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# Morphology of the female genitalia in libellulid dragonflies [*Acisoma panorpoides* (Rambur 1842), *Brachythemis contaminata* (Fabricius 1793) and *Crocothemis servilia* (Drury 1770)] with special reference to the mechanism of sperm competition during copulation (Odonata; Anisoptera)

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## Abstract

The males of many insects, including some libellulid dragonflies, compete with one another after copulation to fertilize the eggs in the female's post ovarian genital complex by removing the sperm of rivals, and/or by placing their own sperm in the most advantageous position. To understand the morphological compatibility for this mechanism of sperm competition, the sperm storage organ of the three species of dragonflies (*Acisoma panorpoides*, *Brachythemis contaminata*, *Crocothemis servilia*) was correlated with the penis head of the male. In *Acisoma panorpoides* and *Crocothemis servilia*, the sperm storage organ is large with a bulbous bursa copulatrix. The penis head bears large inflatable lobes which can be used to push and repack the rivals sperm far from the site of fertilization. The layered sperm material in the bursa copulatrix of *Acisoma panorpoides* indicate that flushing out of rival's sperm is also used as a mechanism of sperm displacement. In *Brachythemis contaminata* the bursa copulatrix and spermathecae are very small and form a common duct before opening into the bursa copulatrix. The penis head of *Brachythemis contaminata* is equipped with a long flagellum with barbed tips which can help in physical removal of sperm from the sperm storage organs.

**Keywords:** bursa copulatrix, sperm competition, spermatheca, sperm storage organ

## Introduction

According to Eberhard (1985) [3] and Shapiro & Porter (1989) [15] morphological diversification of genitalia is very wide-spread among animals with internal fertilization. The genital morphology differs greatly among closely related species. This differentiation is also one of the most poorly understood evolutionary trends in insects. They explained species specific genital morphology in terms of mechanical isolation during reproduction The males of many insects, including some dragonflies, compete with one another after copulation to fertilize the eggs in the female's post ovarian genital complex by removing the sperm of rivals, and/or by placing their own sperm in the most advantageous position (Birkhead & Hunter, 1990; Fincke, 88; McVey & Smittle, 1984; Miller, 1987, 88, 90, 91, 95; Siva-Jothy, 1987a; Sivinski, 1984; Waage, 1979, 84, 86a,b) [1, 7, 9, 16, 22]. Females may gain superior mates from multiple matings and sperm competition, but may have to pay a cost in terms of time, energy and possible damage (Fowler & Partridge, 1989) [5].

Waage (1986a) [25] stated that "the relationship between male and female morphology, copulation duration and the extent of sperm removal or repositioning remains delightfully complex". In Odonata, sperm competition is achieved most conspicuously by sperm displacement, which entails morphological specialization not only of the penis but also complementary modifications in the female's post ovarian genital complex (Waage, 1979) [22]. Sperm competition by sperm displacement can be inferred in odonates on the basis of four criteria- multiple mating by the female; appropriate sperm-storage organs in female; appropriate modification of male genitalia and female's post ovarian genital complex.

A striking feature of the odonate's post ovarian genital complex is the amount of interspecific variation in the volume and number of their sperm-storage organs which directly corresponds to the micro structural changes of the penis head (Walker, 1980; McVey & Smittle, 1984; Waage, 1979, 82, 84 1986a, b; Siva-Jothy & Tsubaki, 1989; Andrew & Tembhare, 1994, 1997; Verma, 2017) [7, 22, 20, 19]. To understand the morphological compatibility for the mechanisms of sperm competition, the sperm storage organ of the three species of dragonflies [*Acisoma panorpoides* (Rambur 1842), *Brachythemis contaminata* (Fabricius 1793), and *Crocothemis servilia* (Drury 1770)] were correlated with the penis head of the male. The mechanism of sperm competition proposed here is therefore based on the morphological and anatomical compatibility of these two components, i.e. the spermatheca and bursa copulatrix of the female as observed in the present investigation and the penis head of the male which has already been described by previous workers (Miller, 1990; Kirti & Singh, 2004) [11, 6].

## Material and Method

The libellulid dragonflies were collected from the water bodies around the campus of Hislop College, Nagpur and dissected in Ringer's saline solution under the stereoscopic binocular stereo microscope (Magnus- MS 24).

For histological studies, the post ovarian genital complex (POGC) was removed from the abdomen and immediately fixed in aqueous Bouin's fixative for 24 h. The POGC was washed in running water for half an hour and later dehydrated in a series of ascending grades of aqueous alcohol, cleared in xylene, embedded in molten paraffin wax at 62ºC for block preparation. The paraffin blocks were mounted on the block-holder of a Rocking microtome (Radical Cambridge type) and sections of 6 µm 10 µm thicknesses were cut in longitudinal and transverse sections and spread on glass slides. The sections were stained with Delafield Haematoxylin Eosin. Selected sections were photographed at required magnifications with the help of Cat Cam Microscope Eyepiece digital camera (CC 130) under the Carl Zeiss microscope (Primostar- 37081).

For whole mount studies, the POGC after dissection was treated to 10 % KOH and boiled for 10 minutes in order clear the organ. It was stained with water soluble Eosin or Acetocarmine and mounted in cavity slide in DPX after dehydration in ascending grades of alcohol and clearing in xylene.

## Observations and Discussion

The discovery that the odonate penis both inseminates the female and displaces rival sperm (Waage 1979) has proved to be an invaluable aid in interpreting odonate reproductive behaviour. The morphology and histology of the female sperm storage organ (Figs. 1A-C) and penis-head of the male complement each other for this performance.

### *Acisoma panorpoides*

In *Acisoma panorpoides* the sperm storage organ is composed of only the bursa copulatrix as the spermathecae are absent. The bursa copulatrix is a large bulbous spheroid organ located along the mid-region above the apical end of the vagina. The large voluminous bursa copulatrix is able to store sperm from several mating as it has been observed that the sperm material is layered with tiers from different mating. The penis-head of *Acisoma panorpoides* is composed a single medial process and a pair each of apical and lateral lobes. The apical lobe is large, inflatable and spiny while the lateral lobes are large and flat. The medial process bears three pairs of inflatable spiny lobes (Miller, 1990) [11].

In *Acisoma panorpoides* the probable mechanism of sperm competition is as follows- during copulation the penis enters the vagina and the penis-head comes to lie below the bursa copulatrix. Erection of penis-head during this stage causes the medial process and the lateral lobes to enter the bursa copulatrix and inflate the three lobes attached to it. Inflation of these lobes causes the rival's sperm material present in the bursa copulatrix to be pushed against the wall. Some sperm material is also trapped in-between the spiny lobes of medial process and lateral lobes which are removed when the penis-head is withdrawn. This results in the formation of an empty space in the bursa copulatrix. Before retracting, the dorsally placed sperm tube injects the sperm in the space created by the penis-head (Figs. 2A-B).

### *Brachythemis contaminata*

In *Brachythemis contaminata* the paired spermathecae are small spherical, bulbous bodies with a common 'T' shaped spermathecal duct. The bursa copulatrix too is a small pear shape sphere with small sperm storage capacity. The penis-head of *Brachythemis contaminata* is composed of an apical lobe which is large, inflatable and bear multiramous bristles on its surface. The lateral lobes are large, sclerotized and flat while the inner lobes are small. The medial process bears inflatable spiny lobes, a pair of crescent shape cornua with bristles and smooth flagellum which is barbed at the tip (Miller, 1990) [11].

During copulation as the penis enters the vagina and the apical lobes comes to lie inside the anterior region of vagina, pressure on the medial process pushes it in the bursa copulatrix. Inside the bursa copulatrix the medial process inflates the lobes. The flagellum enters one of the spermatheca through the common oviduct. The barbed tip 'hooks-out' the rival's sperm from the spermathecae and its duct. The sperm are trapped on the barbed process of flagellum and are removed during withdrawal of penis. The cornua scrap the sperm material from the bursa copulatrix, trap it in between the lobes and remove them completely during withdrawal of penis. As the male withdraws the penis-head from the bursa copulatrix, the short sperm tube which is ventrally place ejects sperm material in the now empty spermatheca and bursa copulatrix. Since the sperm storage organs has a very small storage capacity, the male can even flush out almost the rival's sperm from the bursa copulatrix and spermathecae (Figs. 3A, B).

### *Crocothemis servilia*

The post ovarian genital complex of *Crocothemis servilia* consist of two sperm storage organs, a pair of very large prominent bean-shaped spermatheca which opens into a small bursa copulatrix. The bursa copulatrix is sunk between the two spermathecae (Verma & Andrew, 2015) [21]. In *Crocothemis servilia* the penis-head consist of a pair of spiny, large and inflatable apical lobes, small and flattened lateral lobes, large and prominent medial process and a pair of broad inflatable inner lobes (Miller, 1990; Kriti & Singh, 2004).

During copulation the penis-head enter the vagina and comes to rest under the bursa copulatrix. The apical lobes support and grip the vagina and erection forces the medial process to enter the spermatheca and inflate the lobes.

Further pressure results in inflation of the lateral and inner lobes and the medial process enlarges. This causes pushing of the sperm material further down the lateral and apical end of spermathecae. The broad inflatable inner lobes position themselves around the spermathecae. The sperm tube injects sperm material in the space created by the repositioning of the rival's sperm material and places his own sperm at a place of advantage, just near the bursa communis **(**Figs. 4A, B**).**

Sperm competition has probably resulted from selection pressure on the male relating to the need to maximize paternity (Alcock & Gwynne, 1991). In Odonata, sperm competition is achieved most conspicuously by sperm displacement which entails morphological specialization of the penis and complementary modification in the female post ovarian genital complex (Waage, 1979, Peretti & Aisenberg, 2014) [22, 14]. In shape and size, the sperm storage organ often correspond to penis structure in ways that indicate how effectively rivals sperm in each storage organ can be reached and manipulated during copulation (Corbet, 1999) [2]. The current hypothesis regarding mechanism of displacement of rival's sperm may be accomplished by the following ways. The first type (Type I) is physical removal of sperm by proximally directed armatures on the penis cornua or barbed flagella or spiny lobes, the second type (Type II) is repositioning or packing rival's sperm within the sperm storage organs by expanding the lobes and pushing the rival's sperm far away from the bursa communis and in the third type (Type III) the rival's sperm is flushed by diluting it by own semen during copulation.

Comparing the penis morphology with the female sperm storage organ of the three libellulid dragonflies, it is found that in *Brachythemis contaminata* the bursa copulatrix and spermathecae are very small and form a common duct before opening into the bursa copulatrix. The sperm storage capacity is small and can probably store only 3-5 ejaculates as reported in *Sympetrum rubicundulum* (Waage, 1984) [24]. The penis head of *Brachythemis contaminata* is equipped with a long flagellum with barbed tips. During copulation this flagella enters one of the spermatheca and 'hooks-out' the rival sperm and its duct. The same is repeated in the corresponding spermatheca. Similar physical removal of sperm has been well-documented in *Brachythemis lacustris, Pantala flavescens, Sympetrum danae* and *Potomracha congener* (Miller, 1988, 91; Michiels, 1989; Miller, 1991) [10, 8, 12].

In *Acisoma panorpoides* and *Crocothemis servilia*, the sperm storage organ is large with a bulbous bursa copulatrix. The penis head bears large inflatable lobes which can be used to push and repack the rivals sperm (Type II) far from the bursa communis. The layered sperm material in the bursa copulatrix of *Acisoma panorpoides* indicate that flushing out of rival's sperm (Type III) is also used as a mechanism of sperm displacement in this species and similar mechanism of sperm removal has been documented in *Erythemis simplicicollis, Sympetrum danae* and perhaps *Orthetrum coerulescens* (McVey & Smittle, 1984; Michiels, 1989; Miller, 1990) [7, 8, 11].

After evaluating and correlating the shape and size of the sperm storage organs and the penis head structure of the three libellulid female and the penis-head of the male, it is proposed that sperm competition is a complex activity in these libellulid dragonflies. They probably use more than one type of mechanism concurrently to reposition/remove rival's sperm and to carry out the process of sperm competition.

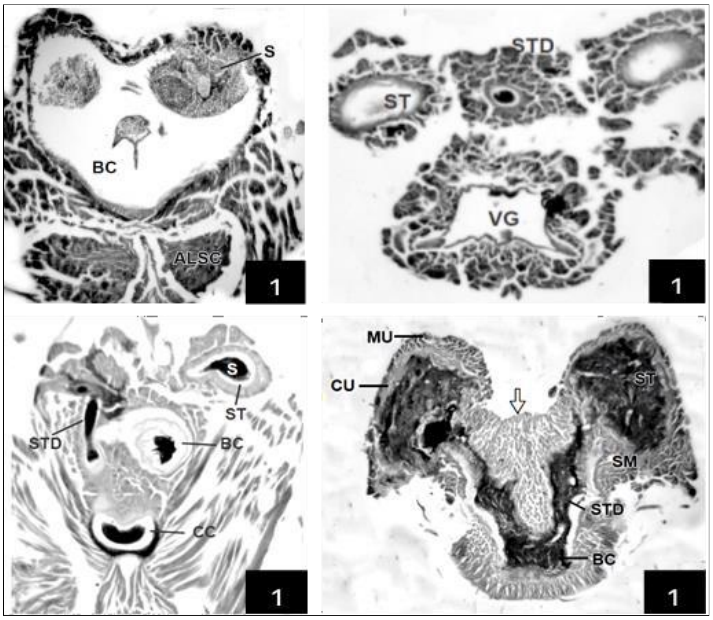

**Fig 1:** 1A-1D: Transverse section passing through the post ovarian genital complex showing bursa copulatrix. Fig.1A. *Acisoma panorpoides:* bulbous and globular bursa copulatrix (BC) showing seminal fluid along with spermatozoa in tiers (arrow head) surrounded by muscles (ALSC) (HE x100). Figs. 1B-1C. *Brachythemis contaminata:* Spermatheca (ST) and its duct (STD) above the vagina (VG) and bursa copulatrix (BC) filled with sperm material (S). Fig. 1D. *Crocothemis servilia:* Spermatheca showing spermathecal duct (STD) and bursa copulatrix (BC) surrounded by muscles (MU) and cuticular intima (CU) (HE x300).

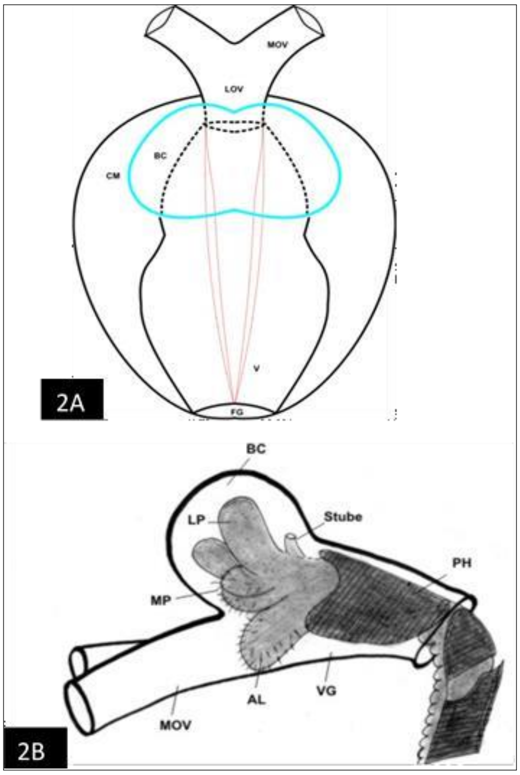

**Fig 2:** 2A-2B: *Acisoma panorpoides*. Fig. 2A: The Post Ovarian Genital complex- Anatomy: BC- Bursa Copulatrix, FG- Female Gonopore- Lateral Oviduct, MOV-Median Oviduct and V-Vagina. Fig. 2B: Diagrammatic representation showing the position of penis head and its lobes in the sperm storage organs: AL- Apical Lobe, BC- Bursa Copulatrix, LL-Lateral Lobes, MOV-Median Oviduct, MP-Medial Process, PH- Penis Head, Stube- Sperm Tube, and VG- Vagina.

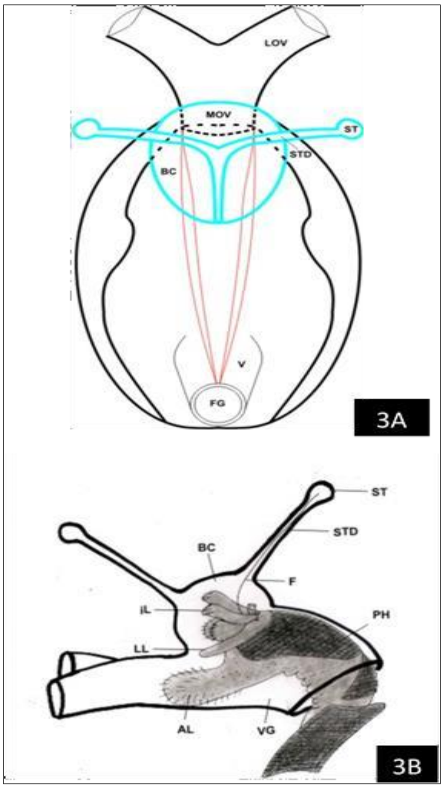

**Fig 3:** 3A-3B: *Brachythemis contaminata*: Fig. 3A: The Post Ovarian Genital complex- Anatomy: BC- Bursa Copulatrix, FG- Female Gonopore- Lateral Oviduct, MOV-Median Oviduct, ST- Spermathecae, STD- Spermathecal Duct and V-Vagina. Fig. 3B: Diagrammatic representation showing the position of penis head and its lobes in the sperm storage organs: AL- Apical Lobe, BC- Bursa Copulatrix, C- Cornua, F- Flagellum, LL- Lateral Lobes, MOV-Median Oviduct, MP-Medial Process, PH- Penis Head, ST- Spermathecae, STD- Spermathecal Duct TUBE- Sperm Tube, and VG- Vagina.

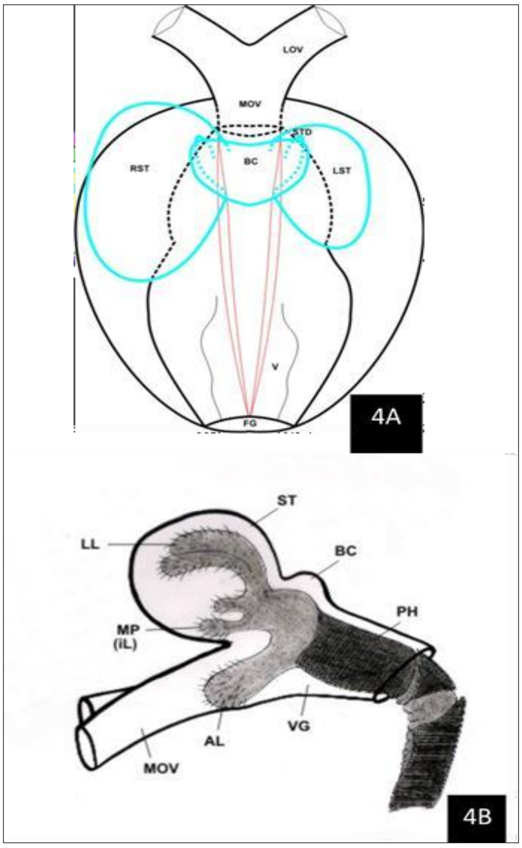

**Fig 4:** A-4B: *Crocothemis servilia*: Fig. 4A: The Post Ovarian Genital complex- Anatomy: BC- Bursa Copulatrix, FG- Female Gonopore- Lateral Oviduct, LST- Left Spermathecae MOV-Median Oviduct, RST-Right Spermathecae, STD- Spermathecal Duct and V-Vagina. Fig. 4B Diagrammatic representation showing the position of penis head and its lobes in the sperm storage organs: AL- Apical Lobe, BC- Bursa Copulatrix, iL-Inner Lobes, LL-Lateral Lobes, MOV-Median Oviduct, MP-Medial Process, PH- Penis Head, ST-Spermathecae, STD- Spermathecal Duct TUBE- Sperm Tube, and VG- Vagina.

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