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ELDA GAINO* - ATTILIA FAVA**

Egg general morphology and eggshell fine organization of the grain weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)°

ABSTRACT

Eggs of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) mature into telotrophic ovarioles and are laid into grains. Each egg is oviposited in a tunnel excavated by the female rostrum and closed at its top by a mucous plug. The relationships and the organization of the egg envelopes have been investigated by scanning and transmission electron microscopy (SEM, TEM) in both ovary eggs and laid eggs. Peripheral ooplasm of growing eggs is enriched by microvillar projections which presumably act as a guide-system for vitelline envelope deposition. The result is an electron-dense network penetrating the oolemma and coalescing to form a homogeneous electron-dense, outermost stratum. The chorion consists of two layers, the endochorion, characterized by wavy electron-dense parallel laminae loosely arranged, and the exochorion showing a homogeneous texture. An outermost electron-dense border delimits the exochorion. The exochorion is rippled all around the ovary eggs and stretches along with egg elongation. Laid egg shows a polar cap and a different eggshell architecture. The vitelline envelope is more compact; the exochorion is widely detached from the vitelline envelope and forms ruffles on the egg surface. The space between the exochorion and the vitelline envelope tends to increase with the disappearance of the endochorion. A serosal cuticle appears underneath the vitelline envelope and gradually extends towards the polar cap, the central region of which contains flocculated material.

Key Words: egg envelopes, ultrastructure, follicle cells.

1. INTRODUCTION

Three species of the genus *Sitophilus, S. granarius* (L.), *S. oryzae* (L.) and *S. zeamais* Mots, have economic importance because they damage stored grains. The biology of these grain pest species has been critically reviewed by LONGSTAFF (1981).

Some aspects of the reproductive biology of *S. granarius* have been highlighted by RICHARDS (1947). The gross anatomy of the reproductive system of *Sitophilus* has been illustrated in *S. oryzae* by NARDON (1978). Data on the

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* Institute of Zoology, University of Perugia, Via Elce di Sotto, 06100 - Perugia (Italy).
** University of Genova, Via Balbi 5, 16126 Genova (Italy).
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functional anatomy of the female apparatus have been reported in *S. granarius* and *S. zeamais* by KHAN & MUSGRAVE (1969). The ultrastructural organization of both spermatheca and sperms have been described in *S. granarius* by TOMBES & RAPPEL (1972).

S. granarius affects wheat in temperate regions of the world, in particular the Mediterranean region. It is restricted to high altitude in warmer regions (CHAMP & DYTE, 1976).

According to STEFFAN (1963), females bore the grains with their rostrum and lay one egg per pit. Afterwards, they deposit a mucous plug on top of each egg, thus providing the external marker for grain infestation. It has been recently proved that each female tends to lay from 2 to 10 eggs (FAVA & SPRINGHETTI, 1991) and that mated females lay more eggs than unmated ones (SPRINGHETTI & FAVA, 1994).

No ultrastructural aspects of oogenesis have been reported for *S. granarius*. Considering the economic impact of these insects on stored grains we have illustrated some ultrastructural details of their eggs before and after deposition. Egg envelope organization could be of some interest for the selection of ovicides acting on the ovary eggs or after their deposition in grains.

2. MATERIALS AND METHODS

Specimens of *Sitophilus granarius* (L.) were reared on wheat grains at ambient environmental conditions. Before dissecting ovaries, adult females were anesthetized by 70% ethyl alcohol (10-20 sec) and transferred to physiological saline medium.

Infested grains were identified with the acid-fuchsin medium according to FRANKENFELD'S method (1948), slightly modified. Egg plugs stain red. Laid eggs were extracted cutting grains at the level of the plugs.

For transmission electron microscopy (TEM), selected material were fixed in KARNOVSKY'S fixative (1965), in pH 7.2 HCl-sodium cacodilate buffer for 1 h and postfixed in 1% osmium tetroxide for 1 h. Ovaries were dehydrated in an ethyl alcohol series and embedded in Araldite resin. Sections were collected on formvar coated copper grids, doubled stained with uranyl acetate and lead citrate, and observed with a Philips EM 300 electron microscope.

For scanning electron microscopy (SEM), ovaries and eggs were fixed following the same procedure used for TEM investigation, rinsed in cacodilate buffer, dehydrated in an ascending series of ethanol alcohol, and critical-point dried in a Bomar apparatus using CO_2. Selected material was mounted on stubs and coated with gold-palladium in a Balzers Union spatter unit. Observations were performed with a Philips 515 SEM.

Explanation of symbols used in the figures:

CD = common oviduct
EE = egg envelopes
Eg = egg
En = endochorion
ES = egg surface
Ex = exochorion
FC = follicle cell
LD = lateral oviduct
MB = microvillar border
Nu = nucleus
Oo = oocyte
Ov = ovarioles
SC = serosal cuticle
St = spermatheca
VE = vitelline envelope
Vg = vagina

3. RESULTS

The female reproductive system of *S. granarius* (fig. 1a) consists of paired ovaries. Each is constituted of a pair of ovarioles merging into a paired sack-like genital oviduct (lateral oviduct), where eggs accumulate before reaching the common oviduct. From this latter, eggs enter the vagina, which presents the spermatheca. According to the taxonomic position of this species, ovarioles belong to the telotrophic type and result from suspensory ligament, germarium and vitellarium.

Red spots on the grains, after acid-fuchsin treatment, indicate the areas affected by oviposition. Cross sections of grains in these areas show that eggs are laid along the main axis of the grain, farthest from the embryo.

SEM observations of grains fragments, including the egg lodging (fig. 1b), show that eggs are surrounded by an irregularly and slightly wrinkled coat (fig. 1c).

When eggs are extracted from grains (fig. 1d), they reveal the occurrence of a polar bulge that forms a short cap. The presence of a rippled coat surrounding the egg is evident.

The eggs of *S. granarius* measure 600 μm in length in average, including the 75-μm cap, and 270 μm in width.

Mature ovary eggs, observed under SEM in cross fractured ovaries, are cylindric; this unusual shape is due to the flattening of both polar regions (fig. 2a). The egg surface displays a greatly wrinkled coat (figs. 2b, c).

TEM sections of ovarioles show young oocytes regularly surrounded by follicular epithelium. The oolemma form microvilli of various length protruding toward the epithelium (fig. 2d). The microvilli are abundant and may be

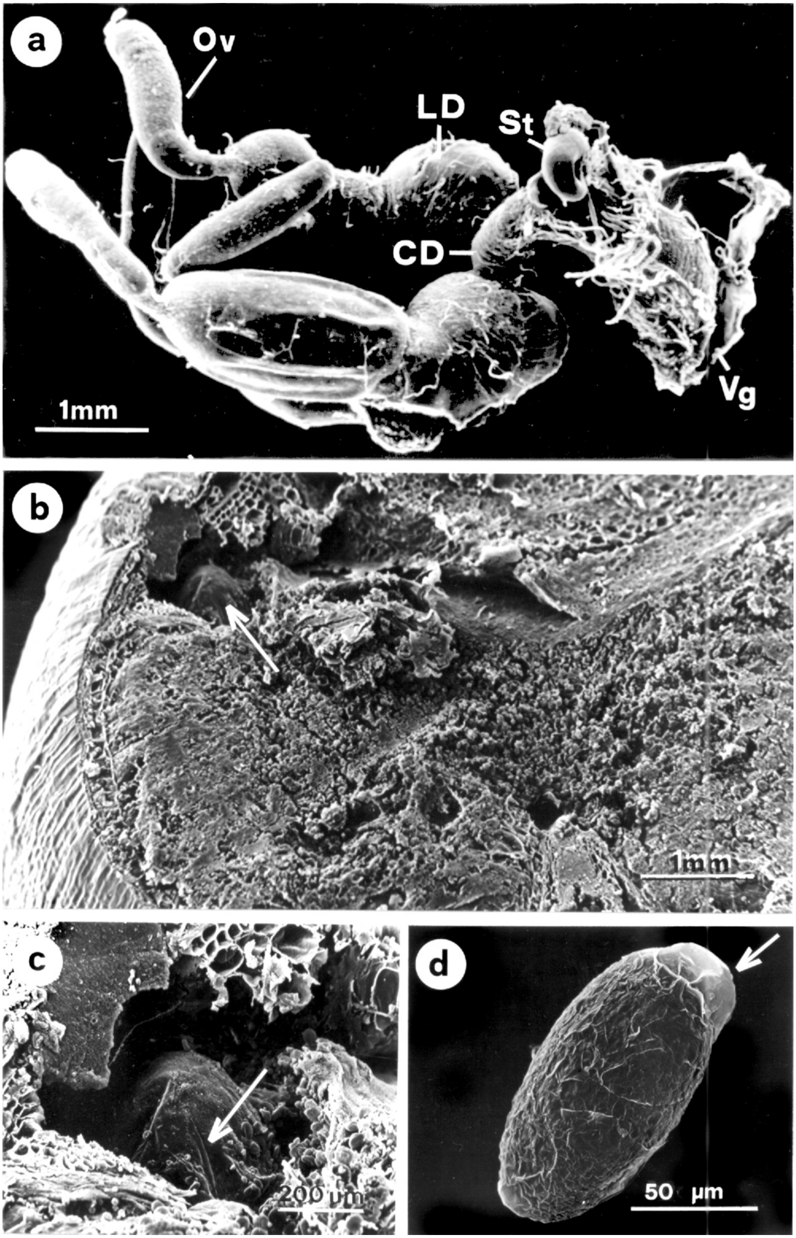

Fig. 1 - SEM view of a) female reproductive apparatus of *Sitophilus granarius*; b) egg (arrow) inside a broken grain; c) detail of the fig. b showing the anterior egg pole with the outermost coat slightly folded; d) egg extracted from a grain. Note the ruffled coat and the polar bulge at the anterior pole (arrow).

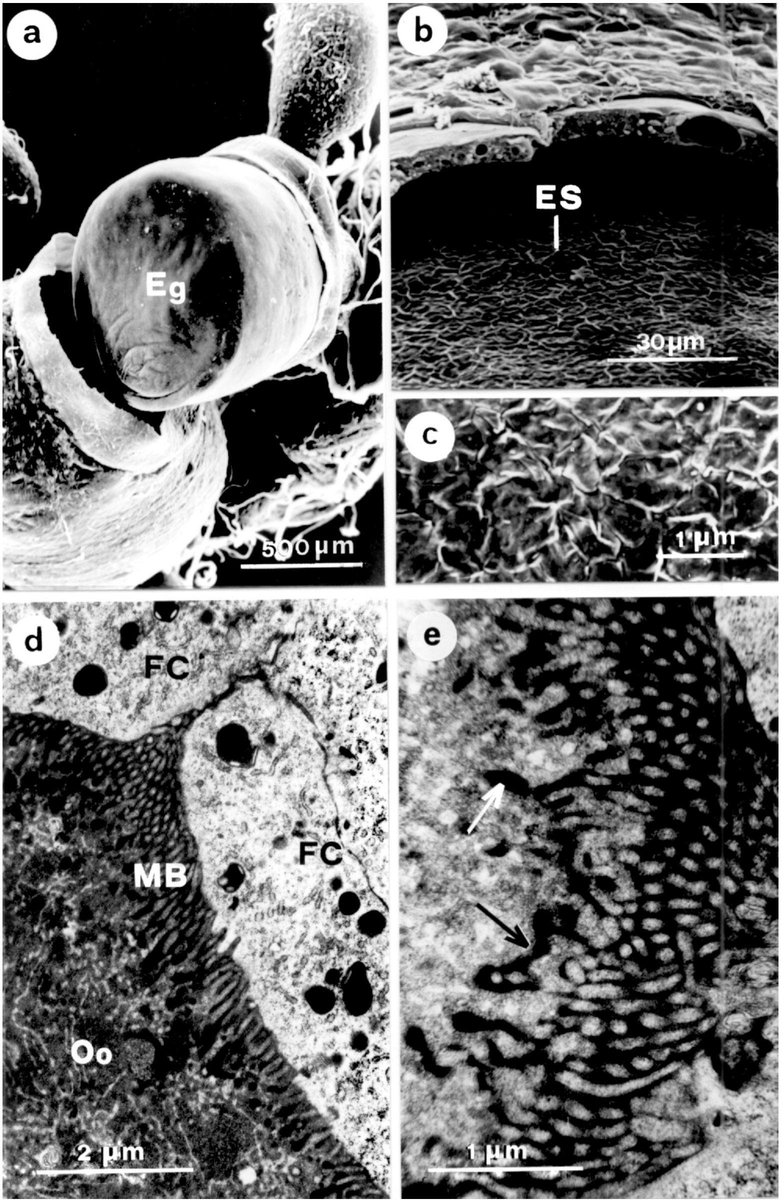

Fig. 2 - SEM (a, b, c) and TEM (d, e) view of a) cylindrical shape of an egg in cross fractured ovary; b) portion of outermost egg surface; c) detail of the egg surface showing its wrinkled appearance; d) detail of a section from a growing oocyte that shows the microvillar border entering the follicle cell interspace; e) deposition of electron-dense material (arrows).

organized in bundles to penetrate the interstices between adjacent follicle cells. Electron-dense material deposits along the microvillar border and accumulates in its basal region (fig. 2e).

As it is known in insects, follicle cells are responsible for egg envelope deposition and persist as a continuous coat until the end of eggshell elaboration (fig. 3a). At the end of choriogenesis, follicle cells tend to detach from the egg surface (fig. 3b). This event parallels the increase in cytoplasmic vacuolization.

The oolemma consists of a homogeneous matrix since stored material tends to accumulate inwards, as shown by SEM images of cross sectioned eggs (fig. 3c).

From the inside out, the shell consists of the vitelline envelope, the endochorion and the exochorion (fig. 3d).

The vitelline envelope measures about 0.2 µm in thickness. It appears as a dense and homogeneous sheet along its outermost surface whereas inwards it gives rise to a meshwork penetrating the oolemma (inset fig. 3b).

The endochorion consists of several wavy laminae that constitute a discontinuous coat measuring 0.15 µm in average thickness (fig. 3d).

The exochorion has approximately the same thickness as the endochorion but presents a uniform electron-transparent texture with an electron-dense border (fig. 3b).

Sections of laid eggs examined under TEM, 24 to 48 hours after deposition in grains, reveal a wide space between the endochorion (fig. 4a) and the exochorion (fig. 4b). The endochorion loses its multi-layered organization and tends to disappear. Some remnants can be occasionally seen associated with the vitelline envelope and/or the exochorion (figs. 4a, b). Eventually, an empty space separates the exochorion from the vitelline envelope (Figs 4d, e). A serosal cuticle appears underneath the vitelline envelope, which shows an organization more uniform than that of ovary eggs. The cuticle enlarges considerably towards the anterior egg pole (fig. 4c), thus contributing to form the polar cap seen in the eggs extracted from grains and examined under SEM (fig. 1d). The polar cap roughly results from two distinct components: a loose central region with flocculated material and a peripheral border strengthened by the serosal cuticle (fig. 4c).

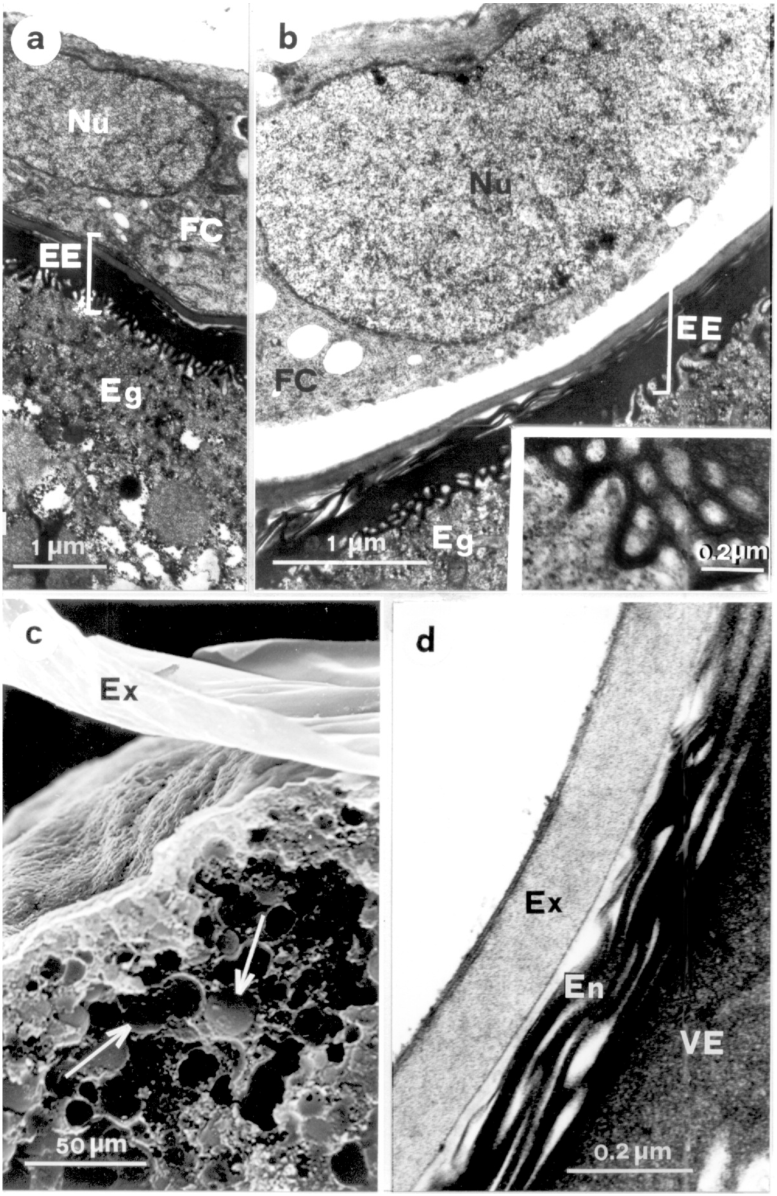

Fig. 3 - TEM (a, b, d) and SEM (c) view of a) oocyte/follicle cell tight adhesion in ovarioles; b) detachment of a follicle cell from the egg surface. The inset is a zoom of the vitelline envelope meshwork; c) egg cross section showing stored material accumulated inside cytoplasm (arrows); d) sequence of the egg envelopes from the inside out: vitelline envelope, endochorion, exochorion.

4. DISCUSSION

In a comparative analysis on the evolution of the egg envelopes, Mazzini *et al.* (1984) have given an extensive overview of their increasing complexity among living organisms. In insects particular attention has been paid to the relationship between eggs and follicle cells for their involvement in vitelline and chorionic layer deposition trough subsequent secretory cycles. This feature has been repeatedly proved in several insect groups both entognathans (Bilinski, 1995) and ectognathans (Anderson & Spielman, 1973; Beams & Kessel, 1969; Cummings, 1972; De Loof, 1971; Favard-Séréno, 1971; King & Kich 1963; Matheu & Rai, 1975; Norton & Vinson, 1982).

Polyphagous Coleoptera, to which *S. granarius* belongs, have telotrophic ovarioles (Büning, 1979 a, b) characterized by the retention of all nurse cells in the anterior trophic chamber (Stys & Bilinski, 1990; Büning, 1993), so that the only cellular sheath that surrounds the growing eggs is constituted by follicle cells. Presumably, as for most insects, these are cells devoted to egg envelope deposition also in *S. granarius*. The secretory paths of this process, however, have not been reconstructed in this species. The images of vitelline envelope organization support this notion. Electron-dense material deposition takes place at the follicle cell/oocyte interface along the microvillar egg border that acts as a mould for vitelline envelope organization.

In laid eggs, along with the disappearance of the endochorion, the vitelline envelope becomes more compact and the distance between this layer and the exochorion gradually increases. Similar events have been evidenced as well in eggs of a cecidomiid (Isidoro & Lucchi, 1989).

The exochorion is more wrinkled in the eggs contained in the ovary than in laid eggs. Presumably, it stretches as the egg increases in volume by elongation. This plasticity may be related to the lack of tight adhesion to the sheath underneath. This feature together with the disappearance of the endochorion in the laid eggs allows the exochorion to ruffle around the egg.

At deposition, females prefer grains with a certain degree of humidity (Holloway, 1985), a parameter that may be important for further development of embryo. On this account, the serosal cuticle constitutes a barrier protecting the embryo from desiccation.

Among living organisms, insects show the most remarkable variety in eggshell organization (review in Hinton, 1981). Different groups have differentiated chorionic decorations whose morphology can be useful for taxonomic and phylogenetic purposes. In addition, eggshell decorations give information on the function of chorionic devices in improving egg survival after deposi-

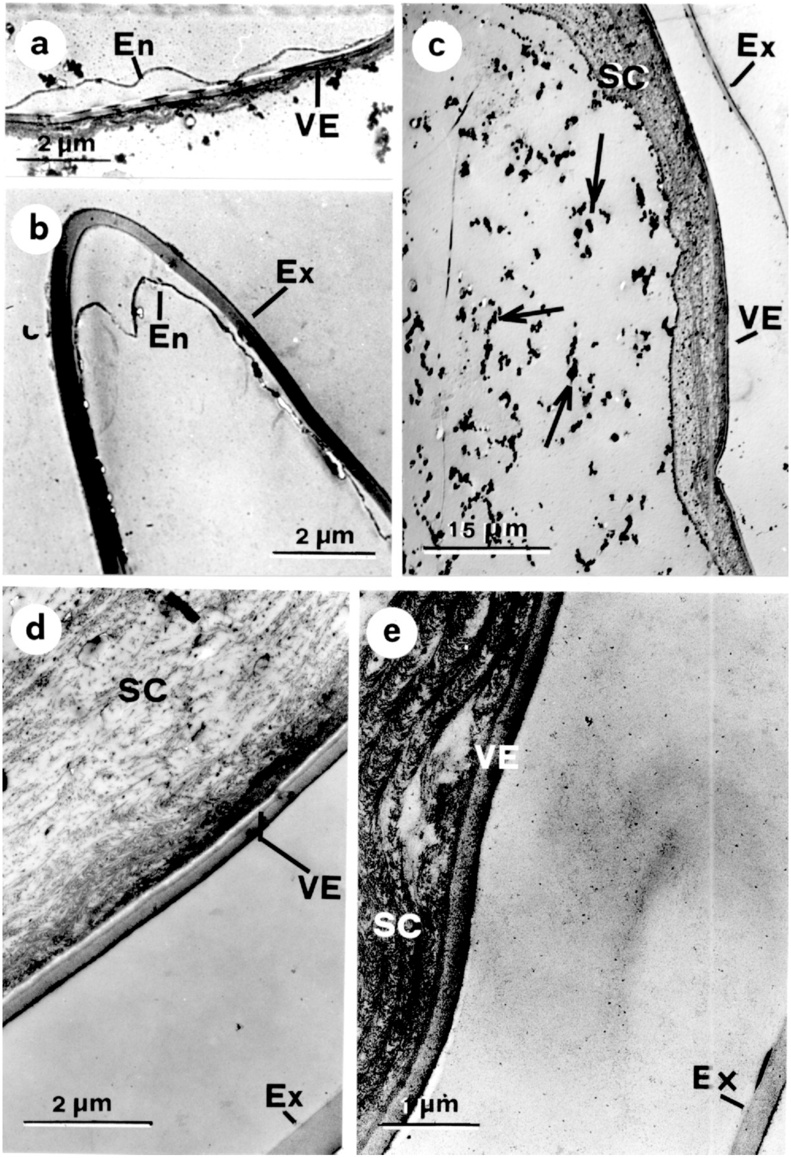

Fig. 4 - TEM view of a laid egg a) feature of the endochorion associated with the vitelline envelope; b) the same associated with the exochorion; c) polar cap of the egg. Note the wide space between exochorion and vitelline envelope, and the serosal cuticle delimiting the region with flocculated material (arrows); d) detail of the serosal cuticle in the polar cap; e) organization of the serosal cuticle along the egg body.

tion (CHAUVIN & CHAUVIN, 1980; GAINO & MAZZINI 1987, 1988; FEHRENBACK, 1989; GAINO *et al.*, 1989; ROSCISZEWSKA, 1991; FAUSTO *et al.*, 1992; MAZZINI *et al.*, 1992, 1993; KAMBYSELLIS, 1993). The eggshell of *S. granarius* lacks peculiar features of differentiation.

Plugs close the tunnels after egg deposition into the grain. The plugs exercise a mechanical function but may also act as a signal for other females in preventing them from ovipositing in the same area. Another possibility is that they could protect eggs from the aggression of environmental micro-organisms. Indeed, it has been demonstrated that in the dipteran *Ceratitis capitata* this protective function is performed by peptides, with lytic and/or antibacterial activity, present in the secretion of the female reproductive accessory glands (MARCHINI *et al.*, 1993).

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6. RIASSUNTO

MORFOLOGIA GENERALE DELL'UOVO E FINE ORGANIZZAZIONE DEGLI INVOLUCRI DI *SITOPHILUS GRANARIUS* (L.), PUNTERUOLO DEL FRUMENTO (COLEOPTERA: CURCULIONIDAE)

Le uova di *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) maturano in ovarioli di tipo telotrofico e vengono deposte all'interno delle cariossidi del grano, dopo che la femmina vi ha scavato con il rostro una piccola galleria. Essa ne occlude successivamente l'apertura secernendo un tappo mucoso. Le indagini in microscopia elettronica a scansione e a trasmissione, condotte su uova ovariche e deposte, hanno permesso di studiare l'organizzazione degli involucri ovulari, i loro rapporti e le modificazioni che seguono all'ovideposizione. L'oolemma degli ovociti in accrescimento è caratterizzato da una ricca rete di microvilli che sembra fungere da stampo per l'elaborazione dell'involucro vitellino. Tale involucro, infatti, mostra uno strato basale reticolare i cui elementi fortemente elettrondensi si approfondano nel sottostante oolemma. Il corion consta di due parti: l'endocorion, a tessitura lassa e plurilamellare, e l'esocorion, omogeneo e delimitato verso l'esterno da un bordo elettrondenso. L'esocorion, inizialmente molto ripiegato su se stesso, si distende con l'allungamento dell'uovo. Le uova deposte presentano un rigonfiamento al polo anteriore ed una diversa organizzazione degli involucri. L'involucro vitellino è più compatto; l'endocorion tende a ridursi fino a scomparire, lasciando un ampio spazio tra l'esocorion e la membrana vitellina. Ciò permette all'esocorion di formare pliche sulla superficie dell'uovo. Una cuticola sierosa compare al di sotto dell'involucro vitellino e gradualmente si estende verso la protuberanza polare, la cui regione centrale contiene materiale flocculato.

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