Odonatologica 23(3): 255-269 *September 1, 1994*

ULTRASTRUCTURE OF THE EGGSHELL AND MICROPYLAR APPARATUS IN *SOMATOCHLORA METALLICA* (VANDER L.), *ORTHETRUM CANCELLATUM* (L.) AND *SYMPETRUM SANGUINEUM* (MÜLL.) (ANISOPTERA: CORDULIIDAE, LIBELLULIDAE)

G. SAHLÉN

Section of Entomology, Department of Zoology, University of Uppsala, Villavägen 9, S-752 36 Uppsala, Sweden

Received December 8, 1993 / Revised and Accepted March 22, 1994

Eggshells are studied using light, scanning electron and transmission electron microscopy. The nomenclature of the eggshell layers is revised. The vitelline envelope (vitelline membrane) is the innermost layer. Its thickness is dependent on the length of the egg stage, and it is believed to play the dominant protective role in the eggshell. The endochorion is thin and multilayered. The exochorion is filamentous and synonymous with the jelly-layer. The jelly has two main functions, one is to camouflage the egg, the other is to enhance the protection provided by the vitelline envelope. Non-expanded jelly is dense and found in the overwintering eggs of *S. sanguineum*. The expansion of the jelly is water-dependent, but the jelly in *S. sanguineum* is unable to expand, regardless if water is present. Expanded jelly seems to be of about the same thickness in many species, even when the size of their eggs differ. The thin (24 nm) threads in the jelly are of the same size and structure in all three species investigated, and are therefore believed to be a basic structure in odonate egg-jelly. No micropylar atrium or pedicel is present. The micropylar chutes lead the sperm directly into the oocyte, and no sperm-storage chamber exists. No extrachorionic material is present in the eggshells.

INTRODUCTION

A number of exophytic dragonfly eggs have thus far been described (e.g. TILLYARD, 1917; ROBERT, 1958; ANDO, 1962; MILLER & MILLER, 1985; MILLER, 1987; IVEY et al., 1988; BECNEL & DUNKLE, 1990; TRUEMAN, 1990a, 1990b, 1991; ANDREW & TEMBHARE, 1992) with light or scanning electron microscopy. Eggs of libelluloid dragonflies have been reviewed by

TRUEMAN (1991), using scanning electron microscopy (SEM) and a freeze--fracture method to propose a standardised nomenclature for the chorion (meaning "eggshell"). SEM is an excellent technique for studying egg surfaces (cf. HINTON, 1981), but the resulting images must be interpreted with a certain scepticism, since the fixation and critical point drying necessary for SEM sometimes distort soft and fragile tissues (CARR & TONER, 1982). One way to get around these difficulties is to use transmission electron microscopy (TEM) as a complement to SEM, since the methods for TEM-fixation do not dry out the preparation. Also, many of the egg descriptions tell us nothing about the functions of the structures described. Therefore it is necessary to compare the morphology of the egg with the known ecology, and the environmental inpact on the egg-stage of each species described.

This study is designed to describe the ultrastructure of the eggshell and micropylar apparatus of 3 species of libelluloid dragonflies with different egg ecology, using both SEM and TEM. The nomenclature introduced by TRUEMAN (1991) is evaluated.

ECOLOGY OF SPECIES

Somatochlora metallica (Vander L.), *Orthetrum cancellatum* (L.) and *Sympetrum sanguineum* (Müll.) are common during late summer in many parts of Europe. They are found in a number of different habitats, mainly stagnant waters, such as lakes, ponds and dams. *S. metallica* also inhabits bogs and rivers. The eggs of these 3 species have previously been described by ROBERT (1958) using light microscopy. All 3 species lay their eggs exophytically. The female of *S. metallica* uses her vulvar scale to deposit the eggs into the mud or *Sphagnum* moss just above or in the water (TORKA, 1909; MÜNCHBERG, 1932; ROBERT, 1958). A gelatinous layer develops around the eggs, to which loose particles and other debris quickly adhere, acting as an effective camouflage. According to SCHIEMENZ (1953) and ROBERT (1958) the eggs hatch after 4 to 14 weeks, but they can overwinter if they are deposited late in the season. The female of *O. cancellatum* deposits her eggs by dipping her abdomen repeatedly into the water while slowly flying over the surface. The eggs sink to the bottom, and a gelatinous layer develops. The eggs hatch after about 6 weeks (ROBERT, 1958). In *S. sanguineum*, the eggs are deposited while flying in tandem over the ground just at the edge of the water. They overwinter and hatch the following spring (ROBERT, 1958), after being submerged from late autumn, when heavy rain raises the waterline.

MATERIAL AND METHODS

Female *S. metallica* (N = 4), *O. cancellatum* (N = 3) and *S. sanguineum* (N = 5) were caught in

different localities in Sweden and Italy during July and August. They were made to oviposit by dipping their abdomens into small glass vials containing de-ionized water. The eggs were left in the water overnight (20-24 h) to allow any gelatinous coating to develop. They were then transferred to a 0.1 mol x dm^{-3} phosphate buffer, pH 7.4, in which they were stored at 4°C for up to 10 days.

For SEM-fixation the eggs were placed for 24 h in a solution of 18 parts 80% alcohol, 1 part formaldehyde (34-38%) and 1 part concentrated isoacetic acid. This fixative preserved the external features well, but the interior of the eggs collapsed to some degree. They were dehydrated for 15 min each in a series of ethanol concentrations from 70 to 100%, critical point dried, mounted, sputter--coated with gold-palladium, and examined in a JEOL JSM 35 scanning electron microscope.

For TEM-fixation the eggs were transferred to a solution of 2.5% glutaraldehyde in 0.1 mol x dm^{-3} phosphate buffer, pH 7.4, 4°C overnight. They were rinsed in a 0.1 mol x dm^{-3} cacodylate or phosphate buffer, pH 7.4, and postfixed for 2 hr in 1% osmium tetroxide in the same buffer. They were again rinsed in cacodylate or phosphate buffer and dehydrated in alcohol (15 to 30 min each in 50-100%, 0.5% uranyl acetate added to the 70% solution), transferred to acetone, infiltrated with (overnight) and mounted in Agar 100 for ultrathin sectioning.

Transverse serial sections through the micropylar apparatus (of *O. cancellatum*) and sagittal sections of the eggshell (of all species) were cut in a LKB 2088 Ultrotome V ultramicrotome (50-80 nm sections), and mounted on formvar-coated slot grids. The sections were stained with 4% uranyl acetate for 20 min and Reynold's lead citrate for 5 min, and examined in a Philips CM 10 transmission electron microscope.

Measurements on a series of whole eggs from all the collected females were made using a Wild M10 stereo microscope and an ocular micrometer. Measurements of details were made on the photographs.

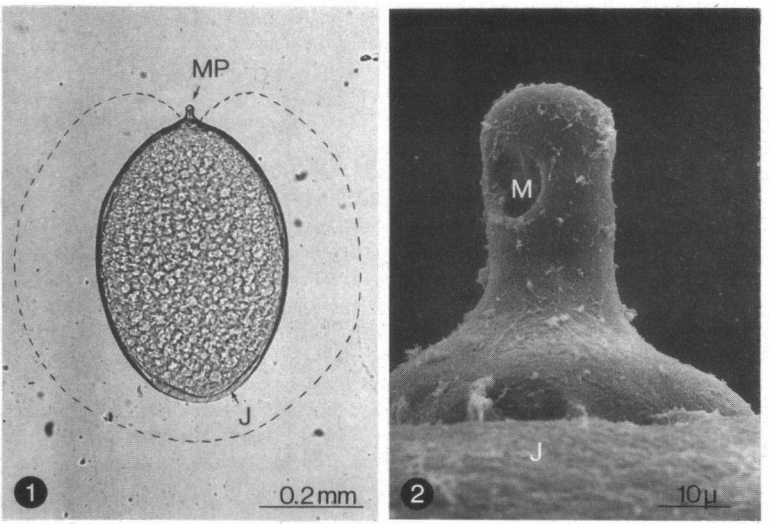
SOMATOCHLORA METALLICA

The egg measures 0.59 ± 0.08 x 0.42 ± 0.03 mm (N = 32) (Fig. 1). The gelatinous layer when fully expanded is about 0.25 mm thick, which increases the total diameter of the egg to 0.94 mm. The micropylar process is the only part of the eggshell that exposes a bare surface (Fig. 2). The rest of the egg is covered with the fibrous jelly.

On the TEM-micrographs a number of layers can be distinguished outside the oocyte. The vitelline envelope (VE) measures 2.9 μm in thickness around the entire oocyte. The layer is electron dense, homogenous in structure, and difficult to penetrate by fixatives.

The endochorion (EN) varies in thickness around the perimeter of the egg. It consists mainly of thin sub-layers that are oriented in an irregular manner (Fig. 3), about 0.35 μm in thickness. During the preparation, the VE shrank back from the EN, pulling out the inner sub-layers to long elastic threads (Fig. 4).

The inner part of the exochorion (EX) is a thin basal layer of the same texture as the EN, attached to the outside of the EN (Fig. 3). It measures about 70 nm in thickness. The rest of the EX forms the gelatinous substance which surrounds the entire egg except the micropylar projection (Figs 3, 5). The jelly consists of thin threads, 24 nm in diameter, that are attached to the basal layer. In the inner part of the layer, they are oriented in bundles which point in different directions, causing the very twisted appearance of the jelly. The threads are more numerous

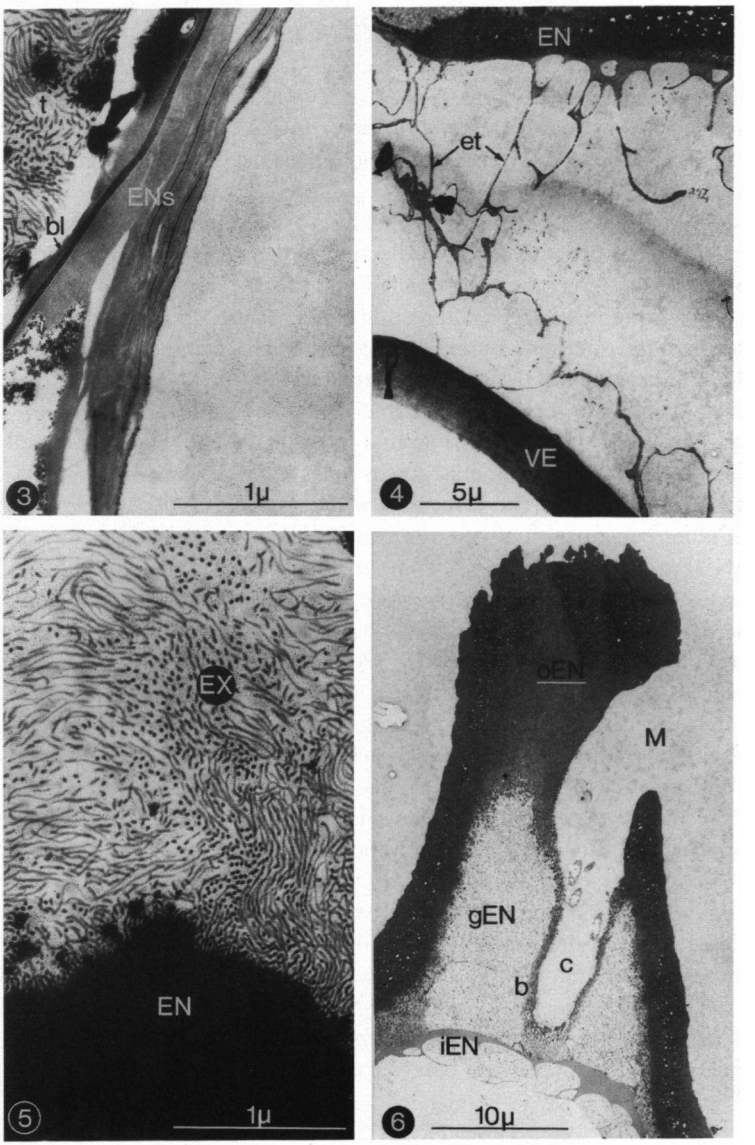


Figs 1-2. *S. metallica*: (1) Light micrograph of egg, jelly-layer (J) not fully expanded due to fixation [dotted line indicates approximate size of fully expanded jelly; micropylar process (MP) on top]; – (2) Scanning electron micrograph of micropylar projection showing surface of fibrous jelly (J) and opening of micropyle (M).

close to the micropylar projection than on the rest of the egg.

In the area under the micropylar projection the EN is separated into two different portions, an inner one which is very elastic, and an outer one which constitutes the major part of the projection. In the outer part a complex meshwork with empty spaces can be seen (Fig. 6). The EX is merged with the EN over the entire structure and the basal layer is absent. Two micropyles open on the surface of the apparatus, their chutes leading down through the merged EX and EN. They are successively reduced in size and an electron-dense barrier always surrounds the two chutes (Fig. 6). Between the thin barrier and the EN, a granulated, not very dense, endochorionic material appears. Neither a micropylar atrium (an empty space under the micropylar projection) nor a pedicel (a ring-shaped ridge in the VE) is to be found.

Figs 3-6. Transmission electron micrographs of *S. metallica* egg: (3) Endochorion consisting of several sub-layers (ENs) and inner part of exochorion, consisting of basal layer (bl) and numerous threads (t); – (4) Endochorion (EN) and vitelline envelope (VE) separated during preparation, pulling out inner sub-layers to long, elastic threads (et); – (5) Homogenous endochorion near micropylar process (EN) and filamentous exochorion (EX) without basal layer [Note how the threads are oriented in bundles pointing in different directions. The threads are more dense in this area than



on other parts of the egg]; – (6) Micropylar process with micropylar opening (M), and micropylar chute (c) leading down towards the VE. An electron-dense barrier (b) surrounds the chute. Endochorion divided into an outer part (oEN) with a complicated meshwork containing empty spaces and an inner, elastic part (iEN); oEN is merged with a non-fibrous exochorion. A granulated endochorionic material (gEN) fills out the space between oEN and iEN.

ORTHETRUM CANCELLATUM

The egg measures 0.49 ± 0.07 x 0.31 ± 0.04 mm (N = 45). The expanded gelatinous layer increases the total diameter to 0.93 - 1.08 mm. The micropylar process exposes a bare surface, while the rest of the egg is covered with fibrous jelly.

The VE measures 1.6-1.7 μm in thickness around the entire oocyte, except in the area close to the micropylar projection, where it is thicker (up to 2.1 μm) and pierced by a large number of canals (Figs 7-8). Thin strands of a tissue that does not incorporate as much contrasting agents as the VE as a whole, suggesting the canals in the micropylar area, appear irregularly over the rest of the VE (Fig. 9). The layer is electron dense and, apart from the canals, rather homogenous in structure. A granular texture can be traced in some parts.

The EN is very thin (0.1 μm) around most of the egg, and it is lamellated in the inner two thirds. 14 lamellae, each measuring 4.8 nm in thickness can be distinguished (Fig. 11). The layer as a whole is electron dense (Figs 8-11).

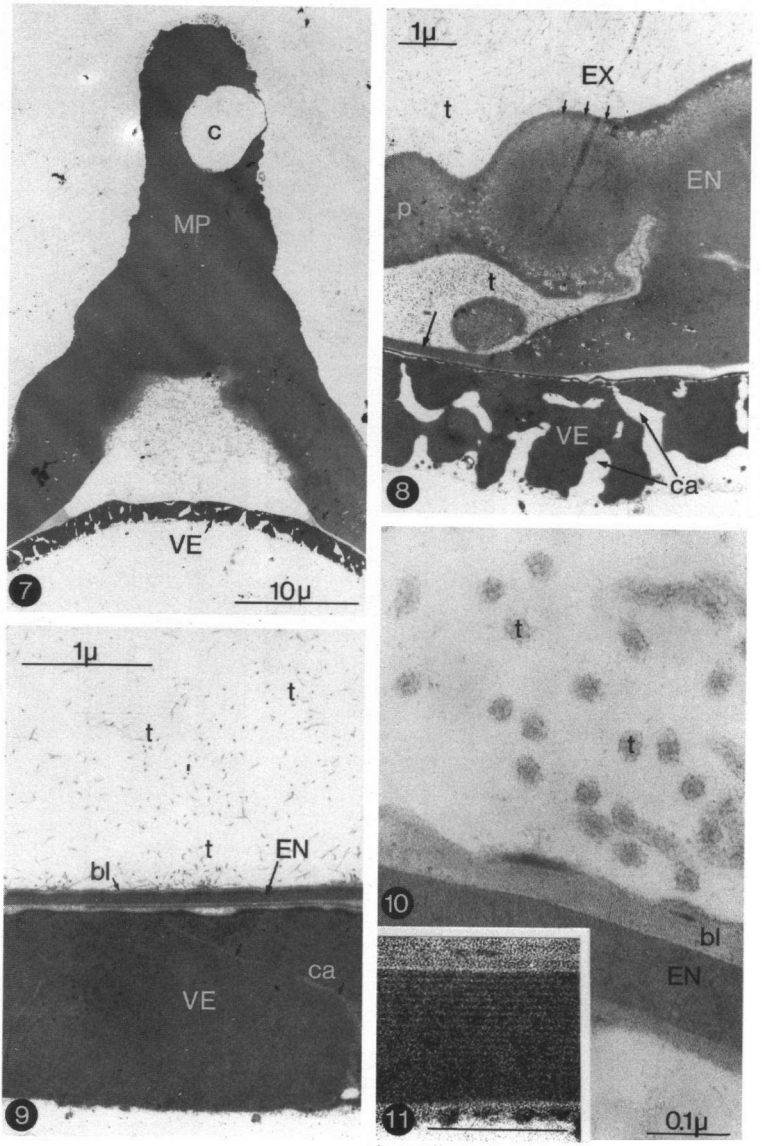
The inner part of the EX is a very thin basal layer attached to the outside of the EN (Fig. 10), measuring 35 nm in thickness. The rest of the EX is the gelatinous substance that surrounds the egg (Figs 10, 12, 13). The jelly is formed out of the same kind of thin threads as in *S. metallica*. The threads have an electron dense center, surrounded by a less dense tube, and another dense layer on the outside (Fig. 10). The threads are numerous close to the basal layer, becoming fewer further out from it (Figs 10, 12, 13).

The micropylar apparatus is formed mainly from the EN, which expands many times in thickness at the anterior end of the egg (Figs 7-8). The basal layer of the EX is thicker, and almost merged with the EN over the entire structure. The micropylar chutes lead down through the merged EX and EN. They are successively reduced in size and merged with the surrounding matrix. However, as in the former species a thin electron-dense barrier surrounds the two chutes and associated structures down to the VE (Figs 14-16). Outside the thin barrier, and inside the massive EN, only thin strands of chorionic material appear. No micropylar atrium and pedicel is to be found. Since the VE is pierced by a number of canals under the micropylar projection, no special canals presumed to act as the continuation of the micropylar chutes can be distinguished.

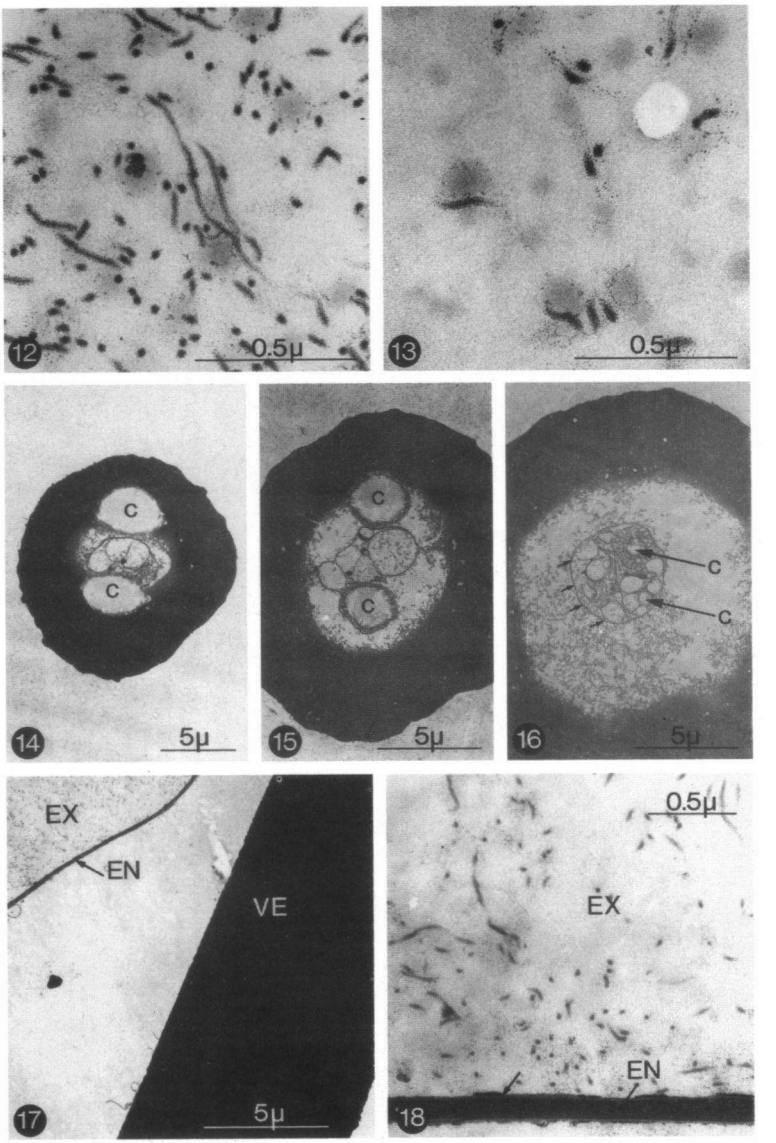
SYMPETRUM SANGUINEUM

The size of the egg is 0.74 ± 0.08 x 0.62 ± 0.06 mm (N = 73). A gelatinous layer increases the diameter slightly, most markedly in the area closest to the micropylar process, where the layer is 0.10-0.13 mm thick.

The VE is thick, about 10 μm around the entire oocyte (Fig. 17). The layer is electron dense and rather homogenous in structure. The fixatives could seldom



Figs 7-11. Transmission electron micrographs of *O. cancellatum* egg: (7) Micropylar process (MP) with micropylar chute (c) and vitelline envelope (VE); – (8) Vitelline envelope (VE) with many canals (ca) near the micropylar process; the endochorion (EN) expands from a thin sheath (arrow) to a very thick structure, the outside of which contain exochorionic material (EX); – (9) Vitelline envelope (VE), endochorion (EN), exochorionic threads (t) and basal layer (bl). Thin strands of a different tissue appear in the VE suggesting the canals near the micropylar process (ca); – (10) The lamellated endochorion (EN) with basal layer (bl) of exochorion and exochorionic threads (t); – (11) Close-up of lamellated endochorion.



Figs 12-18. Transmission electron micrographs of *O. cancellatum* (Figs 12-16) and *S. sanguineum* (Figs 17-18) egg: (12) Expanded exochorionic jelly near basal layer; – (13) Expanded exochorionic jelly near its periphery showing distinctly less density of threads; – (14-16) Cross sections of micropylar process showing micropylar canals (c) diminishing in size going towards oocyte, but always surrounded with electron dense barriers; – (17) Thick vitelline envelope (VE) and thin endochorion with exochorionic threads (EX) on the outside. [Layers separated due to fixation]; – (18) Endochorion (EN) without lamellae and exochorion (EX). [Arrow indicates thin basal layer].

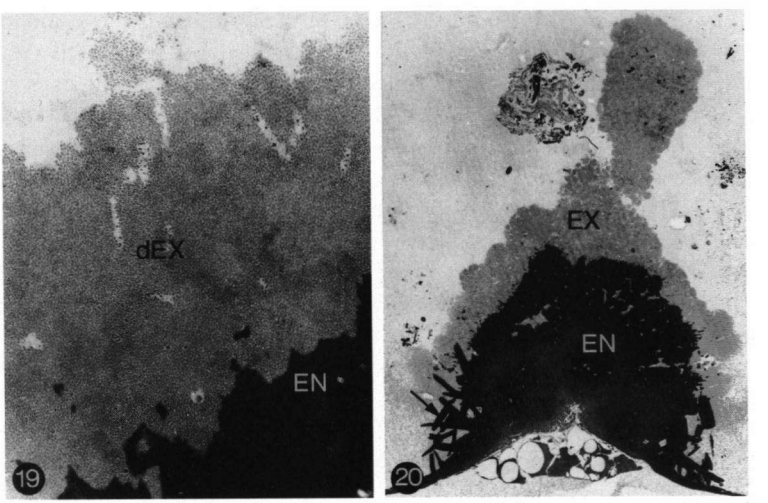
penetrate this layer.

The EN is very thin (0.1 μm) around most of the egg, and it has a homogenous texture, a little less electron dense than the VE (Figs 17-18).

The inner part of the EX also in this species is a thin basal layer attached to the outside of the EN (Fig. 18), measuring only 12 nm in thickness. The rest of the EX forms the gelatinous substance (Figs 17-20). The jelly consists of the same type of thin threads as in the other two species, 24 nm in diameter, attached to the basal layer (Fig. 18). As in the former species, the threads are numerous close to the basal layer, becoming fewer further away from it. However, the layer as a whole is much thinner and the threads are more densely packed.

The micropylar apparatus is formed of the EN and the EX, the EN increasing many times in thickness, forming a 15 μm thick structure (Fig. 20). The EN is not homogenous here, but consists of different elongated sections that are oriented in alternate directions. These sections occupy the entire interior of the structure, and no empty space exist. The EX in this area consists of densely packed threads, arranged in a 3.2 μm thick layer over the entire micropylar projection (Figs 19--20). Two micropyles open in the EX. Their chutes pass through the EX and EN, tapering gradually going inwards. The chutes are the only empty spaces in the micropylar projection.

The EN is separated from the VE in all preparations, so it is not possible to investigate the existence of a micropylar atrium. The outer surface of the VE is smooth, and no pedicel or continuation of micropylar chutes is present.



Figs 19-20. Transmission electron micrographs of *S. sanguineum* egg: (19) Endochorion (EN) and densely packed exochorionic threads (dEX) near micropylar process; – (20) Micropylar process showing endochorion (EN) which fills out most of the structure. Densely packed exochorion (EX) on the outside. [Arrow indicates area where elongated endochorionic sections are oriented in alternate directions].

DISCUSSION

The standarised nomenclature proposed by TRUEMAN (1991) for libelluloid eggs must be revised. He stated that the vitelline membrane (= vitelline envelope in this work) was the innermost part of the EN, seen as a dense layer, while the rest of the EN was considered to be fibrous. In his figure a thin membrane, marked by a single line, is shown inside the EN. However, the VE is not a simple cell-membrane, as has been pointed out by several authors (e.g. BEAMS & KESSEL, 1969; MARGARITIS, 1985), but a relatively thick layer formed of proteins secreted by the rough endoplasmic reticulum in the nurse cells (BEAMS & KESSEL, 1969). The VE looks like an ordinary membrane in light microscopy, hence the former term "vitelline membrane". The only membrane surrounding an oocyte is the oocyte membrane, which is situated inside the VE (MARGARITIS, 1985). The 'EN' of TRUEMAN (1991) must therefore be the VE and the rest of the layers in his figure have to be moved one step accordingly.

Since this revised nomenclature reduces the EX into a very thin sheath and a fibrous jelly, the possible presence of extrachorionic material, 'spumaline', must be considered. MILLER (1987) dissected eggs of *Sympetrum danae* from the ovary, after which they were found to develop a normal layer of jelly (= EX). No part of the jelly is therefore secreted by the female during the oviposition. Until recently it was stressed that spumaline glands have yet to be described from the Odonata (HINTON, 1981; MARGARITIS, 1985). However, SRIVASTAVA & SRIVASTAVA (1992) found accessory glands in the zygopteran *Ischnura rufostigma*, an endophytic species. Here, the two glands were said to bathe the eggs in a frothy secretion prior to laying. The question is whether this secretion is anything more than a lubricant to facilitate the insertion of the eggs into the stems of waterplants. If this is the case, it may disappear shortly after laying. Since exophytic species have little use for any lubricant for egg-laying, it can be assumed that no such secretion takes place in these species. Supporting this hypothesis is the fact that accessory glands have yet to be reported from exophytic species. There is also the possiblity that part of the jelly is secreted as an extrachorionic material higher up in the oviduct, as e.g. in fleas (ROTHSCHILD, et al., 1986), but all of the jelly has the same basic structure and should therefore be derived from the same source.

Strand jelly, which appears in egg-strands, such as those of the corduliids *Epitheca bimaculata* (ROBERT, 1958), *Tetragoneuria* sp. (CORBET et al., 1960) and *Hemicordulia* sp. (ARMSTRONG, 1958; ROWE, 1987; TRUEMAN, 1991) might also be of extrachorionic origin. However, a specialisation of the EX would be more likely to evolve than a completely new extrachorionic devise, since no evidence exists for such structures in the Odonata. Further studies on eggs with strand-jelly might provide us with the answer to that question.

ROBERT (1958) stated that no jelly was present in the egg of *S. sanguineum.*

However, those species of *Sympetrum* in which he found no egg-jelly lay their eggs beside the water, while the species which develop a normal layer of jelly lay their eggs into the water. What Robert interpreted as absence of jelly in *S. sanguineum*, could in fact have been jelly that didn't expand, since no water was available. The layers might have been difficult to identify using only light microscopy. When such eggs are submerged later in the season, the jelly might expand as in the other species. But of the main purpose of the jelly is to provide a sticky surface whereto particles can adhere for camouflage, which is the general opinion, this can be achieved even if it has not expanded. Another possibility is that *S. sanguineum* uses the non-expanded jelly as an extra protection for overwintering. ROBERT (1958) reported that the eggs of *S. sanguineum* were resistant to freezing and a certain amount of drying up. This could be achieved partly through the very thick VE in this species (see below), as it is sometimes impermeable even to fixatives. A dense, non-expanded jelly will obviously enhance the protection. Jelly that stays non-expanded over a long period might on the other hand be unable to expand when the egg is finally submerged. As all the eggs I studied were deposited in water and the jelly did not expand, it can be assumed that the jelly of *S. sanguineum* is in fact unable to expand at all. GARDNER (1950) also observed egg-laying of this species where the eggs were deposited directly into the water in the autumn. Non-expanded jelly might serve the same purpose in the water as on land as there is no guarantee that eggs deposited into water always will stay submerged until spring. The possibility that ions in the water might play a role in the expansion of the jelly must also be considered. Since the investigations described in this essay were carried out in de-ionised water, it is possible that lack of ions prevented jelly-expansion in *S. sanguineum*. However, the jelly of the other two species investigated expanded normally in the same kind of water.

The use of the jelly as a protective camouflage is probably its major role in *O. cancellatum*, even though the layer, when expanded, is very thick. Another function might be to stick the eggs to various objects in the water. In *S. metallica* the layer is equally thick. ROBERT (1958) reported the jelly-layer to be between 0.11 and 0.35 mm in 7 European libelluloid species. The thickness of the fully expanded EX of *S. metallica* and *O. cancellatum* comes just under the highest values, while the non-expanded EX of *S. sanguineum* is close to the lowest value. One can therefore assume that the measurements given by ROBERT (1958) are derived from species with both expanded and non-expanded jelly layers. Considering the difference in size between the eggs of *S. metallica* and *O. cancellatum*, and their jelly-layers being of relatively equal thickness, one can suspect that the EX's of libelluloid eggs are of equal thickness regardless of the size of the eggs. The measurements given by ROBERT (1958) do point this way, but further studies are needed in order to confirm this relationship.

Long thin thread-like structures are common in different tissues throughout

the animal kingdom, and normally they are formed from various proteins, as e.g. in the reproductive tract of *Drosophila melanogaster* (BAIRATI, 1966, 1968; PEROTTI, 1971). BEAMS & KESSEL (1969) reported of the formation of the EX in *Aeshna* sp. that the so called 'prechorion substance' secreted by the follicle cells had the shape of long, coiled threads. The entire layer was formed from such densely packed threads (BEAMS & KESSEL, 1969), looking exactly like the EX in the micropylar projection of *S. sanguineum* (Fig. 15). Here the EX has not expanded, and the threads are tightly coiled into a more compact layer. The identical EX-threads in the 3 unrelated species *S. metallica, O. cancellatum* and *S. sanguineum* also suggest these long filaments to be a common basic structure in libelluloid eggs.

Another type of filamentous threads have also been described from the Odonata, namely the long, 2 μm thick filaments in the posterior cone of the egg of *Ictinogomphus rapax* (ANDREW & TEMBHARE, 1992), and corresponding structures in other related gomphids (GAMBLES, 1956; GAMBLES & GARDNER, 1960; CORBET, 1962; TRUEMAN, 1990a). The position of these filaments on the surface of the eggshell indicates that they should be related to the thin threads of the EX described in this essay. To confirm this assumption the sectioning of the filaments for TEM is necessary.

The EN consists of sub-layers, or is lamellated in *S. metallica* and *O. cancellatum*. This is not the case for *S. sanguineum*. An EN consisting of several sub-layers is known from other species of dragonflies. In *Hemianax ephippiger* the EN consists of many layers which increase in thickness from inside and outside. (DEGRANGE, 1971). This is also the case in *Aeshna juncea* (SAHLEN, 1994). In other orders, e.g. Diptera, the EN is two-layered in *Drosophila melanogaster* (MARGARITIS, 1985), and clearly lamellated in *Culex theileri* (POLLARD, et al., 1986) and *C. pipiens* (SAHLÉN, 1990). It would seem that this layer of the eggshell often has two or more sub-layers. The absence of such layers in *S. sanguineum* could therefore be due to poor fixation. In the Odonata, the EN generally seems to be multilayered.

In the micropylar projection of all three species studied, the EN constitutes the major part. The layer is not lamellated here, but in *S. sanguineum* it is divided into small sections which point in alternating directions. These I consider to be some sort of a stabilizing device.

The VE in *S. metallica* and *O. cancellatum* is rather thin compared to the very thick and impermeable layer in *S. sanguineum*. The many canals in the VE of *O. cancellatum* have no corresponding counterparts in the other 2 species. Since the egg of *S. sanguineum* always overwinters, a thick and impermeable VE might be necessary to withstand the desiccation as well as the mechanical disturbance that acts on the eggs during the winter. As for *O. cancellatum* no corresponding protective layer is needed for a short egg stage. The eggs of *S. metallica* are said to overwinter if they are deposited late in the season (ROBERT, 1958). Normally,

they hatch after 4 to 14 weeks, i.e. sometimes even earlier than those of *O. cancellatum* (SCHIEMENZ, 1953; ROBERT, 1958). Since all the eggs used in this study were collected during July and August, it is impossible to know if the thickness of the eggshell layers varies with the age of the female, as is the case with the size of the eggs (see below). It is possible that the VE of *S. metallica* is thicker in eggs that are laid late in the season and have to overwinter. Until eggs of other overwintering and non-overwintering species have been investigated, we can tentatively assume that the VE plays the dominant protective role in the eggshell of exophytic dragonfly eggs and that its thickness is dependent on the duration of the egg stage.

TRUEMAN (1991) tentatively identified shallow depressions on the surface of the VE within the pedicel, under the micropylar projection, as the micropyles. But as micropyles, according to HINTON (1981) and MARGARITIS (1985), are composed of exochorionic, endochorionic, as well as vitelline envelope parts, the two openings on the micropylar projection (atrial openings of Trueman) and the chutes associated with them are the micropyles. The shallow depressions on the VE observed by TRUEMAN (1991) could be the continuation of the micropylar chutes, the VE part of them having closed after fertilization or during the fixation of the eggs. Micropylar chutes through the VE of unfertilized eggs of *Hemianax ephippiger* stay open after fixation (DEGRANGE, 1971), while those of fertilized eggs of *Aeshna juncea* are closed (SAHLÉN, 1994).

TRUEMAN (1991) considered the interior of the micropylar projection to contain a hollow space, above the so-called micropylar atrium, where sperm could be collected after the egg had been laid, and from where the spermatozoa later entered the 'micropyles' in the VE and fertilized the oocyte. However, the micropylar chutes of *S. metallica* and *O. cancellatum* lead straight down to the VE, and although the interior of the micropylar projection is somewhat hollow, a dense barrier coats the chutes. I therefore believe that the sperm does not leave the chutes to occupy the hollow space, but is transported directly into the oocyte. This is more obvious in *S. sanguineum*, where the micropylar projection does not contain any hollow space at all.

The absence of a pedicel and micropylar atrium in the species investigated indicates that the ring-shaped ridge should not be included among the basic structures of the libelluloid egg as suggested by TRUEMAN (1991), since it only seems to appear in certain groups of species within the superfamily.

The size measurements for the eggs of all 3 species come within those reported by ROBERT (1958). It should be stressed that the size of an aquatic insect egg always varies depending on how soon after the egg-laying the measurements are made. All aquatic eggs, according to HINTON (1980) and MARGARITIS (1985), increase successively in size after laying due to an uptake of water. Measurements of total egg size should therefore be accompanied with notes on the time passed since the deposition. It has also been shown that the size of eggs deposited

diminish with increasing age of the female in some species (WATANABE & ADACHI, 1987; HIGASHI & WATANABE, 1993). If this is a common condition which occurs in all species of dragonflies, the size of an egg reported in any description is no longer of high importance if the age of the egg-laying female is unknown.

ACKNOWLEDGEMENTS

I thank Professor CHRISTINE DAHL for helpful suggestions concerning the manuscript, and my wife ANNA for linguistic assistance. I also thank Mr GARY WIFE for his never-ending work in the photo-lab along with Mr LARS-ERIK JÖNSSON. Miss GUNILLA OLSSON and Mrs. INGMARIE OLSSON made excellent embedding for TEM, and they, along with Mrs GUN-BRITT ÖRLANDER took care of the extensive sectioning. Dr STEFAN GUNNARSSON came up with good suggestions concerning fixation and embedding. This study was funded by Stiftelsen för Zoologisk forskning, Uppsala University.

REFERENCES

ANDO, H., 1962. *The comparative embryology of Odonata with special reference to the relic dragonfly Epiophlebia superstes Selys*, Japn Soc. Promot. Sci., Tokyo.

ANDREW, R.J. & D.B. TEMBHARE, 1992. Surface ultrastructure of the egg chorion in the dragonfly, Ictinogomphus rapax (Rambur) (Odonata: Gomphidae). *Int. J. Insect Morph. Embryol.* 21: 347-350.

ARMSTRONG, J.S., 1958. The breeding habitats of the Corduliidae (Odonata) in the Taupo district of New Zealand. *Trans. R. Soc. N.Z.* 85: 275-282.

BAIRATI, A., 1966. Filamentous structures in spermatic fluid of Drosophila melanogaster Meig. *J. Microscopie* 2: 71-80.

BAIRATI, A., 1968. Structure and ultrastructure of the male reproductive system in Drosophila melanogaster Meig. II. The genital duct and accessory glands. *Monit. zool. ital.* (N.S.) 2: 105-182.

BEAMS, H.W. & R.G. KESSEL, 1969. Synthesis and deposition of oocyte envelopes (vitelline membrane, chorion) and the uptake of yolk in the dragonfly (Odonata: Aeshnidae). *J. Cell Sci.* 4: 241-264.

BECNEL, J.J. & S.W. DUNKLE, 1990. Evolution of micropyles in dragonfly eggs (Anisoptera). *Odonatologica* 19: 235-241.

CARR, K.E. & P.G. TONER, 1982. *Cell structure. An introduction to biomedical electron microscopy.* 3rd ed. Livingstone, Edinburgh.

CORBET, P.S., 1962. *A biology of dragonflies.* [reprint 1983]. Classey, Faringdon.

CORBET, P.S., C. LONGFIELD & N.W. MOORE, 1960. *Dragonflies.* Collins, London.

DEGRANGE, C., 1971. L'oeuf de Hemianax ephippiger (Burmeister) 1839 (Odonata, Anisoptera, Aeschnidae). *Trav. Lab. Hydrobiol.* 62: 131-145.

GAMBLES, R.M., 1956. Eggs of Lestinogomphus africanus (Fraser). *Nature, Lond.* 177: 663.

GAMBLES, R.M. & A.E. GARDNER, 1960. The egg and early stages of Lestinogomphus africanus (Fraser) (Odonata: Gomphidae). *Proc. R. ent. Soc. Lond.* 35: 12-16.

GARDNER, A.E., 1950. The life-history of Sympetrum sanguineum Müller (Odonata). *Ent. Gaz.* 1: 21-26.

HIGASHI, T. & M. WATANABE, 1993. Fecundity and oviposition in three skimmers, Orthetrum japonicum, O. albistylum and O. triangulare (Odonata: Libellulidae). *Ecol. Res.* 8: 103-105.

HINTON, H.E., 1981. *Biology of insect eggs*. vols 1-3. Pergamon Press, Oxford.

IVEY, R.K., J.C. BAILEY, B.P. STARK & D.L. LENTZ, 1988. A preliminary report of egg chorion features in dragonflies (Anisoptera). *Odonatologica* 17: 393-399.

MARGARITIS, L.H., 1985. Structure and physiology of the eggshell. *In*: G.A. Kerkut & L.I. Gilbert, [Eds], Comprehensive insect physiology, biochemistry and pharmacology, Vol. 1., pp. 153-230, Pergamon Press, Oxford.

MILLER, P.L., 1987. Oviposition behaviour and eggshell structure in some libellulid dragonflies, with particular reference to Brachythemis lacustris (Kirby) and Orthetrum coerulescens (Fabricius) (Anisoptera). *Odonatologica* 16: 361-374.

MILLER, P.L. & A.K. MILLER, 1985. Rates of oviposition and some other aspects of reproductive behaviour in Tholymis tillarga (Fabricius) in Kenya (Anisoptera: Libellulidae). *Odonatologica* 14: 287-299.

MÜNCHBERG, P., 1932. Beiträge zur Kenntnis der Biologie der Libellenunterfamilie der Corduliinae Selys. *Int. Revue ges. Hydrobiol. Hydrogr.* 27: 265-302.

PEROTTI, M., 1971. Microtubules as components of Drosophila male paragonia secretion. An electron microscopic study with enzymatic tests. *J. submicr. Cytol.* 3: 255-282.

POLLARD, S.R., R.M. MOTARA & R.H.M. CROSS, 1986. The ultrastructure of oogenesis in Culex theileri. *S. Afr. J. Zool.* 21: 217-223.

ROBERT, P.-A., 1958. *Les libellules*. Delachaux & Niestlé, Neuchâtel.

ROTHSCHILD, M., Y. SCHLEIN & S. ITO, 1986. *A colour atlas of insect tissues via the flea*. Wolfe Publishing, London.

ROWE, R., 1987. *The dragonflies of New Zealand*. Auckland Univ. Press, Auckland.

SAHLEN, G., 1990. Egg raft adhesion and chorion structure in Culex pipiens (L.) (Diptera: Culicidae). *Int. J. Insect Morph. Embryol.* 19: 307-314.

SAHLÉN, G., 1994. Ultrastructure of the eggshell of Aeshna juncea (L.) (Odonata: Aeshnidae). *Int. J. Insect Morph. Embryol.* 23: [in press].

SCHIEMENZ, H., 1953. *Die Libellen unserer Heimat*. Urania, Jena.

SRIVASTAVA, V.K. & B.K. SRIVASTAVA, 1992. Morphohistology and the functional aspects of the components of the '8th complex' in female Ischnura rufostigma Selys (Zygoptera: Coenagrionidae). *Odonatologica* 21: 181-194.

TILLYARD, R.J., 1917. *The biology of dragonflies*. Cambridge Univ. Press, Cambridge.

TORKA, V., 1909. Eierablage des Weibchens von Cordulia metallica Linden. *Z. wiss. InsektBiol.* 5: 405.

TRUEMAN, J.W.H., 1990a. Unusual eggshell structures in Ictinogomphus australis (Selys) (Anisoptera: Gomphidae). *Odonatologica* 19: 293-296.

TRUEMAN, J.W.H., 1990b. Eggshells of Australian Gomphidae: Plastron respiration in eggs of stream-dwelling Odonata (Anisoptera). *Odonatologica* 19: 395-401.

TRUEMAN, J.W.H., 1991. Egg chorionic structures in Corduliidae and Libellulidae (Anisoptera). *Odonatologica* 20: 441-452.

WATANABE, M. & Y. ADACHI, 1987. Number and size of eggs in the three emerald damselflies, Lestes sponsa, L. temporalis and L. japonicus (Odonata: Lestidae). *Zool. Sci.* 4: 575-578.