

**TRANSMISSION ELECTRON MICROSCOPY
OF THE EGG SHELL IN FIVE DAMSELFLIES
(ZYGOPTERA: COENAGRIONIDAE,
MEGAPODAGRIONIDAE, CALOPTERYGIDAE)**

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The previously undescribed eggshell ultrastructure of 5 spp. is discussed in relation to other known eggshells within the order. Whereas the vitelline envelope has the same structure in all species, the endo- and the exochorion are diverse. Endochorionic sublayers are present, being few and uniform in the smaller eggs but numerous and increasing in thickness from the inside in the larger eggs. The exochorion varies from a compact layer via a layer with pores to a fibrous layer acting as a jelly. It is proposed that endophytic eggs as a group are not so homogeneous as previously believed. Eggshell differences are possibly influenced by different egg-laying substrates, i.e. various living and decaying plants.

INTRODUCTION

In recent years a number of papers have been published dealing with the eggs and eggshells of dragonflies (DEGRANGE, 1971; MILLER & MILLER, 1985; MILLER, 1987; IVEY et al., 1988) by means of scanning electron microscopy (SEM). In some cases also fractured eggshells have been studied by SEM to obtain knowledge about the parts of the eggshell that are below the surface (BECNEL & DUNKLE, 1990; TRUEMAN, 1991).

Perhaps a better technique to study the different layers in the eggshell is transmission electron microscopy (TEM). The advantage over SEM is that the preparation is never dried and therefore many artefacts due to shrinking are avoided (SAHLÉN, 1994a, 1994b).

Two egg types exist in the order: those laid freely on or near the water surface (exophytic eggs) and those inserted into living or decaying plant tissues using an

ovipositor (endophytic eggs) (CORBET et al., 1960; CORBET, 1962). Although our knowledge of dragonfly eggshells has increased lately, much remains to be investigated. One such example is the zygopteran eggshell, which is always endophytic. The shape and size of these eggs have been known for a long time (e.g. TILLYARD, 1917; ROBERT, 1958), but no ultrastructural descriptions have been published. A few studies dealing with the micropyles and hatching of these eggs do exist (ANDO, 1962; DEGRANGE, 1960, 1971, 1974), and a study of crystalline sheaths in the chorion has been made (FURNEAUX & MACKAY, 1972). The formation of the eggshell within the ovary has been studied in endophytic eggs belonging to the Anisoptera (BEAMS & KESSEL, 1969).

In this paper, I describe the ultrastructure of the eggshell layers in five zygopteran species using TEM, and thus enhance our knowledge of the general variations in the dragonfly eggshell.

MATERIAL AND METHODS

Females of *Coenagrion hastulatum* (Charp.), *Ischnura elegans* (Vander L.), *Pyrrosoma nymphula* (Sulz.) and *Calopteryx virgo* (L.) from central Sweden and *Rhipidolestes hiraoui* Yamamoto from Shikoku, Japan, all regarded as endophytic, were placed in small plastic jars containing wet toilet paper. The jars were kept in a shady place until eggs were deposited. The eggs were left in the wet paper over night (approx. 10 h) before being transferred to a 0.1 mol x dm⁻³ phosphate buffer, pH 7.4.

General measurements were taken on whole eggs through a Wild M5 stereo microscope. Fixation, embedding and sectioning for TEM were carried out as described in SAHLÉN (1994a). The nomenclature used is also based on SAHLÉN (1994a). A series of eggs from more than one female was used in all investigations. The drawings are based on photographs.

RESULTS

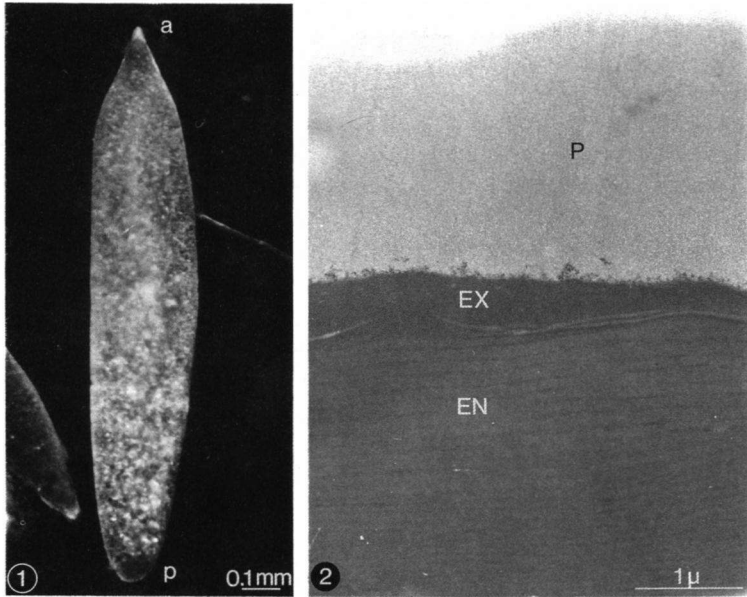
The eggs are very similar in shape, typically endophytic and spindle-shaped with the anterior end pointed and the posterior end rounded (Fig. 1). General measurements of the eggs (length and width) are shown in Table I. The surfaces are faintly reticulated except close to the anterior pole where numerous polygonal impressions are seen. The micropyles are situated just below the apex of the anterior end, their openings being between the polygonal impressions and the rounded micropylar process.

There are more clearly defined species-specific differences in the ultra cross-sections. The eggshell layers are expressed as follows:

C. HASTULATUM (Fig. 3). – Midway on the egg the vitelline envelope (VE) is

Table I
Egg length and width ($\bar{x} \pm \text{sd}$ for 30 eggs from at least 2 different females)

Species	Length (mm)	Width (mm)
<i>C. hastulatum</i>	1.19±0.12	0.22±0.08
<i>I. elegans</i>	0.98±0.06	0.21±0.06
<i>P. nymphula</i>	1.13±0.04	0.26±0.03
<i>R. hiraoui</i>	1.43±0.07	0.27±0.06
<i>C. virgo</i>	1.37±0.08	0.28±0.05

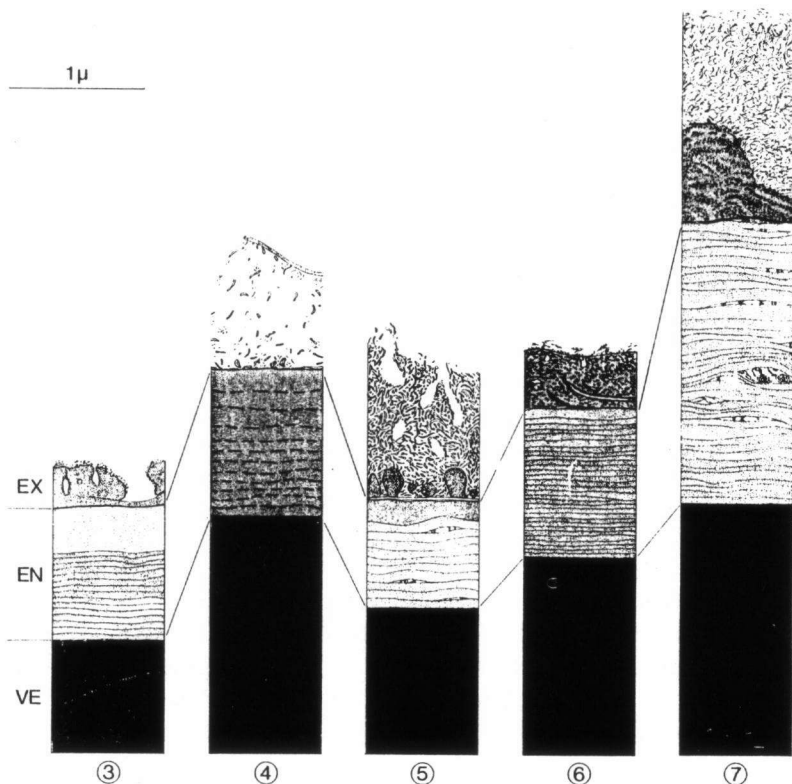


Figs 1-2. *Rhipidolestes hiraoui*: (1) Whole egg representing general zygopteran egg shape; anterior end (a) pointed and posterior end (p) rounded; – (2) Part of many-layered endochorion (EN), exochorion (EX) and porous substance (P) near anterior end.

0.86 μm , the endochorion (EN) 1.0 μm , and the exochorion (EX) around 0.29 μm thick. The VE is homogeneous, while the EN is clearly lamellated with between 12 and 16 lamellae, all but the outermost being of equal thickness (0.04–0.05 μm). The one facing the EX comprises about one third of the layer. This section of the EN takes up less contrasting agents than the rest. The EX has a very irregular outside. In the anterior third of the egg, small pores can be observed in this layer, but in the rest of the shell the pores are less conspicuous and appear less frequently. In some areas the EX is reduced to nothing but a very thin sheath. Near the anterior end of the egg all eggshell layers increase in thickness, the VE reaching 3.09 μm , the EN 1.24 μm , and the EX 0.79 μm .

I. ELEGANS (Fig. 4). – In this species the layers are less variable in thickness. The VE is 1.8 μm , only increasing marginally at the anterior end. It is homogeneous as in the previous species. The EN is about 1.1 μm and homogeneous, except at the anterior end where it separates into a variable number of sub-layers, thus increasing the thickness of the EN by adding empty spaces between the sub-layers. The EX is only a thin sheath which further expands into a kind of coarse jelly-layer. The expansion varies around the perimeter of the egg, but is best expressed at the anterior end. No fixed measurements on the thickness of the layer can thus be given. The jelly is formed from small filamentous structures, measuring about 10–50 nm in diameter. No pores are present in the EX.

P. NYMPHULA (Fig. 5). – The VE is a homogeneous, very electron-dense layer, measuring $1.1\ \mu\text{m}$ midway along the egg. At the anterior end its thickness increases to $2.4\ \mu\text{m}$. The EN is $0.82\ \mu\text{m}$, reaching $1.52\ \mu\text{m}$ at the anterior end. The EN is lamellated with 10-12 lamellae of equal thickness (approx. $0.06\ \mu\text{m}$) and an outer more dense layer, as thick as 2-3 lamellae. This outer layer varies in thickness, seemingly compensating for any inclusion of empty spaces between the lamellae. The EX is approximately $1.0\ \mu\text{m}$ thick. At its base, small electron-dense droplets, surrounded by a fibrous matrix, face the EN but the two layers are rarely attached to each other. The outer portion of the EX is only a fibrous material, gradually decreasing in compactness going outwards. Thin strands of fibrous material stretch out from it at irregular intervals. In some areas, large empty spaces are incorporated.



Figs 3-7. Schematic drawings of shell in (3) *Coenagrion hastulatum*; – (4) *Ischnura elegans*; – (5) *Pyrrhosoma nymphula*; – (6) *Rhipidolestes hiraoui*; – (7) *Calopteryx virgo*. – [Vitelline envelope (VE), endochorion (EN) and exochorion (EX) indicated with lines]

R. HIRAOI (Fig. 6). – The VE is about 1.5 μm thick and homogeneous as in the previous species, likewise increasing in thickness up to 2.5 μm at the anterior end. The EN is 1.1 μm thick midway along the egg, consisting of 25–35 lamellae of increasing thickness from the inside outwards (the outermost being maximally about 0.17 μm). At the anterior end the thickness of the EN increases to 2.8 μm , but the number of lamellae is the same. The EX is 0.45 μm thick midway along the egg. The layer is rather electron-dense (more so than the EN) and its outside as well as its internal structure is irregular. Some empty spaces are incorporated at irregular intervals but no pores or pore-canal are present. Near the anterior end the layer becomes more electron-dense and homogeneous, but on the outside a new, very soft and porous-looking substance appears (Fig. 2). This new layer is up to 3.4 μm thick in some places and its inside is nowhere attached to the rest of the EX.

C. VIRGO (Fig. 7). – The VE is 1.9 μm thick midway along the egg. The layer is completely homogeneous and rather electron-dense. Near the anterior end the layer is thicker (up to 4.0 μm). No openings or channels through the layer are seen. The EN is 2.1 μm , increasing to 3.3 μm at the anterior end. The layer is intermediate in density, having numerous (approx. 30–40) irregularly arranged sub-layers of almost equal thickness. Near the anterior end the sub-layers start to separate, enclosing numerous empty spaces within the layer. The EX is 1.6 μm thick and rather irregular with an inner, electron-dense part and an outer part of less density. The inner part is sometimes only one tenth of the layer in thickness, but in some areas it constitutes more than half of the layer. The outer part is irregular, its texture somewhat fibrous. Near the anterior end the EX is up to 2.5 μm thick, more or less integrated to the EN.

DISCUSSION

The general shape and structures are similar in the eggs of all the 5 species. They are also similar to those of *Aeshna juncea* (L.) (SAHLEN, 1994a). The major difference between the somewhat smaller eggs from the zygopterans studied here and the larger ones from *A. juncea* seems to be overall size and complexity of the chorionic layers, whereas the VE is a homogeneous medium-dense layer in all the hitherto investigated dragonfly species, including the exophytic ones (SAHLÉN, 1994b). However, some interesting differences occur in the EN and the EX.

The EN seems to have an increasing number of sub-layers the larger the egg is. For *C. hastulatum*, *I. elegans* and *P. nymphula*, the number of sub-layers is between 10 and 20 (if discernable) while the larger eggs of *R. hiraoi* and *C. virgo* have between 20 and 40 layers. In the even larger eggs of *A. juncea* there are never less than 70 sub-layers (SAHLÉN, 1994a). The same is true for *A. grandis* (L.) (Sahlén, unpubl.). In the smaller eggs, all but the outermost of the sub-layers are of equal thickness, while the thickness of the layers increases continually from the inside to the outside in the larger eggs. The inner part of the EX in *A. juncea* con-

sists of a crystalline-like layer of about 50 densely packed sub-layers (SAHLEN, 1994). Crystalline layers have also been reported from *C. virgo* (FURNEAUX & MACKAY, 1972), but no such structures were found in this species by me. With the exception of the two studies mentioned above, no crystalline layers have been reported from any other dragonfly eggs. Crystalline layers should therefore not be considered as basic structures in endophytic eggs of dragonflies.

The EX is very variable between the five species. In *R. hiraoui* the layer has two parts, an inner, more compact part, and an outer less dense one, but neither has any particular sub-structure. The inner part of the layer in *C. virgo* is also rather compact, but here it is possible to discern traces of a fibrous texture in the outer part. In *C. hastulatum* pore-canals, characteristic of aeshnid eggs (DEGRANGE, 1971; SAHLEN, 1994a) are present, although less numerous and not as well defined as in the larger anisopteran species. Some hints of fibrous texture exists in the layer. In *P. nymphula* the fibrous texture is dominating, but in the inner part of the layer small droplets of a non-fibrous substance are present. Pores still exist, but they are very poorly defined. In *I. elegans* the fibrous structure is well developed, the layer expanding like the jelly layers of the exophytic dragonfly eggs (SAHLÉN, 1994b). Here, the entire layer consists of thin threads and no trace of any pores or any basal layer can be found. The jelly-threads in *I. elegans* have a variable diameter (10-50 nm), and not the standardized 24 nm as have been found in other dragonfly eggs (SAHLÉN, 1994a, 1994b).

Thus, in these species, a transition from compact layers via layers with pores into fibrous layers acting as a jelly can be seen. However, taking into account the proposed systematic position of the investigated species (e.g. CARLE, 1982; DAVIES & TOBIN, 1986), no clear evolutionary line in chorionic development can be seen. For instance, *C. hastulatum*, *P. nymphula* and *I. elegans* are placed in the same subfamily, but nevertheless they show great differences in the EX - at least as great as the differences between *R. hiraoui* and *C. virgo*, which are in different families. Investigations on other, closely related species may show if the differences are generic.

As the EN, and more so the EX of the zygopterans seem to vary to a great extent despite the standardized niche occupied by endophytic eggs (i.e. inside living or decaying plant tissue), it seems as though the endophytic group may not be so homogeneous as was previously believed. A possible reason might be adaptations to compete with the chemical defence of different plants. BUCHWALD (1992) showed that *Ceriagrion tenellum* (de Vill.) actively chooses the type of vegetation in which to oviposit, more so than choosing the type of water to lay eggs in. The anisopteran *Aeshna viridis* Eversm. is known to prefer plants of *Stratiotes aloides* as an egg-laying substrate (e.g. ROBERT, 1958). HELLMUND & HELLMUND (1991, 1993) reported findings of fossil zygopteran eggs from the upper Oligocene, about 25 million years ago. This gives evidence for an old association between plants and eggs (although the association is probably much older as the fossil record

of species with an exophytic mode of oviposition shows) and thus, substances secreted by the tissues of specific plant species may have triggered species-specific responses in the eggs, which in time may have lead to differences in eggshell morphology.

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