*Odonatologica 16(4): 393-399* *December 1, 1987*

# ON THE ZYGOPTERAN SPERM MATERIAL, WITH REFERENCE TO THE SPERMATOPHORE

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*Received June 3, 1986 / Revised and Accepted April 6, 1987*

The histology of the male internal genitalia (testes, vasa deferentia, sperm sac, ejaculatory duct) of 9 Indian spp. was investigated. Special attention is given to sperm material reaching up to the sperm-sac and, subsequently, to the vesicula spermalis of the secondary copulatory apparatus. Though generally known to students of odon. sperm competition, it is here for the first time histologically evidenced and explicitly stated that true spermatophores do not occur in the order.

## INTRODUCTION

The occurrence of spermatophores (sperm masses or sperm capsules) in Odonata has been reported by many workers viz. MARSHALL (1914), TILLYARD (1917), WHEDON (1918), CHOPARD (1949), BRINCK (1962, CORBET (1962, 1974), ENGELMANN (1970), MIDTTUN (1974), MATSUDA (1976), and RICHARDS & DAVIES (1977). Some of them have even described the presence of spermatophores in one or the other part of the male system (MARSHALL, 1914; TILLYARD, 1917; WHEDON, 1918; CHOPARD, 1949; MIDTTUN, 1974; RICHARDS & DAVIES, 1977). However, none of these reports agrees with the definition of insect spermatophores as given by SNODGRASS (1935). On the other hand, B.K. SRIVASTAVA (1963a, 1963b, 1979), TUXEN (1970), PFAU (1971), WIGGELSWORTH (1972), V.K. SRIVASTAVA (1980), and SRIVASTAVA & SRIVASTAVA

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(1986) do not mention the occurrence of spermatophores in the group. Recent workers on sperm competition, like WAAGE (1979, 1982, 1984), MILLER (1982, 1984), MILLER & MILLER (1981) and SIVA-JOTHY (1984, 1986), believe that spermatophores as such do not exist in odonates and hence have not used the term. We investigated the sperm material produced in testes, transported via vasa deferentia to sperm sac, translocated to the male vesicula spermalis (vesicle), and transferred therefrom to the female through the penis, in zygopteran odonates.

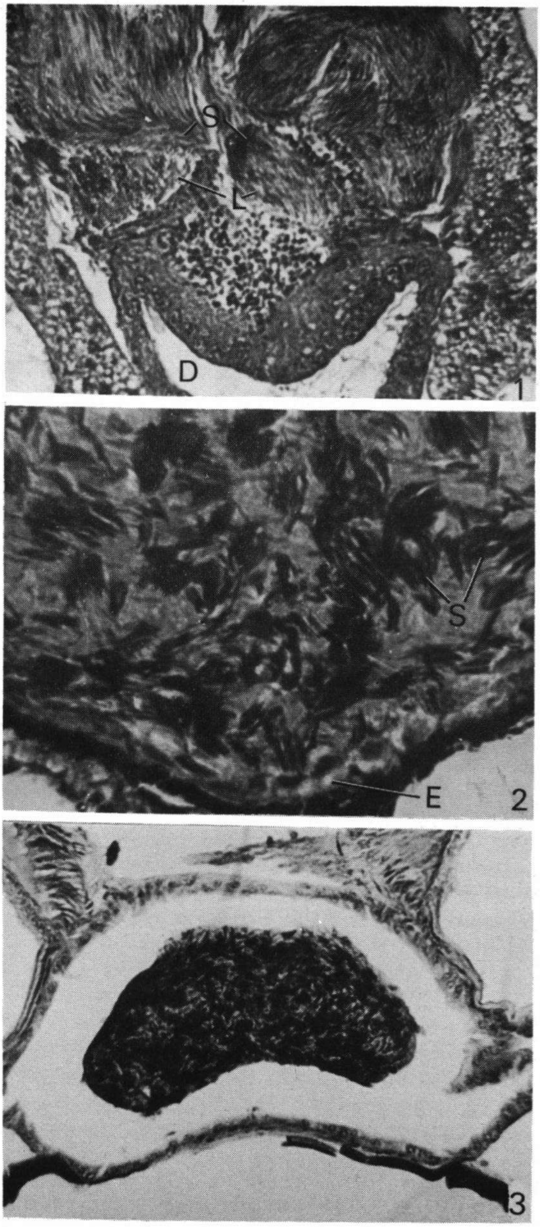
## MATERIAL AND METHODS

Pre-mated, mating and mated males of *Pseudagrion rubriceps* Sel., *P. decorum* (Ramb.), *Ceriagrion coromandelianum* (Fabr.), *Ischnura senegalensis* (Ramb.), *I. rufostigma* Sel., *Agriocnemis pygmaea* (Ramb.), *Cercion malayanum* (Sel.), *Enallagma parvum* Sel. and *Copera marginipes* (Ramb.), were captured during 1977/1980 from the area of Sagar Lake, Sagar (M.P.). Segments bearing the reproductive organs as well as the reproductive organs dissected, were fixed in aqueous Bouin's fluid for 18 hours. For sectioning, paraffin embedding and the celloidin-paraffin double embedding methods were employed. Sections were cut, 6-8 μm thick, and stained with Delafield's haematoxylin and eosin. Some were also treated with Mallory's triple stain. Whole mounts of the secondary copulatory apparatus and its components were prepared with picro-indigo-carmine as stain. The contents of the sperm-sac and the vesicula spermalis were also examined in fresh and alcohol preserved specimens.

## OBSERVATIONS AND DISCUSSION

Male accessory reproductive glands in insects usually perform two functions. They act as sperm reservoir to provide a nourishing medium to sperms, and secrete material for spermatophore formation. Accessory glands have been reported absent from the male reproductive system of Zygoptera and Anisoptera (BERLESE, 1909; MARSHALL, 1914; TILLYARD, 1917; WHEDON, 1918; GEORGE, 1929; CHOPARD, 1949; MIDTTUN, 1974; RICHARDS & DAVIES, 1977; B.K. SRIVASTAVA, 1979; V.K. SRIVASTAVA, 1980), but they have been recorded in the anisozygopteran *Epiophlebia superstes* (Sel.), where the sperm sac (seminal vesicle) is absent (ASAHINA, 1954). However, histological and functional details of the organ are lacking. According to MATSUDA (1976), sperm sacs can be considered to be present in *Epiphlebia* in the form of accessory glands. He suggested that the structures reported by ASAHINA (1954) as accessory glands, are in fact enlarged sperm-sacs.

The damselfly testis consists of numerous lobules opening into a common lateral duct. The lobules produce spermatozoa, mostly in bundles or groups (Fig. 1), which are later discharged into the common lateral duct. The sperm groups descend down the common lateral duct and the distally connected vas deferens to reach the sperm sac. The sperm sac, into which both vasa deferentia open, stores spermatozoa till translocation takes place through the ejaculatory duct. Newer sperm groups produced are temporarily stored in the vasa deferentia themselves when the sperm-sac becomes empty. In the absence of accessory glands in zygopteran odonates, their functions seem to have been taken over by the vasa deferentia and the sperm sac. These two organs not only serve to store spermatozoa, but have also assumed the glandular function of the accessory glands. The epithelial cells of the wall of the vasa deferentia and the sperm-sac are known to exhibit secretory activity (MIDTTUN, 1974; B.K. SRIVASTAVA 1979; V.K. SRIVASTAVA,



Figs 1-3. Histology of the zygopteran male internal genitalia: (1) Transverse section through the testis of *Ischnura rufostigma*, showing the lobules (L) containing sperm groups (S) produced (x 300). The sperm is discharged into the common lateral duct (D) of the testis which is distally connected with the vas deferens. — (2) Transverse section through the sperm-sac of *Cercion malayanum*, showing contained sperm groups (S) embedded in secretion, and the secretory epithelium (E) of the wall (x 400). — (3) Transverse section through the vesicula spermalis of *Ceriagrion coromandelianum* showing contained seminal fluid having embedded sperm groups (x 100).

1980; SRIVASTAVA & SRIVASTAVA, 1986). In the proximal region of the vas deferens, secretory activity has been found to be of relatively low intensity in Zygoptera. The epithelial cells of the vasa deferentia and the sperm sac (Fig. 2) in fact secrete a thick mucilaginous fluid, which is utilized for providing viability and a fluid medium for mobility of sperms.

CHAPMAN (1971) and RICHARDS & DAVIES (1977) have reported that only one bundle of sperms is derived from a single testicular lobule. On the other hand, after investigating a large number of odonate species (B.K. SRIVASTAVA, 1963a, 1963b, 1979; V.K. SRIVASTAVA, 1980), we have noticed that, besides a few isolated spermatozoa, many more than a single group of sperms is produced and is present inside a single testicular lobule (Fig. 1). This observation is similar to those of PRASAD & SRIVASTAVA (1960, 1964), OMURA (1957) and KALLA & NATH (1970). It has been believed that a sperm bundle resolves into separate spermatozoa upon reaching the sperm sac (KALLA & NATH, 1970), or the vas deferens (CHAPMAN, 1971), in odonates. In our view, the sperm groups, after discharge from the lobules into the common lateral duct of the testis, descend as such, unresolved down the vas deferens and reach the sperm sac; and on the way get embedded in a mucilaginous secretion (Fig. 2). It is for this reason that sperms in the present study have been noticed mostly in the form of groups or bundles throughout the testes, vasa deferentia and sperm sac. Similar groups of sperms were also noticed by BALLOWITZ (1916, 1918), TILLYARD (1917) and ASAHINA (1954) in the regions of the vasa deferentia and seminal receptacle or seminal vesicle (the sperm sac). Sperm bundles have been recently observed also in some aeshnids by SIVA-JOTHY (1984). The sperm groups were termed as "spermiozeugmen" by BALLOWITZ (1916, 1918), and "spermatodesms" by CHAPMAN (1971). It is likely that these unusual bundles of sperms, found embedded in the mucilaginous secretion, have been referred to by earlier workers as "spermatophores".

In Zygoptera, sperm material (containing sperm groups and seminal fluid) has not been found enclosed within any covering in the vasa deferentia or in the region of the sperm sac. From the sperm sac the material is translocated via the ejaculatory duct to the vesicula spermalis of the secondary copulatory apparatus where it is stored temporarily in the lumen (Fig. 3), and from where it is passed into the female during copulation. Mechanical compression of the vesicula spermalis releases through its orifice the sperm in thick viscous masses. Perhaps that is also a reason why earlier workers believed in the existence of spermatophores in Odonata. The present study has revealed that the sperm material has no covering around it in the vesicula spermalis either, and that the sperms are still present in groups. Dr H.K. Pfau (pers. comm., 1979) had also found only accumulation of sperms in the vesica (vesicula) spermalis "but no spermatophore". MILLER & MILLER (1981) also are of the opinion that sperm are not enclosed in a spermatophore in the sperm store (the vesicula spermalis).

As in other odonates, in Zygoptera sperm transfer from the male vesicula spermalis to the female bursa copulatrix occurs through the penis of the secondary copulatory apparatus, lying close to the vesicula spermalis in the second segment of the abdomen. But unlike in Anisoptera, where the penis has an internal central canal as sperm conduit, the zygopteran penis is provided with an external dorsal groove running medially (SRIVASTAVA, 1980; SRIVASTAVA & SRIVASTAVA, 1986), along which sperm enter the female. Similar external grooves in the penis of other zygopterans were recorded by PFAU (1971), MILLER & MILLER (1981) and WAAGE (1979, 1984). For sperm transfer the male damselfly inserts its penis through the female genital aperture so as to reach the bursa copulatrix. The vesicula spermalis is then compressed by muscular action, releasing the sperm material through its orifice. The sperm material flows into the appositely placed external groove of the penis, and is deposited into the bursa copulatrix. A covering around the sperm material would certainly be a hindrance and disadvantage to such complex mechanisms of sperm translocation and sperm transfer. Moreover, both being external processes, the sperm material would be liable to various hazards including spilling, if not organized properly. Perhaps to avoid the loss of sperms by spilling during such activities, zygopterans have a mucilaginous seminal fluid of such consistency that can contain the sperm groups, and yet flow smoothly.

In view of the above facts, it is evident that the sperm material produced, translocated and transferred in Zygoptera is not enclosed within any covering, sac or wall, nor is there any need to have it around. Thus, the structure of the sperm material of Zygoptera does not agree with that in the definition of a spermatophore given by SNODGRASS (1935). It is well established that the mode of sperm transfer in the order is an "indirect" one (TILLYARD, 1917; BRINCK, 1962; CORBET, 1962, 1974; ENGELMANN, 1970; JOHNSON, 1972; RICHARDS & DAVIES, 1977; WAAGE, 1984); and "indirect" sperm transfer is generally associated with spermatophores (SCHALLER, 1971). Yet we suggest that true "spermatophores" do not occur in dragonflies.

## ACKNOWLEDGEMENTS

The authors are thankful to the Head of Zoology Department, Dr Hari Singh Gour University, Sagar (M.P.) for providing facilities to carry out the laboratory work.

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