

## **Histology of the Post Ovarian Genital Complex of the Dragonfly *Pantala Flavescens* (Fabricius, 1798) (Odonata: Libellulidae)**

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**Abstract:** In the dragonfly, *Pantala flavescens* (Fabricius, 1798), the post ovarian genital complex (POGC) consists of a pair of long thin spermatheca with bulbous tips, a small, spherical dorsal bursa copulatrix and a large ventrally placed vagina. The wall of the POGC is basically composed of an outer muscle layer, middle epithelial layer resting on a basement membrane and an internal layer of cuticle. It is externally covered in muscle bands. The internal lining of ST is annulated with cuticular rings and is formed of 3-4 layers of cuticle consecutively undergoing sclerotization. The bursa copulatrix is small and spherical with a thick layer of folded cuticular intima. The fertilization pore is in the form of a valve covered with cuticular spines. The bursa communis is a tunnel like structure formed by three plates, a median-dorsal plate and paired lateral plates. The vagina is divided into anterior and posterior regions- the anterior region bears large number of small flat finger-like processes or stubs while the posterior region is a long, large, laterally folded, sac like structure which tapers into the female gonopore.

**Keywords:** *Pantala flavescens*, POGC, bursa copulatrix, spermathecae, vagina.

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### **1. INTRODUCTION**

The female reproductive system in insects usually consists of a pair of ovaries, the lateral oviducts and the common oviduct which opens into a post ovarian genital complex (POGC). The POGC consist of the sperm storage organs in the form of bursa copulatrix and spermatheca and a vagina which is the extension of the median oviduct or the involution of the female gonopore. The male deposits his sperms in the sperm storage organs during copulation, where it is stored until it is needed. The spermathecal gland along with other sex glands produces nutrients in order to keep the sperm alive in the spermatheca, where sperm can survive for weeks, months or even years.

In Odonata, Tillyard (1917), Asahina (1954), Prasad & Srivastava (1961) and Bjånes (1974) initiated the study of the POGC. Recent studies on the POGC indicate that in most Odonata, it comprises typically the spermathecae, bursa copulatrix and the vagina on the 8<sup>th</sup> abdominal sternum (Midttun, 1976; Miller, 1982a, b, 1984, 1987; Waage, 1982, 1984, 1986; Siva-Jothy, 1987; Michels, 1989; Andrew & Tembhare, 1994, 1996, 1997). However, Prasad & Srivastava (1961) and Srivastava & Srivastava (1986) referred to the POGC as the 8<sup>th</sup> complex on the basis of its location.

Miller (1982a, 1984) described the gross structure of the female genitalia in *Brachythemis lacustris*, *Nesciothemis farinose*, *Nesciothemis farinose* and *Orthetrum chrysostigma* with reference to possible copulatory event and mechanism. Waage (1986) described the genital complex of *Celithemis elisa* and *Erythemis simplicicollis* while studying the phenomena of sperm displacement and copulatory duration. Siva-Jothy (1984) gave a brief structural description of the genital complex in *Crocothemis erythraea* and *Orthetrum cancellatum*. Srivastava and Srivastava

(1988) described the 8<sup>th</sup> complex of the damselfly, *Ischnura rufostigma*. Andrew & Tembhare (1994, 96, 97) described the POGC of the libellulid, *Tramea virginia*, aeshnid *Anax guttatus* and zygopteran *Ischnura aurora*.

The available literature, moreover, reveals that various morphological and physiological aspects of the POGC are still unexplored although Prasad and Srivastava(1961) studied the female reproductive system of *Pantala flavescens*, the present work was undertaken to study in details the histology of the POGC of the dragonfly, *Pantala flavescens*.

## 2. MATERIAL AND METHODS

The dragonflies *Pantala flavescens* (Fabricius, 1798) (Fig.1) were collected from the playground of Hislop College, Nagpur, dissected in Ringers saline solution under the stereoscopic binocular stereo microscope (Magnus- MS 24). The post ovarian genital complex (POGC) was dissected from the abdomen and processed for fixation (Tembhare, 2008). The POGC and associated structures were immediately fixed in Bouin's Fixative (Aqueous) for 24 hours.

The POGC was washed, dehydrated in running water for half an hour and dehydrated in a series of ascending grades of aqueous alcohol, treated with xylene, embedded in molten paraffin wax at 62 degrees for block preparation. The paraffin blocks were mounted on the block-holder of a Rocking microtome (Radical Cambridge type) and sections of 6  $\mu\text{m}$  – 10  $\mu\text{m}$  thicknesses were cut in longitudinal and transverse sections and spread on slides. The sections were double stained with Delafield Haematoxylin – Eosin.

Selected sections were photographed at required magnification with the help of Cat Cam Microscope Eyepiece digital camera (CC 130) under a Carl Zeiss microscope (Primostar- 37081).



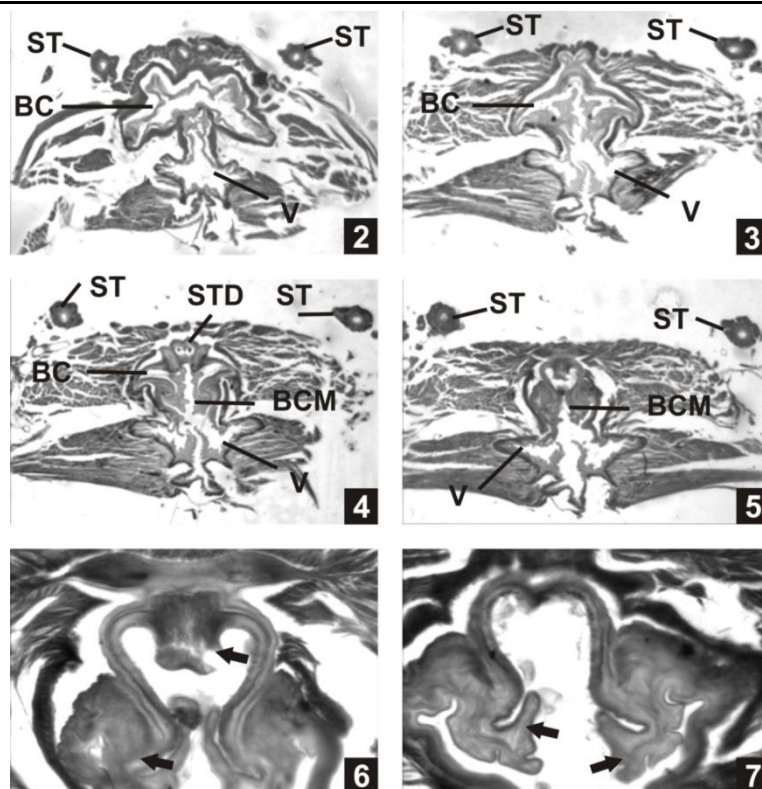
**Fig1.** *Pantala flavescens* female

## 3. OBSERVATION

In the matured adult *Pantala flavescens*, the post ovarian genital complex (POGC) is well developed. It lies in the mid-region of the eight sternum below the hind-gut vestibule just behind the last abdominal ganglion. It consists of a pair of long thin spermatheca (SC) with bulbous tips, a dorsal bursa copulatrix (BC) and a large ventrally placed vagina. A rectangular median cuticular plate with arms embedded in muscles lies mid-dorsally on the POGC.

The ST is situated antero-laterally to the complex. They open separately through their well distinct ducts into the mid-dorsally situated spherical BC. The BC is a small round structure lying on the vagina. The vagina occupies a large part of the complex and is firmly attached to the eight sternums by strong muscles. The vagina receives the median oviduct anteriorly and posteriorly it opens outside through a well defined female gonopore.

The wall of the POGC is basically composed of an outer muscle layer, middle epithelial layer resting on a basement membrane and an internal layer of the cuticle (Figs. 2-17)



**Figs2-7.** Transverse section passing through the anterior region of the post ovarian genital complex of the dragonfly *Pantala flavescens*. **Fig. 2.** The small bursa copulatrix is located above the tubular vagina. **Figs. 3-5.** Cuticular opening of the bursa copulatrix, the bursa communis into the vagina. **Figs. 6-7.** Heavy cuticular deposition of the bursa communis (arrow) (BC- Bursa copulatrix; BCM- bursa communis; ST- spermatheca; STD- spermathecal duct; V- vagina).(Figs 2-5, HE X50; Figs. 5-7, HE X400)

### 3.1. Spermathecae (St)

The epithelium of the ST is composed of tall columnar cells with distinct cell wall and centrally placed nuclei. The internal lining of ST is annulated with cuticular rings and is composed of 3-4 layers of cuticle consecutively undergoing sclerotization. The thickness of the cuticular lining is about 5.7  $\mu\text{m}$  (Figs. 2-5; 2-14).

Each opens into the BC antero-laterally by a well-defined short spermathecal duct (STD) (Fig. 14). Histologically it resembles the ST except that the inner cuticular lining is thinner. In the copulated females, the lumen of ST and STD is filled with variable quantity of spermatozoa (Figs. 13-14).

### 3.2. Bursa Copulatrix (BC)

The BC is a small and spherical. The internal lumen is lying predominantly with a thick layer of cuticle followed by a single layer of columnar epithelial cells. The whole body of BC is externally covered with muscles (Figs 2, 3). The cuticular layer of the dorsal wall of BC is thinner than the lateral and ventral wall. In the dorsal region of BC the thick ventral cuticular lining causes a great reduction in the internal lumen. As a result, the dorsal region is modified into a valve, the fertilization pore (FP).

#### 3.2.1. Fertilization Pore (FP)

In the FP cuticular teeth are present on the inner and outer surface of the valve. Variable amount of spermatozoa is present in the BC. The lateral wall of the BC is grooved and contains cuticular folds running in the antero-posterior direction (Figs. 10, 11). The BC communicates with the genital chamber (vagina) through a well defined valve, the bursa communis.

#### 3.2.2. Bursa Communis (BCM)

The BCM is formed due to heavy thickening of the cuticle and enlargement of the epithelial cells.

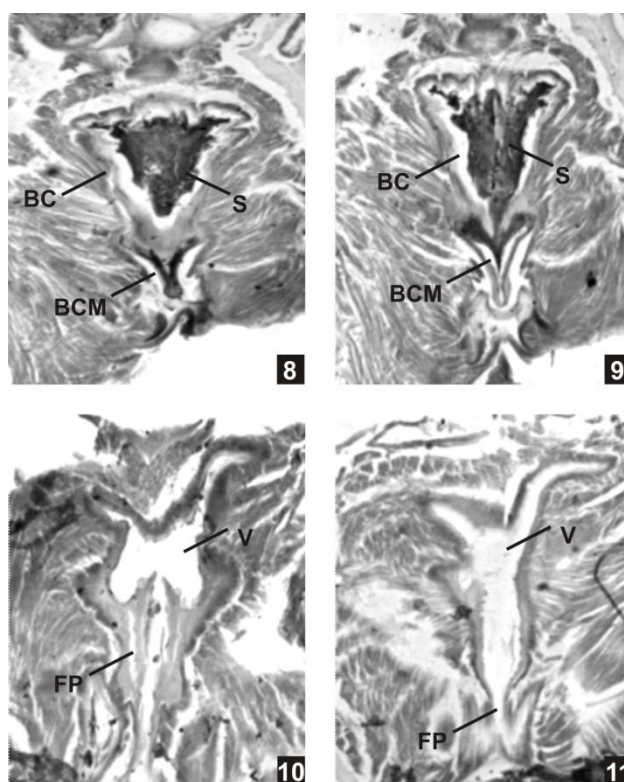
It comprises three plates, a median-dorsal plate and paired lateral plates (Figs 4-7). The lateral plates represent a structure similar to that of the BC. The dorsal plate initially projects out of the complex and lies on the surface of vagina. It forms a roof joining the two ventral plates. The median dorsal plate is initially innervated by a thick mass of muscles suggesting that the opening and closing of the BCM is controlled by muscles. The cuticle is lateral wall of the BCM possesses internally large number of fine spines. In the rest of the region the cuticles of all the three walls bear smaller cuticular projections. The entire lumen of the valve is internally lined with spines (Figs 8,9).

### 3.3. Vagina (VG)

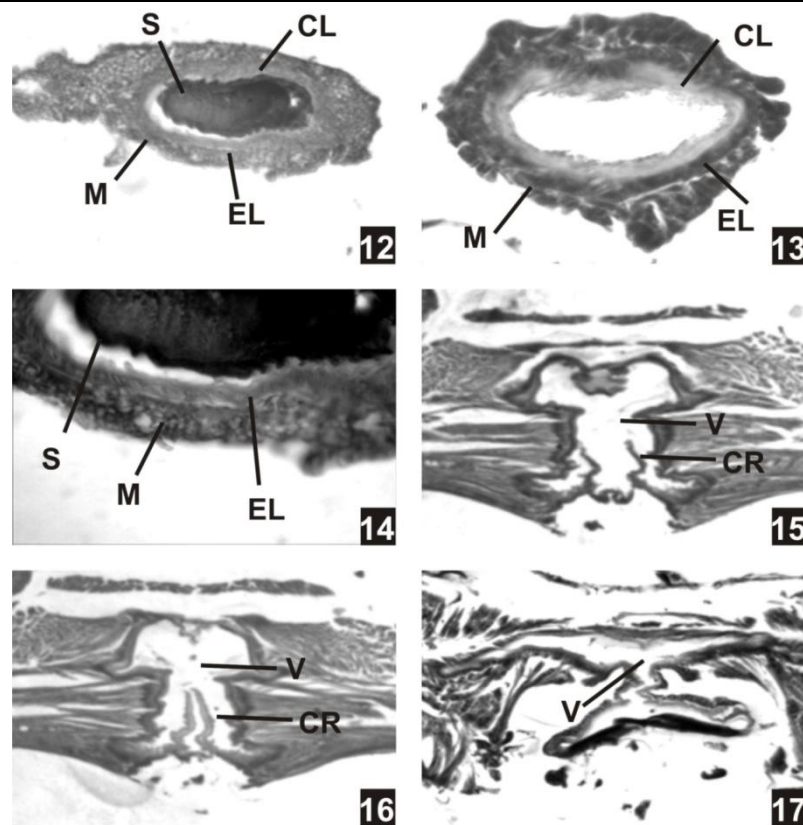
It can be divided into anterior and posterior regions-

a) *The anterior vagina (AVG)*: The AVG is histologically composed of an outer muscle, middle epithelial and inner thick cuticle layers. It is wrapped on both the lateral sides by mass of muscles. The surface of the lumen is lined with a thick cuticle extending a large number of small flat finger-like processes or stubs. The cuticular layer becomes extremely thickened at the antero-posterior and lateral regions. The AVG extends on either ventro-lateral site of the BCM in the form of distinct pouches. These pouches run below on either side but obliterate in the mid- region (Figs. 2-3; 10-11).

b) *The posterior vagina (PVG)*: The PVG is a long, large, laterally folded, sac like structure. Anteriorly the lumen of the PVG is voluminous rectangular and extensively elongated at the antero-lateral and postero-lateral regions. The cells of the dorsal region of the wall of the PGC are very tall while the cells of lateral regions of the wall are smaller. The cuticle though heavily sclerotized is thin. The dorsal region of the wall of the PVG is internally lined with smooth cuticle but the lateral regions of the wall contain highly wrinkled cuticle. The middle and posterior parts of the lumen of the PVG modify into a T-shaped structure. A pair of thin cuticular ridges are present on the floor of the vagina (Figs 15-17). The PGC tapers posteriorly into the female gonopore (FG). The FG is situated mid-ventrally in the anterior region of the 9<sup>th</sup> abdominal sternite. It is covered by the two lateral fusiform genital plates. These plates are basally attached to the FG and control the opening and closing of the FG by their movements.



**Figs.8-11.** Serial Longitudinal section passing through the bursa communis of the post ovarian genital complex of the dragonfly *Pantala flavescens*. Note the bursa copulatrix filled with sperm trickling down the bursa communis and the cuticular fertilization pore. (BC-Bursa copulatrix; BCM- bursa communis; FP- fertilization pore; S- sperm; V- vagina). (HE X200).



**Figs12-17.** Section passing through various components of the post ovarian genital complex of the dragonfly *Pantala flavescens*. **Fig. 12.** Section through the spermatheca and (**Fig. 13**) spermathecal bulb showing innermost cuticular layer, single layered epithelial layer and outer muscle covering in magnified view (**Fig. 14**). **Figs. 15-17.** Transverse section passing through the posterior vagina. Note the large T shaped lumen and pair of cuticular ridges on the floor (arrow). (CL-cuticular layer; CR- cuticular ridge; EL-epithelial layer; M- muscles; S- sperm; V- vagina). ( Figs. 12-13, HE X300; Fig. 14, HE X400; Figs. 15-17, HE X50).

#### 4. DISCUSSION

Rathke (1832) initiated the study of POGC in Odonata. Later, Siebold (1840) and Marshall (1914) described the POGC in some dragonflies. Tillyard (1917) described a pair of accessory glands on either side of the genital complex of *Petalura*. Asahina (1954) reported that in *Epiophlebia superstes*, the ST is absent and the ST glands are attached to the vagina. According to Siva-Jothy (1987) the T-shaped spermathecal gland of *Epiophlebia superstes* reported by Asahina (1954) are in fact a pair of ST fused to form a single ST duct opening into the BC. In *Pantala flavescens*, the POGC consists of a vagina, a single bursa copulatrix (BC) occupying the antero-dorsal position and receiving the ducts of the paired lateral spermathecae (ST). The account given by Matsuda (1976) on the POGC that only a single median ST is attached to anterior end of the vagina and is provided with paired spermathecal glands in Odonata therefore needs reinvestigation. Richards and Davies (1977) mentioned that in Odonata the two oviducts are very short and open into a large pouch-like ST in the eighth segment; but in *Pantala flavescens* both the lateral oviducts join and finally opens into the vagina as reported in some oriental dragonflies (Andrew & Tembhare, 1996; 97).

Among Odonata, there is a great variation in the size, number and structure of the ST. There are generally paired ST in Anisoptera and single in Zygoptera (Tembhare & Andrew, 1986; Srivastava and Srivastava, 1986a, 88; Siva-Jothy, 1987). In the dragonfly *Brachythymis leucosticta* and *B. lacustris* the paired ST unite to form a common STD which opens into the BC (Miller, 1982a). In *Pantala flavescens*, the ST are paired, thin with small bulbous tips. Each ST is provided with independent duct opening into the lateral region of the BC.

The BC differs in shape and size in most of the anisopteran dragonflies. It may be large as in *Trithemis arteriosa*, *Crocothemis erytraea*, *Sympetrum rubicundalum* (Waage, 1984),

*Nesciothemis farinosa* (Miller, 1984) and *Sympetrum depressiusculum* (Siva-Jothy, 1987); of median size as in *Brachythemis leucoticta*, *Celithemis eponina* and *Brachythemis lacustris* (Miller, 1982a) or of a very small size as in *Orthetrum cancellatum* and *O. chrysostigma*. In Zygoptera, moreover the BC is quite large triangular or spherical in shape (Waage, 1982; Miller, 1987). In *Pantala flavescens* the BC is small and spherical as found in *Orthetrum* species.

It is of a general occurrence in dragonflies that the paired oviducts unite to form a median oviduct before entering the vagina (Matsuda, 1976). The median oviduct opens into the vagina anteriorly in *Brachythemis lacustris*, *Nesciothemis farinosa* (Miller, 1982a,b); *Lestes vigilax* (Waage, 1982); *Orthetrum chrysostigma* (Miller, 1984); *Ischnura elegans* (Miller, 1987) and *Orthetrum cancellatum* (Siva-Jothy, 1987) while in *Somatochlora arctica* (Midttun, 1976) and *Celethemis elisa* (Waage, 1984), the median oviduct after running ventrally for a short distance opens into the vagina mid-ventrally. In *Pantala flavescens*, the median oviduct opens anteriorly although in *Tramea virginia* it runs below the vagina and opens into the lumen of the vagina ventrally (Andrew & Tembhare, 1994).

In *Pantala flavescens*, the BC is divided into an anterior region with voluminous round lumen and the posterior tapering region. No division of BC is, however, reported in *Sympetrum danae* (Bjanes, 1974) and *Somatochlora arctica* (Midttun, 1976). The BC of *Somatochlora arctica* is lined by cuticle with intricate folds (Midttun, 1976). Similar folding is observed in the BC of *Pantala flavescens*.

In *Somatochlora arctica*, Midttun (1976) stated “occupying the central area of the floor (BC) are two elevated structures lying close together and separated by a groove, which receives furrows from these structures. The groove and furrows, in addition to the ducts bursae, represents the connection with the vagina”. This is termed as the ventral slit by Miller (1984) and bursa communis by Siva-Jothy (1987). In *Pantala flavescens*, the BCM and the fertilization pore are well-distinct and these structures contain cuticular spines resembling that of other dragonflies (Midttun, 1976; Miller, 1984; Siva-Jothy, 1987; Andrew and Tembhare, 1994, 1996, 1997).

Siva-Jothy (1987) reported the presence of an extension of the vagina anteriorly under the BC in *Orthetrum cancellatum* and termed it as the anterior vaginal pouch, while in *Pantala flavescens* the vagina extends anteriorly and is termed as the anterior genital chamber. In *Somatochlora arctica*, the median oviduct opens into the mid-vagina through a slit like opening encircled with a cuticular rim (Midttun, 1976). This opening is situated just below the fertilization pore. In *Pantala flavescens*, the vagina receives the median oviduct anteriorly and posteriorly it opens outside through a well defined female gonopore.

## 5. CONCLUSION

The post ovarian genital complex (POGC) of the dragonfly, *Pantala flavescens* (Fabricius, 1798) is located in the 8<sup>th</sup> abdominal segment. It consists of a pair of long thin spermatheca with bulbous tips, a small, spherical dorsal bursa copulatrix and a large ventrally placed vagina. The POGC is ectodermal in origin with outer muscle layer, middle epithelial layer resting on a basement membrane and an internal layer of cuticle. It is externally covered in muscle bands. The internal lining of ST is annulated while that of the bursa copulatrix is folded. In the connection between the bursa and vagina is located the fertilization pore is in the form of a valve covered with cuticular spines. The vagina also contains a bursa communis which is a tunnel like structure formed by three plates, a median-dorsal plate and paired lateral plates. The vagina is divided into anterior and posterior regions showing variation in their cuticular intima.

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