteriorly and rounded posteriorly. The chorionic respiratory system is well defined on the dorsal surface as two patches consisting of several aeropyles at the posterior and anterior ends (Fig. 1). The number of aeropyles at the anterior end varies from eighteen to thirty-five (Fig. 2), while those at the posterior end vary from eight to fifteen (Fig. 3). Each aeropyle is semicircular to oval measuring 2.5-3.5 μm in length and 2.0-2.5 μm in width, containing an irregular respiratory network inside (Fig. 4). However, no difference in the structure of aeropyles was observed between the anterior and posterior regions. The dorsal surface of the chorion of laid eggs is well demarcated by several pentagonal and hexagonal shell imprints (Fig. 3). The ventral surface of the egg is flat and thin. The anterior group of aeropyles is surrounded by circular hatching lines (Fig. 2) measuring 55-65 μm in length and 45-55 μm in width. The maggot emerges by cutting open the membranous anteroventral surface and then penetrating into the silkworm body.

An extensive search was made of the anterior portion of several oviposited eggs (washed in bleaching powder and KOH) revealed that the micropyle is located at the extreme antero-dorsal end which is not encircled by shell imprints (Fig. 5). A large number of fertilized eggs at different chorion developmental stages were dissected from the oviduct of Uzi flies of different ages. In a few eggs (three days after fertilization), the anterior chorionic respiratory system was observed to be comprised of only a few grouped aeropyles (varying from five to ten) without hatching lines. The shell imprints were not well demarcated (Fig. 6). However, the posterior groups of aeropyles were as well developed as in mature eggs (five days after fertilization).

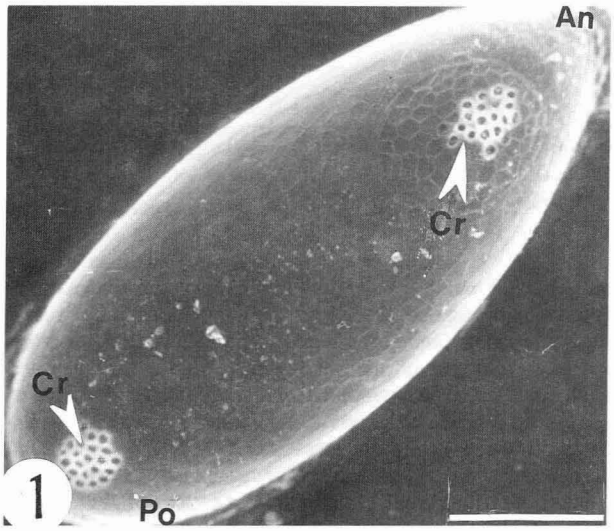

Fig. 1. Scanning electron micrograph of a laid mature egg of *Exorista bombycis* showing the chorionic respiratory (Cr) system at the anterior (An) and posterior (Po) end. Scale bar = 70 μm.

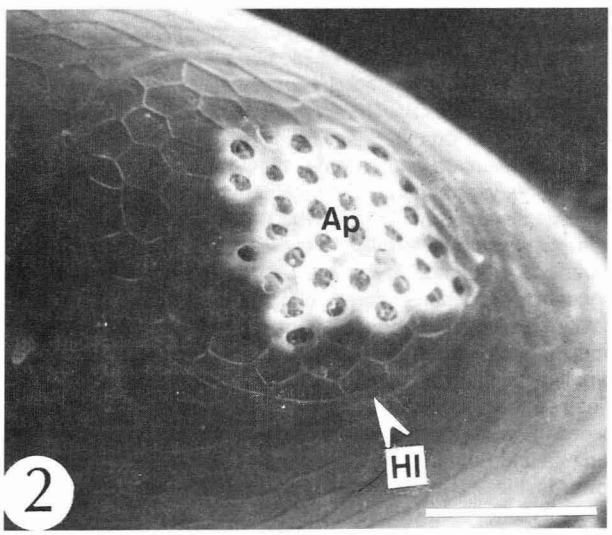

Fig. 2. Anterior end showing aeropyles (Ap) and hatching lines (Hl). Scale bar = 30 μm.

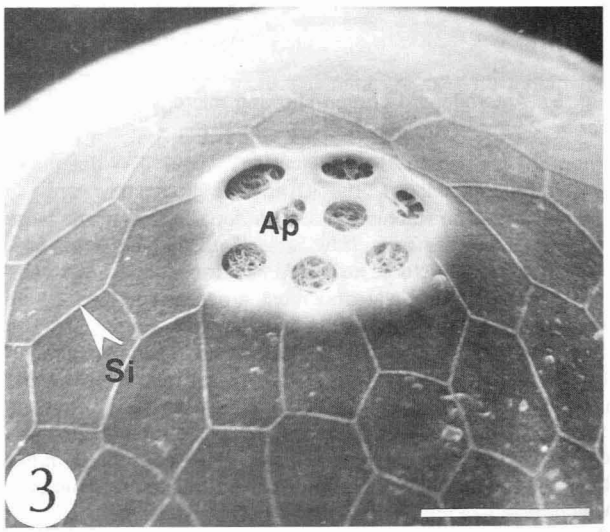

Fig. 3. Posterior end showing aeropyles (Ap) and shell imprints (Si). Scale bar = 15 μm.

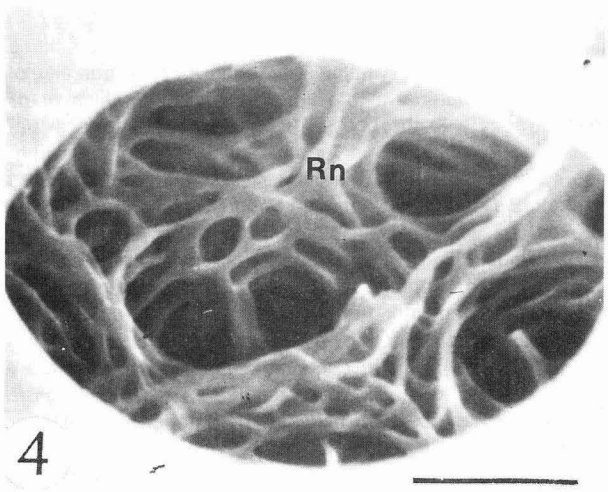

Fig. 4. Single aeropyle showing irregular respiratory network (Rn) inside. Scale bar = 1 μm.

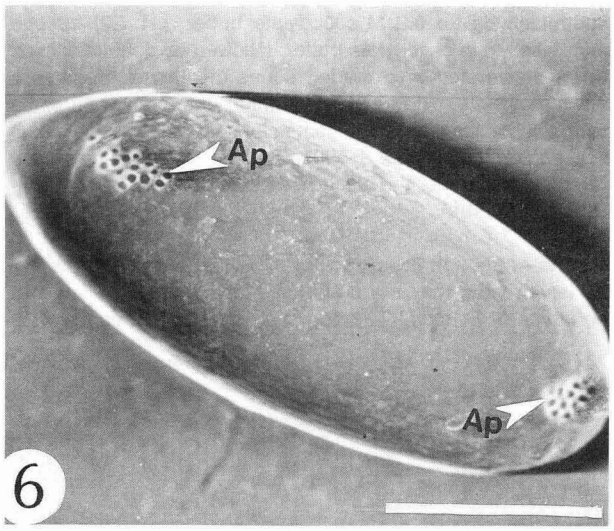

Fig. 6. The egg (third day after fertilization), dissected out from oviduct, showing aeropyles (Ap) at the anterior and posterior end. Scale bar = 100 μm.

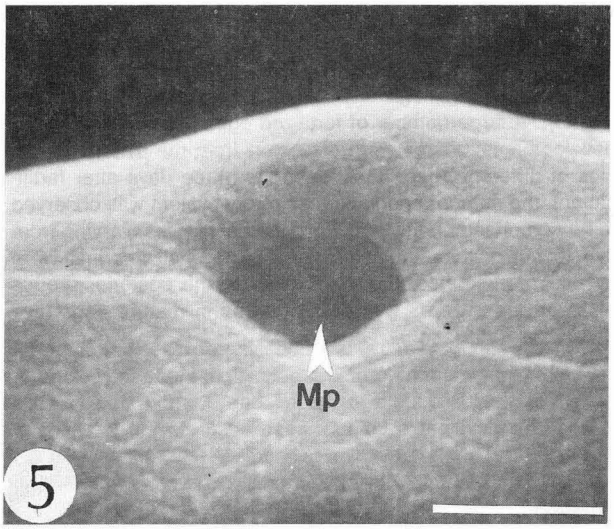

Fig. 5. Antero-lateral portion showing micropyle (Mp). Scale bar = 2 μm.

Discussion—The tachinids are endoparasites of a large number of insects especially caterpillars and adult beetles. The chorionic structures of the family Tachinidae have been investigated superficially and most are characterized by an asymmetrical shape and by the location of the aeropyles. The distribution of aeropyles in *E. bombycis* resembles that of *Euphorocera* (now *Austrophorecera*), a close relative of *Exorista* (Thompson 1968). Salkeld (1980) has investigated twenty-one species of the family Tachinidae, by ultrastructural analysis of the eggshell of *Eucexorista futilis*. The eggs of *E. futilis* have thick dorsal parts bearing air-filled trabecular structures which resemble the posterior poles of *Drosophila melanogaster* (Margaritis 1985). However, such structures have not been observed on the dorsal side of the *E. bombycis* egg. The tachinid eggs vary in their chorionic structure depending on habit and habitat. The chorion of *E. bombycis* is not modified for hatching, i.e., the eggs are considered indehiscent, like the eggs of *Centeter*, *Meigenia* and *Trichopoda*. In these the larva cuts through the anteroventral side of the shell and enters the host directly. Alternately, the eggs of some Tachinids like *Bessa*, *Podotachina* and *Winthermia* are classified as dehiscent. In these the shell possesses a hatching line at the anterior end through which the larva emerges into the open (Hinton 1981). Mellini (1960) observed that *Bessa selecta* Meig. (dehiscent eggs) makes use of the preformed hatching line. However, the maggot may sometimes cut through the ventral side of the chorion and then enter directly into the host. The chorion of *E. bombycis* has a lid that is well defined by a hatching line. However, it does not appear to play any important function in the hatching from the egg. After completion of hatching it has been observed that the lid had sometimes been pushed up mechanically by larva while hatching, but at other times, the lid, along with the hatching line, was found to be intact. The use of the compound microscope and visual observations on hatching behaviour of maggots and chorionic structures have been made by Siddappaji (1985) and Siddappaji and ChannaBasavanna (1990). The thick imprints on the dorsal side, the antero-dorsally located aeropyles and the smooth ventral surface in tachinid eggs of *Patelloa pochypyga* as observed by Salkeld (1980) under the scanning electron microscope are similar to those of *E. bombycis*. The impression of the micropylar opening at the anterior end and finger-like projections laterally directed towards the micropylar cavity were observed under a light microscope by Manjunatha and Puttaraju (1993) in *E. bombycis*. In the present study, a prominent micropylar opening without any finger-like projections was resolved under the scanning electron microscope. It appears to us that the finger-like projections observed by Manjunatha and Puttaraju (1993) may be the shell imprints directed towards the micropylar opening. Further, the anterior and posterior chorionic respiratory systems on the dorsal side of the eggshell were labelled incorrectly as operculum by Manjunatha and Puttaraju (1993).

While investigating the insect chorion, the type of chorionic

respiratory system, shape, size, and location of aeropyles and architecture of follicular epithelial imprints have been the main focus of many workers. The chorionic respiratory system consists of the aeropyles and/or plastron-bearing respiratory horn or holes and has been studied in different families of Diptera. These include Tephritidae = Trypedidae – aeropyles are located at the anterior end but are few in number (Hinton 1981); Psilidae – have conspicious ring of large aeropyles (micropyles ?) on a circular elevation (Ashby and Wright 1946); Scimyzidae – most of the genera bear plastrons (Hinton 1981); Dryomyzidae – have a pair of plastron-bearing respiratory horns, and in some genera (Cyclorrhapha) respiratory holes at the posterior end (Hinton 1960); Coelopidae – have a pair of long plastron-bearing respiratory horns (Egglishaw 1960); Lonchaeidae and Palopteridae – have scattered aeropyles; Helomyzidae – body surface has holes; Sphaeroceridae (Borboridae) – has plastron-bearing respiratory horns (Hinton 1981); Calliphoridae – has a plastron network (Richards and Morrison 1972); and Sarcophagidae – has a well developed plastron medially or in between hatching lines (Hinton 1963, 1981). The location of a well developed respiratory system on the dorsal surface of *E. bombycis* resembles that of the eggs of Drosophilidae.

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家蠶*Bombyx mori* Linn.內寄生蠅－Uzi 蠅*Exorista bombycis* (Louis) (雙翅目屬，寄生蠅科)卵殼表面形態

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從掃描電子顯微鏡對Uzi蠅卵殼表面之研究中，對其卵殼結構行爲上可能發生之機能與發育已獲得進一步之瞭解。本研究顯示出形態學與生理學上之重要結構，係氣孔、受精孔、具圓形蓋而明顯的孵化縫與卵殼呼吸網等。Uzi蠅的卵是不開裂型的卵群，幼蟲孵化時，卵殼不會裂開，而從卵的腹面孵化出來，然而在Uzi蠅，通常發現在寄生蠅的不開裂型卵上有一被蓋子環繞而明顯的孵化縫係用來孵化，此外，寄生蠅科昆蟲的卵殼呼吸系統在本文中亦作詳細之討論。

關鍵詞：卵殼，卵殼呼吸系統，孵化縫，Uzi蠅，掃描電子顯微鏡。

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