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EGGSHELL FINE STRUCTURE OF FUNGUS-GNATS *NEOEMPHERIA LINEOLA* (MEIG.) AND *N. STRIATA* (MEIG.) (DIPTERA MYCETOPHILIDAE, MYCOMYINAE)

INTRODUCTION

The insect eggshell is synthetized and laid down around the oocyte by the follicle cells in the last stages of the egg maturation. It has a very complex structure with both radial and regional complexity (MARGARITIS *et al.*, 1980) consisting respectively of various layers (vitelline envelope, waxy layer, endo and exochorion) and regional specialization (micropyle, respiratory and adhesive chorionic appendages, collar, operculum and others).

This complex chorionic structure is very interesting because of its important and specific protective functions for the mature egg and its diagnostic value for insect taxonomy and phylogenetic relationships. The importance of the chorion as a taxonomic character in insects has been firmly established by many scanning electron microscope (SEM) studies, most of them concerning dipteran eggs (HINTON, 1968, 1981; HINTON and SERVICE, 1969; HORSFALL *et al.*, 1970; MATSUO *et al.*, 1974a, b; KAMBYSELLIS, 1974; MAZZINI, 1974; WARD and READY, 1975; MAZZINI, 1977; ZIMMERMAN *et al.*, 1977; HILLEN and SOUTHERN, 1979; COGLEY and ANDERSON, 1983; GASC *et al.*, 1984; ISIDORO and LUCCHI, 1989). SEM investigation has also provided good evidence of species-specific chorionic patterns in Mycetophilidae (PLACHTER, 1981; MAZZINI and SANTINI, 1983; SANTINI and MAZZINI, 1983; 1989; MAZZINI *et al.*, 1990, 1992; SANTINI *et al.*, 1990).

The aim of this study was to investigate the eggshell of two fungus-gnats of the genus *Neoempheria*, *N. lineola* and *N. striata* (Diptera,

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Mycetophilidae), by scanning and transmission (TEM) electron microscopy. These species, included by TUOMIKOSKI (1966) in the subfamily Mycomyinae, belong to a very small group with palearctic distribution. They are present in Central and Boreal Europe (SEGUY, 1940). The relationships between the genera in the subfamily Mycomyinae are not properly understood (VAISANEN, 1984). Further morphological data could therefore be useful for taxonomic and phylogenetic purposes. The study may also facilitate the comprehension of structure-function relationships.

MATERIALS AND METHODS

Mature eggs were taken from adult females collected during the autumns of 1987 and 1988 on the wooded plain of San Rossore-Migliarino (Pisa) for *N. striata* and on the hilly wooded area of Montefoscoli (Pisa) for *N. striata* and *N. lineola*.

For scanning electron microscopy, the eggs were immersed in KARNOVSKY's fixative (1965) in 0.1 M, pH 7.2 sodium cacodylate buffer for 2 h at 4°C and dehydrated in a graded ethanol series. The eggs were dried by the critical point method using liquid CO_2 in a Balzers CPD 020 apparatus, attached to specimen holders with double sticky tape and coated with a layer (80 nm) of gold in a MED 010 Sputter Coater (Balzers Union). Egg surface observations were carried out with a Jeol JSM 5200 scanning electron microscope operating at 15 kV.

For transmission electron microscopy eggs were fixed and dehydrated as above, then embedded in Epon-Araldite mixture. Ultra-thin sections were cut with a Reichert Ultracut and an LKB Nova ultramicrotome, stained with uranyl acetate and lead citrate, and observed with a Jeol JEM 1200 EX II transmission electron microscope.

RESULTS

The eggs of the two species of the genus *Neoempheria*, *N. lineola* and *N. striata*, measure about 0.53 mm and 0.44 mm in length, and 0.20 mm and 0.16 mm in width respectively. They have a pear-like shape the apical pole of which is more sharply pointed than the posterior one (fig. I, 1,3). The apical pole contains a single micropyle.

In both species the micropylar area does not occupy the entire

anterior pole but is located slightly laterally. This makes it possible to recognize two different egg faces: dorsal face and ventral face. The latter, opposite to the micropyle, is characterized by a central plate and is more flatten than the dorsal face, which has sinuous morphology with the anterior part flat and the other convex. The micropylar area, in which the micropyle is centrally located, has the same shape in both species, but different basal chorionic morphology. It has fine granular structure in *N. lineola* (fig. I, 2), and in *N. striata* is covered by spherical appendages (fig. I, 4).

The external morphology of the egg surface is formed by a complex network of forked interconnecting ridges (fig. II, 1). Each ridge is raised on columns of different sizes (fig. II, 2), while the interridge area consists of a network of cord-like structures. This organization forms a complex system of intercommunicating chambers (fig. II, 1, 2).

The ventral plate, about 25 μm in diameter in both species, is circular in shape, and separated from the surrounding egg surface by a clear boundary. In both species, a thin cord-like structure divides it into several areas (fig. II, 3,4) characterized by a central polygon in *N. striata* (fig. II, 4).

TEM observations, shows that the eggshell consists of four layers: a well developed vitelline envelope, a waxy layer, a thin endochorion and an exochorion (fig. III, 1). The vitelline envelope is 1.2 μm thick and consists of homogeneously electrondense material crossed by a network of thin and irregular filaments (fig. III, 2 inset). The waxy layer consists of several membranes interposed between the vitelline envelope and the endochorion. The latter is a thin homogeneous layer supporting the network of columns and cord-like structures of the exochorion. A thin layer of mucous material coats the exochorion (fig. III, 1).

DISCUSSION

The eggs of Mycetophilidae have been divided into four types according to eggshell structure (PLACHTER, 1981). Eggs characterized by a plastron, a very complex chorion consisting of more than one layer and an outer surface covered by longitudinal ridges belong to the first type. Eggs with a plastron, a complex chorion and an outer surface with hexagonally distributed fine structures belong to the second type. Eggs without plastron and with a uniform, thick or very thin chorion (usually one-layered) belong to the third and fourth types respectively. PLACHTER

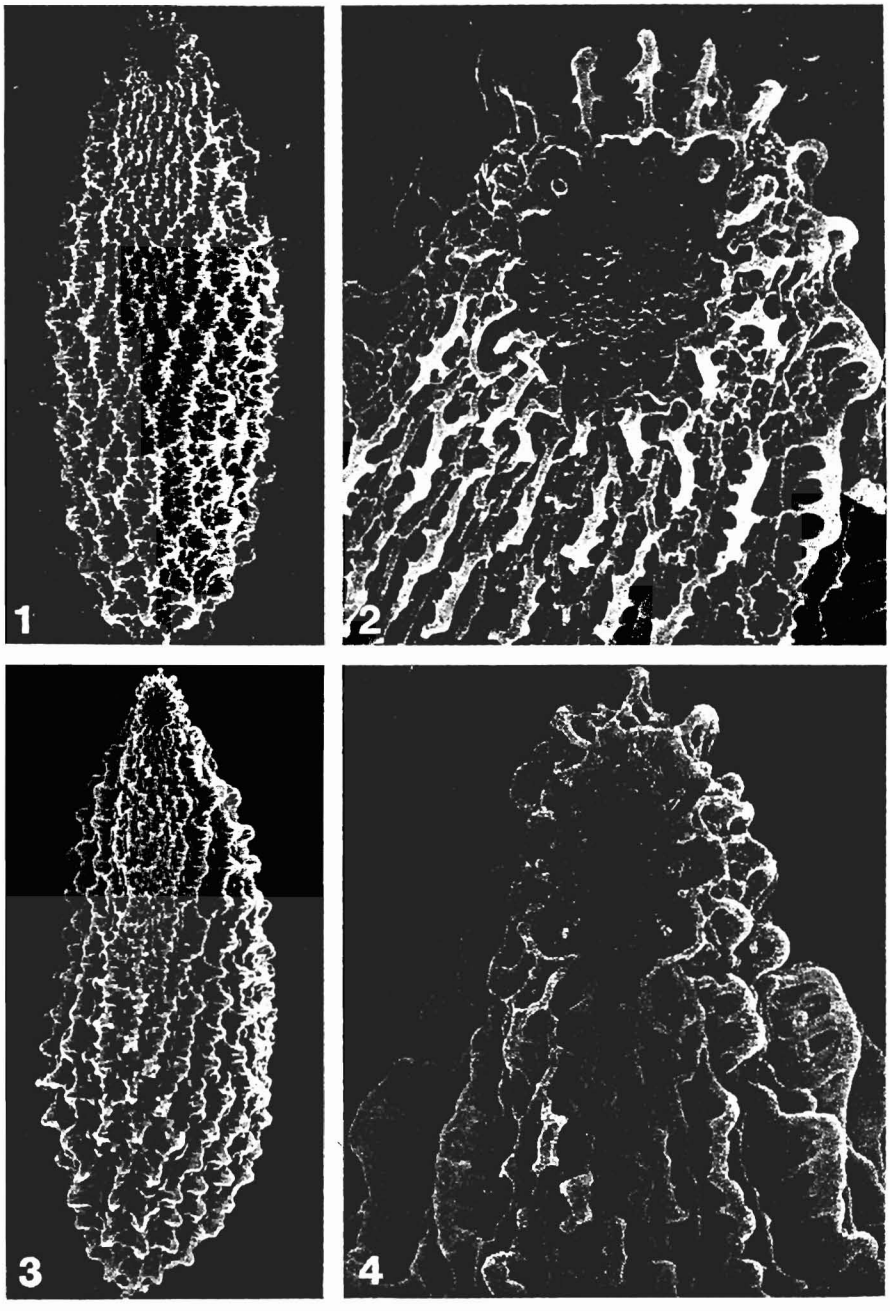

Fig. I

Dorsal face of the egg of *Neoempheria lineola* (1) and *N. striata* (3) with the respective micropylar areas (2,4) with a single centrally-located micropyle (arrow). The chorionic basal layer shows fine granular structure in *N. lineola* (2) and spherical appendages in *N. striata* (4). 1 × 170; 3 × 200; 2, 4 × 1500.

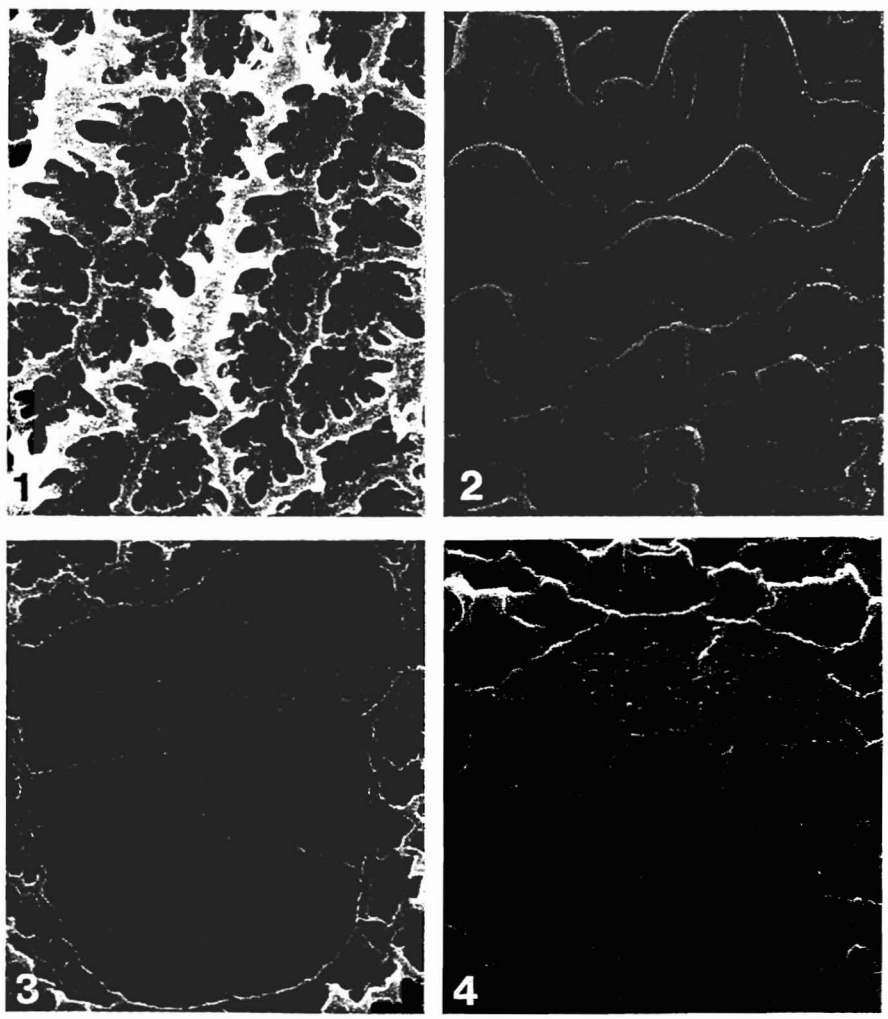

Fig. II

Outer egg surface of *N. lineola* showing the complex pattern of the interconnecting ridges (1) and the ridge outline supported by columns of different size (2). Ventral plate with irregular polygonal pattern in *N. lineola* (3) and with a central polygon in *N. striata* (4). 1 × 1500; 2 × 2000; 3,4 × 2000.

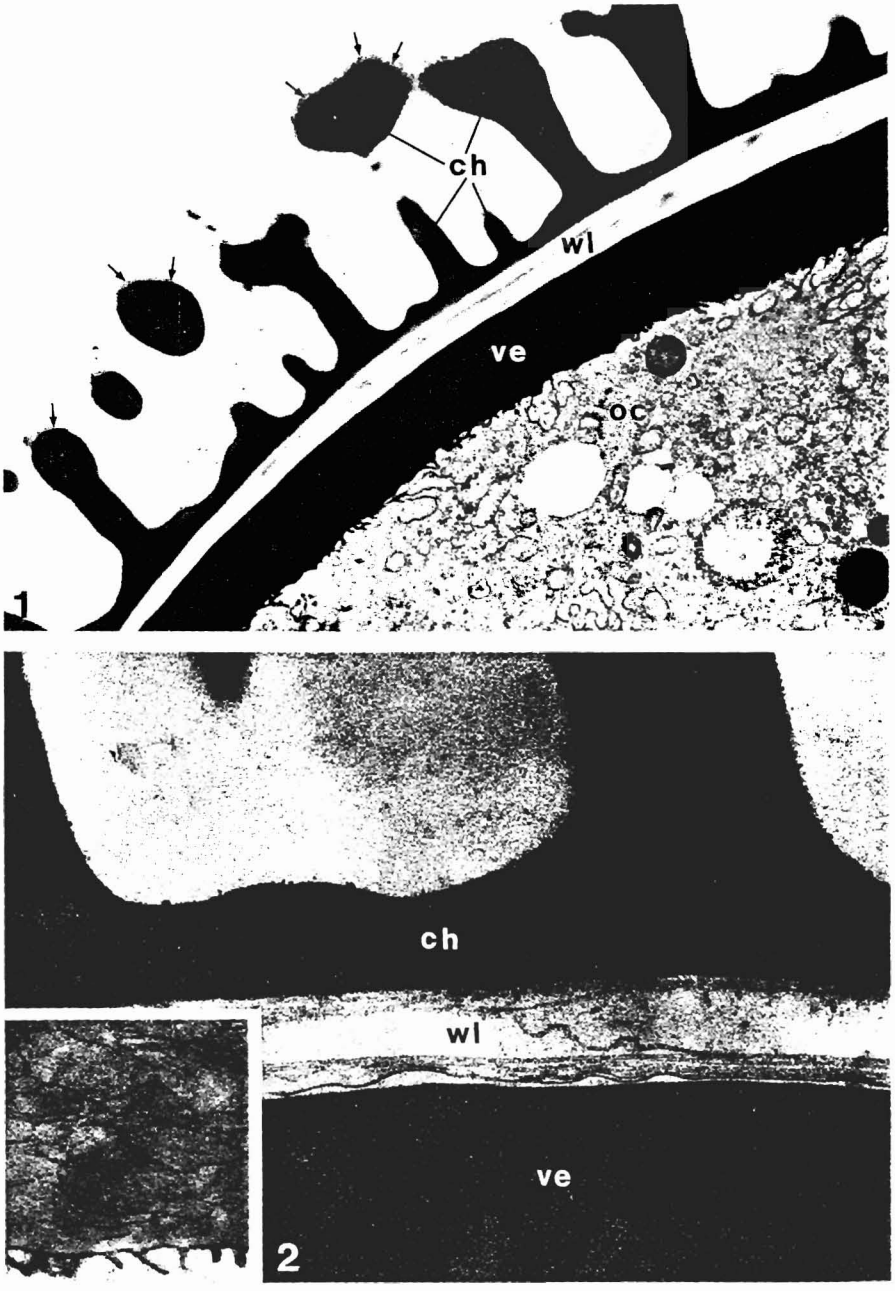

Fig. III

Transmission electron micrographs of the eggshell of *N. lineola* (1,2) showing the oocyte (oc) surrounded by vitelline envelope (ve), waxy layer (wl), outer chorion (ch) and thin layer of mucous material covering the external surface (arrows). Inset shows a detail of vitelline envelope by irregular network of electrondense filaments. 1, × 10000; 2, × 50000; inset × 30000.

(1981) regards the first type as characteristic of the subfamily Mycomyinae, with the exception of *Speolepta dissona* which has eggs with a very thin, one-layered, vitreous chorion. According to PLACHTER (1981), from the general features point of view, the eggshell structure of *N. lineola* and *N. striata* can be classified as belonging the first type, but some morpho-functional and ultrastructural differences are recognizable. The complex chorion of Mycomyinae eggs was interpreted by PLACHTER (1981) to consist of an exochorion, a one-layered endochorion and a thick «condensed sheet», homologous to the vitelline envelope of other insects.

TEM observations show that the eggshell ultrastructure of *N. lineola* and *N. striata*, like that of *Mycomya occultans* (MAZZINI *et al.*, 1990), consists of four layers interpreted as vitelline envelope, waxy layer formed by several membranes, endochorion and exochorion. The same ultrastructural organization has been observed in the eggshell of other Mycetophilidae belonging to a different subfamily (SANTINI *et al.*, 1990).

The respiratory plastron in Mycomyinae eggs was interpreted by PLACHTER (1981) as the internal portion of the longitudinal ridges. MAZZINI *et al.* (1992) interpret as plastron the ventral plate found for the first time in three species of *Mycomya*, in both the present species and in another species belonging to Leinae (our unpublished data).

Unlike other Mycomyinae species, the outer egg surfaces of *N. lineola* and *N. striata* show a complex pattern formed by the network of interconnected longitudinal ridges. These data confirm the close relationships between structure and functions ensuring exchanges between the environment and the developing embryo in the two *Neoempheria* species, like in the related *Mycomya* (MAZZINI *et al.* 1990, 1992) and other Mycetophilidae (MAZZINI and SANTINI, 1983; SANTINI and MAZZINI, 1983, 1989, SANTINI *et al.*, 1990). These functions include the regulation of gas exchanges, protection against desiccation, adhesion to the substrate and resistance to crushing.

Although both eggs are pear-shaped, and have a micropylar area characterized by a single centrally-located micropyle and a common external chorionic pattern consisting of a network of irregular cord-like structure, from ootaxonomic point of view some morphological characters seem to be species-specific. The eggs of *N. lineola* and *N. striata* are different in size, have different basal chorionic sculptures in the microplylar area (fine granular in *N. lineola* and spherical appendages in *N. striata*) and different morphological design of the ventral plate.

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SUMMARY

The eggshell structure of two fungus-gnat species, namely *Neoempheria lineola* (Meig.) and *N. striata* (Meig.) (Diptera Mycetophilidae, Mycomyinae), was studied by scanning (SEM) and transmission (TEM) electron microscopy. The eggs of both species are pear-shaped and have a single micropyle located at the centre of a well defined micropylar area. In both species, the latter occupies a dorsal position at the anterior pole of the egg. The outer surface has a complicated pattern of forked and interconnecting ridges and a network of cord-like structures in the interridge areas. The ventral plates, interpreted as a respiratory plastron, show a polygonal pattern. Species-specific characters include the egg size, the different chorionic basal layer of the micropylar area and the design of the ventral plate.

These features are compared with those of other dipterans and especially other Mycomyinae.

KEY WORDS: Ootaxonomy, Egg, Chorion, Micropyle, Mycetophilidae.

RIASSUNTO

FINE STRUTTURA DELLE SCULTURE CORIONIDEE DELL'UOVO DI DUE SPECIE DI NEOEMPHERIA, N. LINEOLA *E* N. STRIATA *(DIPTERA MYCETOPHILIDAE, MYCOMYINAE)*

La fine struttura del guscio delle uova di due specie di micetofilidi micomiini, *Neoempheria lineola* (Meig.) e *N. striata* (Meig.) è stata esaminata ai microscopi elettronici a scansione (SEM) ed a trasmissione (TEM). In entrambe le specie le uova sono piriformi e mostrano un singolo micropilo localizzato al polo anteriore, al centro di una ben delimitata area situata dorsalmente. La superficie esterna è caratterizzata da una morfologia complessa dovuta alla presenza di rilievi longitudinali biforcati alle estremità ed interconnessi. Le piastre ventrali, interpretate come piastroni respiratori, mostrano un pattern poligonale. Le dimensioni delle uova, le decorazioni dello strato basale dell'area micropilare e delle piastre ventrali, sono caratteri di rilevante interesse ootassonomico.

I dati ottenuti sono stati comparati con quelli relativi ad uova di altri ditteri ed in particolare di altri Micomiini.

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