

FINE STRUCTURE OF THE EGG SHELLS OF *HABROPHLEBIA FUSCA* (CURTIS) AND *H. CONSIGLIOI* BIANCHERI (EPHEMEROPTERA : LEPTOPHLEBIIDAE)*

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Abstract—The eggs of 2 mayflies, *Habrophlebia fusca* and *H. consiglioi* (Ephemeroptera : Leptophlebiidae) were observed with scanning and transmission electron microscopes. The external surface of the eggs in both species had longitudinally oriented costae. The chorion of *H. fusca* had different structures in its costal and intercostal zones. Three distinct layers could be recognized: an inner layer close to the vitelline coat, consisting of electron-dense lamellae perpendicular to the egg surface; an intermediate layer, consisting of loosely structured fibrillar material; and an outer highly electron-dense layer, consisting of 2 separate laminae, divided by an electron-transparent line. In the egg of *H. fusca*, the costal area of the chorion shows a columnar structure. The columns merge distally to create wide chambers. This organization has been observed with the SEM in *H. consiglioi* as well. The chambers are interconnected and communicate with the exterior through openings along the costal edges. Masses of mucus-like substance are present both in the chambers and outside the chorion; they show fibrillar material and electron-dense bodies with a paracrystalline structure.

Index descriptors (in addition to those in title): Ootaxonomy, chorion, vitelline envelope, micropyle, adhesive substance, scanning electron microscopy, transmission electron microscopy.

INTRODUCTION

NUMEROUS scanning electron microscope (SEM) studies have been performed on insect eggs (see Hinton, 1981). Most of these works have revealed diagnostic characteristics useful in the assessment of phylogenetic relationships and have advanced our knowledge of the mechanism of fertilization.

Previous SEM studies of ephemeropteran eggs have included Baetidae (Kopelke and Müller-Liebenau, 1981a,b; 1982), Caenidae (Kopelke, 1980; Malzacher, 1982), Heptageniidae (Flowers, 1980; Kopelke, 1980; Landa and Soldán, 1982), Leptophlebiidae (Kopelke, 1980; Pescador and Peters, 1982; Gaino and Mazzini, 1984) and Tricorythidae (Kopelke, 1980). These studies have revealed features of the chorionic sculpturing, micropyle, and attachment structures that can be useful in understanding the phylogeny of Ephemeroptera.

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However, no information on the ultrastructure of the Ephemeroptera egg shell has yet been published. We present here an SEM analysis of the eggs of *Habrophlebia fusca* and *H. consiglioi*. We also present the first report of the fine morphology of the egg envelope of *H. fusca*, as seen with the transmission electron microscope (TEM).

Ephemeroptera lay their eggs in water with different oviposition mechanisms. In particular, *H. fusca* lays eggs in masses; when the abdomen of the mayfly contacts the water, the eggs separate from one another, and fall down to the bottom (Grandi, 1960).

MATERIALS AND METHODS

Source of eggs

For SEM, eggs were obtained by dissecting mature nymphs and imagos of *Habrophlebia fusca* (Curtis) collected in Piemonte and imagos of *Habrophlebia consiglioi* Biancheri from Sardegna.

For TEM analysis, eggs were taken from mature nymphs of *H. fusca* gathered in the Erro stream (Piemonte).

In both species only unfertilized eggs were studied.

Scanning electron microscopy

Eggs were stored in 80% ethanol or fixed for 2 hr in 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2 at 4°C (Karnovsky, 1965), dried by the critical-point method, using liquid CO₂ in a Bomar apparatus, attached to specimen holders by silver conducting paint, coated with gold in a Balzers Union evaporator and observed with a JEOL JSM 2 or a Coates & Welter Cwiskan 106 A field emission scanning electron microscope. Some eggs were observed without critical-point treatment.

Transmission electron microscopy

Eggs were fixed for 1 hr in Karnovsky's (1965) fixative, placed for 12 hr in cacodylate buffer, post-fixed in 1% OsO₄ for 1 hr, dehydrated in a graded ethanol series and embedded in Epon 812 and Epon - Araldite. Sections were cut either with LKB or Reichert OM U2 ultratome, mounted on grids, stained with uranyl acetate and lead citrate, and observed with a Philips EM 300 or EM 301 at the Institute of Zoology of the University of Siena.

RESULTS

The external surface of eggs belonging to the genus *Habrophlebia* is characterized by a series of raised costae whose arrangement, as observed under the SEM, could be used as a taxonomic characteristic to determine species (Gaino and Mazzini, 1984). In *H. fusca*, the longitudinal costae, are spaced well apart from each other but sometimes converge (Fig. 1). Each of these costae shows a series of small pores on its dorsal surface and a sinuous pattern owing to projections and indentations along its outside margins (Fig. 2).

Although the chorion shows differences between the costa and the intercostal areas, it can be subdivided into 3 distinct layers according to its morphology in sections.

The chorion in the intercostal areas

The chorion shows an inner, 0.3 μm thick layer adjacent to the vitelline coat. It is characterized by a periodic distribution of electron-dense lamellae displaying variable forms and disposed more or less perpendicular to the egg surface (Fig. 3). Each lamella, about 17nm thick, is separated from another by 10nm. The superimposed intermediate chorionic layer consists of loosely structured fibrillar material, which is sometimes organized in an irregular lattice (Fig. 3). This coat supports the outermost layer of the chorion, 0.3 μm thick, which is highly electron-opaque and consists of 2 laminae lying one upon the other and separated by a sinuous electron-transparent stratum (Figs. 3;4). The outermost lamina has an irregular surface (Figs. 3;4), which imparts a granular appearance to the chorion coat and forms small protrusions that are especially well developed in the intercostal areas (Fig. 2). The electron-transparent innermost layer, the vitelline coat, about 0.4 μm thick, separates the chorion from the underlying ooplasm (Figs. 4;5).



FIG. 1. Scanning electron micrograph of a whole *H. fusca* egg. $\times 500$.

FIG. 2. An SEM view of external surface with sinuous costae (*c*) showing pores (arrows) and intercostal areas (*ic*) (*H. fusca*). $\times 3,000$.

FIG. 3. Transmission electron micrograph of chorion in intercostal area; *il* = inner layer; *iml* = intermediate layer; *ol* = outer layer consisting in 2 superimposed laminae (*H. fusca*). $\times 84,000$.

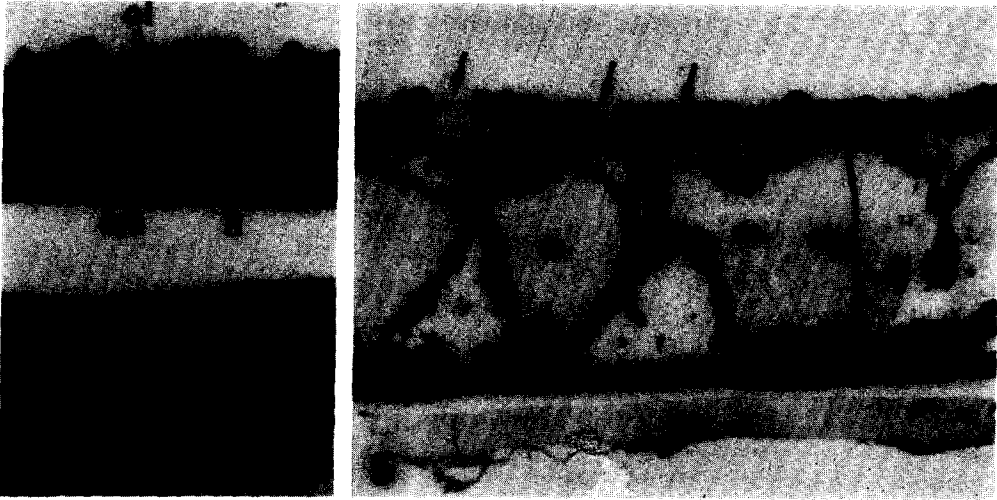


FIG. 4. Transmission electron micrograph of egg envelopes in intercostal area; *co* = cortical ooplasm; *il* = inner layer; *iml* = intermediate layer; *ol* = outer layer; *ve* = vitelline envelope (*H. fusca*). $\times 38,000$.

FIG. 5. Transmission electron micrograph of egg at costal area. Note organization of chorion (*ch*) with external cavities (arrows), mucus (*m*) inside chambers, and vitelline envelope (*ve*) (*H. fusca*). $\times 12,000$.

FIG. 6. Transmission electron micrograph of chorion showing superimposed mucous adhesive layer (*m*) externally delimited by fibrillar projections (arrows). $\times 38,000$.

FIG. 7. Enlarged view of mucous material with dense bodies having paracrystalline structure, and fibrillar material. $\times 38,000$.

The chorion in the costal areas

The organization of the costal areas is complex, compared with that of the intercostal spaces. In fact, the chorion consists of columnar structures arising vertically from the innermost chorionic layer (Fig. 5). These formations are composed of the same kind of matrix that characterizes the intermediate layer of the chorion of the intercostal zones (Fig. 4). The presence of the columnar layer increases the thickness of the chorion to 2.6 μm . The distal parts of the columns converge into a plane parallel to the egg surface, thus creating wide chambers (Fig. 5) that are interconnected along the axis of the costa and communicate with the exterior through openings along the edges.

Each column is covered by an electron-dense substance, 0.1 μm thick, continuous with the internal chorionic lamina of the intercostal spaces. This lamina also supports the outer lamina, which forms a 0.3 μm thick layer covering the costae. Small cavities, corresponding to the pores observed with the SEM, make the outer surface of the costae more irregular (Fig. 5).

The chambers situated among the columns contain a mucus-like substance (Fig. 5) that forms an adhesive layer covering the outer surface of the chorion (Fig. 6). It also penetrates the small sac-like cavities that are situated on the dorsal surface of the costae (Fig. 5). The mucous mass is limited by a thicker edge from which emerge filiform projections with a length of about 0.4 μm (Figs. 6;7). Inside the mucous mass, there are electron-dense bodies of fibrillar material with a paracrystalline structure (Fig. 7).

The extent and thickness of the mucous layer are variable: its consistency generally allows the underlying layers to be seen when eggs are observed under SEM. In some cases, however, as in the intercostal areas of *H. consiglioi*, the mucus may be more compact and the underlying chorion seen only in limited areas (Fig. 8). SEM investigations also show that adhesive substances can be found along the outer edges of the micropyle sperm guide both in *H. consiglioi* (Fig. 9) and in *H. fusca* (Fig. 11).

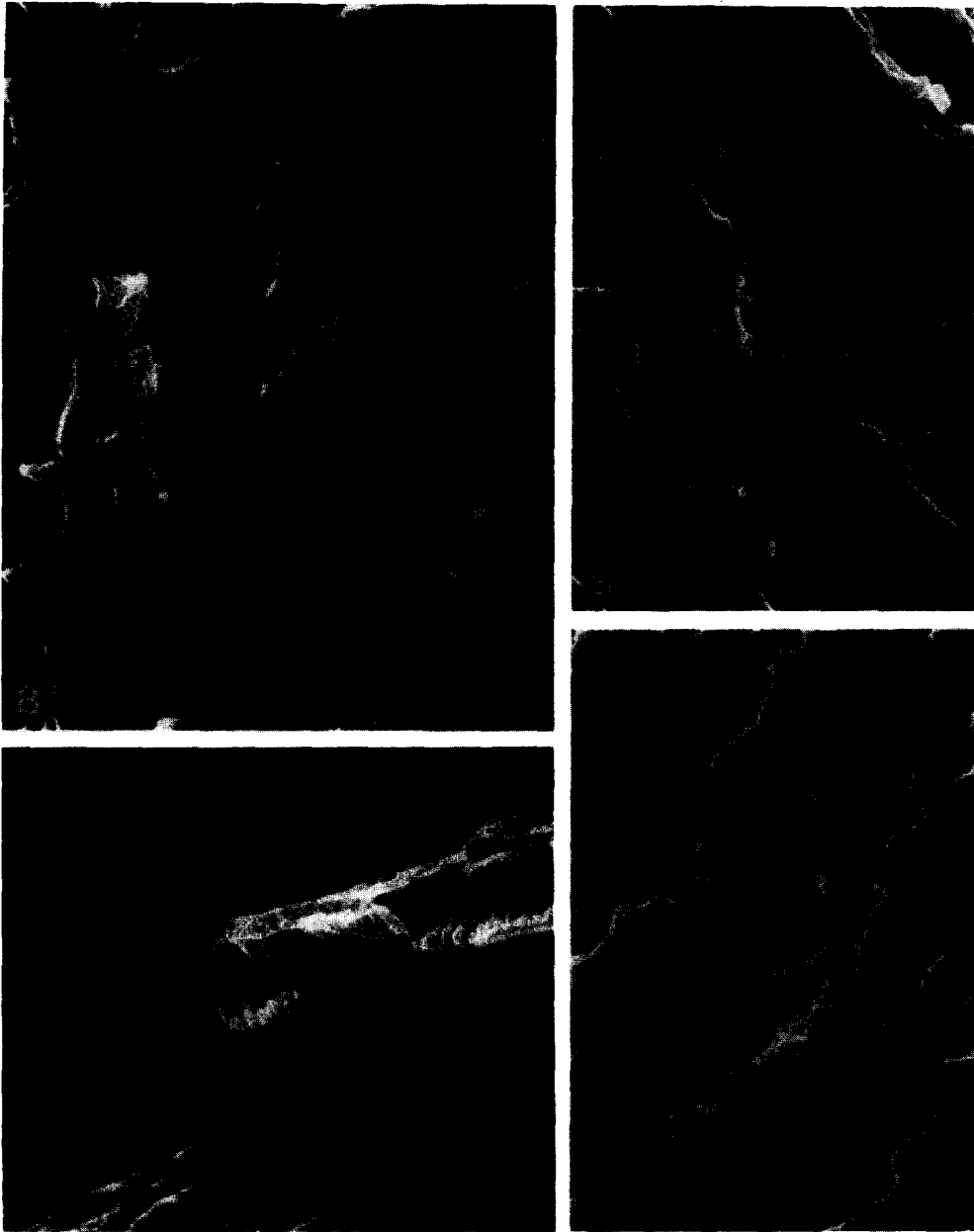
The costae of *H. consiglioi* show a structure of columnar systems similar to that of *H. fusca*. Such structure is evident in eggs with mechanically broken shells (Fig. 10). In the costae of *H. consiglioi*, the chorion is about 4.2 μm thick and seems to consist of 3 superimposed layers. The intermediate layer has a columnar structure and consists of elements of 1.2 μm in diameter (Fig. 10).

DISCUSSION

Figure 12 shows schematically the structure of the egg coats of *H. fusca* at the level of the costae and intercostal zones. The chorion is made up of 3 morphologically distinct layers, which wrap the vitelline coat and which are in turn covered with a mucus-like substance. This substance seems to be secreted during the aquatic life.

The chorionic layer adjacent to the vitelline coat has a peculiar structure; it is characterized by electron-dense lamellae with a periodicity of 10 nm, whose orientation is perpendicular to the egg surface. A similar periodic pattern has recently been observed in the chorion of the ichneumonid, *Campoletis sonorensis* (Norton and Vinson, 1982). Polycrystalline layers with a periodic structure and composed mainly of proteins are known from several insect orders (Furneaux and Mackay, 1972).

The presence of columnar systems in the chorion, interpreted as respiratory plastron, characterizes the eggs of many insect species (see Hinton, 1981). In *H. fusca*, however, the columnar system is limited to the costal region, whereas in the intercostal areas the chorion appears compact even though made up of 3 layers. The presence of costae seems



FIGS. 8 and 9. Scanning electron micrographs of external surface of egg at level of chorion (8) and micropyle (9). Note mucous material (*m*) that covers chorion whose pattern is clearly observed only at some points (arrows) (*H. consiglioi*). $\times 3,000$.

FIG. 10. An SEM view of cross-broken egg. Observe chorion layer with a columnar structure (*H. consiglioi*). $\times 3,000$.

FIG. 11. *H. fusca* external surface at micropyle level. Note traces of mucous material (arrows) in different point of chorion and micropyle. $\times 3,000$.

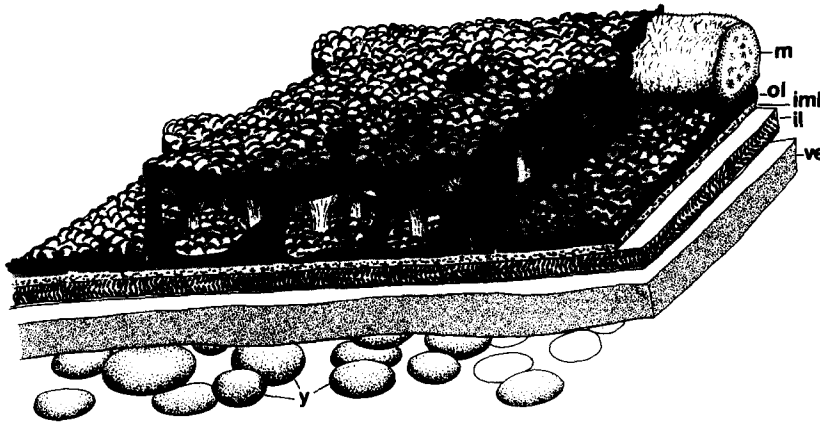


FIG. 12. Schematic drawing of egg envelopes of *H. fusca* at level of costae and intercostal zones; *il* = inner layer; *iml* = intermediate layer; *m* = mucus; *ol* = outer layer; *ve* = vitelline envelope; *y* = yolk.

to be typical of most ephemeropteran species belonging to the genera *Habrophlebia* and *Habroleptoides* (Gaino and Mazzini, 1984). The columnar structure of costae is therefore likely to be found also in other species of these genera.

Pores on the dorsal face of the costae (Gaino and Mazzini, 1984) might be considered aeropyles, such as are typical of most terrestrial eggs (Arbogast *et al.*, 1980; Cummings and O'Halloran, 1974; Klug *et al.*, 1974; Matheny and Heinrichs, 1972; Mazzini, 1974; Salkeld, 1976) but also have been found in Plecoptera (Stark and Szczytko, 1982), which lay eggs in water.

The gonads of Ephemeroptera have no accessory glands (Brinck, 1957); nevertheless the eggs of many genera show adhesive layers (Degrange, 1960; Koss, 1968; 1970; Koss and Edmunds, 1974; Soldàn, 1979; Kopelke and Müller-Liebenau, 1981a,b; 1982). Since no secretory activity has been observed in the epithelial cells of the oviduct (Soldàn, 1979), it is probable that the adhesive layers are elaborated by the follicular cells after the various egg coats have been secreted (Koss, 1970).

Mucous coats have frequently been observed on the chorionic surface of insects such as Coleoptera (Biemont *et al.*, 1981), Lepidoptera (Barbier and Chauvin, 1974a,b; Chauvin *et al.*, 1974), and Diptera (Mazzini and Santini, 1983). However in *H. fusca*, the adhesive layer not only covers those surfaces that are in direct contact with the outside, but it also penetrates the wide chambers among the columns. The internal location of the mucus confers to this layer a greater stability as opposed to the mucus present on the chorionic surface, which is exposed externally during the accumulation of eggs in the oviduct. This would explain the different levels of consistency and development observed in this mucous layer even in eggs taken from the same individual. On the other hand, the mucus contained inside the chambers among the columns would serve as a reserve to aid in the adhesion of cells to the substrate when eggs are laid. This is made possible by way of the communications existing between the chambers and along the lateral edges of the costae.

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