#### SHORT COMMUNICATIONS

# EGG CHORIONIC ULTRASTRUCTURE OF THE DRAGONFLY TRAMEA VIRGINIA (RAMBUR) (ANISOPTERA: LIBELLULIDAE)

### R.J. ANDREW

Department of Zoology, Shri Shivaji ESA's Science College, Congress Nagar, Nagpur-440012 (MS), India

Received May 28, 2000 / Revised and Accepted September 12, 2001

SEM studies reveal that the egg chorion of *T. virginia* is divided into an outer soft exochorion and an inner tough endochorion. The exochorion expands into a jelly-like, sticky coat in water, while the endochorion is smooth, thin and unsculptured. The apically situated micropylar apparatus is formed of a large, dome-shaped, sperm-storage chamber and a small, flat, micropylar stalk which contains a pair of circular micropylar orifices. The micropylar apparatus is encircled by an exochorionic collar. The chorion is modified in accordance with the aquatic (still-water) mode of oviposition exhibited by this species while the micropylar apparatus is shaped to fit in the fertilization pore of the vagina.

## INTRODUCTION

The egg chorion in insects is not a simple enveloping layer of the egg but exhibits various regional and radial complexities. Scanning electron microscopic (SEM) observations have further disclosed that the chorion, not only protects the growing embryo but also undergoes various morphological modifications to assist in fertilization and oviposition (HINTON, 1981; MARGARITIS, 1985). The eggs of Odonata have only been recently examined ultrastructurally (IVEY et. al., 1988; TRUEMAN, 1991; ANDREW & TEMBHARE, 1992, 1995, 1996; MAY, 1995; SAHLEN, 1995) and is the least understood stage of this group (NORLING & SAHLEN, 1997). The present investigation was undertaken to study the chorionic ultrastructure in relation to fertilization and oviposition in *Tramea virginia*.

#### MATERIAL AND METHODS

Tramea virginia is a large libellulid dragonfly, found sexually active near small water tanks in fields

172 R.J. Andrew

and gardens in India during the post-monsoon period. Ovipositing females were collected near garden tanks at Brahmapuri and Nagpur (Maharashtra State, India). Egg dumping was initiated by passing a thin needle through the thorax (ANDREW & TEMBHARE, 1995). The live fertilized, unwetted eggs were collected on a clean cloth and the eggs were immediately picked up with a wet needle at the basal region and fixed in Bouin's fluid. In preparation for examination under the stereoscan 250 MK III Cambridge Scanning Electron microscope (SEM), the eggs were dehydrated in alcohol, cleared in acetone, air-dried, mounted on stubs and gold coated in a Poloron Automatic unit (E 5200). Some completely wetted eggs were also processed and observed under the SEM. All measurements given below are means of not less than ten readings along with ± standard error. The female reproductive organs consist of a pair of long ovaries and a compact post-ovarian genital (spermatheca-bursa-vagina) complex (ANDREW & TEMBHARE, 1994). The terminology of TRUEMAN (1991) is used to describe the various regions of the egg.

## RESULTS AND DISCUSSION

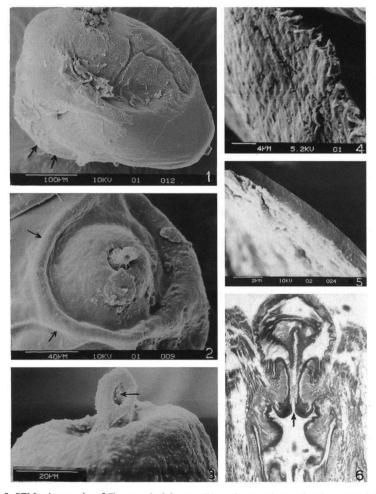
Tramea virginia oviposits in small, still-water bodies like cement tanks and ornamental garden ponds. The unwetted eggs are light yellow, oval and have linear dimension of  $340\pm15\times250\pm12~\mu m$ . The oval shape facilitates quick sinking of the egg. Further, the oval eggs can easily settle in small recesses on the floor of the water body (CORBET, 1962). The egg chorion is divided into an outer soft exochorion (EX) and an inner tough endochorion (EN) (Figs 1-5). The EX in unwetted condition is thin, soft, smooth, non-porous and unsculptured. As soon as it comes in contact with water, it swells and expands into a jelly-like, spongy coat which remains soft throughout the incubation period. The linear dimension of the egg at this stage is  $450\pm20\times260\pm15~\mu m$ . This change provides a sticky surface to which particles adhere thereby camouflaging the egg; also the eggs become firmly glued to various objects on the floor of the water body.

The EN is thin (1.5±0.6 μm), smooth and does not undergo any post-oviposition changes in water. In *Bradinopyga geminata* and *Landona deplanta*, the EN is composed of large flat plates (IVEY et at., 1988; ANDREW & TEMBHARE, 1996), but such plates are not found in *T. virginia*.

The apical tip of the chorion is modified into a complex micropylar apparatus (MA) (Figs 2, 3). The MA is lodged in a circular collar which is formed by a fold of the EX. The collar has a diameter of  $75\pm10\,\mu m$  and a height of  $10\pm1.5\,\mu m$ . It probably protects the MA from mechanical injury. The MA consists of a large dome-shaped, sperm-storage chamber, 40  $\mu m$  in diameter. A small, plate-like, rectangular micropylar stalk arises from the center of the storage chamber. The stalk is  $18\,\mu m$  long and  $6\,\mu m$  wide. A pair of micropylar orifices are present sub-apically on the flat surface of the stalk. Each orifice has a diameter of 4-5  $\mu m$ . In libellulid dragonflies, the MA generally consists of a convex/concave or hyperboloid stalk and a small storage chamber (TRUEMAN, 1991; MAY, 1995). The dome-shaped chamber and small flat stalk has so far been recorded only from T. virginia.

The fertilization pore (FP) in the vagina of T. virginia is narrow, spiny, tunnel-like

and formed from a mid-dorsal and paired lateral cuticular plate (Fig. 6). The FP lies just above the female gonopore (ANDREW & TEMBHARE, 1994). The eggs in the median oviduct of *T. virginia* are not randomly orientied as found in *Orthetrum chrysostigma* (MILLER, 1984), but the MA is always directed upwards as the eggs



Figs 1-5. SEM micrographs of *Tramea virginia* egg: (1) partly wetted egg, showing expanded sticky exochorion at the wetted basal region (arrows) and unwetted smooth region at the anterior end; — (2) anterior region of the egg, showing micropylar apparatus encircled by the collar (arrows); — (3) lateral view of the above, showing the micropylar orifice (arrow) on the projecting micropylar stalk; — (4) fractured section of the unwetted egg, showing thin, soft exochorion; — (5) fractured section of the wetted egg, showing thin, tough endochorion; — (6) longitudinal section of the vagina, showing the narrow spiny fertilization pore (arrow) [x 90].

174 R.J. Andrew

move down the oviduct into the vagina through the female gonopore. The MA therefore, comes to lie just below the FP.

In *T. virginia* the structure of the MA of the egg is complementary to the shape of the FP. During fertilization, the stalk of the MA enters the FP. Sperm trickles down the spiny FP due to muscular contraction of the bursa (SIVA-JOTHY, 1987; MILLER, 1991) and enters the MA through the orifices of the stalk. The structure of the MA of *T. virginia* supports the two-step fertilization mechanism as proposed by TRUEMAN (1991). He postulated that "[the] sperms [is] mechanically scooped into the atrium during oviposition but only later penetrat[es] the endochorion". This aids rapid fertilization, which is necessary since the vagina is too small to hold more than two or three eggs at a time and *T. virginia* exhibits a high rate of oviposition (8-12 egg/sec.).

#### ACKNOWLEDGMENTS

I am deeply indebted to Dr D.B. TEMBHARE for constant encouragement. This work is a part of UGC minor research project (23-382 / 99 WRO). The help rendered by S.V.RAO (RSIC, Nagpur University) and BABA SARODEY in SEM studies and photomicrography is gratefully acknowledged.

#### REFERENCES

- ANDREW, R.J. & D.B. TEMBHARE, 1992. Surface ultrastructure of the egg chorion in the dragonfly, Ictinogomophus rapax (Rambur) (Odonata: Gomphidae). Int. J. Insect Morphol. Embryol. 21: 347-350.
- ANDREW, R.J. & D.B. TEMBHARE, 1994. The post-ovarian genital complex of the dragonfly, Tramea virginia (Rambur) (Anisoptera: Libellulidae). *Odonatologica* 23: 239-340.
- ANDREW, R.J. & D.B. TEMBHARE, 1995. Ultrastructural post-oviposition changes in the egg-chorion of the dragonfly, Zyxomma petiolatum Rambur (Odonata: Libellulidae). *Int. J. Insect Morphol. Embryol.* 24: 235-238.
- ANDREW, R.J. & D.B. TEMBHARE, 1996. Surface ultrastructure of the egg chorion of Bradinopyga geminata (Rambur) and Rhyothemis variegata variegata (Linn.) (Anisoptera: Libellulidae). Fraseria (NS) 3: 1-5.
- ANDREW, R.J. & D.B. TEMBHARE, 1997. The development and structure of the ovaries in the dragonfly Tramea virginia (Rambur) (Libellulidae: Odonata). *J. adv. Zool.* 18: 86-95.
- CORBET, P.S., 1962. A biology of dragonflies. Witherby, London.
- 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, 1998. A preliminary report of egg chorion features in dragonflies (Anisoptera). *Odonatologica* 17: 393-399.
- MARGARITIS, L.H., 1985. Structure and physiology of 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., 1984. The structure of the genitalia and the volumes of sperm stored in male and female Nesciothemis farinosa (Foerster) and Orthetrum chrysostigma (Burmeister) (Anisoptera: Libellulidae). Odonatologica 13: 415-428.
- MLLER, P.L., 1991. The structure and function of the genitalia in the Libellulidae (Odonata). Zool. J. Linn. Soc. 102: 43-73.
- MAY, M.L., 1995. Comparative notes of micropyle structure in "cordulegastroid" and "libelluloid" Anisoptera. Odonatologica 24: 53-62.

- NORLING, U. & G. SAHLEN, 1997. Odonata, dragonflies and damselflies. *In:* A.N. Nilsson, [Ed.], *Aquatic insects of North Europe*, Vol. 2, pp. 13-65. Apollo Books, Stenstrup, Denmark.
- SAHLEN, G., 1995. The insect eggshell: ultrastructure, organisation and adaptive traits in Odonata and Diptera. Ph.D. thesis, Uppsala Univ.
- SIVA-JOTHY, M.T., 1987. The structure and function of the female sperm-storage organs in libellulid dragonflies. *J. Insect Physiol.* 33: 559-567.
- TRUEMAN, J.W.H., 1991. Egg chorionic structures in Corduliidae and Libellulidae (Anisoptera). Odonatologica 20: 441-452.