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Morphology of follicle cells of Libellulidae (Odonata)

Kamilla Koch[a]*, Nadine Fuchs[a] and Göran Sahlén[b]

[a]*Department of Ecology, University of Mainz, Becherweg 13, 55128 Mainz, Germany;* [b]*Ecology and Environmental Sciences, Halmstad University, PO Box 823, 30118 Halmstad, Sweden*

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In libellulids, mature oocyte size varies within and between individual ovaries. The regulating mechanism is not yet understood. Variations in the contents of the follicle cells, and thereby their ability to secrete material into the oocyte, might explain some of the observed differences in oocyte size. We therefore investigated the follicle cell surface, the interstitial space width between follicle cells and between follicle cells and oocytes, the number of nuclei, and the cell compartment proportions using scanning and transmission electron microscopy. In all investigated species, the follicle cells were covered by a basal lamina. We found cytoplasmic microvilli and septate junctions. As we could not find any pores or other structures on the cell surface, endocytosis seems to be the only mechanism transporting material into the follicle cells. Larger follicle cells had larger interstitial gaps between follicle cells and oocytes, larger nuclei and a larger mitochondrial area. Larger interstitial spaces between follicle cells and oocytes may afford more room that can be filled with material from the follicle cell layer. More mitochondria could provide more energy/ATP needed for the transport of the material. The quantity of free ribosomes and the mean number of nuclei seemed to be even more important to the productivity of the follicle cell. All these variations in cell contents cause productivity differences among follicle cells and may explain some of the size differences between oocytes within individual ovaries in libellulids.

Keywords: Odonata; dragonflies; ovary; oocyte size variation; follicle cell components; follicle cell surface; scanning electron microscopy; light microscopy; transmission electron microscopy

Introduction

Dragonfly eggs and their structures are comparatively well investigated (e.g., Corbet, 1999; Ivey et al., 1988; Miller & Miller, 1985; Sahlén, 1992, 1994a, 1994b, 1995a, 1995b), at least for certain groups. Most studies deal with the nomenclature of the different eggshell layers (e.g. Sahlén, 1992, 1995a, 1995b), and describe species-specific patterns of the micropyles and other surface structures on the eggshell (e.g. Sahlén, 1994a, 1994b, 1995a, 1995b). As in other insects, dragonflies have paired ovaries, each one consisting of numerous separate ovarioles (e.g. in libellulids a range of 22 to 254; Karlsson et al., 2010), which are longitudinally arranged in the abdomen (Tillyard, 1917). This is where oogenesis takes place (Gaino et al., 2008; Tillyard, 1917). Each ovariole can be subdivided into three sections: the terminal filament, which connects the anterior end of the ovarioles to the body wall; the germinal area, where the oocytes are formed by cleavage; and the vitellarium area (Snodgrass, 1935; Tillyard, 1917), where the oocytes mature and store

*Corresponding author. Email: Kochka@uni-mainz.de

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energy reserves and RNA during vitellogenesis (Brennan et al., 1982; Bünning, 1994). At the posterior end of the ovarioles, mature oocytes surrounded by the chorion (eggshell) are ready for fertilization and deposition.

Dragonflies have panoistic ovaries, in which the immature oocytes are surrounded by a single follicle cell layer (Matsuzaki, 1971; Schwalm, 1988; Tillyard, 1917). The follicle cells secrete the material required to build up the oocytes during vitellogenesis. In many groups of the Neoptera separate nurse cells have evolved for this purpose, and follicle cells are retained merely as a stabilizing layer (Brennan et al., 1982; Dettner & Peters, 2003; Snodgrass, 1935). Yolk precursors are produced in the female's fat body and transported via the haemolymph to the ovaries, where they are taken up by follicle cells and finally released into the interstitial space between the follicle cells and the oocyte (Hagedorn & Kunkel, 1979). In addition, mRNA, rRNA and energy reserves such as lipids and glycogen are produced by follicle cells and likewise released into the interstitial space (Brennan et al., 1982). From there, the maturing oocyte can take up all products via endocytosis (Dettner & Peters, 2003; Müller & Hassel, 2003). Other functions of the follicle cells are construction of the egg integument, production and release of all egg integument components (Hinton, 1970; Margaritis, 1985; Martins & Serrão, 2002) and determination of the egg shape (Sahlén, 1992). Follicle cells often cause species-specific patterns on the surface of the mature egg integument (Beament, 1946; Fehrenbach et al., 1987; Hinton, 1969, 1970).

Like all eukaryotic cells, follicle cells have organelles, which are important for the synthesis and transport of products (cf. Munk, 2000). In most cases there is only one nucleus per follicle cell, but in libellulids there are frequently more than one (Karlsson et al., 2010) possibly as a result of ongoing mitosis. The endoplasmic reticulum (ER) produces export proteins, membrane proteins and membrane lipids. The rough ER with many ribosomes on the surface produces proteins, whereas the smooth ER synthesizes lipids and lipophilic compounds. For that reason, tissues active in protein synthesis contain mainly rough ER (Bielka & Börner, 1995). Mitochondria supply most of the ATP required by the cell. Their number and the shape of the mitochondrial membranes are closely correlated with the oxidative metabolism of the tissue; the energy requirements of the cell are proportional to the number of cristae of the mitochondrial membranes.

In libellulids it is known that oocyte and egg size varies not only among species and within species, but also within individual ovaries (Koch et al., 2009) and egg clutches (Schenk & Söndgerath, 2005; Schenk et al., 2004). Some species deposit the largest eggs first followed by successively smaller ones, while others continuously mix egg sizes (Schenk et al., 2004). The mechanism regulating egg size difference is not yet understood, but Koch et al. (2009) found variations in oocyte size and follicle cell dimensions during oogenesis. Taking knowledge one step further, it is reasonable to look at the ultrastructure of both the follicle cells and the growing oocytes. Variations in the organelles of the follicle cells as well as in the contact between cells and thereby their ability to secrete material into the oocyte might explain some of the differences in oocyte size observed. We therefore investigated the follicle cells: their surface, proportion of cells with more than one nucleus, cell compartment proportions (organelles, etc.) in each cell, contact area to other follicle cells, and the potential interstitial space width between follicle cells and oocytes (IOF) using light, scanning and transmission electron microscopy.

Materials and methods

Scanning electron microscopy (SEM)

We captured females of *Crocothemis erythraea* (Brullé, 1832) ($n = 15$), *Leucorrhinia dubia* (Vander Linden, 1825) ($n = 16$), *Pantala flavescens* (Fabricius, 1798) ($n = 7$), and *Sympetrum striolatum* (Charpentier, 1840) ($n = 5$) and preserved them in 70% ethanol. Their abdomens were

opened ventrally and postfixed for 60 minutes in 10% formaldehyde. Afterwards, we removed the paired ovaries. Only the middle section (for more details see Koch et al., 2009) was used, because this is the region where vitellogenesis takes place and where the oocytes are totally surrounded by follicle cells (Büning, 1994). After preparation, the ovary parts were dehydrated for 30 minutes each in a series of ethanol concentrations from 50 to 100% and then transferred into acetone. Some ovary parts were treated with freeze-fracturing to get many small fragments showing cells in cross section: they were transferred into liquid nitrogen, broken with tweezers and then returned to acetone. Alternatively, the ovary pieces were cut by fine scalpels without liquid nitrogen treatment (i.e. only fracturing). The small ovary fragments were mounted on aluminium stubs with adhesive carbon film and dried at room temperature. Finally, the preparations were sputter-coated (adding 6–7 nm) with gold (Flegler et al., 1995) and the follicle cell surfaces were examined in a JEOL JSM-6480LV scanning electron microscope.

Light microscopy (LM) and transmission electron microscopy (TEM)

Females of *C. erythraea* ($n = 3$), *L. dubia* ($n = 3$) and *S. striolatum* ($n = 6$) were captured and transported alive into the laboratory. The ovary parts were dissected and transferred for 60 minutes to a solution of 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. They were rinsed in 0.1 M cacodylate buffer, pH 7.4 and postfixed for 60 minutes in 2% osmium tetroxide in the same buffer. After rinsing in water, the ovary parts were dehydrated for 10 minutes each in a series of ethanol concentrations from 30 to 100% and then transferred for 15 minutes into propylenoxide. They were then infiltrated over night with a mixture of propylenoxide and araldite (1:1), stored for another night in pure araldite and finally embedded with pure araldite in a rubber mould and polymerized for two nights at 60–65°C. For LM, sections of 1 μm were made with an ultramicrotome Ultracut S (Leica) using a glass knife. The sections were transferred to microscope slides, stained with methylene blue azure II according to Richardson et al. (1960) and finally examined with a light microscope. For TEM, ultrathin sections of 50–70 nm were made with the same ultramicrotome using a diamond knife and mounted on formvar-coated copper grids. The sections were stained with 2% uranyl acetate in 50% ethanol and lead citrate according to Hayat (2000) and examined in a Technai 12 transmission electron microscope.

In the LM we counted the number of nuclei per follicle cell (*C. erythraea* $n = 369$ cells, *L. dubia* $n = 254$, *S. striolatum* $n = 1154$). In the TEM we examined two oocytes per specimen and evaluated four follicle cells per oocyte. Our aim was to compare equally mature oocytes. The nuclei were counted, and the respective surface areas of the entire cell, the nuclei and the mitochondria were measured, using the free software ImageTool 3.0. The width of the interstitial space between the oocyte and the follicle cells (IOF) was determined by using the mean value of three measurements per follicle cell. We also estimated the area (in % of cell surface) of the ER and the ribosomes. The Golgi apparatus and vesicles were difficult to distinguish, and we summarized the area of these organelles as "residual area". Finally, for all three species we ran linear regression analyses with follicle cell surface as the independent variable and interstitial space width and nuclear area, mitochondrial area, rough endoplasmic reticulum (ER) area, ribosome area and residual area as dependent variables in order to test whether or not there was a correlation between follicle cell size and follicle cell parameters.

Results

Scanning electron microscopy (SEM)

SEM provided a clear three-dimensional impression (Figures 1–4) of the oocyte arrangement inside the ovarioles, the micropylar area (Figures 2–3) and the follicle cell layer surrounding

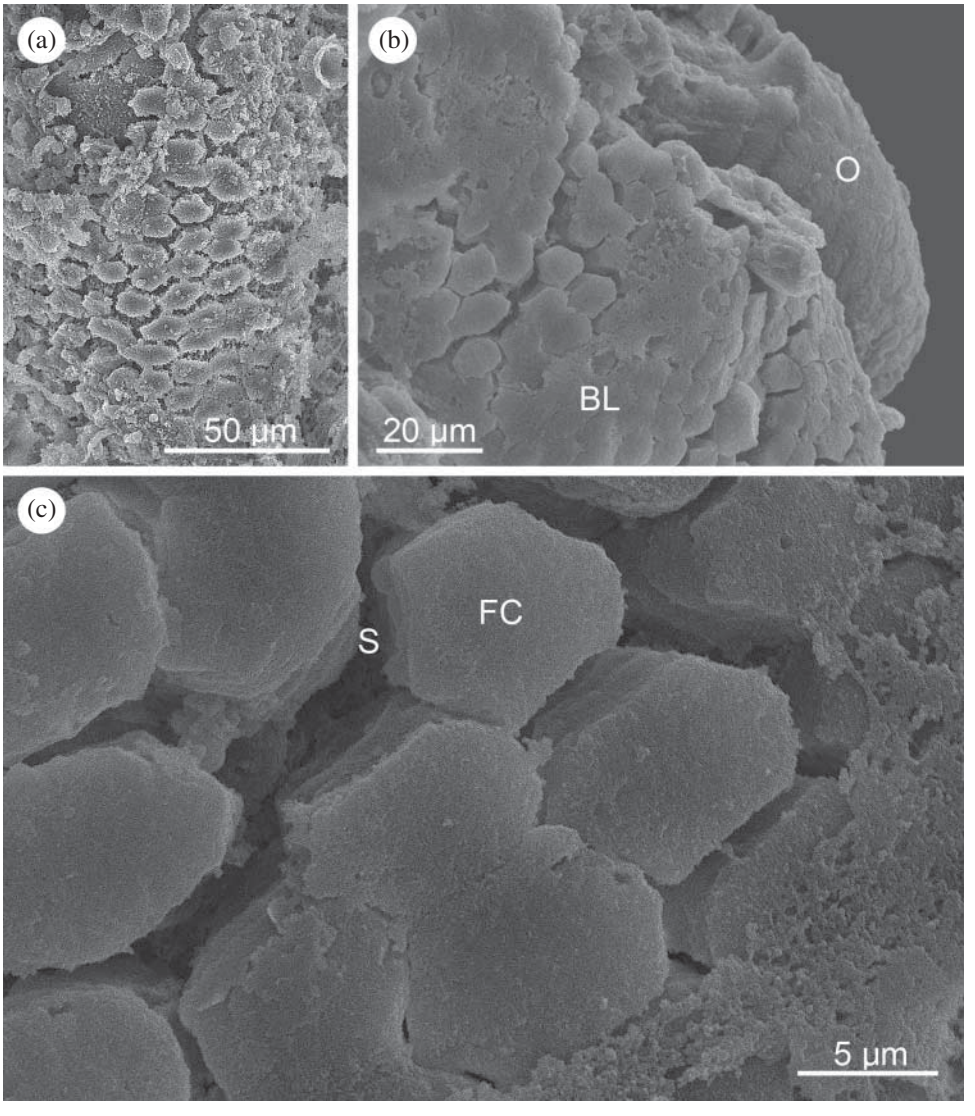

Figure 1. SEM of *Crocothemis erythraea* oocyte surfaces. (a) Oocyte with follicle cells seen in external view. Separation between follicle cells is an artefact resulting from tissue shrinkage caused by the preservation or by the dehydration process. (b) Two oocytes (O) in external view with only moderate shrinkage of follicle cells in some areas; some of them with basal lamina (BL). (c) Close-up of (b) showing polygonal follicle cells (FC) and separation by shrinkage (S) in external view.

each individual oocyte. In *Crocothemis erythraea*, *Leucorrhinia dubia* and *Pantala flavescens*, the SEM cross sections showed that the oocytes from individual specimens varied a lot with regard to maturity, size, and appearance. In *Sympetrum striolatum* the intrinsic variation in oocyte size and maturity were smaller.

Depending on the direction of the fracture, we could see the follicle cell layer in cross section or in external view (from the outside of the maturing egg). In all species, the follicle cell layers were covered by a basal lamina (Figures 1–4). Follicle cell shape was in most cases clearly visible through this layer, and many of the cells had a hexagonal shape (Figures 1–4). In all SEM pictures we observed a separation between the follicle cells surrounding the oocyte (e.g.

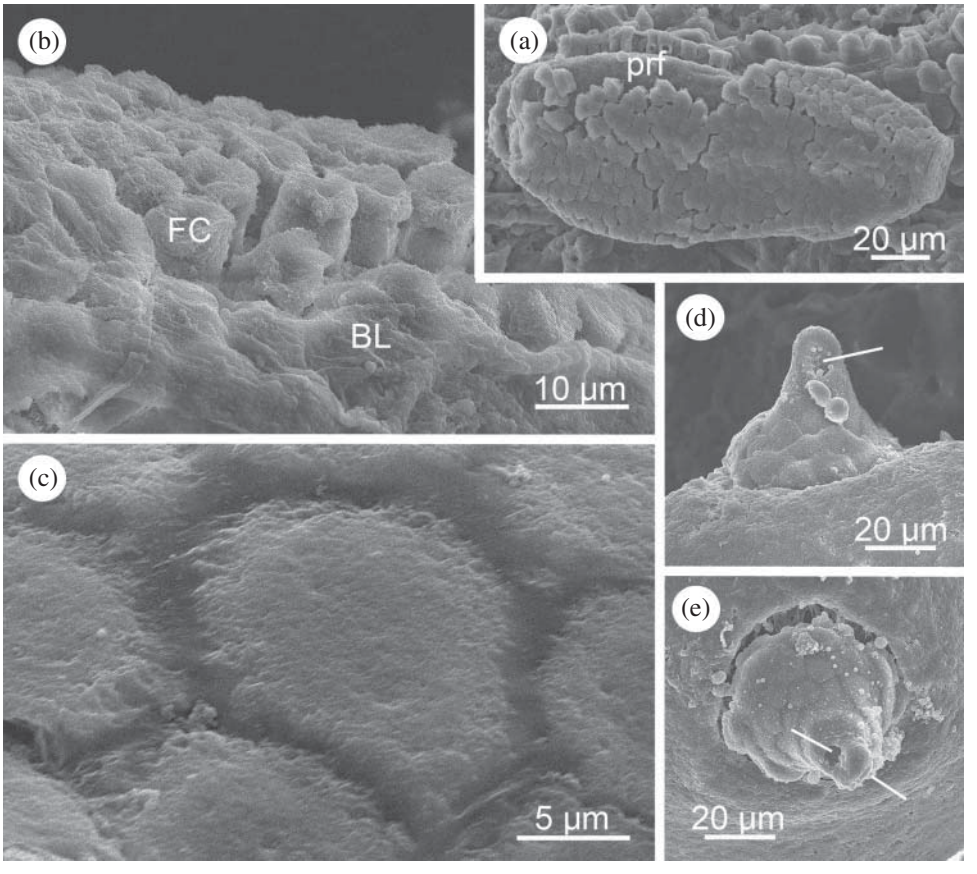

Figure 2. SEM of *Leucorrhinia dubia* oocyte surfaces: (a) whole oocyte with partly removed follocle cells (prf) and remaining follicle cells sometimes slightly separated (artefact); (b) close-up of separated follicle cells (FC), some with basal lamina (BL); (c) close-up of polygonal follicle cells with basal lamina; (d) and (e) micropylar projection in external view. Line indicates micropylar opening.

Figures 1, 2b, 3c, 4a). This separation was, however, absent in all TEM pictures (Figure 5). We therefore conclude that this separation was an artefact resulting from tissue shrinkage and caused by the preservation in ethanol or by the dehydration process and therefore not interesting for analysis.

Leucorrhinia dubia (Figure 2) showed the biggest variation in the appearance of the cell surface. Some cells had a smooth external side, while others were covered with protuberances. In *P. flavescens* (Figure 3) the external surface of the follicle cells was very smooth, whereas the internal side showed a distinctive microstructure (Figure 3c). In *S. striolatum* (Figure 4) the shape and height of follicle cells varied between and within single cells without any clear patterns.

Light microscopy (LM) and transmission electron microscopy (TEM)

The proportion of follicle cells with more than one nuclei varied both within and between the species (*C. erythraea*: 12.7% two nuclei; *L. dubia*: 48.8% two nuclei; *S. striolatum*: 20.6% two nuclei).

An IOF was observed in all species examined (*C. erythraea* 1.91 µm ± 1.01 standard deviation (s.d.), *L. dubia* 0.2 µm ± 0.47, *S. striolatum* 1.41 µm ± 1.27). On the external side, the follicle

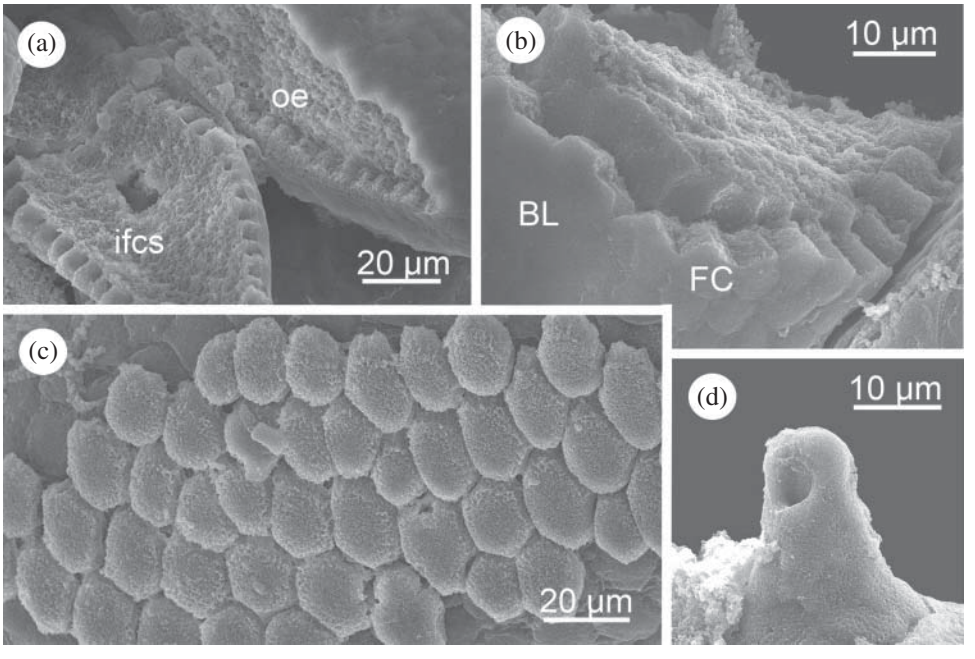

Figure 3. SEM of *Pantala flavescens* oocyte surfaces: (a) part of the follicle cells removed showing internal follicle cell surface (ifcs) and oocyte exterior (oe); (b) oocyte sectioned at egg equator showing follicle cell (FC) arrangement and basal lamina (BL); (c) polygonal arrangement of follicle cells on oocyte; here separated due to tissue shrinkage; (d) micropylar projection with one micropylar opening visible.

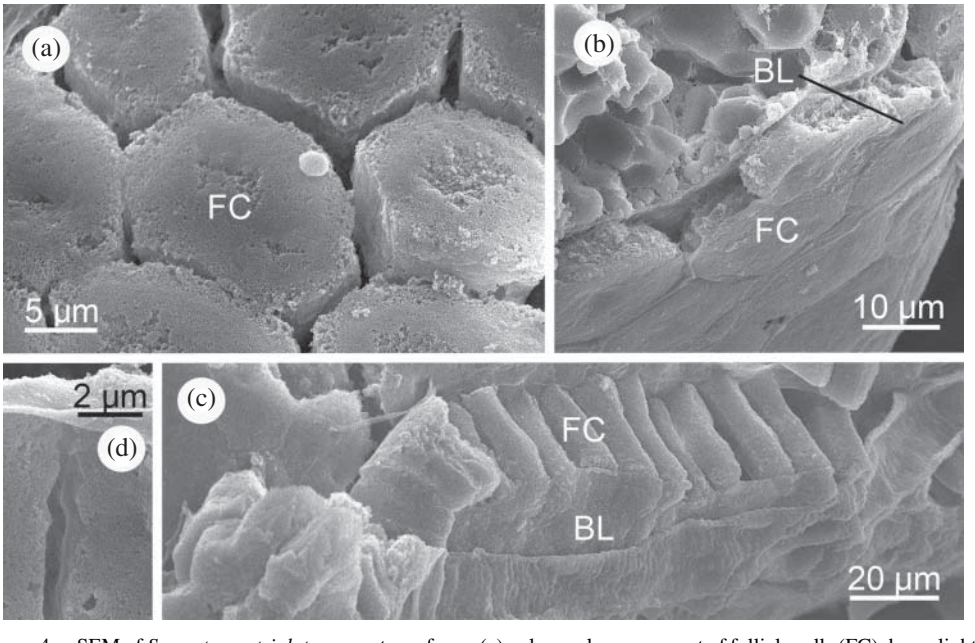

Figure 4. SEM of *Sympetrum striolatum* oocyte surfaces: (a) polygonal arrangement of follicle cells (FC); here slightly separated due to tissue shrinkage; (b) oocyte sectioned at equator showing flat and wide follicle cells (FC) and basal lamina (BL); (c) narrow and high follicle cells (FC) near anterior end of egg; also basal lamina (BL); (d) close-up of two follicle cells with thin, shrunken cavity between them. Basal lamina on top.

cell layer was always covered by a basal lamina (Figure 5). There were septate junctions between neighbouring follicle cells in *C. erythraea*, oocytes with cytoplasmic microvilli in *S. striolatum*, and finger-shaped cytoplasmic extensions between neighbouring follicle cells as well as between the follicle cells and the oocyte in all species (Figure 6).

Inside the follicle cells nuclei, mitochondria, ER, free ribosomes and microtubules were visible (Figures 5, 6). The size of nuclei, mitochondria, ER and free ribosomes in relation to the total cell area varied between the three species (Table 1). *Crocothemis erythraea* and *S. striolatum* were comparable, but *L. dubia* in general had smaller follicle cells with smaller nuclei, less ER and more free ribosomes (Table 1). The mean thickness of the interstitial space between follicle cell layer and oocyte membrane was greatest in *C. erythraea*, smaller in *S. striolatum* and smallest in *L. dubia* (Table 1). In general, larger follicle cells had a wider IOF, and proportionally larger nuclei and mitochondrial surface area (Table 2). Only the percentage of ER, free ribosomes and the residual components followed no clear pattern (Table 2).

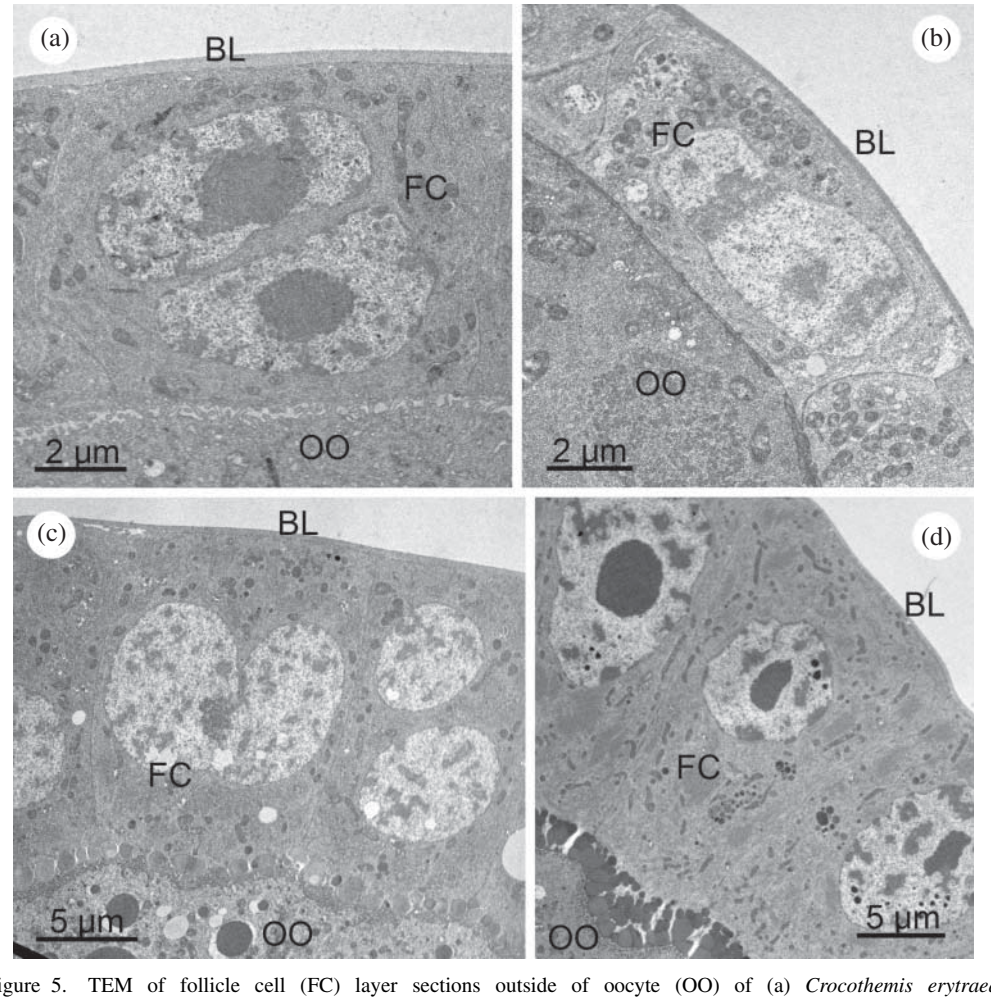

Figure 5. TEM of follicle cell (FC) layer sections outside of oocyte (OO) of (a) *Crocothemis erytraea*; (b) and (c) *Leucorrhinia dubia*; (d) *Sympetrum striolatum*. The follicle cells are always covered by a basal lamina (BL).

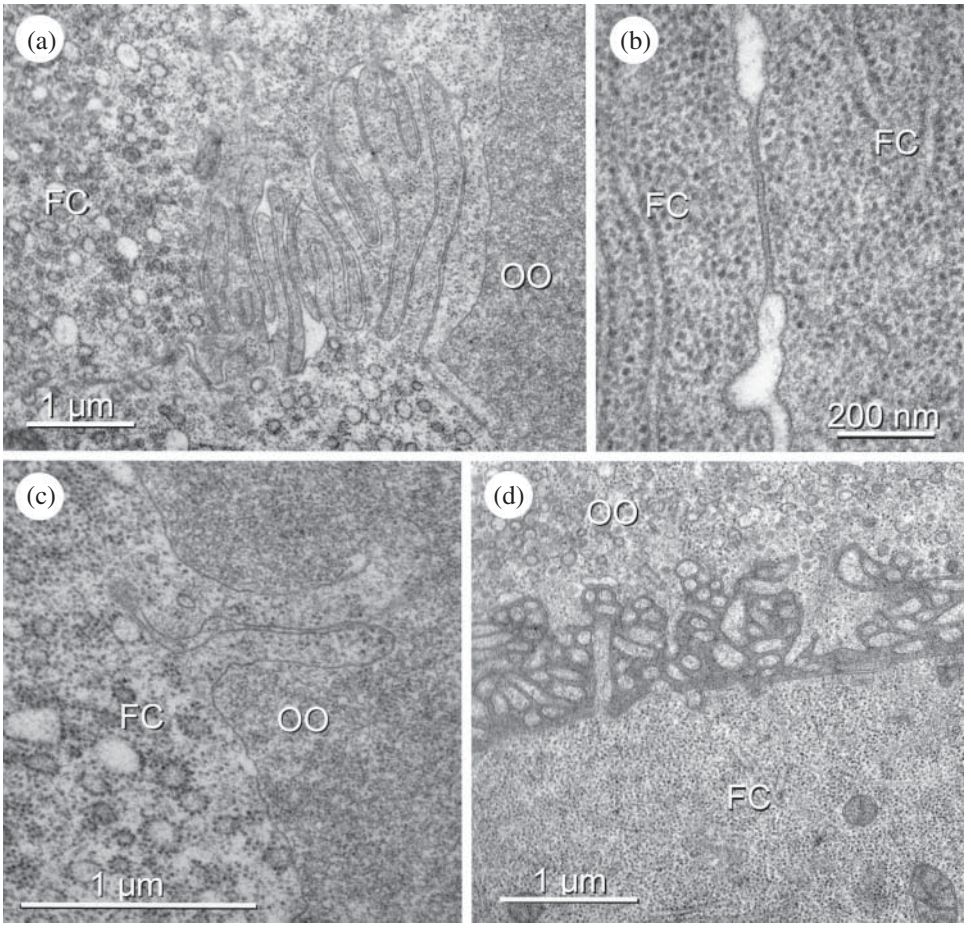

Figure 6. TEM of follicle cell layer: (a) cytoplasmic extensions between neighbouring follicle cells (FC) in *Leucorrhinia dubia*, oocyte (OO) on right; (b) septate junctions between neighbouring follicle cells (FC) in *Crocothemis erythraea*; (c) cytoplasmic extensions between follicle cell (FC) and oocyte (OO) in *Crocothemis erythraea*; (d) oocyte (OO) and follicle cell (FC) border with microvilli in *Sympetrum striolatum*.

Table 1. Follicle cell characters (follicle cell area and percentage of nuclei, mitochondrial, ER, ribosomal and residual area, respectively) in the Libellulids *Crocothemis erythraea* (three females and six follicle cells), *Leucorrhinia dubia* (three females and six follicle cells) and *Sympetrum striolatum* (six females and 12 follicle cells).

	Crocothemis erythaea		*Leucorrhinia dubia*		*Sympetrum striolatum*	
	Mean	±s.d.	Mean	±s.d.	Mean	±s.d.
Follicle cell area [μm^2]	156.92	75.20	59.87	71.62	203.62	117.53
Nuclear area [%]	29.73	8.88	48.81	11.33	31.77	14.23
Mitochondrial area [%]	5.20	1.38	4.64	2.35	4.76	0.91
ER area [%]	55.42	7.06	34.21	14.59	57.59	14.42
Ribosomal area [%]	4.79	2.02	6.83	11.69	3.27	2.51
Residual area [%]	4.50	1.72	4.79	3.05	1.94	0.48

Table 2. Regression analyses for *Crocothemis erythraea* (24 follicle cells), *Leucorrhinia dubia* (24 follicle cells) and *Sympetrum striolatum* (48 follicle cells) for follicle cell surface against interstitial space width between follicle cell and oocyte, nuclei, mitochondria, ER and ribosome area as well as the rest area (see methods).

	Crocothemis erythraea			*Leucorrhinia dubia*			*Sympetrum striolatum*		
	R^2	*p*	Slope	R^2	*p*	Slope	R^2	*p*	Slope
Follicle cell surface/interstitial space width	0.45	<0.0001	+0.51	0.91	<0.0001	+0.16	0.23	0.001	+0.37
Follicle cell surface/nuclei area	0.79	<0.0001	+6.27	0.94	<0.0001	+2.67	0.61	<0.0001	+2.75
Follicle cell surface/mitochondria area	0.83	<0.0001	+0.24	0.94	<0.0001	+0.05	0.7	<0.0001	+1.59
Follicle cell surface/ER area	0.04	0.32	+52.28	0.11	0.12	+30.18	0.26	<0.0001	+44.53
Follicle cell surface/ribosome area	0.17	0.04	+3.03	<0.01	0.96	−6.93	0.17	0.003	−5.08
Follicle cell surface/residual area	0.05	0.33	+3.60	0.01	0.91	−4.84	0.01	0.73	−4.84

Discussion

The SEM cross sections through the ovaries confirmed that *C. erythraea*, *L. dubia* and *P. flavescens* belong to the species which produce oocytes continuously, whereas *S. striolatum* belongs to the species with a stepwise oocyte production (cf. Karlsson et al., 2010). Outside the follicle cell layer, the oocytes of all species were surrounded by a basal lamina, which provides some mechanical stability (Moll & Moll, 2003). The basal lamina is permeable to various molecules through diffusion (Czihak et al., 1996). We could also confirm the more or less regular hexagonal shape of the follicle cells (Koch et al., 2009); also visible as impressions in mature eggs (Sahlén, 1994b) where they are obscured by the expanded jelly layer after deposition in water (Sahlén, 1995b). The micropylar area at the anterior end of the oocytes (Dettner & Peters, 2003) looked similar in all species, having two openings as described by Sahlén (1994b); two openings seem to be a general trait in Corduliidae and Libellulidae (cf. Trueman, 1991). At fertilization the sperm enters the oocyte via the micropylar canals (Sahlén, 1994b; Snodgrass, 1935; Zarani & Margaritis, 1991). As all investigated oocytes within the middle part of the ovary already had fully developed micropyles, the formation of these structures seems to occur quite early within the oogenesis.

All oocytes showed cytoplasmic microvilli, extensions between neighbouring follicle cells, cytoplasmic extensions between follicle cells and oocytes, and septate junctions between neighbouring follicle cells. Septate junctions are intercellular junctions found in invertebrate epithelia, which are characterized by a ladder-like appearance that provides structural strength and serves as a barrier to diffusion of solutes through the intercellular space (e.g. Garavito et al., 1982). We could, however, find no pores or other structures on the follicle cell surface. We can therefore confirm the exchange process to be endocytosis and exocytosis only (cf. Dettner & Peters, 2003; Müller & Hassel, 2003). In panoistic ovaries the oocytes themselves are able to produce yolk. Most of the yolk, mRNA, rRNA and energy reserves (lipids and glycogen) do, however, come from the haemolymph and the follicle cell layer (Van Antwerpen et al., 2005), whence they are released into the interstitial space (Brennan et al., 1982). Good mechanisms for transport into the oocytes are therefore necessary.

All species differed in the appearance of the follicle cell surface, in the IOF and the proportions of cell content per follicle cell. With regard to cell compartment proportions per follicle cell and number of nuclei per follicle cell *C. erythraea* and *S. striolatum* differed markedly from *L. dubia*. The latter species had the smallest follicle cells and the smallest IOF. However, *L. dubia* had a higher amount of free ribosomes and a much higher ratio of two nuclei per follicle cell than the other species. We suggest that these features partly explain the high productivity of the

relatively small follicle cells of *L. dubia*, as they produce the largest eggs of the investigated species (egg circumference: *C. erythraea* ($n = 802$) 2.22 mm ± 0.08 s.d.; *L. dubia* ($n = 562$) 2.88 mm ± 0.14 s.d.; *P. flavescens* ($n = 4488$) 2.41 mm ± 0.25 s.d.; *S. striolatum* ($n = 3038$) 2.86 mm ± 0.11 s.d.; Koch & Suhling, 2005; Koch unpubl. data).

The presence of two nuclei per follicle cell in some of the cells of all species also supports the idea that mitosis continues in follicle cells throughout oocyte formation (Ando, 1962; Koch et al., 2009). Ongoing mitosis will affect the quantity of organelles present in the cells as the stages in the cell cycle change over time. Therefore, our conclusions are preliminary and need to be followed by further sampling of follicle cells at various stages of the cell cycle.

In general, we found that larger follicle cells had greater IOFs, larger nuclei and a larger mitochondrial area. Due to the greater IOF, more space is available which can be filled with material excreted from the follicle cells. More mitochondria can provide more energy/ATP needed to transport the material. We assume, however, that the number of free ribosomes is the most important characteristic for the productivity of the follicle cell. All these differences in cell contents taken together may well be the cause of productivity differences between follicle cells, and hence the reason behind oocyte size differences within individual ovaries and egg clutches. We conclude that the differences in cell component proportions between individual follicle cells, and thereby in their ability to secrete material into the oocyte, may at least partly explain the oocyte size differences previously observed in libellulids. However, all cell compartments go also through cyclical variation over time. Therefore, it will be important in future work to compare follicle cells in the same developmental phases.

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References

Ando, H. (1962). The comparative embryology of Odonata with special references to a relic dragonfly *Epiophlebia superstes* Selys. Tokyo: The Japan Society for the Promotion of Science.

Beament, J.W.L. (1946). The formation and structure of the chorion of the egg in an hemipteran *Rhodnius prolixus*. *Quarterly Journal of Microscopical Science 87*, 393–429.

Bielka, H., & Börner, T. (1995). *Molekulare Biologie der Zelle*. Heidelberg: Springer-Verlag.

Brennan, M.D., Weiner, A.J., Goralski, T.J., & Mahowald, A.P. (1982). The follicle cells are a major site of vitellogenin synthesis in *Drosophila melanogaster*. *Developmental Biology*, *89*, 225–236.

Büning, J. (1994). *The insect ovary. Ultrastructure, previtellogenic growth and evolution*. Cambridge, UK: Chapman & Hall.

Corbet, P.S. (1999). *Dragonflies – behavior and ecology of Odonata*. Colchester, UK: Harley Books.

Czihak, G., Langer, H., & Ziegler, H. (1996). *Biologie*. Berlin und Heidelberg: Springer.

Dettner, K., & Peters, W. (2003). *Lehrbuch der Entomologie*. Heidelberg: Spektrum Akademischer Verlag.

Fehrenbach, H., Dittrich, V., & Zissler, D. (1987). Eggshell fine structure of three lepidopteran pests: *Cydia pomonella* (L.) (Tortricidae), *Heliothis virescens* (Fabr.) and *Spodoptera littoralis* (Boisd.) (Noctuidae). *International Journal of Insect Morphology & Embryology*, *16*, 201–219.

Flegler, S.L., Heckmann, J.W., & Klomparens, K.L. (1995). *Elektronenmikroskopie – Grundlagen, Methoden, Anwendungen.* Berlin: Spektrum Akademischer Verlag.

Gaino, E., Piersanti, S., & Rebora, M. (2008). Egg envelope synthesis and chorion modification after oviposition in the dragonfly *Libellula depressa* (Odonata, Libellulidae). *Tissue and Cell*, *40*, 317–324.

Garavito, R.M., Carlemalm, E. Colliex, C., & Villiger, W. (1982). Septate junction ultrastructure as visualized in unstained and stained preparations. *Journal of Ultrastructure Research*, *80*, 344–353.

Hagedorn, H.H., & Kunkel, J.G. (1979). Vitellogenin and vitellin in insects. *Annual Review of Entomology*, *24*, 475–505.

Hayat, M.A. (2000). *Principles and techniques of electron microscopy: Biological applications*. Cambridge, UK: Cambridge University Press.

Hinton, H.E. (1969). Respiratory systems of insect eggshells. *Annual Review of Entomology*, *14*, 343–368.

Hinton, H.E. (1970). Insect eggshells. *Scientific American*, *223*, 84–91.

Ivey, R.K., Bailey, J.C., Stark, B.P., & Lentz, D.L. (1988). A preliminary report of egg chorion features in Odonata (Anisoptera). *Odonatologica*, *17*, 393–399.
Karlsson, M., Sahlén, G., & Koch, K. (2010). Continuous and stepwise oocyte production in Libellulidae (Anisoptera). *Odonatologica*, *39*, 107–119.
Koch, K., Quast, M., & Sahlén, G. (2009). Morphological differences in the ovary of Libellulidae (Odonata). *International Journal of Odonatology*, *12*, 147–156.
Koch, K., & Suhling, F. (2005). Do behavioural and life history traits vary with mate-guarding intensity in libellulid odonates? *Canadian Journal of Zoology*, *83*, 1631–1637.
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). Oxford, UK: Pergamon Press.
Martins, G.F., & Serrão, J.E. (2002). Notes on ovary structure in four species of adult dragonflies (Anisoptera: Gomphidae, Libellulidae). *Notulae odonatologicae*, *5*, 109–116.
Matsuzaki, M. (1971). Electron microscopic studies on the oogenesis of dragonfly and cricket with special reference to the panoistic ovaries. *Development, Growth and Differentiation*, *13*, 379–398.
Miller, P.L., & Miller, A.K. (1985). Rates of oviposition and some other aspects of reproductive behaviour in *Tholymis tillarga* (Fabricius) in Kenya (Anisoptera: Libellulidae). *Odonatologica*, *14*, 287–299.
Moll, K.J., & Moll, M. (2003). *Atlas Anatomie*. München. Müller: Elsevier.
Müller, W.A., & Hassel, M. (2003). *Entwicklungsbiologie*. Springer-Verlag, Heidelberg.
Munk, K. (2000). *Grundstudium Biologie – Biochemie, Zellbiologie, Ökologie, Evolution*. Heidelberg: Springer-Verlag.
Richardson, K.C., Jarret, L., & Fincke, E.H. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technology*, *35*, 313–323.
Sahlén, G. (1992). Formation, structure and function of the insect eggshell, with special reference to Ephemeroptera, Odonata and Diptera: Culicidae. Introductory Research Essay No. 9, Department of Zoology, University of Uppsala, Sweden.
Sahlén, G. (1994a). Ultrastructure of the eggshell of *Aeshna juncea* (L.) (Odonata: Aeshnidae). *International Journal of Insect Morphology & Embryology*, *23*, 345–354.
Sahlén, G. (1994b). Ultrastructure of the eggshell and micropylar apparatus in *Somatochlora metallica* (Vander L.), *Orthetrum cancellatum* (L.) and *Sympetrum sanguineum* (Müll.) (Anisoptera: Corduliidae, Libellulidae). *Odonatologica*, *23*, 255–269.
Sahlén, G. (1995a). Transmission electron microscopy of the eggshell in five damselflies (Zygoptera: Coenagrionidae, Megapodagrionidae, Calopterygidae). *Odonatologica*, *24*, 311–318.
Sahlén, G. (1995b). The insect eggshell – ultrastructure, organisation and adaptive traits in Odonata and Diptera. Comprehensive summaries of Uppsala dissertations. Acta Universitatis Upsaliensis. Uppsala, Schweden.
Schenk, K., & Söndgerath, D. (2005). Influence of egg size differences within egg clutches on larval parameters in nine libellulid species (Odonata). *Ecological Entomology*, *30*, 456–463.
Schenk, K., Suhling F., & Martens, A. (2004). Relation between egg distribution, mate guarding intensity and offspring characteristics in dragonflies (Odonata). *Animal Behaviour*, *68*, 599–606.
Schwalm, F.E. (1988). Insect morphogenesis. In H.W. Sauer (Ed.), *Monographs in developmental biology*. Basel: Karger.
Snodgrass, R.E. (1935). *Principles of insect morphology*. New York, USA: McGraw-Hill.
Tillyard, R.J. (1917). *The biology of dragonflies (Odonata or Paraneuroptera)*. Cambridge, UK: Cambridge University Press.
Trueman, J.W.H. (1991). Egg chorionic structures in Corduliidae and Libellulidae (Anisoptera). *Odonatologica*, *20*, 441–452.
Van Antwerpen, R., Pham, D.Q., & Ziegler, R. (2005). Accumulation of lipids in insect oocytes. In K.G. Adiyoudi & R.G. Adiyoudi (Eds.), *Reproductive biology of invertebrates*, Volume XII, Part B, Progress in Vitellogenesis. Plymouth, UK: Science Publishers.
Zarani, F.E., & Margaritis, L.H. (1991). The eggshell of *Drosophila melanogaster*. VII. Formation of the micropylar canal and the role of the paracrystalline structure. *Developmental Biology*, *200*, 95–103.