

ODONATE EJACULATE STRUCTURE AND MATING SYSTEMS

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This paper presents the results of a preliminary structural survey of the ejaculates of representatives of all major odonate taxonomic groupings. Members of the Zygoptera, Libellulidae and the Corduliidae transfer individual spermatozoa whilst males of the remaining taxa inseminate females with spermatodesms (aggregated sperm) and varying degrees of free spermatozoa. The distribution of spermatodesm use across the reviewed taxa shows a relationship with various aspects of male and female reproductive behaviour. A function for odonate spermatodesms based on this relationship is suggested, and preliminary evidence consistent with the major prediction from this hypothesis is provided.

INTRODUCTION

WAAGE's (1979) identification of the mechanism of sperm competition in *Calopteryx maculata* spawned a plethora of studies that sought to find mechanistic links between reproductive anatomy, reproductive physiology and the evolution of odonate reproductive behaviour (e.g. MILLER, 1982; FINCKE, 1984; McVEY & SMITTLE, 1984; SIVA-JOTHY, 1984; MICHIELS & DHONDT, 1988; SIVA-JOTHY & TSUBAKI, 1989; CORDERO & MILLER, 1992; HOOPER & SIVA-JOTHY, 1996). Almost all of the work on odonate reproduction prior to, and as a consequence of, WAAGE's (1979) study focused on three odonate families, the Coenagrionidae, the Calopterygidae and the Libellulidae. These odonates are ideal study organisms for behaviourists because they are relatively large, easy to mark and observe, tend to remain in the vicinity of the reproductive site and have complex reproductive behaviour. Many of the sperm competition studies of members of these groups revealed that these odonates did not transfer spermatophores (effectively parcels of spermatozoa: see MANN, 1984) but transferred free spermato-

zoa during copulation. However, anatomists had already examined the reproductive structures associated with ejaculation in other odonates and described "hairy" associations of sperm which they termed "Spermiozeugma" (BALLOWITZ, 1895; ASAHINA, 1954). These sperm aggregations occurred in taxonomic groups that were not amenable to field study, and consequently no link was made between their occurrence and the behaviour and ecology shown by the taxa that utilised them.

The phenomenon of aggregated sperm is not unique to the Odonata. During spermatogenesis in nearly all insects, a single spermatogonium produces the contents of a follicular cyst. The cyst subsequently undergoes one (often more) mitotic divisions followed by a meiotic division (DUMSER, 1980; SIVINSKI, 1984) to produce several protogametes. In any one cyst the protogametes are at the same stage of development (SNODGRASS, 1935; WIGGLESWORTH, 1965) and because of their relationship with the primary spermatogonium they all have a haploid share of the diploid genome of the spermatogonium. The number of sperm present in a cyst is dependent on the number of post stem-cell differentiation divisions (DE WILDE & DE LOOF, 1973) and in insects where many sperm are derived from a single cyst it is common for their heads to be embedded in a hyaline cap (GILMOUR, 1970; SIVINSKI, 1984; RETNAKAREN & PERCY, 1985): this effectively bonds together the spermatozoa produced from a single spermatogonium. However, in most cases the hyaline cap disappears before the sperm enter the vas deferens (see NABI & HARRISON, 1983). Only a few insects transfer "bonded sperm" to the female during insemination. These include the Orthoptera (CANTACUZENE, 1968), Hemiptera (NUR, 1962; ROBISON, 1966; FOLLIOT & MAILLET, 1970), Thysanura (BAWA, 1964; DALLAI & AFZELIUS, 1984), Coleoptera (MACKIE & WALKER, 1974; DALLAI & AFZELIUS, 1985), Lepidoptera (DRUMMOND, 1984) and Mantidae (LAWRENCE, 1991). Associations of bonded sperm resulting from single testicular follicles are here termed "spermatodesms".

In this paper I present the results of a preliminary survey of the nature of ejaculates in odonates and couple this with a survey of the fine-structure of the hyaline cap in those species with spermatodesms. I also examine the dynamics of spermatodesm storage and breakdown in the Aeshnidae, and finally formulate a working hypothesis for the taxonomic distribution of these structures within the Odonata and present preliminary observations consistent with a prediction from that hypothesis.

I have not attempted an exhaustive structural survey of all representatives at all taxonomic levels. This paper is intended to draw attention to major observable traits. Given that it is not exhaustive, it is likely that some of the generalisations I make will not be applicable to all odonates: my intention in this paper is to provoke investigation. I have not provided a detailed description of spermatodesm or spermatozoan fine structure. Instead I describe relevant detail and generalisations in order to illustrate differences. Students of ultra-structure, and its many important

Table I
A list of species, with dates and place of capture, used in the survey of odonate ejaculate and spermatodesm structure

Epiophlebitidae	Gomphidae	Cordulegasteridae	Aeshnidae	Corduliidae	Macromiidae	Petaluridae	Libellulidae	Platycnemididae	Coenagrionidae	Lesiidae	Calepterygidae
<i>Epiophlebia superstes</i> 1987 Sekigahara, Japan	<i>Davidius nanus</i> 1986-1988 Nagoya, Japan	<i>Anisogaster sieboldii</i> 1986-1988 Nagoya, Japan	<i>Aeshna grandis</i> 1984 Oxford, UK	<i>Cindalia aenea</i> 1987 Nagoya, Japan	<i>Epophthalmia elegans</i> 1987 Suzaka, Japan	<i>Tanypterus pryeri</i> 1986 Nagoya, Japan	<i>Orthemis concolorum</i> <i>O. albivittulum</i>	<i>Platycnemis longipennis</i> 1985 Comarque, France	<i>Enallagma cyathigerum</i> <i>Erythronia najas</i> 1984 Oxford, UK	<i>Letes sponsa</i> 1982-1994 Comarque, France	<i>Culex cornutus</i> 1987 Fukuoka, Japan
	<i>Trigomphus ogumai</i> 1984 Cevenne, France	<i>Cordulegaster boltonii</i> 1990 Cevenne, France	<i>Aeshna mixta</i> 1984 Oxford, UK							<i>Cylindrolestes bominensis</i> 1987 Nagoya, Japan	<i>Mnais pratinosa</i> 1986 Nagoya, Japan
	<i>Gomphus melanops</i> <i>Ictinogomphus pertinax</i> 1986-1988 Nagoya, Japan		<i>Anax parthenope</i> <i>Anax imperator</i> <i>Hemianax ephippiger</i>				<i>Sympetrum depressiusculum</i> <i>Sympetrum striolatum</i> <i>Crocothemis erythraea</i> 1982-1985 Comarque, France		<i>Erythronia viridulum</i> 1991 Cevenne, France		<i>Mnais contalis</i> 1995 Tsukuba, Japan
			<i>Anaxaetchna isonectes</i> <i>Aeshna affinis</i> 1985 Comarque, France				<i>Rhytilentis variegata</i> <i>Nannophya pygmaea</i> <i>Libellula quadrimaculata</i> 1986-1988 Nagoya, Japan				<i>Calepteryx splendens</i> 1990-1996 Cevenne, France
		<i>Coryphaesha</i> spp. 1978 Everglades, USA									<i>Calepteryx virgo</i> 1994-1996 Derby, UK
											<i>Mnais basilaris japonica</i> 1996 Okinawa, Japan

branches, should find enough information to conduct their own, more informed and detailed studies.

MATERIAL AND METHODS

COLLECTION OF SAMPLES. – Post-translocation males of the species listed in Table 1 were collected, and their secondary sperm storage organs removed after decapitating the male. Sperm in the seminal vesicle and/or vas deferens were then either prepared for examination with a compound microscope, or were fixed for examination with transmission electron microscopy (TEM).

PREPARATION FOR LIGHT MICROSCOPY. – An ejaculate collected from the male's secondary genitalia was placed in PBS solution on a glass slide. All preparations were made and examined within 1 hr of capture of the male. A simple assay for determining the strength of the physical bond between sperm heads in a spermatodesm was carried out by gently displacing the coverslip back and forth three times over the sample (5mm lateral displacement). The surface tension of the saline in which the spermatodesms were suspended provided the only downward force on the coverslip.

PREPARATION OF SAMPLES FOR TEM. – Material for TEM investigation was fixed in 2% glutaraldehyde in cacodylate buffer (pH 7.3, with 7% sucrose) at 4°C for 2-3 hr. Material was then washed in cacodylate buffer for 24 hr, post-fixed in 1% osmium tetroxide for 1hr, dehydrated through a 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 (STEMPAK & WARD, 1964) and lead citrate (REYNOLDS, 1963) they were viewed and photographed with a Phillips EM400T at 80KV.

SPERMATODESM BREAKDOWN RATES. – Male aeshnids were captured after sperm translocation and the primary and secondary genitalia sealed with New Skin (Germolene) to avoid further transfer or ejection. Males were then placed in a clear plastic container provided with a perch and damp tissue paper (to maintain a high humidity) maintained at 16°C and 12:12 L/D. Males were hand-fed with fourth instar *Locusta migratoria* twice a day (morning and afternoon). A sample of ejaculate from the secondary genitalia was collected every 24 hr for a total of 96hr after capture by first removing the NewSkin and then gently squeezing the first segment of the penis until it inflated. By maintaining pressure a sample of ejaculate would seep from the ejaculatory duct after a few seconds. The pressure was released after the sample was collected and the NewSkin reapplied.

Female aeshnids that had just finished copulation in the field were caught with their mates. The male was dissected to determine whether he had transferred an ejaculate (100% of samples). Females were kept in an identical environment and under the same feeding regimes as males. At the end of a given period females were sacrificed and the sperm in the sperm storage organs prepared for TEM examination. Only the most intact spermatodesms were examined, so the measure of breakdown rate estimated from this technique is an under-estimate.

ANATOMY OF EJACULATES IN DIFFERENT ODONATE TAXA

When viewed with a compound microscope two important structural divisions in the sperm and ejaculate morphology of odonates are apparent. First, the spermatozoa themselves are either short (~15 µm), im-motile, lanceolate structures (e.g. Fig. 1a), or they are long (~25 µm), mobile structures with a distinct head and flagellum (e.g. Fig. 1b). Table II shows the distribution of taxa across these two categories. The second pattern that is readily observable is that there are at least three patterns of spermatodesm usage: some taxa transfer structurally intact spermatodesms, some transfer single sperm as well as spermatodesms, whilst others never transfer spermatodesms. Table III shows the taxonomic distribution of

these spermatodesm usage patterns.

Table II
The taxonomic dichotomy of spermatozoan types in the Odonata

EPIOPHLEBIIDAE	Short, lanceolate, immotile sperm	Long, clearly flageolate, motile sperm
<p>The long (450 μm), filamentous spermatozoid of <i>Epiophlebia superstes</i> (Fig. 1c) consisted of two, longitudinally aligned hyaline strips (Fig. 2). The spermatozoa were angled into each strip. Longitudinal sections through the hyaline strips revealed that the sperm are embedded in rows running perpendicular to the long axis of each strip (Fig. 3) with 11.25 ± 1.6 ($n=10$ spermatodesms from 3 males; range = 9-12) sperm per row. Individual spermatozoa were extremely rare in ejaculates collected from male secondary genitalia and spermatodesms were difficult to physically disrupt.</p>	<p>Libellulidae Cordulidae Macromiidae</p>	<p>Epiophlebiidae Gomphidae Petaluridae (?) Cordulegastridae Aeshnidae Zygoptera</p>

GOMPHIDAE

Several gomphid species were examined, and all showed morphological differences in the gross anatomy of the spermatodesms (see Figs 1d, 1e) stored in the secondary genitalia. Despite the differences, they showed important similarities: the general form of the spermatodesm was shuttlecock-like, with a distinct hyaline cap into which the sperm heads were embedded. TEM examination revealed the cap had no obvious structure: the sperm heads were embedded in a homogeneous matrix (Figs 4, 5).

Careful preparation of light microscope samples revealed very few individual spermatozoa. The spermatodesms of some species (e.g. *Trigomphus ogumai* [Fig. 1d]) were much easier to disrupt than those of others (e.g. *Davidius nanus* [Fig. 1e]).

Table III

The taxonomic distribution of spermatodesm types in the Odonata. - [* denotes families which have more than one type (see text): the position in the table reflects the predominant pattern]

Intact spermatodesms	Non-intact spermatodesm	Individual spermatozoa only
Epiophlebiidae	Cordulegastridae	Zygoptera
Aeshnidae	Macromiidae	Corduliidae
Gomphidae*	Petaluridae	Libellulidae

CORDULEGASTRIDAE

The ejaculates collected from the secondary sperm stores of male cordulegastrids contained free sperm as well as spermatodesm fragments and apparently whole spermatodesms (Fig. 1f). The ultrastructure of the spermatodesms from *Anotogaster*

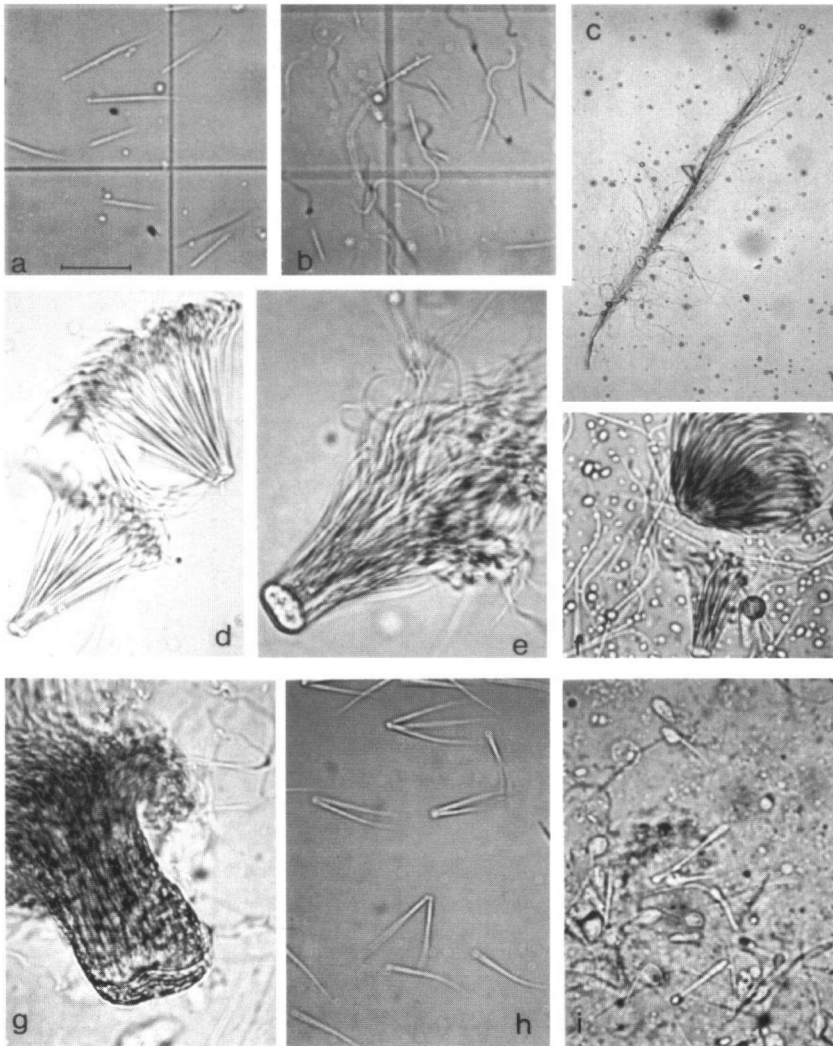


Fig. 1. Ejaculate structure in representative odonates [all to same scale (scale bar in Fig. 1a = 10 μ m. All photomicrographs phase-contrast except Fig. 1c.): (a) the typical spermatozoa of Libellulidae (sample = *Orthetrum cancellatum*); – (b) the typical spermatozoa of Zygoptera (sample = *Mnais pruinosa*); – (c) the spermatodesm of *Epiophlebia superstes*; – (d) the spermatodesm of *Trigomphus ogumai*; – (e) the spermatodesm of *Davidius nanus*; – (f) the spermatodesms and free sperm of *Anotogaster sieboldii*; – (g) the spermatodesm of *Aeshna mixta*; – (h) the ejaculate of *Epophthalmia elegans*; – (i) the ejaculate of *Tanypteryx pryeri*.

sieboldii was essentially the same as that found in the *Gomphidae* examined. The spermatodesms in ejaculates broke up easily upon physical disruption.

AESHNIDAE

All aeshnids examined produced a physically robust spermatodesm, typically with a discoid shaped hyaline cap when viewed with a compound microscope (Fig.1g). Despite careful collection, a few individual spermatozoa were always found in an ejaculate, but disintegrating spermatodesms were not observed at this stage.

The fine structure of the hyaline cap of the aeshnid spermatodesm showed a meshwork of tubes (~0.5µm in diameter), each attached to the head of a single spermatozoan (Fig. 6). It appears that the structural integrity of this type of spermatodesm is maintained by contact between these tubes, since there is no evident contact between spermatozoa in the structure. Aeshnid spermatodesms were very difficult to disrupt.

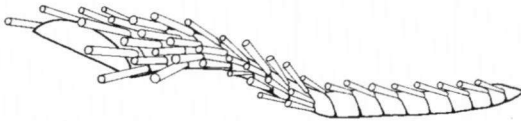


Fig. 3. A diagrammatic reconstruction of part of one of the proteinaceous strips that make up the hyaline cap in *Epiophlebia superstes* to show the structure of this unusual spermatodesm. The spermatozoa are embedded in rows perpendicular to the long axis of the spermatodesm. The first ten rows only show a single spermatozoan attached to the 'segment', and only the first 19 segments are illustrated.

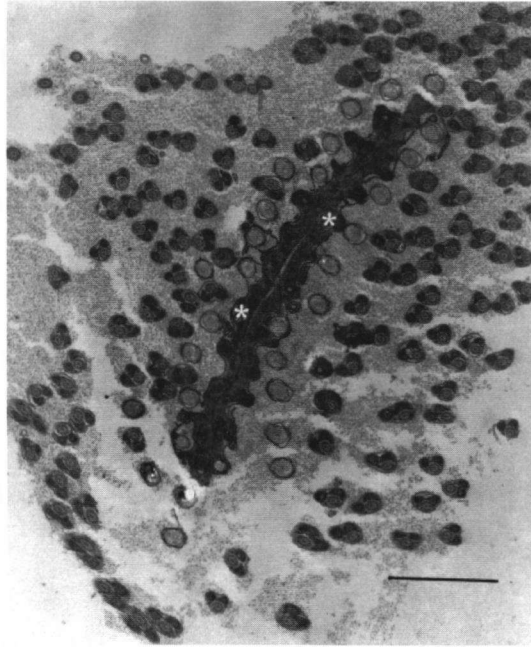


Fig. 2. A transverse TEM section through the spermatodesm of *Epiophlebia superstes*. The hyaline cap consists of two proteinaceous sheets (*) that run the length of the spermatodesm. Because the sperm are embedded in rows in these sheets, the sections through spermatozoa in the micrograph progress down the length of the spermatozoa as they get further out from the protein strips. – [Scale bar = 1 µm]

CORDULIIDAE

Cordulid males of the species examined never translocated spermatodesms or sperm aggregations. The spermatozoa in an ejaculate were indistinguishable from libellulid sperm when examined with a compound microscope and

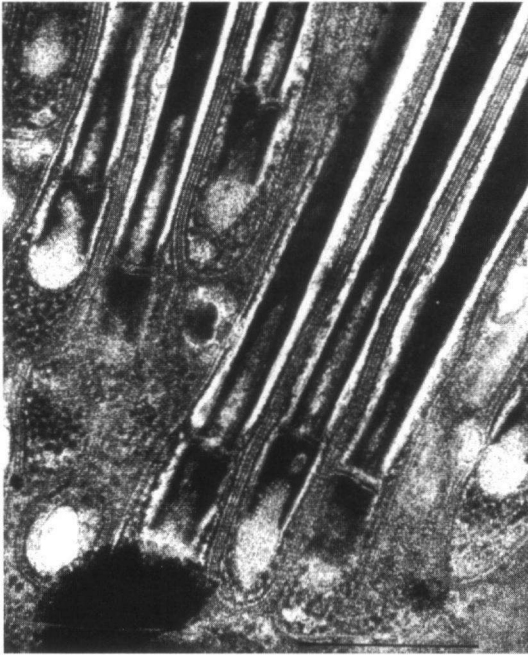


Fig. 4. A longitudinal section through the hyaline cap of a spermatodesm from *Trigomphus ogumai*, showing the sperm heads lying adjacent to each other, with no obvious membrane associations, or structural component to the cap. – [Scale bar = 1µm]

contained mainly motile individual spermatozoa, with a few small fragments of spermatodesms. Under light microscope examination these appeared to be atypical odonate sperm, with large swollen areas associated with some sperm and not others (Fig. 1i). TEM examination revealed these areas to be self-assembling lipid monolayers, and a range of other non-gametic structures. Moreover, the seminal fluid was structurally very complex with large numbers of electron opaque inclusions (Fig. 8). Examination of spermatodesms in the vas deferens revealed spermatodesms whose hyaline caps consisted of the association between adjacent tubes of material attached to each sperm head (Fig. 9). Moreover, the seminal fluid did not contain any electron opaque inclusions or electron lucent droplets containing lipid monolayers. The non gametic structures found in ejaculates are therefore either secreted into the seminal fluid before it is transferred to the secondary genitalia, or they result from the breakdown of the hyaline tubes attached to the sperm heads in the spermatodesm.

where suspended in a featureless seminal fluid (e.g. Fig. 1a).

MACROMIIDAE

The ejaculate of *Epo-phthalmia elegans* contains small aggregations of spermatozoa as well as individual sperm (Fig. 1h). There were rarely more than six spermatozoa in an aggregation. These sperm associations were very easy to disrupt, and TEM examination revealed that associations were maintained only by a small area of membrane contact between adjacent sperm heads (Fig. 7).

PETALURIDAE

The ejaculate in the secondary genitalia of the petalurid *Tanypteryx pryeri*

LIBELLULIDAE

All libellulids examined transferred individual spermatozoa in a featureless seminal fluid (Fig.1a). TEM examination of the sperm revealed that approximately half of the total length (15 μ m) of a spermatozoan is the head (nucleus and acrosome) and half the flagellum.

ZYGOPTERA

All Zygoptera examined transferred individual spermatozoa in a featureless seminal fluid (Fig.1b) with the exception of the lestids, where the seminal fluid appears 'granular' (similar to Fig. 1i) when examined with the light microscope. In all cases the sperm were about 25-30 μ m long with a distinct head, and sinusoidal flagellum.

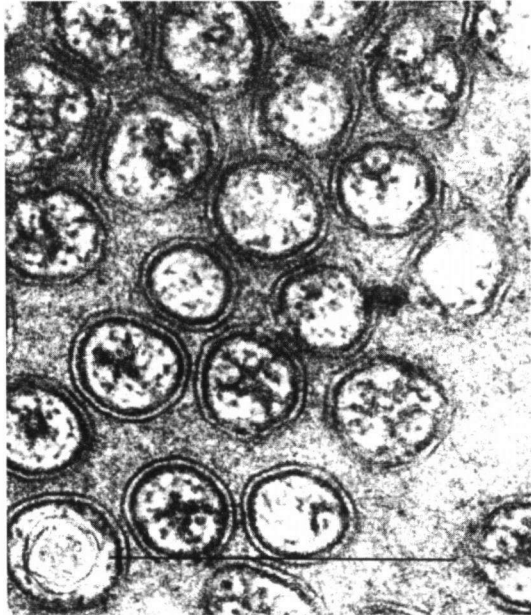


Fig. 5. A transverse section through the hyaline cap of a spermatodesm from *Trigomphus ogumai*. – [Scale bar = 1 μ m]

SPERMATODESM BREAKDOWN IN *AESHNA MIXTA*

MALE. – The fine structure of the spermatodesms from the secondary genitalia did not change in any identifiable way over the 96 hr sample period (n=4) and showed no discernible difference to the fine structure of spermatodesms removed from the primary genitalia of male *A. mixta*.

FEMALE. – Sperm removed from the sperm storage organs of sacrificed female *A. mixta* showed a marked structural change over 96 hr. Up to 48 hr after copulation spermatodesm structure remained relatively unchanged (n=2). 72 hr (n=3) after copulation spermatodesms were still intact, but the lipo-protein-like layers and droplets associated with the cap were absent. In some spermatodesms there were indications of the onset of breakdown in the cap: the interwoven tubules were beginning to separate from each other (Fig. 10d) and clumps of sperm, still attached by their heads, were beginning to break away from the spermatodesm. The structure of the cap tubules was not appreciably different from fresh spermatodesms. After 96 hr (n=2) all the spermatodesms showed considerable physical breakdown and myelin-forms began to appear in the seminal fluid (Fig. 10c). Moreover, aggregations



Fig. 6. A longitudinal section through the hyaline cap of a spermatodesm from *Aeshna mixta*, showing the sperm heads attached (open arrows) to the proteinaceous tubes (solid arrows) that make up the discoideal hyaline cap. – [N=nucleus, a=acrosome, *=inclusions in the seminal fluid]

pattern of spermatodesm usage revealed in this survey, those species that use spermatodesms are significantly larger in body length (49.0 ± 12.2 mm, $n=66$) than those that do not use spermatodesms (31.2 ± 8.9 mm, $n=88$) (MWU test; $U_{\text{prime}}=5117$; $Z=-8.11$, $P=0.0001$) (Fig. 11)

REPRODUCTIVE BEHAVIOUR AND SPERMATODESM USE

In this section I will consider those odonates that use free sperm separately from those that use spermatodesms in any form (i.e. I will consider the taxa in columns 1 and 2 from Table III together).

TAXA THAT UTILISE FREE SPERM

The reproductive behaviour of these taxa is extremely well-documented: consequently I will only briefly review the pertinent points of their reproductive biology in the context of sperm use.

of bacterioids (Fig. 10a) were commonly observed in the vicinity of regions of spermatodesm breakdown.

Females caught during oviposition always contained free sperm (i.e. they never contained spermatodesms alone). TEM examination of a female that contained only free sperm revealed that some of the free spermatozoa showed a loss of physical integrity (Fig. 10b) and that such spermatozoa were often associated with bacterioids (Fig. 10e).

BODY SIZE AND SPERMATODESM USE

Using the measurements of body length for Japanese odonates (ISHIDA, 1984), and assuming that each family predominantly shows the

Almost all studies of odonate reproductive biology have centred on zygopteran (e.g. PAJUNEN, 1966; WAAGE, 1979, 1984; SIVA-JOTHY & TSUBAKI, 1989; MARDEN & WAAGE, 1990; GIBBONS & PAIN, 1992; SIVA-JOTHY et al., 1994; SIVA-JOTHY & HOOPER, 1995; PLAISTOW & SIVA-JOTHY, 1996) or libellulid (e.g. JACOBS, 1955; MILLER, 1983; TSUBAKI & ONO, 1987; KOENIG, 1991; TSUBAKI et al., 1994) taxa. The proximate reason is probably that species in these taxa tend to have their reproductive activity centred around a spatially restricted resource. Reproductively active females converge on restricted, predictable sites, and males are territorial at those sites.

Nearly all of what we know about the mechanistic basis of paternity assurance and repro-

ductive physiology in relation to the evolution of odonate mating systems is restricted to studies of these taxa. The coenagrionids have also been an important group in these studies (FINCKE, 1984; MILLER & MILLER, 1981; MILLER, 1982, 1987; CORDERO, 1990; CORDERO & MILLER, 1992), and although not 'classic' resource defence polygamists, they too utilise spatially restricted resources, where males and sexually receptive females aggregate.

Relatively few studies have been carried out on the mating system of cordulids, but those that have show that this group is similar to libellulids in many respects. Males defend and patrol distinct spatio-temporal territories which females visit to copulate and oviposit (KORMONDY, 1959; TAKETO, 1959, 1960a; UBUKATA, 1975, 1984; SAKAGAMI et al., 1974; HILTON, 1983; ROWE, 1987).

Although males are territorial, they show several important departures from the typical libellulid mating system (which the cordulid system superficially resembles). First, when a male and female enter tandem, they leave the male's territory

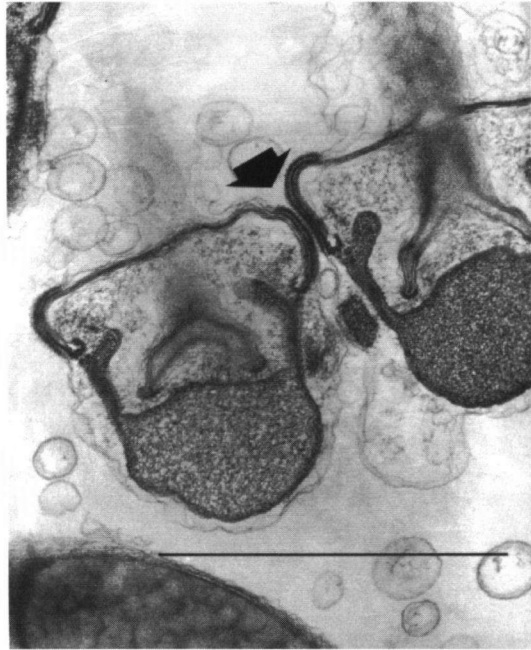


Fig. 7. An oblique longitudinal section through an association between two spermatozoa in the ejaculate of *Epophthalmia elegans*. The nuclei have only just been incorporated in the section and can be seen as 'shadows'. The inverted bell-shaped structures are the acrosomal heads. The only physical association between the heads is a region of the membrane on the widest part of the sperm head that shows clear membrane specialisation (solid arrow). – [Scale bar = 1µm]

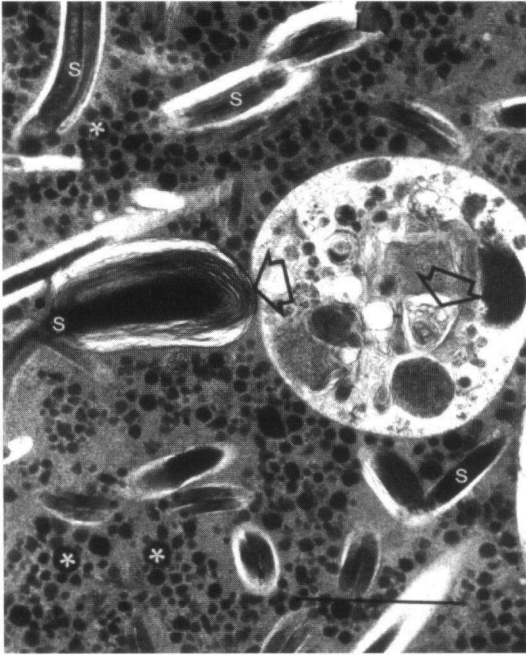


Fig. 8. A section through the ejaculate of *Tanypteryx pryeri* showing the complex nature of the seminal fluid, with electron opaque inclusions (*) and self-assembling lipid monolayers (arrows). Sperm nuclei and flagellae are indicated with an 's'. - [Scale bar = 1 μ m]

is true resource defence polygyny (sensu EMLÉN & ORING, 1977) is not clear. When a male captures a female at a larval site the pair settle nearby, or at the point of capture (TAKETO, 1960b; WOLFE, 1953; ROWE, 1987). It appears that males will enter tandem with females released nearby (DUNKLE, 1981) and that females may be able to avoid interactions with males by ovipositing at a different time of day (CLEMENT & MEYER, 1980). Oviposition is unguarded (TILLYARD, 1909; SVIHLA, 1960; CLEMENT & MEYER, 1980; DUNKLE, 1981; ROWE, 1987) but, in those descriptions that mention it, appears to follow copulation (WOLFE, 1953; TAKETO, 1960b; ROWE, 1987).

In short these 'free-sperm' utilising taxa have the following common mating system features. The sexes come together at spatially predictable and often restricted (i.e. defensible) resources. Copulation tends to occur at those resources, and oviposition usually follows copulation immediately.

and copulate some distance away. Second, males rarely guard their mates after copulation. Third, ovipositing females are cryptic and appear to have an effective rejection display. These facts make it difficult to assess if female cordulids oviposit after copulation: most studies are not explicit about this, but SAKAGAMI et al., (1974) report that 9/14 copulations in *Hemicordulia ogasawarensis* were followed by oviposition. Whether this is a general pattern is unclear.

Descriptions of petalurid reproductive behaviour suggest that males are also associated with a spatially restricted resource (the bogs in which the larvae develop) (TAKETO, 1960b; CLEMENT & MEYER, 1980; ROWE, 1987). Whether this

TAXA THAT UTILISE
SPERMATODESMS

In contrast to taxa that transfer free spermatozoa during copula, the reproductive biology of spermatodesm-utilising groups is relatively poorly understood. The proximate reason is almost certainly that these taxa tend to be wide-ranging, free-flying species, whose reproductive activity is not restricted to predictable, localised sites. This makes these species hard to mark and observe and presumably accounts for their poor representation in the literature. I will deal with each family in turn, reviewing or describing their reproductive biology, before summarising the information at the end of this section.

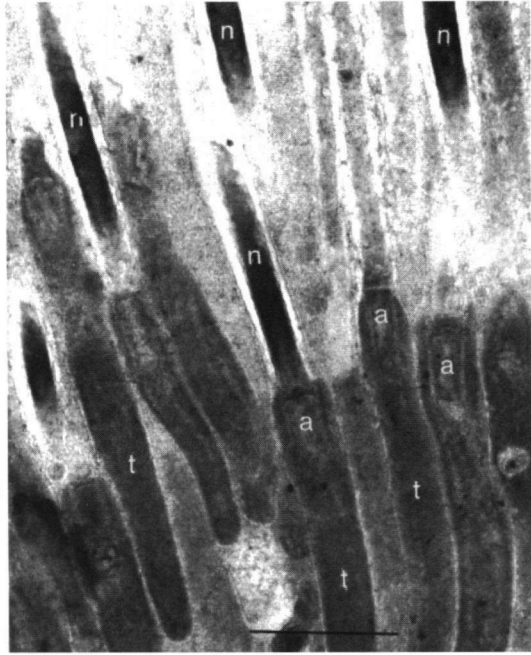


Fig. 9. A longitudinal section through the hyaline cap of a spermatodesm in the vas deferens of *Tanypteryx pryeri* showing the sperm heads (n=nucleus, a=acrosome) embedded in the short, longitudinally arranged proteinaceous tubes (t) that make up the cap. – [Scale bar = 1µm]

EPIOPHLEBIIDAE

This description of the reproductive behaviour draws from observations made at a site near Sekigahara, Japan (Y. Tsubaki, pers. comm. and pers. obs.).

Males patrolled in a sun-lit clearing above a precipitous mountain stream flowing through dense coniferous forest from mid-day onwards. The stream was spring-fed and fast flowing: along the margins were numerous aquatic and semi-aquatic angiosperms and males alternated between patrolling high above the stream, and searching amongst these water-side plants. Females arrived singly from the direction of the forest and began ovipositing immediately without a guardian male. These oviposition bouts were extremely cryptic, and males often flew over females without detecting them. In general, males only caught females that were in flight (usually only females that flew conspicuously above the stream without any apparent prior oviposition, or occasionally, females at the end of a bout of oviposition). There was no apparent courtship, and once the male had grasped the female in tandem, they flew into the tree-tops around the stream where copulation occurred.

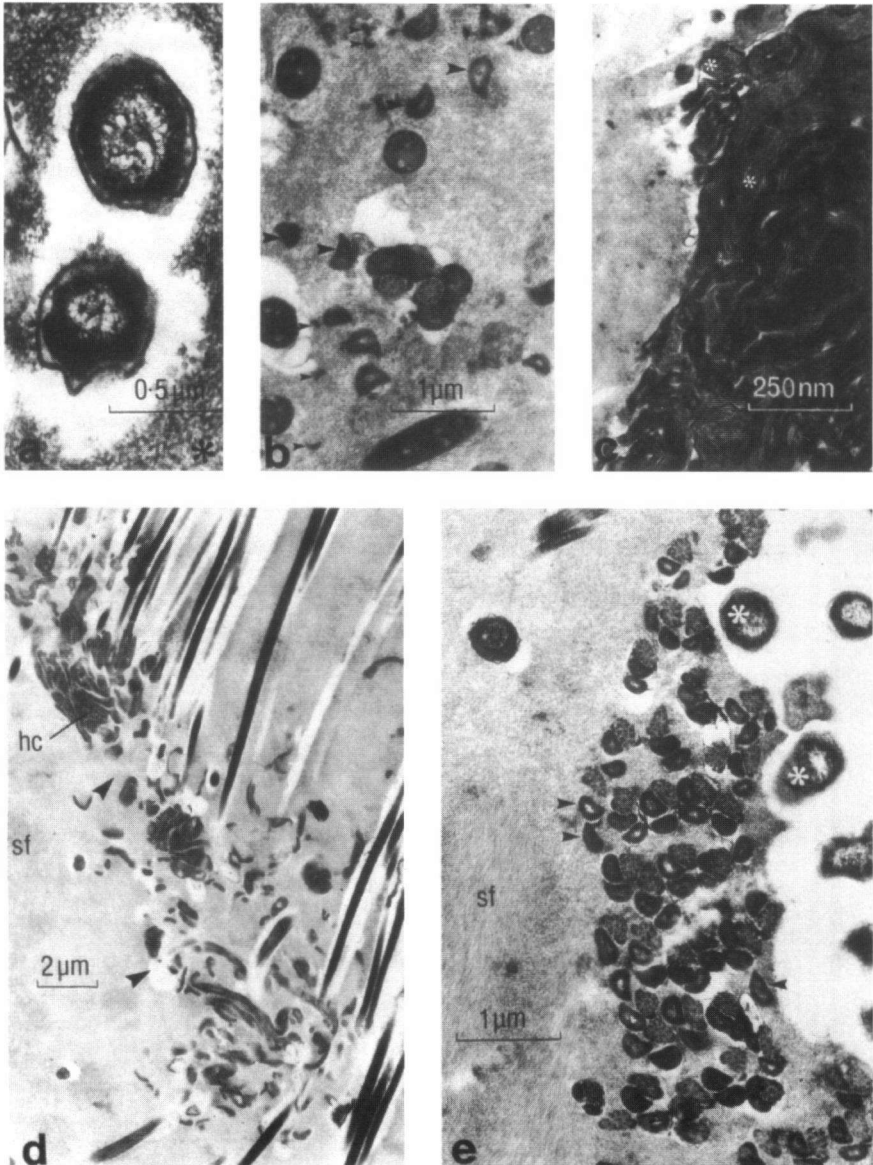


Fig. 10. Sections through ejaculates from the sperm storage organs of female *Aeshna mixta*. (a) Two bacteroid organisms within in the seminal fluid (*); – (b) evidence of spermatozoan breakdown within the female's sperm storage organs. The arrows show sections through dissociated mitochondrial derivatives normally attached to the flagellum of spermatozoa; – (c) self-assembling lipid monolayers (*) in the seminal fluid; – (d) a longitudinal section through a disintegrating hyaline cap (hc): the arrows show the sites of breakdown. sf=seminal fluid; – (e) a section showing spermatozoan breakdown (arrows) in the vicinity of bacteroids (*) within the seminal fluid (sf) in the female's sperm storage organs.

After copula, the male flew straight back to the stream and recommenced patrolling, whilst the female flew into the forest.

Observations from a site near Tokyo (OKAZAWA & UBUKATA, 1978) suggested that ovipositing females avoided males by ovipositing after male patrolling behaviour had ceased.

My observations suggest that females either arrived at the stream to copulate or to oviposit, and rarely did both. I never observed a female arrive, copulate and then commence oviposition. Females were not guarded during oviposition, and copulation was cryptic (i.e. away from the oviposition and encounter site(s) and in a place hidden from easy view).

GOMPHIDAE

Gomphids that utilise riverine habitats have a characteristic mating system. Females oviposit alone (ARAI, 1981; MILLER & MILLER, 1985; pers. obs.), and in some species are ignored by reproductively active males (MILLER & MILLER, 1985). There are some indica-

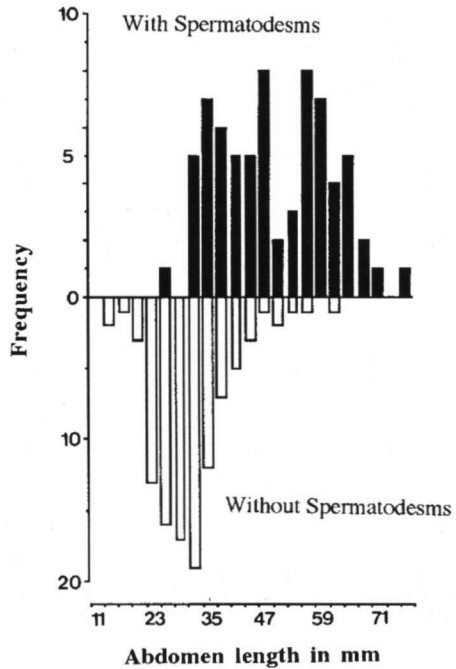


Fig. 11. The frequency distribution of body sizes of Japanese odonates with respect to the pattern of sperm use during insemination.

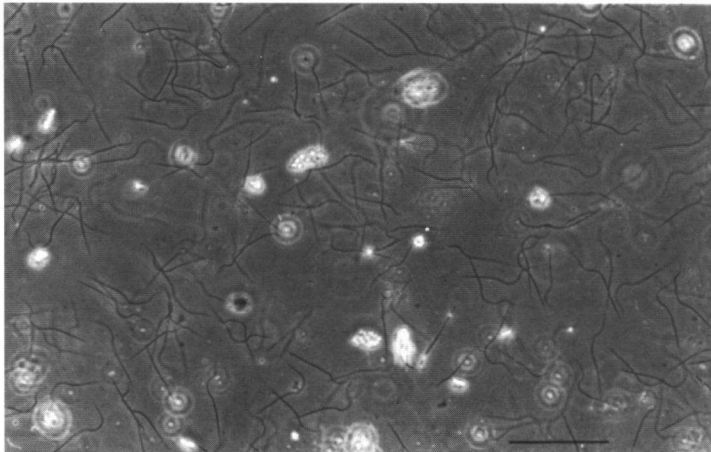


Fig. 12. A phase-contrast micrograph of the ejaculate of *Ictinogomphus pertinax* showing free spermatozoa. — [Scale bar = 15µm]

tions of temporal separation between oviposition behaviour and male reproductive activity (AIDA 1973; ARAI, 1981; pers. obs. of *Onychogomphus uncatatus*). Males tend to patrol long stretches of river but do not show territoriality (e.g. they do not defend a distinct boundary) despite the fact that there are frequent intraspecific clashes. Male sexual behaviour usually occurs within clearly defined temporal limits (MILLER & MILLER, 1985; EDA, 1958). When a male captures a female the pair immediately depart from the river (MILLER & MILLER, 1985) and the male returns some time later on his own (EDA, 1958; MILLER & MILLER, 1985). Females do not appear to oviposit after copulation (pers. obs.).

AESHNIDAE

The aeshnids are perhaps the best studied of all the spermatodesm-utilising species. There are several different patterns of reproductive behaviour in this family. The males of many species patrol riverine habitats (e.g. KAISER, 1974a, 1974b; ROWE, 1987) searching for sexually receptive females. Females can avoid male interference by ovipositing at times of day when males do not patrol (KAISER, 1974a, 1985) or by signalling unreceptivity (JURZITZA, 1967). When pairs enter copula they fly high into surrounding trees (KAISER, 1974a; JURZITZA, 1967) after which the female may, or may not, return to oviposit (KAISER, 1974a; pers. obs. of *Aeshna eremita* and *A. palmata*).

As well as the behaviours described above, some species appear to defend a territory from a central perch (ROWE, 1987), and some species of aeshnid show strong contact guarding after copulation (HADRYN et al., 1992, 1993; ROWE, 1987) during which the female lays eggs.

MACROMIIDAE

Macromid males patrol riverine habitats for females (d'AGUILAR et al., 1985). Observations of *Epopthalmia elegans* near Nagoya, Japan in 1986 and 1987, and *Macromia splendens* in the Cevenne, France in 1994, revealed that females oviposited unaccompanied by males, and that once in tandem pairs flew high into the surrounding tree tops. Whether females oviposited after copulation was not observed, but males returned to patrol without any evidence of the presence of their recent mate.

CORDULEGASTRIDAE

These notes are based on personal observations of the behaviour of *Anatogaster sieboldii* at mount Sanage Japan 1987-1988.

Males patrolled beats up and down stretches of suitable streams (i.e. suitable larval/oviposition habitats), and inspected suitable oviposition sites for the pres-

ence of females. Females oviposited alone and, if found by a male, rapidly left the oviposition site in the direction of the nearest dense vegetation (n=5). The start of copulation was never observed, but two tandem pairs were seen to fly high into the tree-tops near a suitable stream, and were lost from view. These observations suggest that mate encounter is relatively rare, and once it occurs the post-encounter behaviour is cryptic.

SUMMARY OF GENERAL PATTERNS IN REPRODUCTIVE BEHAVIOUR IN SPERMATODESM-UTILISING ODONATES

In general, spermatodesm-utilising taxa show little post-copulatory guarding, tend to copulate well-away from the encounter/oviposition site, rarely appear to commence oviposition after copulation, and often show temporal separation between oviposition and copulation. These taxa also tend to utilise reproductive resources that are distributed in an undefendable pattern within the environment. Consequently their reproductive behaviour is 'cryptic', perhaps explaining why these groups are so poorly studied.

THE WORKING HYPOTHESIS

There are exceptions to the general classification presented above. For example, there are a number of aeshnid species which break the general mating system trends observed in other spermatodesm-utilising groups (see HADRYN et al., 1992, 1993).

Notwithstanding these anomalies, a pattern that emerges from the information I have presented is that species that utilise non-defendable reproductive resources and have, what I call 'cryptic' reproductive behaviour, tend to use spermatodesms. On the other hand territorial taxa, or taxa that are restricted to predictable resources where receptive females and males congregate, tend to utilise free sperm. If these two variables are causally related we would expect any member of a spermatodesm-utilising taxa that utilised a defendable resource to utilise free sperm, even if its riverine relatives did not.

An obvious exception to the general mating system pattern shown by gomphids is the highly territorial, pond utilising genus *Ictinogomphus*. My hypothesis predicts that, unlike its riverine relatives, this genus should utilise free sperm.

In 1988 I collected three territorial male *Ictinogomphus pertinax* from ponds near Nagoya University, Japan, and sampled sperm from their primary genitalia and from the seminal vesicle of one male that had translocated sperm. As predicted, this species has atypical gomphid ejaculate morphology in that males transfer free sperm to females during copula (Fig. 12). In addition to my observations, TEMBHARE & THAKARE (1982) report that the "sperm bundles" of *I. rapax* break down in the seminal vesicle of this species.

DISCUSSION

There are several hypotheses regarding the function of "grouped" sperm in other taxa.

SEXUALLY SELECTED SPERM AGGREGATIONS. – This hypothesis proposes that the grouped sperm are better able to reach the site of fertilisation than single sperm. In other words, the "metazoan" grouped sperm outcompete single sperm (BALLOWITZ, 1895; FRETTER, 1953; NUR, 1962, COHEN, 1975).

SPERM AGGREGATIONS PROVIDE DIRECT BENEFITS FOR FEMALES. – This hypothesis proposes that the hyaline cap provides a nutrient investment by the male which increases female fitness (HANSON et al., 1952; FAIN-MAUREL, 1966; BRELAND & SIMMONS, 1970; MACKIE & WALKER, 1973). This hypothesis does not exclude any of the other hypotheses in this section.

SPERMATODESMS AVOID TOXINS SELECTED BY INTRASEXUAL SELECTION. – If males transfer general toxins that incapacitate sperm stored in the female, then they need to protect the sperm in their own ejaculate. One way they could do this would be to transfer a labile toxin that only incapacitated free sperm, but lost its activity before the spermatodesms in the self-ejaculate broke down. This mechanism is more likely to be important in insects (and invertebrates) where there is almost no ability to recognise allogeneic antigens (LACKIE, 1983).

SPERM AGGREGATIONS INCREASE THE LONGEVITY OF THE SPERM. – This hypothesis proposes that the hyaline cap (i) provides nutrients either directly, via its breakdown, or indirectly, via the seminal fluid, for the sperm (CANTACUZENE, 1968; MACKIE & WALKER, 1974) or (ii) protects the delicate acrosome (PHILLIPS, 1970; BEDFORD et al., 1984).

CANTACUZENE (1968) has carried out a detailed structural, histochemical and enzymatic study of the spermatodesms of Orthoptera. She suggests a trophic function for the mucoproteic cap of which bears a great deal of structural similarity to the cap of aeshnids. There is some evidence supporting the idea that the orthopteran cap breaks down extra-spermatodesm compounds and provides the sperm with nutrients. It is interesting that the spermatodesms of Orthoptera can remain structurally constant for up to 7 months in the male, but begin to break down soon after transfer to the female. Many analogies can be drawn between this system and that of aeshnids.

Spermatodesm usage appears to occur in odonate taxa where the males do not defend oviposition resources, do not mate at the oviposition resource, and do not guard their mates after copulation; females of these taxa do not, in general, oviposit immediately after copulation. These taxa tend to be large odonates that utilise riverine habitats.

By contrast, the males of free-sperm utilising taxa are mainly resource defence, or scramble competition, polygynists (EMLEN & ORING, 1977). Males show

strong mate-guarding and females oviposit immediately after copulation. These species tend to be small odonates that utilise spatially restricted resources.

It appears that larger (and therefore rarer (ELTON, 1927; PIANKA, 1970)) odonates, that utilise non-defendable resources tend to use spermatodesms. Because of their size, relative rarity, and the structure of the habitat they utilise for reproduction, males will tend to have a relatively low, unpredictable encounter rates with females. By contrast small, numerous odonates that aggregate at defendable resources tend to use free-sperm. Resource holding males in these taxa will tend to have very high, predictable encounter rates with females.

I propose that the ecological aspects of the mating systems of odonates I have outlined above are causally linked with the patterns of sperm usage in this ancient order. Interestingly, the resource-defending *Ictinogomphus pertinax* utilises free-sperm, despite the fact that the riverine members of its family examined in this study utilise spermatodesms. Examining the nature of the ejaculates of more representatives of spermatodesm-utilising families in the context of their reproductive ecology will, no doubt, shed more light onto this intriguing dichotomy.

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