

**SPERM COMPETITION AND PENIS STRUCTURE  
IN SOME LIBELLULID DRAGONFLIES  
(ANISOPTERA)**

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*Received March 4, 1994 / Reviewed and Accepted April 5, 1994*

Within and between species the copulation of libellulid dragonflies varies in duration (seconds to >1 h), situation (perched or in flight) and in the amount of copulatory movement that occurs (from no visible activity to persistent rocking movements of the base of the male's abdomen). Three ways in which sperm may be removed from the spermathecae of females are considered. In the first, in species which make long copulations accompanied by bouts of very small but fast rocking movements, sperm may be gradually filtered from the ejaculate by rows of flagellar spines as the flagellum is oscillated back and forth within the duct. In the second, strong but slower copulatory movements occur which may drive the spiny cornua in and out of the spermathecal ducts, filtering sperm as they do so. In the third, in which there are also marked copulatory movements, the grooved cornua lack spines and the male's own ejaculate, or a diluted fraction of it, may be used to wash out rival sperm. Finally, in a fourth group of species in which copulation is brief and aerial, and the flagella lack spines but possess large terminal hooks, there is probably less sperm removal from the spermathecae.

**INTRODUCTION**

Tony Watson's interest over many years in dragonfly genitalia, from both a functional and a taxonomic point of view, has been an inspiration to many odonatologists, and this paper is dedicated to his memory. The genital morphology of libellulid dragonflies varies greatly between species, as is the case in many other groups of animals (EBERHARD, 1985). Recently it has been suggested that sexual selection can explain much of this variability, either through providing further opportunities for female choice, possibly by sensory exploitation and a Fisherian run-away process (EBERHARD, 1993), or as a result of sperm competition (WAAGE, 1984; MILLER, 1991a). Sperm competition is

important in the reproduction of many libellulid dragonflies. Good evidence for last-male precedence exists for several species, and, in some, it is achieved at least in part by the removal of rival sperm from the female's storage organs (McVEY & SMITTLE, 1984; WAAGE, 1984, 1986; SIVA-JOTHY, 1987a; MICHIELS & DHONDT, 1988; MICHIELS, 1992). However the mechanism is not fully understood, although specialised parts of the penis are probably involved (MICHIELS, 1989; MILLER, 1991a).

In some species the penis bears one or more long fishing rod-like structures on the fourth segment, which are of the appropriate length to reach into the female's spermathecae. They are extended in various ways when the penis is inflated by fluid displacement from the first segment (MILLER, 1990, 1991a). They take the form of a single medial flagellum, bifurcated towards the tip in some species, or, in others, of paired cornua or inner lobes, often armed with spines. Libellulid copulation takes place either in flight or after perching, and it varies considerably in duration both within and between species. Longer copulations have been shown in several species to lead to greater sperm precedence either because more ejaculate can be delivered or because more rival sperm can be removed from the female's storage organs (SIVA-JOTHY, 1987a; WOLF et al., 1989; MICHIELS, 1992). During the copulation of some species, rocking movements of the genital segments occur at various frequencies and amplitudes, while in others there may be no detectable movement. However in no libellulid has it been possible to identify copulatory stages similar to those known in some Zygoptera (CORDERO & MILLER, 1992), nor has a comparison of copulatory activity with genital structure been previously attempted in libellulids.

The aim of the present paper is to describe some further aspects of penis structure, mainly of the flagella, and to review ways in which rival sperm might be removed from the spermathecae during copulation.

#### MATERIAL AND METHODS

Genital structure has been examined in the following species: *Orthetrum cancellatum* (L.), *O. coerulescens* (Fabr.), *O. chrysostigma* (Burm.), *O. sabina* (Dru.), *O. pruinosum* (Burm.), *O. trinacria* (Sel.), *Pantala flavescens* (Fabr.), *Potamarcha congener* (Ramb.), *Tholymis tillarga* (Fabr.), *Trithemis stictica* (Burm.) and *T. furva* (Karsch). Preserved specimens were prepared for scanning electron-microscopy as previously described (MILLER, 1984). Some further species have been examined in the field and laboratory as indicated in the following sections.

#### RESULTS AND DISCUSSION

The species examined fall into four categories according to their copulatory activity, genital morphology and the suggested mechanisms used in sperm competition.

## CATEGORY 1

In all *Orthetrum* species examined, copulation is normally completed after perching (*O. cancellatum* occasionally makes brief aerial copulations) and is variable in duration (from ca 15 s to >1 h). During copulation there is either no visible movement of the genital segments, or bouts of rapid but very shal-

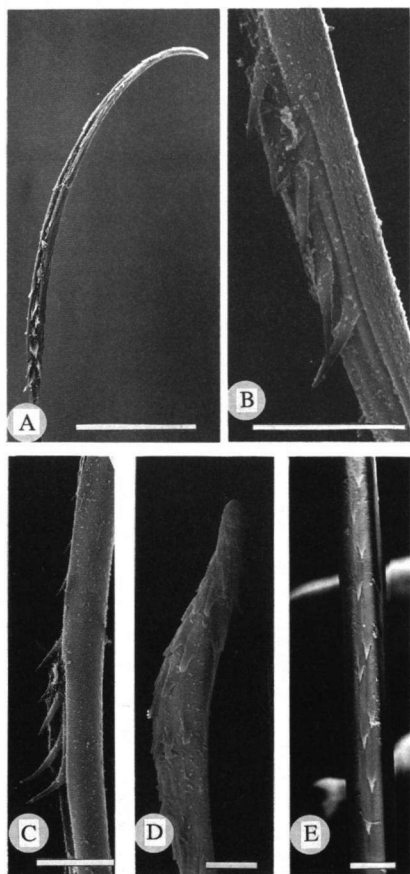


Fig. 2. (A-C) *Orthetrum sabina*, flagellum: (A) distal part of flagellum; – (B-C) longer spines in mid-region of shaft; – (D-E) *O. pruinosum*, flagellum; – [Scales: (A) = 50  $\mu$ m; others = 10  $\mu$ m].

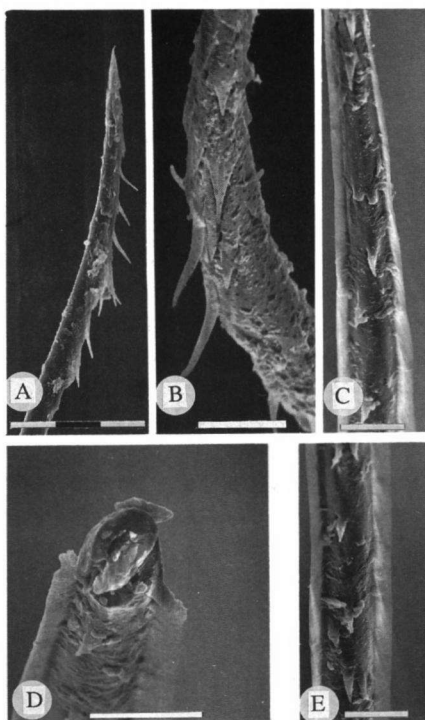


Fig. 1. The flagellum of *Orthetrum coerulescens*: (A-B) tip of flagellum; – (C) groove in mid region; – (D) cut shaft showing groove; – (E) basal region. – [Scales: (B) = 5  $\mu$ m; others = 10  $\mu$ m].

low movements appear, sometimes at >5Hz, which could be due to the activity of either partner (MILLER, 1983, 1990, 1991a).

The penis bears a medially situated flagellum which protrudes posteriorly in the resting position. With inflation of the penis by muscular compression of the basal segment, the medial process expands and rotates, sometimes by as much as 180°, so that the flagellum points upwards and forwards. Scanning electron micrographs have shown that short, broad spines, pointing proximally, occur

usually along one side of the flagellum, lying in a slightly recessed groove. In *O. coerulescens* there are additional, longer and thinner spines, not in the groove, which stand out near the tip (Fig. 1). Examining the cut end of a shaft shows the groove to be shallow with thickened margins (Fig. 1D). The structure is similar in *O. sabina* (Fig. 2A-C) and *O. chrysostigma* but the longer spines occur more proximally, while in other species examined they are absent. In *O. cancellatum* (Fig. 3) and *O. pruinatum* (Fig. 2D-E) the groove is deeper and contains larger, sometimes double-headed, spines, with further stubby spines distributed round the tip. Finally in *O. trinacria* no groove can be distinguished but the small spines are restricted to one margin, being pressed flat against the shaft. The females of all *Orthetrum* species examined possess small storage organs with paired spermathecae at the ends of narrow ducts (sperm-storage indices, 0.6-3.0; MILLER, 1991a; see also MILLER, 1984; SIVA-JOTHY, 1987b).

The flagellar groove may provide a passage for the proximal movement of pre-

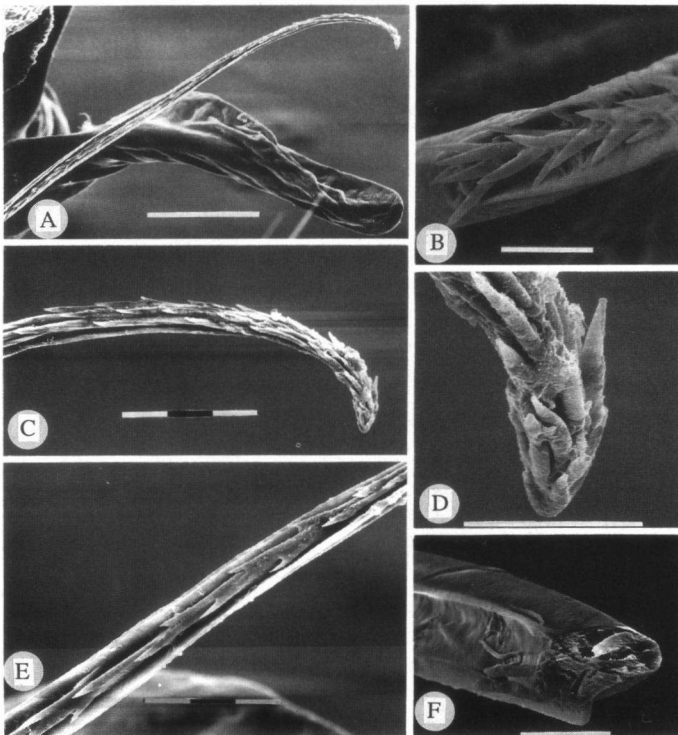


Fig. 3. *Orthetrum cancellatum*, flagellum: (A) whole flagellum; - (B) spines at the base of the groove; - (C-D) tip of the flagellum; - (E) longer spines within the groove; - (F) cut shaft showing groove. - [Scales: (A) = 100 µm; others = 10 µm].

-existing ejaculate which is displaced by the entry of the flagellum into the spermatheca. Rapid copulatory movements could then cause the flagellum to oscillate within a spermathecal duct, gradually working rival sperm proximally along the spines. The spines might act more effectively as filters if they stood out during withdrawal and were folded when the flagellum was thrust towards the spermatheca. In addition the ejaculate could be driven proximally along the flagellar groove by spermathecal contractions, possibly resulting from the excitation of vaginal receptors by the penis as previously suggested (MILLER, 1990), and this too would assist the filtering action. The flagellum would need to be inserted into each spermathecal duct in turn.

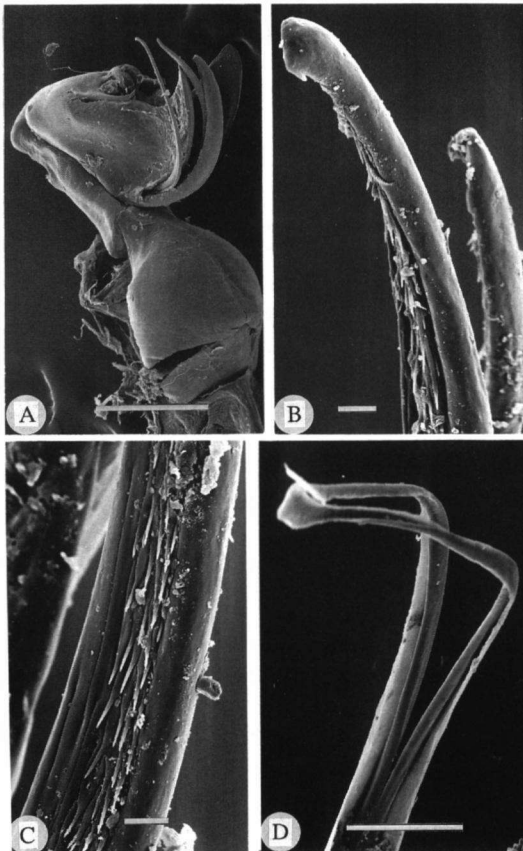


Fig. 4. (A-C) *Trithemis stictica*: (A) whole penis; - (B) tips of the cornua; - (C) shaft of one cornu, showing groove containing spines; - (D) *Tholymis tillarga*: flagellum, showing grooves in the distal bifurcated region. - [Scales: (A) = 500  $\mu$ m; (B-C) = 10  $\mu$ m; (D) = 50  $\mu$ m].

#### CATEGORY 2

*Celithemis*, *Sympetrum* and some *Trithemis* species make perched copulations of relatively long duration (>5 min). In *S. striolatum*, *S. depressiusculum*, *S. danae*, *S. sanguineum*, *C. eponina* and *T. stictica*, they are accompanied by strong, regular, rocking movements of the male's second and third abdominal segments at 0.5-2 Hz. In *C. elisa* they may occur only during the early part of copulation (WAAGE, 1984), whereas in *T. stictica* and other *Sympetrum* species they continue throughout the greater part of copulation (MICHIELS, 1989; MILLER, et al., 1984).

In *T. stictica* the cornual groove bears many proximally pointing spines (Fig. 4A-C). When the penis is inflated the groove is everted and the spines stand out from the cornua. A similar mechanism has been seen in *T. furva* (Fig. 6D) and possibly also in *T.*

*annulata*. Comparable eversible regions carrying densely packed and proximally directed spines occur on the inner lobes of *Zygonyx torrida*, *Brachythemis leucosticta*, *B. lacustris* and *Zyxomma petiolatum* (MILLER, 1991a, 1991b). In *S. striolatum* the cornua bear many spines which are fixed along one margin, not within a groove. The cornua of *S. fonscolombi* and *S. sanguineum* carry a few similarly fixed spines.

The vigorous copulatory movements known to occur in some of these species may drive the cornua or inner lobes back and forth within the relatively broad spermathecal ducts, filtering out sperm as suggested for *Orthetrum* species. The spines of *T. stictica* would probably work most effectively if they were everted during the withdrawal movement and folded during the subsequent thrust, requiring full inflation to coincide with withdrawal. But if the penis remained fully inflated, the spines would be partially folded during the forwards thrust by contact with the duct walls and could still operate effectively.

### CATEGORY 3

In some species in this category, a male's own ejaculate may be used to assist the removal of rival sperm by washing it out. In many libellulids inflation and ejaculation may sometimes occur together because they are achieved by the same mechanism – a compression of the first penis segment. Sustained compression more usually leads to ejaculation, whereas brief compressions may expand the penis without ejaculate release (MILLER, 1990). The copulations of *S. danae* last from 6 to >60 min and ejaculate may be transferred to the female throughout copulation at the same time as rival sperm is being removed (MICHIELS, 1989, 1992). Longer copulations lead to the removal of almost all rival sperm, giving near 100% paternity in subsequent clutches until the female re-mates. The cornua of *S. danae*, *S. depressiusculum* and *S. meridionale* lack spines but bear grooves along one margin. A male *S. danae* may force his ejaculate into the spermathecae along the cornual grooves thereby increasing the spermathecal volume and mixing with ejaculate already present (MICHIELS, 1989). Spermathecal contractions may then force the mixture proximally along the groove, carrying with it a fraction of the rival sperm present. Insects such as locusts (PARKER & SMITH, 1975) and leaf beetles (DICKINSON, 1986) are equipped with a tube which is able to enter deep within the female's storage organs acting like a hosepipe, and in these the delivery of fresh ejaculate can at the same time force rival sperm proximally, but with an open groove this is not possible.

Species which carry relatively large ejaculate volumes in the secondary genitalia compared to the volume of the female's storage organs, may be well placed to use the wash-out method. For example a male *O. chryso stigma* carries ten times as much sperm as can be contained in the female's bursa and spermathecae (MILLER, 1984). But in other species in which the female can store more ejacu-

late than a male's seminal vesicle can hold (e.g. *Nesciothemis farinosa*, *Crocothemis erythraea*; SIVA-JOTHY, 1987b), wash-out is unlikely to be employed.

Since sperm production may be costly (BERRIGAN & LOCKE, 1991), the use of a diluted ejaculate for washing out rival sperm would be advantageous, both as an economy and for hydrodynamic reasons. This may account for the observation that sustained compression of the first penis segment in fresh males of *P. flavescens*, *P. congener*, *Tramea basilaris*, *O. coerulescens*, *Brachythemis contaminata* and *Tholymis tillarga* sometimes releases a relatively clear fluid, to be followed later by an opaque sperm-rich ejaculate. The initial clear fluid could perhaps be used to wash out rival sperm. It will be of interest to know if this pattern of release occurs naturally. Males sometimes retain substantial amounts of ejaculate in the secondary genitalia after copulation (MILLER, 1984), but they usually top up with fresh sperm before a subsequent copulation.

#### CATEGORY 4

*Pantala flavescens*, *Tholymis tillarga*, *Zyxomma petiolatum* and *Brachythemis* species, all possess a deeply grooved flagellum with no spines, and a tip crowned with a large hook. Examination of the cut end of the flagellum of *P. flavescens* has shown that the groove is formed from a thickened cuticular rod, suspended between thinner regions of cuticle (Fig. 5B-D). Though the structure suggests that the groove may be eversible, as in the cornua of some *Trithemis* species, manipulation of fresh genitalia has not shown this to be the case.

In these species copulation is brief and on the wing. Observations with a close-

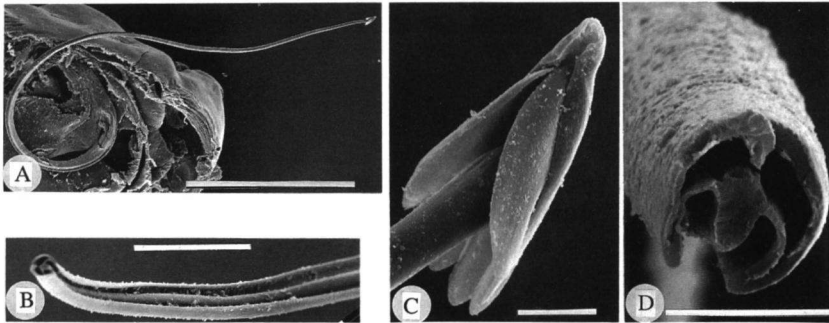


Fig. 5. Flagellum of *Pantala flavescens*: (A) penis head dissected to show the whole flagellum; – (B) part of shaft showing the groove; – (C) tip of the flagellum with the anchor-like hook; – (D) shaft cut to show groove. – [Scales: (A) = 500  $\mu$ m; (B) = 50  $\mu$ m; (C-D) = 10  $\mu$ m].

-focussing monocular of hovering pairs of *P. flavescens* (copulation duration 2-3 min), and of *T. tillarga* (copulation duration 14 s: MILLER & MILLER, 1985), have not shown any marked copulatory movements to be occurring. Female *P.*

*flavescens* possess small storage organs (sperm storage index = 7), comparable to those of *Orthetrum*, while in *Brachythemis* and the other species in this category the storage organs are also relatively small (e.g. storage index in *B. leucosticta* = 12: MILLER, 1991a). The groove could again provide a passage for pre-existing ejaculate to pass proximally when it was displaced from the spermatheca by the flagellum. The large terminal hook could anchor the flagellum into the spermatheca, holding the penis in place, resisting longitudinal movement within the duct and also preventing the expulsion of the penis by ejaculation. The flagellum in *Brachythemis* bears well developed lateral flanges which might hold open the duct and allow the proximal passage of sperm. In *Zyxomma petiolatum* curled

leaf-like lateral extensions of the flagellar shaft, each supported on a rib, could act as valves, trapping sperm when the flagellum was finally withdrawn (MILLER, 1991b). However although there may be some rival-sperm removal, the brevity of copulation together with the relative inaccessibility of the spermathecae in these species suggest that less sperm is removed than in the other categories.

Finally we may consider *Potamarcha congener*, a species which also makes brief aerial copulations. The cornua, which are very flexible and carry no spines (Fig. 6), are formed from strap-like structures rolled into a tube, leaving a longitudinal slit-like opening. They are probably too pliant to be forced into the female ducts and are thus unlikely to have a role in sperm removal, and are perhaps in a vestigial state (MILLER, 1991a).

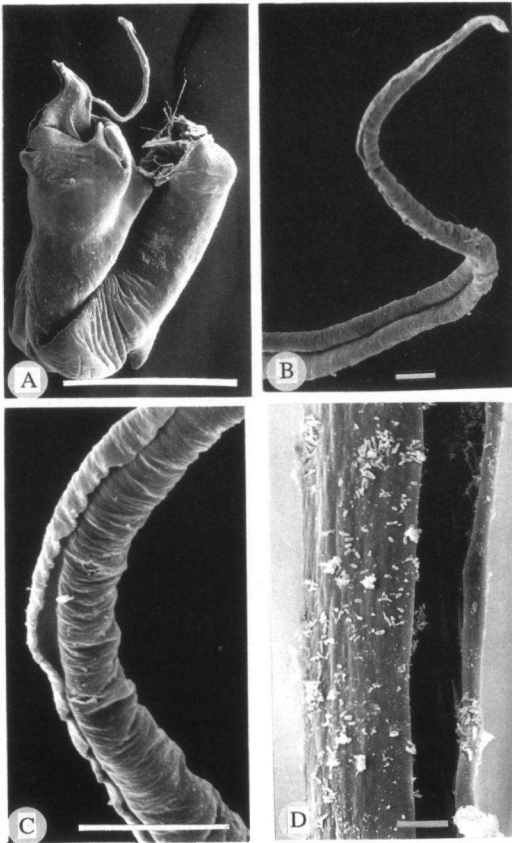


Fig. 6. (A-C) *Potamarcha congener*: (A) whole penis; – (B-C) part of the cornua; – (D) *Trithemis furva*: grooved cornu containing spines. – [Scales: (A) = 500  $\mu$ m; (B-D) = 10  $\mu$ m].



## CONCLUSION

During the relatively long copulations of some species, sperm may be filtered from the ejaculate by the spiny flagella or cornua as they are moved within the spermathecal ducts by the rocking movements of the male. Contractions of the spermathecae might assist by driving ejaculate proximally. Other species which make long copulations but possess cornua without spines may use their own ejaculate, or a diluted fraction of it, to mix with and then wash out rival sperm from the spermathecae, finally removing it from the bursa and vagina with their bristly lateral and apical lobes. Such methods may supplement each other within one species. Some of those species which make brief and aerial copulations have males possessing flagella without spines but with large terminal hooks which may anchor the penis within the spermathecae. Although some sperm removal from the spermathecae may occur in these, it is probably less than in other species.

## ACKNOWLEDGEMENTS

I am most grateful to BARBARA LUKE for her assistance with the scanning electron microscopy.

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