The Bioscan 6(4) : 587-590, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

# SPERMATOGENESIS AND SPERM BUNDLE FORMATION IN THE DRAGON FLY *ANAX GUTTATUS* (BURMEISTER) (INSECTA: ODONATA: AESHNIDAE)

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**KEY WORDS**
*Anax guttatus*
Spermatogenesis
Sperm bundle
Dragon fly

**Received on :**
28.07.2011

**Accepted on :**
21.10.2011

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**ABSTRACT**
In *Anax guttatus*, the freshly moulted adults contain primary spermatogonia to fully-formed spermatozoa indicating commencement of spermatogenesis in the ultimate nymphal stage. All the progametes of a single cyst exhibit a single stage of spermatogenesis. Vigorous process of spermiogenesis occurs in the adult dragonfly, leading to the formation of spermatozoa and sperm bundle. The "shuttle cock" shaped sperm bundles are formed with a conical head cap. The central canal secretes thick viscous seminal fluid around the sperm bundle which facilitates downward migration of sperm bundles from the central canal to the vas deferens. In mature adults, the vasa deferentia, seminal vesicles and sperm sac are completely packed with the sperm bundle embedded in viscous seminal fluid secreted by epithelial cells of the genital ducts. The acellular wall of cyst containing mature spermatozoa undergoes ultrastructural changes which help the sperm bundle to move towards the central canal.

## INTRODUCTION

Formation of sperm bundles and/or spermatophores has proved advantageous in the evolution of insects since they facilitate safe transfer of spermatozoa into the female reproductive tract avoiding the risk of desiccation and predation during copulation (Retnakaran and Percy, 1985). Omura (1955, 1957), reported the formation of sperm bundles by the union of the head region of spermatozoa to form a compact mass while the tails remain free, later on, sperm bundles were reported in *Epiophlebia superstes* (Asahina, 1954) *Ictinogomphus rapax* (Tembhare and Thakare, 1982) and *Aeshna juncea* (Abro, 2003). Bakare and Andrew (2008, 2010) described the male reproductive system and the ultrastructure and biochemistry of the male genital duct of *Anax guttatus*. The present study deals with the process of spermatogenesis and formation of sperm bundle in the aeshnid dragon fly, *Anax guttatus*.

## MATERIALS AND METHODS

The dragon flies were collected from local waterbodies by using insect net or light trap at day and night, respectively. The traditional method used by tribals for the collection was also used to collect them during day time. The latex squeezed from the tender terminal branch of *Ficus bengalensis* was used as adhesive for the collection. The tips of tender branch were cut off and the milky white latex was squeezed in a small bottle. Approximately 1-2 mL vegetable oil was mixed thoroughly with 10 mL of latex. At the time of collection this latex mixture was applied to the terminal end of the long thin wooden stick. The terminal end was then brought near the wings of a flying or settled dragonfly. The dragonflies, flying or settled were trapped by slowly touching the wing by the sticky end of the stick (Bakare and Andrew, 2008).

For light microscopic histological observation, the internal male genitalia were dissected in saline and the testis was fixed in the Bouin's fluid. The fixed tissue was dehydrated in alcohol, cleared in xylene and embedded in paraffin wax at 60-62 ºC. The sections of 4 – 6 $\mu$m thick were cut and stained with Ehrlich's haematoxylin- eosin and Heidenhain's Iron-haematoxylin- orange G. For transmission electron microscopy, the internal male genitalia were dissected in cacodylate buffer and the testis was fixed in 3% glutaraldehyde in cacodylate buffer (pH 7.3) at 4ºC for two to three hours. The material was then washed in cacodylate buffer for 24 h, post fixed in 1% osmium tetraoxide for 1 h, dehydrated through graded series of ethanol and passed through 1, 2-epoxypropane before embedding in EMscope CY212 resin. 80 nm sections were cut on a Reichert OmU3 ultra microtome and mounted on Athene 400 EM grids. After staining with uranyl acetate and lead citrate they were viewed and photographed by the Phillips EM400T scanning electron microscope at 80KV (Tembhare, 2008).

## RESULTS AND DISCUSSION

The testis of *Anax guttatus* is unifollicular and internally filled with a large number of maturing cysts, each exhibiting only a single stage of spermatogenesis. In adults all the stages of

**Table 1: Cell and nuclear diameter (μm) of various stages of spermatogenesis and diameter of maturing cyst (μm) of the dragonfly, *Anax guttatus* (Primary spermatogonia- PSMG; Secondary spermatogonia- SSMG; Primary spermatocyte- PSCT; Secondary spermatocyte- SSCT; Spermatids- SPTD; Spermatozoa- SPMZ; Sperm bundle- SPBD)**

| Stages | PSMG | SSMG | PSCT | SSCT | SPTD | SPMZ | SPBD |
|---|---|---|---|---|---|---|---|
| Cell | 16.0 ± 0.93 | 16.96 ± 1.23 | 10.56 ± 0.93 | 13.7 ± 1.02 | 7.2 ± 0.84 | - | - |
| Nucleus | 1.6 ± 0.01 | 5.12 ± 0.05 | 4.8 ± 0.04 | 5.44 ± 0.05 | 2.6 ± 0.02 | - | - |
| Size of cyst | 68 ± 5 | 110 ± 12 | 78 ± 5 | 65 ± 6 | 78 ± 8 | 42 ± 5 | 42 ± 5 |

**Table 2: Percentage of cysts containing specific stages of spermatogenesis in the dragonfly, *Anax guttatus* (Primary spermatogonia- PSMG; Secondary spermatogonia- SSMG; Primary Spermatocyte- PSCT; Secondary spermatocyte- SSCT; Spermatids- SPTD; Spermatozoa- SPMZ; Sperm bundle- SPBD)**

| Stages | PSMG% | SSMG% | PSCT% | SSCT% | SPTD% | SPMZ% | SPBD% |
|---|---|---|---|---|---|---|---|
| Final instar nymph | 38 | 26 | 12 | 12 | 06 | 06 | 00 |
| Freshly moulted | 30 | 17 | 14 | 20 | 15 | 04 | 00 |
| Mature | 09 | 08 | 11 | 20 | 12 | 20 | 20 |

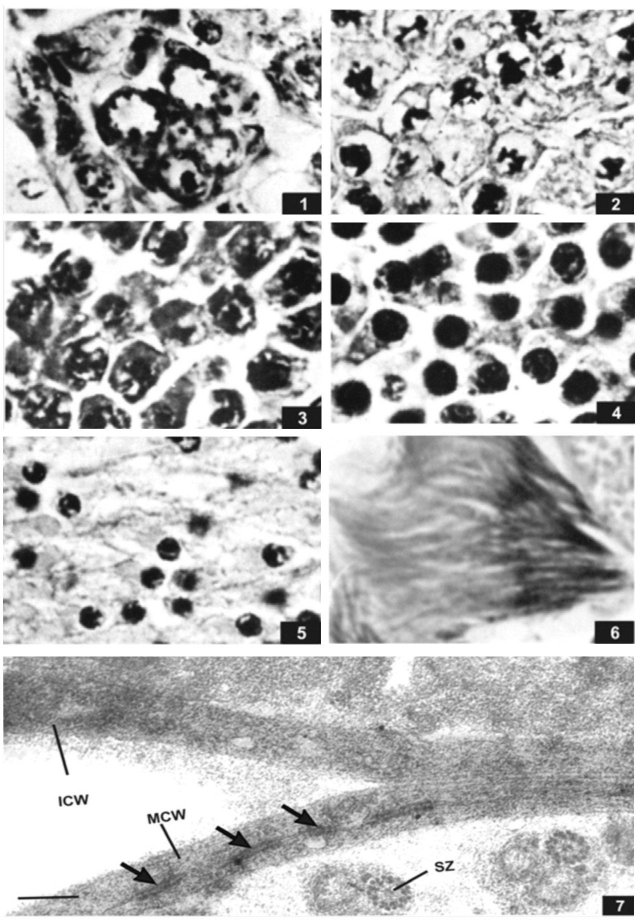


**Figure 1 to 7: Section passing through the testicular cysts showing various stages of spermatogenesis in the dragonfly, *Anax guttatus* (HE x 1500).** (1) Primary spermatogonia; (2) Secondary spermatogonia; (3) Primary spermatocytes; (4) Secondary spermatocytes; (5) Spermatid; (6) Spermatozoa; (7) Transmission electron microscopic structure of the testicular cyst wall showing formation of fibrils (arrows) in cyst containing mature spermatozoa (SZ) (ICM- immature cyst wall; MCW- mature cyst wall; SZ- spermatozoa)

spermatogenesis can be observed, along with formation of the sperm bundles in some cysts.

### Spermatogenesis

The germ cells during spermatogenesis show distinct cytological changes from one stage to another (Table 1; Figs.1 to 6). The number of cysts containing different stages of spermatogenesis varies with the age of the dragonfly (Table 2). As spermatogenesis takes place, there is a gradual reduction in the size of the cysts (Table 1). The acellular cyst wall containing mature spermatozoon also undergo ultrastructural changes. The cyst wall is about 200 nm broad but for movement of the mature cyst towards the central canal, a thin pair of fibril (50 nm thick) develops in the core of the cyst wall containing mature spermatozoa (Fig. 7).

### Sperm bundle formation and transport

After the completion of spermatogenesis, most of the cysts become filled with sperms. The heads of sperms remain embedded in secretory droplets. Once the sperm heads are stuck together there is synchronized lashing of sperm tails in the cyst (Figs. 8, 9). The sperm bundles, thereafter actively rotates inside the cyst giving the appearance of a shuttle cock and start migrating head first towards the vas deferens. They pierce through the wall of the extending branch of central canal and enter its lumen (Figs. 10 to 13). The wave-like peristaltic movements of tail helps during the course of migration from cyst to central canal. The central canal secretes thick viscous fluid around the sperm bundle which facilitates downward migration of sperm bundles from central canal to the vas deferens. In mature adults the vasa deferentia, seminal vesicles and sperm sac are completely packed with the sperm bundle embedded in viscous seminal fluid secreted by epithelial cells of the genital ducts. No cytological changes can be seen in the structure of sperm bundles as it moves down the vas deferentia, seminal vesicles and sperm sac.

In the sperm sac, marked condensation of seminal fluid around the sperm bundle takes place. The compact sperm masses embedded in fluid aggregate in the posterior region of the sperm sac *i.e.* pre-exit chamber until intra male sperm translocation during which, the male ejaculate is transported from the primary to the secondary copulatory apparatus. In *Anax guttatus,* the freshly moulted adults contain primary spermatogonia to spermatozoa indicating commencement of spermatogenesis in the ultimate nymphal and adult stages as reported in the dragonfly *Tramea virginia* (Andrew and Tembhare, 1993).

In Insects, spermatozoa are transferred to the female during copulation in three forms. As "free" spermatozoa in matrix of

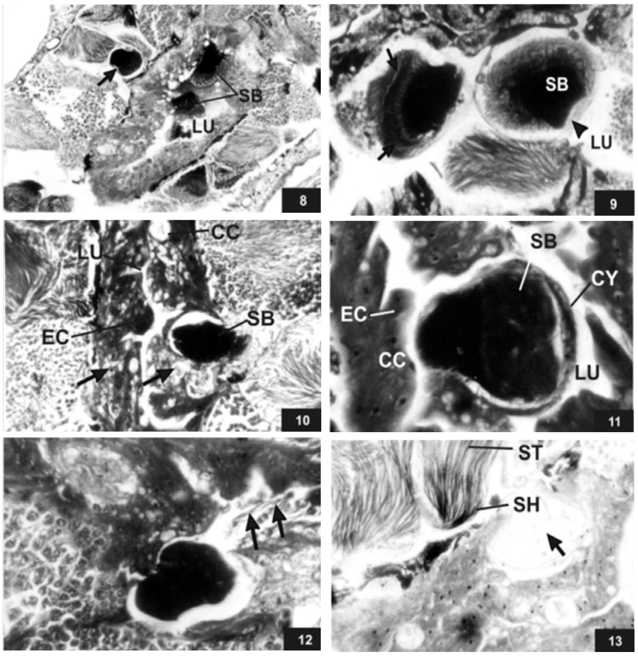


**Figure 8 to 13: (8) Section passing through the testis showing two sperm bundles (SB) in the lumen (LU) of central canal and a third sperm bundle (arrow) trying to enter the canal (HE x200); (9) Section passing through the central canal showing sperm bundle (SB) in the lumen (LU). Note the wavy motion of the sperm tail (arrow) and conical cap (arrow head) of the sperm bundle (HE x500); (10) Section showing the entry of sperm bundle (SB) in the lumen (LU) of central canal (CC). Note the increase in the vacuoles (arrows) of the epithelial cells (EC); (11) Entry of sperm bundle (SB) in the lumen (LU) of the central canal (CC) with its cyst (CY) (HE x 600); (12) Disintegration of the epithelial cells of central canal (arrows) for the entry of the sperm bundle (HE x 500); (13) Empty testicular lumen (arrow) surrounded by the epithelial cells (EC) of the central canal. (SH- sperm head; ST- sperm tail) (IH x 400)**

seminal fluid or as spermatodesmata, binding all the sperm heads in a hyaline cap or as spermatophore packed in a capsule (Siva-Jothy, 1997). Although spermatophores are lacking in Odonata (Srivastava and Srivastava, 1987), sperm bundle shows a wide variation in its organization and structure. In *Epophthalmia elegans,* the male ejaculate contains small aggregation of spermatozoa of not more than six sperm. In *Tanypteryx pryeri,* the sperm heads are embedded in a short longitudinally arranged proteinacious tubes that form a cap. In *Epiophlebia superstes,* the sperm bundle is in the form of long proteinacious strips to which the spermatozoa are embedded in perpendicular rows (Siva-Jothy, 1997). In *Anax guttatus* the typical 'shuttle cock' shaped sperm bundle is well evident, but the hyaline cap is conical whereas in other aeshnids, it is flat (Siva-Jothy, 1997; Abro, 1998, 1999, 2004). The sperm heads are attached by a hyaline cap which is formed of a network of tubes, each attached to the head of a single spermatozoon, and these tubes stick to each other to form the hyaline cap. Such 'shuttle-cock' like sperm bundle have been reported in only two groups of Odonata, the Gomphidae (Tembhare and Thakare, 1982; Siva-Jothy, 1997) and the Aeshnidae (Andrew and Tembhare, 1997; Siva-Jothy, 1997; Abro, 1998, 1999; Bakare and Andrew, 2008, 2010). According to Siva- Jothy (1997) the hyaline cap, holding the sperm head is tube-less and is formed of homogenous matrix in gomphids whereas tubed matrix has been found in the aeshnid *Aeshna mixta*. Abro (1998, 2003) reported that the tubes are formed from the slender cytoplasmic protrusions in front of the acrosomal rodlets/nuclear heads and decomposing droplets of surplus cytoplasm from early spermatids which tend to adhere to the cap of the sperm bundle. Tembhare and Thakare (1982) found that in *Ictinogomphus rapax,* the follicular wall of the testis secretes some dense granular secretion which helps in the formation of the hyaline cap. Dense granular secretion is also noticed around the sperm heads of *Anax guttatus* during sperm bundle formation.

In *Anax guttatus,* the sperm bundles do not break down in the seminal vesicle or the sperm sac to liberate individual sperm. The sperm bundles are broken down in the post ovarian genital complex of the inseminated female by churning mechanism of the cuticular plates of the bursa copulatrix (Andrew and Tembhare, 1997). Siva-Jothy (1997) noticed that in *Aeshna mixta*, bacterioids present in the bursa copulatrix disintegrate the hyaline cap of sperm bundle.

According to some workers (Ballowitz, 1916; Fretter, 1953; Nur, 1962; Cohen, 1975), grouped sperm are better capable to reach the site of fertilization in the female than the individual spermatozoa, while others (Hanson *et al*., 1952; Fain-Maurel, 1966; Breland and Simmons, 1970; Mackie and Walker, 1974) propose that the hyaline cap of sperm bundle provides a nutrient investment by the male which increases female fitness. Cantacuzene (1967), Phillips (1971), Mackie and Walker (1974), Bedford *et al.,* (1984) and Abro (1999) are of the opinion that the hyaline cap provides nourishment to the sperm and protects the delicate acrosome. In *Anax guttatus,* the sperm bundle exists for a long period in the sperm storage organs of the female (Andrew and Tembhare, 1997) before being used for fertilization and therefore, it seems that the hyaline cap not only provides nourishment to the stored spermatozoa but also protects the delicate acrosomes as proposed by the above mentioned workers.

Ishida (1984) reported that species of Japanese dragonflies, significantly larger in body length contain sperm bundle but according to Siva-Jothy (1997), there is direct link between the presence of sperm bundle and reproductive behavior. Sperm bundle are found in those species in which males do not defend oviposition sites, do not mate at oviposition site and do not guard mate after copulation, and where the females do not oviposit immediately after copulation. *Anax guttatus* in one of the largest dragonflies of Indian subcontinent and the male does not guard the female after copulation; neither does the female oviposit immediately after copulation (Kumar and Prasad, 1981) and the contentions of Ishida (1984) and Siva-Jothy (1997) seem to be appropriate for *Anax guttatus.*

## ACKNOWLEDGEMENTS

We are grateful to Prof. D. B. Tembhare for constant guidance. SSB thanks University Grants Commission for partial financial support under the Minor Research Project F-47-169/07.

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