NOTES ON OVARY STRUCTURE IN FOUR SPECIES OF ADULT DRAGONFLIES (ANISOPTERA: GOMPHIDAE, LIBELLULIDAE)

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Abstract – Dragonflies have panoistic ovarioles. Here, some histological aspects are described in *Aphylla theodorina* (Gomphidae), *Orthemis discolor*, *Pantala flavescens*, and *Perithemis mooma* (all Libellulidae) from Brazil. Each ovary consists of a great number of pecnated ovarioles. A typical germarium, with undifferentiated germ cells, was not observed in these spp. In the vitellarium, 3 regions can be distinguished, viz. previtellogenic, vitellogenic and postvitellogenic. A single layer of follicular, often binucleated cells lines each follicle.

Introduction

In insects the ovaries are formed by egg tubes or ovarioles. These are where oocyte development occurs; the number of ovarioles per ovary is variable among different species (CHAPMAN, 1998).

Each ovariole has three principal regions: (1) the terminal filament, which is the most anterior part of the ovariole; (2) the germarium, formed by somatic cells, with a role in oocyte support, as well as containing the germinative cells and (3) the vitellarium, where vitellogenesis and oocyte growth take place, which is the longest part of the ovariole (CHAPMAN, 1998)

The ovaries can be classified as panoistic or meroistic, according to the absence or presence of nurse cells, which are responsible, for supplying the oocyte with cytoplasmic material (BÜNNING, 1994). Panoistic ovarioles are found in representatives of the Odonata, Plecoptera, Orthoptera and Isoptera (BÜNNING, 1994).

Classical studies have been made on the structure of the ovaries with regard to reproduction (for review see CHAPMAN, 1998). Recently, considerations on these structures have also been used in phylogenetic studies in Diptera, Mecoptera, Hemiptera, Coleoptera and Neuropteroidea (BITSCH & BITSCH, 1998; BILINSKI et al., 1998; KUBRAKIEWICZ et al., 1998). In spite of the ecological importance of dragonflies, there are few studies on ovary structure in the Odonata (BÜN-

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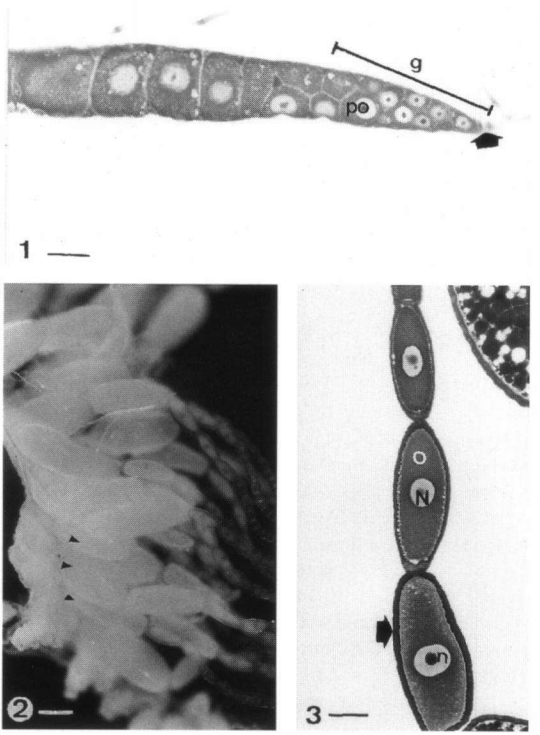
NING, 1994; ANDREW & TEMBHARE, 1997; MATSUZAKI, 1971; BEAMS & KESSEL, 1969). As part of an ongoing study, the present paper deals with the morphology of the ovaries in the adults of four Brazilian species.

Material and methods

The species studied were: *Aphylla theodorina* (Navás) (Gomphidae), *Orthemis discolor* (Burm.), *Pantala flavescens* (Fabr.) and *Perithemis mooma* Kirby (all Libellulidae).

The specimens were captured at the Universidade Federal de Viçosa, MG, Brazil and transferred to the laboratory. The ovaries were dissected in saline solution for insects. Measurements were made of ovariole length and follicle length and width. The last two measurements were taken from mature follicles which, in the species used, were easily distinguished from developing follicles by the whitish colour they acquire during vitellogenesis. The material was then fixed in 4% paraformaldelhyde held at a pH of 7.4 with a phosphate buffer. After dehydration in an ethanol series, the material was embedded in historesin JB-4. Sections 4 µm thick were stained with Dominici solution prepared according to PEARSE (1953).

Some sections were submitted to histochemical tests using the Feulgen reaction and PAS, in order to determine the presence of DNA and neutral polysaccharides, respectively, as described by PEARSE (1953).



Figs 1-3. Ovarioles in *Aphylla theodorina* (Fig. 1), *Orthemis discolor* (Fig. 2) and *Pantala flavescens* (Fig. 3): (1) longitudinal section through the anterior region of ovariole [g: germarium, – Po: primary oocyte; – (arrow), terminal filament; – bar = 10 µm]; – (2) general aspect of ovarioles which are pecnated [arrows indicate ovariole insertion on lateral oviduct; – bar = 5 µm]; – (3) longitudinal section through the vitellogenic region of an ovariole [arrow: follicular epithelium, – n: nucleolus, – N: nucleus, – o: oocyte; – bar = 10 µm].

Results and discussion

The ovaries were quite similar in the four studied species. They were well developed and elongated, running lateral to the digestive tract, almost occupying the entire length of the abdomen. In the anterior part, the terminal filaments from the ovarioles join them (Figs 1-2). In Aeshinidae both ovaries are completely separated (TILLYARD, 1917), while in the species studied here they are united anteriorly, which has also been documented in Libellulidae by ANDREW & TEMBHARE (1997) and MARSHALL (1914). Each ovary consists of a great number of pecnated ovarioles (inserted serially to the lateral oviduct) (Figs 2, 4). This array is similar to other odonate species reported by BÜNNING (1994).

The ovarioles are short, varying from 1.39 mm in *Orthemis discolor* to 2.17 mm in *Pantala flavescens*. The mature follicles were best developed in *Aphylla theodorina* (Tab. I). These results cannot be related to the dragonfly size, since *P. flavescens*, which has

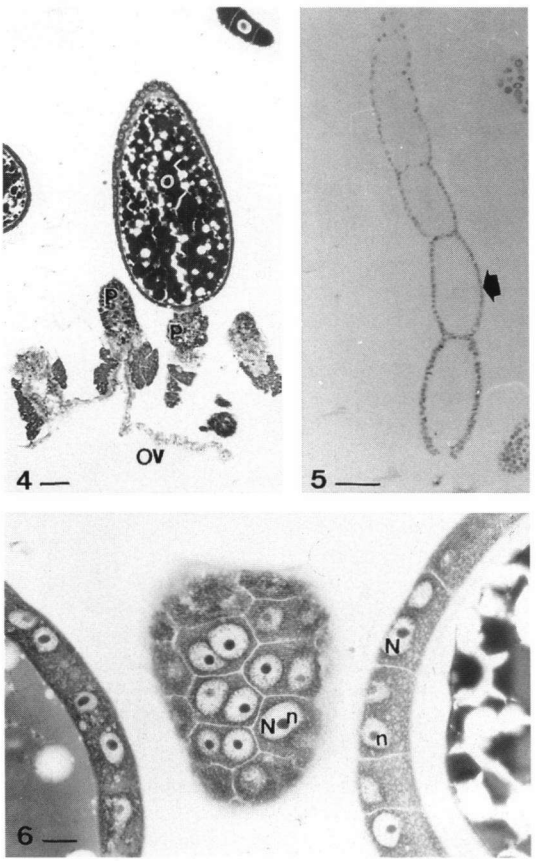
the longest ovarioles, is the smallest species studied.

The panoistic ovarioles have a short terminal filament following a short segment filled with primary oocytes, which can be seen as a germarium (Fig. 1). The vitellarium can be divided into three parts: (a) previtellogenic, formed by small follicles; (b) vitellogenic, where the follicle growth take place, and (c) postvitellogenic, characterized by well developed follicles and the oocyte with the egg shell formed. (Figs 3-4).

In *Tramea virginia* and *Orthetrum chrysis* four regions were reported in the ovarioles: terminal filament, germarium, vitellarium and pedicel (ANDREW & TEMBHARE, 1997; THAKARE & TEMBHARE, 1977). However, it is suggested here that the pedicel is not a morphological region in adult dragonflies because, in mature forms, the plug of cells degenerates (Fig. 4).

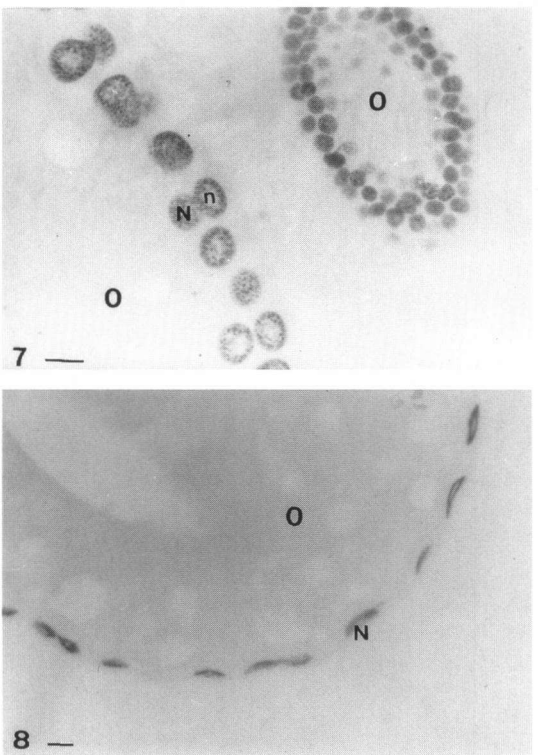
The germarium is a short segment, just below the terminal filament, and it is almost filled with primary oocytes. A typical germarium, where undifferentiated germ cells (oogonia) are present, was not observed in our material. Perhaps this is due to the fact that, in the last larval instar, all oocytes are differentiated, as observed in *Tramea virginia* by ANDREW & TEMBHARE (1997), suggesting that in adults there is only oocyte growth.

In the previtellogenic region, the oocytes have a circular nucleus with very discondensed chromatin and one evident nucleolus (Fig. 3). During oocyte growth in the vitellogenic part they became ovoid (Fig. 3) and, in the postvitellogenic region, yolk granules were easily distinguished (Figs 3-4).



Figs 4-6. Ovarioles in *Pantala flavescens* (Fig. 4) and *Aphylla theodorina* (Figs 5-6): (4) longitudinal section through the mature oocyte (o) in the post-vitellogenic region of an ovariole [ov: oviduct, – p: pedicel; – bar = 20 μm]; – (5) Feulgen reaction of a longitudinal section through the vitellogenic and post-vitellogenic region of an ovariole [note the increase in the number of follicular cells (arrow) along the ovariole; – bar = 10 μm]; – (6) binucleated follicular cells [left and right: saggital section of follicular epithelium, – center: longitudinal section of the same, – N: nucleus, – n: nucleolus; – bar = 6 μm].

A similar situation has been encountered in the panoistic ovarioles of Mecoptera by BILINSKI et al., (1998), which is usual, since panoistic ovariole lack nurse cells, so all cytoplasmic spectra should be produced by the oocyte, which can be enhanced by a well developed nucleolus (Fig. 3). Throughout vitellogenesis only one nucleolus per oocyte was present, contrasting with the nucleolar fragmentation observed in Mecoptera. However, the Feulgen reaction showed the presence of some DNA in the nucleolus, which suggests a process of DNA



Figs 7-8. *Aphylla theodorina*. Feulgen reaction of: (7) the follicular epithelium of a vitellogenic follicle [bar = 2 μm]; – (8) a transverse section of a mature follicle, showing picnotic nucleus [n: nucleolus, – N: nucleus, – o: oocyte; – bar = 4 μm].

amplification, as observed in several insects independently of the ovariole type (BÜNNING, 1994; KLOCK et al., 1995).

A single layer of follicular cells, which in the previtellogenic region are few, lines each follicle. In the more posterior section of this region, there was an increase in the number of follicular cells and the follicle had become well delimited and evident (Fig. 5). In the vitellogenic region, the follicle cells were high and polyhedral, with a circular nucleus, in which a well developed nucleolus can be seen (Fig. 6). In this region the occurrence of binucleated follicular cells was frequent (Figs 6-7). In the postvitellogenic region, the follicular cells were flattened, the nucleolus was not visible and the chromatin became very condensed, acquiring a picnotic aspect (Fig. 8). Follicular cells did not show PAS positive reaction anywhere along the entire length of the vitellarium.

In the previtellogenic region, each oocyte was enveloped by a few follicular cells; in spite of mitosis not having been observed, the increase in their number in the vitellogenic region suggests mitotic activity in these cells, in addition to endomitosis that leads to formation of binucleate cells. A similar situation was observed in other Odonata by ANDREW & TEMBHARE (1997) and SESHACHAR & BAGGA (1963). The negative PAS reaction in follicle cells suggests that yolk precursors are taken from the haemolymph by intercellular, rather than intracellular, transport by follicle cells. Therefore, similarly as in meroistic ovarioles, these cells play a role only in egg shell production, as reported by BÜNNING (1994). The picnosis observed in the nucleus of the follicular cells, in the postvitellogenic region, is a feature that regards cell death, which is an expected result, because each follicle is separated from the preceding and succeeding follicles by intrafollicular tissue, so these cells degenerate in order to provide the passage for the ovulating oocyte.

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Table I – Measurements of ovarioles, follicles and thorax (in mm) in four dragonfly species – (Mean ± SD)

Species	Length of ovariole	Length of follicle	Width of follicle	Width of thorax
Aphylla theodorina	1.99 ± 0.22	0.54 ± 0.038	0.43 ± 0.045	7.50
Orthemis discolor	1.39 ± 0.12	0.36 ± 0.027	0.15 ± 0.031	6.72
Pantala flavescens	2.17 ± 0.20	0.49 ± 0.057	0.23 ± 0.038	3.97
Perithemis mooma	1.90 ± 0.12	0.51 ± 0.060	0.28 ± 0.035	6.50

SZEWSKI & J. NURKOWSKA, 1995, *Folia histochem. cytobiol.* 33: 267-276; – KUBRAKIEWICZ, J., I. JEDRZEJOWSKA & S.M. BILINSKI, 1998, *Folia histochem. cytobiol.* 36(4): 179-187; – MARSHALL, W.S., 1914, *Trans. Wisc. Acad. Sci.* 17: 755-759; – MATSUZAKI, M., 1971, *Dev. Growth Differ.* 13: 379-398; – PEARSE, A.G.E.,1953, *Histochemistry theoretical and applied*, [3rd edn], Vol. 1, Little, Brown & Company, Boston; – SESHACHAR, B.R. & S. BAGGA, 1963, *Growth* 27: 225-246; – THAKARE, V.K. & D.B. TEMBHARE, 1977, *Zoologica Pol.* 26: 557-572; – TILLYARD, R.J., 1917, *The biology of dragonflies*, Cambridge Univ. Press Cambridge.

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