# MORPHOLOGY OF MALE AND FEMALE GENITALIA IN *SYMPETRUM DANAE* (SULZER), WITH SPECIAL REFERENCE TO THE MECHANISM OF SPERM REMOVAL DURING COPULATION (ANISOPTERA: LIBELLULIDAE)

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The female genitalia are characterized by a spherical bursa copulatrix and 2 separate, narrow spermathecae. The fourth segment of the penis is characterized by 2 long cornua, 2 inflatable inner lobes, 2 flap-like lateral lobes and a tube-like apical lobe. Sperm remains were found on most parts of the penis of individuals from interrupted copulas, especially on the apical and inner lobes. — Observations of copulatory movement, supplemented by a reconstruction of the coitus, suggest that sperm may be removed and ejaculated during a single type of movement, throughout the major part of copulation.

## INTRODUCTION

Male dragonflies can use their copulatory organ not only for sperm transfer, but also for displacement of rival sperm in the female's storage organs in favour of their own, a kind of sperm competition that seems widespread in Zygoptera (WAAGE, 1979, 1982, 1984, 1986a; MILLER & MILLER, 1981; FINCKE, 1984; MILLER, 1987a, b) and libellulid Anisoptera (McVEY and SMITTLE 1984; SIVA-JOTHY, 1984, 1987a; WAAGE, 1984, 1986b; MICHIELS & DHONDT, 1988). By doing so, males ensure for themselves a high degree of precedence in fertilizing the batch laid immediately after copulation. Physical sperm removal as found in Odonata is a unique mechanism of sperm competition in the animal kingdom (SMITH, 1984) and is associated with the evolution of a specialized penis. In Zygoptera the presence of hook-, scoop- or flap-like structures on the rigid penis is related to this function (WAAGE, 1986a). In libellulids, the penis is provided with an internal liquid-filled cavity. Contraction

of muscles attached to the first segment raises the internal pressure and erects the penis (PFAU, 1971). This process inflates flexible structures in the 4th, apical segment and everts rigid appendages.

The female sperm storage organs of libellulids usually consist of a bursa copulatrix, connected with the dorsal side of the vagina via the bursa communis — a slit-like valve — and two spermathecae, connected with the lumen of the bursa copulatrix via one common, or two separate, ducts (see detailed description in SIVA-JOTHY, 1987b). Comparison of the inflated penis with the internal morphology of the female genital tract revealed similarities which have been used to predict displacement mechanisms (MILLER, 1981, 1982, 1984; SIVA-JOTHY, 1984; WAAGE, 1984, 1986).

In the small libellulid *Sympetrum danae* we previously demonstrated that males remove 40-80% of the rival sperm during copulation, yielding 95% precedence in immediate fertilization (MICHIELS & DHONDT, 1988a). Sperm removal was especially marked in the first 5 minutes of the copulation and was followed by ejaculation. In this paper I describe male and female genitalia and discuss the possible way in which sperm removal is brought about. The male genitalia of *S. danae* were previously described in detail by SCHMIDT (1915), but based on deformed, fixed material, and by WARINGER (1984), who described the uninflated penis only.

## METHODS

All individuals were collected or observed in August-October 1986-87 near Turnhout (Belgium) under natural and semi-natural conditions (see for details MICHIELS & DHONDT, 1988, 1989).

The male secondary genitalia were dissected from freshly killed individuals. The penis was inflated by squeezing its basal segment with blunt forceps, and drawn at different stages of erection, using a WILD dissection microscope with drawing mirror (6-50x). The presence and location of remains of sperm on the penis was noted in males from interrupted and completed copulations and unmated males. For scanning electron microscopy three inflated penes were plunged into liquid nitrogen, freezedried and coated with carbon. SEM photographs were made with a JEOL 733 Super Probe and later transformed to line-drawings. The female genitalia were dissected after preservation in 70% alcohol for approx. two weeks, facilitating removal of surrounding tissues. I noted the presence and colour of the sperm in the bursa copulatrix and spermathecae. MICHIELS & DHONDT (1988) indicated that fresh sperm could be recognized as more yellowish relative to the whitish old sperm (after preservation). All individuals are identical to those used by MICHIELS & DHONDT (1988) for estimating sperm displacement.

Occasional observations of copulatory movements were also made. If the temperature declined during a copulation, e.g. when the sun was hidden behind a cloud, it was possible to approach mating pairs closely enough to observe the connection of the genitalia with a pocket-lens (10x). However, we failed to collect copulating pairs without disconnection of the genitalia: both freezing with cryogenic gas and decapitation invariably ended copulation within seconds.

Terminology was adopted from WAAGE (1986b) for the male genitalia and from SIVA-JOTHY (1987b) for the female genitalia.

## MORPHOLOGY OF FEMALE SPERM-STORAGE ORGANS

The bursa copulatrix is spherical and dorso-ventrally flattened, depending on the amount of sperm stored (Fig. 1). The two spermathecae are separately attached to the caudal end of the bursa. They are long narrow tubes with a club-like end. Sperm masses could readily be recognized through the transparent wall of both storage organs. Under the bursa communis



Fig. 1: Lateral (above) and dorsal (below) view on the female genital complex of *S. danae:* vl = valvular lamina; — v = vagina; — avp = anterior vaginal pouch; — bc = bursa copulatrix; — st = spermathecae; — co = common oviduct; — ovd = oviduct; — mcp = medial cuticular plate; — 8ster = 8th sternite. — Anterior = right, — dorsal = up in the lateral view.]
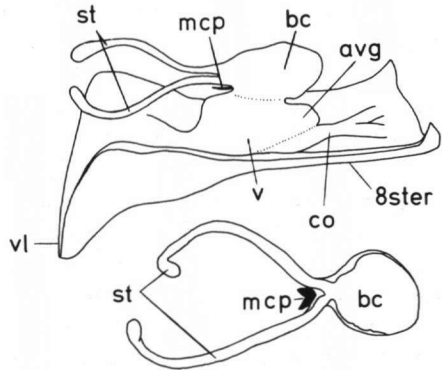
a wide anterior vaginal pouch extends anteriorly and above the common oviduct. The medial cuticular plate is slightly forked. Dissection of females from interrupted mating pairs indicated that yellowish, fresh sperm was first deposited in the spermathecae and in the lumen of the bursa copulatrix near the connection of the spermathecae.

## POSITION AND INFLATION OF THE PENIS

The position of the uninflated penis in the 2nd abdominal segment of the male is illustrated in Figure 2. The hamuli (h) function as "clothes-pegs"
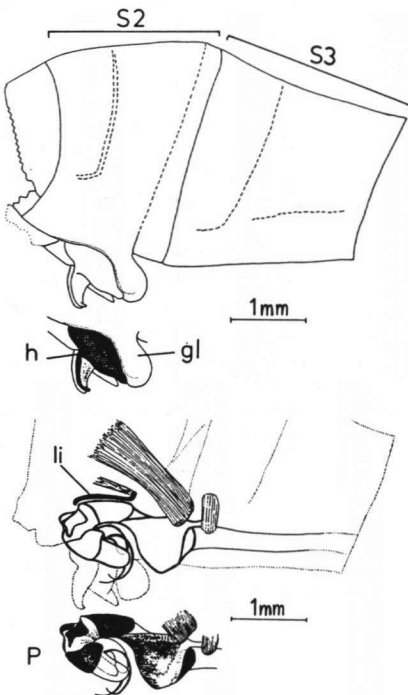


Fig. 2: Lateral view of the 2 nd and 3rd abdominal segments of a male *S. danae*, showing the position of the penis (P): S2, S3 = second and third abdominal segment; — h = hamuli; — gl = genital lobe; — li = ligula. Above: external, — below: internal, — anterior = left, — dorsal = up.

which hold the valvular lamina or subgenital plate of the female during copu-
lation. When uninflated the penis (P) is retracted between them, and rests in the
scoop-formed ligula (li). During copulation, contraction of the muscles attached
to the ligula moves the penis in a ventral and caudal direction (PFAU, 1971).
Gradual compression of the slightly chitinized base segment (s1, Fig. 3a), reveals
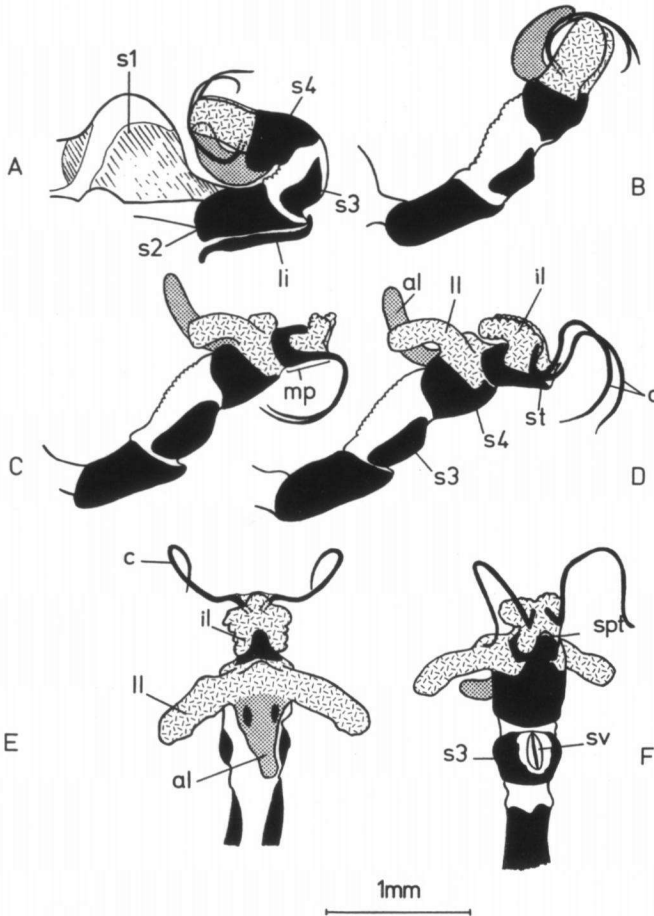three steps of inflation. In the first step, the penis is extended in anterio-ventral



Fig. 3: Gradual inflation of the penis of *S. danae:* uninflated (A), penetration phase (B), expansion
phase (C) and ejaculation phase (D, E, F): s1-s4 = penis segments 1-4; li = ligula; — mp = medial
process; — al = apical lobe; — ll = lateral lobe; — il = inner lobe; — st. = sperm tube; — c = cornua;
— sv = sperm valve. — [Lateral view with anterior = right and dorsal = down in A-D. Ventral view
in E, dorsal view in F with anterior = up. Rigid zones, presumed to be strongly chitinized, are black.
Structures on the 4th segment were hatched and dotted for clarification of positional changes.]

direction (Fig. 3b). If, however, combined with the normal action of the ligula, it would point more caudally than appears from Figure 3. I shall call this stage the "penetration phase". Under increased pressure, the structures on the fourth segment are suddenly flung open (Fig. 3c, "expansion phase"). Only at maximal pressure are all structures fully inflated (Fig. 3d-f). After a few seconds of maintained pressure, sperm release commences. Ejaculation occurs — in vitro — only in this stage, which therefore will be called the "ejaculation phase". PFAU (1971) indicated that ejaculation occurs during decompression of the fourth penis segment. This could not be observed in vitro, but it is possible that under natural conditions, with rhythmical compression-decompression (see below), sperm is ejected in the decompression phase of each cycle.

## PENIS MORPHOLOGY

The first segment (s1) is globular and not as rigid as the other segments. Posteriorly it is attached to the sternite of the 3rd segment. Except for a narrow ventral zone, the second segment (s2) is strongly chitinized, enabling it to be gripped by the ligula. The third segment (s3) has only a small chitinous plate on its dorsal side. Ventrally, its pliant surface is cross-wrinkled. The sperm valve (sv) through which sperm is injected during pre-copulatory intra-male sperm transfer, is located dorsally.

The fourth segment (s4) consists of a rigid central part, bearing one apical lobe (al), two lateral lobes (ll) and one medial process (mp), which in turn comprises two inner lobes (il), two cornua (c) and the sperm tube (spt).

The centrally placed, tube-like apical lobe (al) protrudes caudally and is rather rigid. During inflation its volume remains seemingly unchanged. It is densely covered with proximally oriented spines whose length is about 10 $\mu$m (Fig. 4b). At its base it has two dark, probably more chitinized zones (Fig. 3e). The lateral lobes (ll)
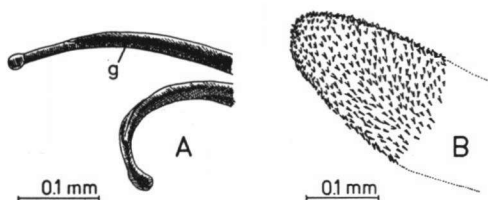


Fig. 4: Details of appendages on the 4th penis segment of *S. danae*: (A) top of both cornua; g = groove; — (B) top half of the apical lobe, covered with proximally oriented spines.

are blade-like, rigid and smooth with a constant volume. During inflation, they open in a lateral direction. The medial process (mp) becomes visible only after expansion and appears as if it were a small, partly chitinized, fifth apical segment. The cornua (c) protrude already in the deflated penis, but they are brought out strongly in a dorsal direction during inflation. A groove runs along their inner side. It is deep at the base, shallows along its course and ends before the

club-formed apex (Fig. 4a). The cornua surface is smooth: apical "hooks", as described by WARINGER (1984), were never found. The inner lobes are weak and sac-like and their volume greatly increases in the "ejaculation phase". At maximal inflation, they are directed towards the base of the medial process. Their surface is covered with thin spines ($<$ 10 $\mu$m), without clear orientation when semi-inflated. This does not, however, preclude the possibility that some orientation is present at maximal inflation, when no good microscopical preparation of the inner lobes could be made. From the sperm tube (spt) at the dorsal side of the medial process, sperm is ejected between the bases of the cornua.

## LOCATION OF SPERM REMAINS

Most males had no, or few, sperm remains attached to their penes before copulation (Tab. I). It is likely that those with sperm remains had copulated previously. During and after copulation masses of sperm accumulate especially around the base of the apical lobe and on the surface of the lateral lobes. Remains were also found in a shallow median depression of the chitinous plate on the dorsum of the 4th segment, on the ventral side of the 3rd segment, and on the medial process, including the cornua.

Table I

Presence of sperm remains on the penis of *S. danae* males before, during and after copulation. Per penis part, the number of individuals with sperm remains on that part, is given.

| Group | Pre-copula | Interrupted copula | Post-copula |
|---|---|---|---|
| Number of individuals | 13 | 18 | 5 |
| *No sperm remains* | 8 | 1 | 0 |
| *Sperm remains observed on* apical lobe | | | |
| — a bit | 3 | 1 | 0 |
| — a lot | 2 | 13 | 4 |
| lateral lobes | 2 | 14 | 3 |
| medial process & cornua | 1 | 0 | 2 |
| dorsum 4th segm. | 1 | 5 | 2 |
| ventrum 3rd segm. | 0 | 2 | 0 |

During external examination of copulating individuals I frequently observed clusters of "sperm" (once including two eggs) stuck in the stiff hairs on the genital lobes on each side of the 3rd abdominal segment of the male (cf. Fig. 2).

## COPULATORY MOVEMENTS

In addition to occasional observations, five copulating pairs were observed in detail, two of which were followed throughout their copulation (Fig. 5). During copulation no different stages could be recognized. After an initial resting pause of varying length, the main activity was a rhythmical movement of the 2nd and 3rd abodminal segments of the male, interrupted by resting pauses (Fig. 5). It may have led to compression of the first penis segment and consequently inflated the penis. In one copulation the female was seen to make movements with her posterior abdominal segments during a small part of the copulation. When copulating, the male also repeatedly made a characteristic movement with his wings. It started with a backward movement of the wings until they were completely folded above the abdomen, as in freshly emerged Anisoptera. In the next step, he moved them maximally forward, as sometimes seen in perched libellulid males. Finally it



Fig. 5: Sequence of copulatory movements in two copulation-wheels (A-B, 16 August 1986) with indication of shade temperature . — [0—0 = onset and end of copulation; cross-lines = wing flips (see text); — points below line = flights; — upper thick line = continuous movements of the 2nd and 3rd abdominal segments of the male.]

ended in the normal, slightly forward oriented position. Such "wing-flips" occurred seemingly more in the beginning of copulation (Fig. 5). Males from not-copulating perched tandem-pairs were also regularly seen to exhibit wing-flips, which may suggest that they are not a different kind of copulatory movement. The link with sexual activity though was firm as wing-flips were never observed in solitary males.

As ejaculation occurs throughout the major part of copulation (MICHIELS & DHONDT, 1988), the observed rhythmical movements probably lead to the ejaculation phase.

## DISCUSSION ON THE MECHANISM OF SPERM REMOVAL AND TRANSFER

Based on these findings, combined with tests using two-dimensional cardboard models of male and female genitalia, I suggest the following copulation mechanism (Fig. 6). In the penetration phase, the penis would enter the vagina
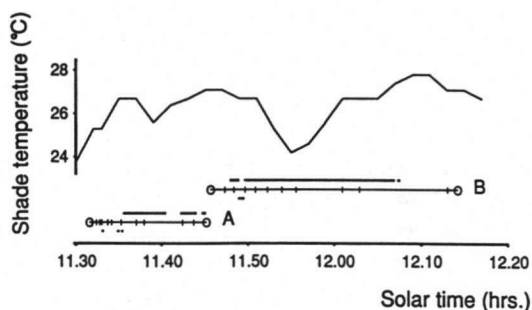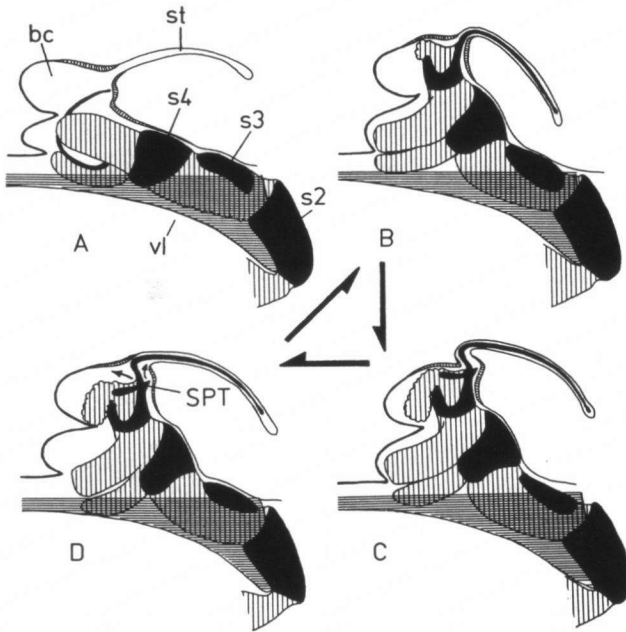
Fig. 6: Suggested mechanism of sperm transfer and removal in *S. danae*. (A) penetration phase; — (B) expansion phase; — (C-D) ejaculation phase. — [Female structures are horizontally hatched (8th sternite with valvular lamina, vl) or clear (bursa copulatrix, bc; — spermathecae, st). The penis of the male is vertically hatched (black when chitinized); — s2-s4 = penis segments 2-4; — SPT = sperm tube. — Anterior = left and dorsal = up in the female, and the opposite in the male. See also Figs 1 and 3. — Arrows in the middle indicate proposed cyclic process during rhythmical copulatory movements. Small arrows in D represent sperm release.]

without being hampered by the structures of the 4th segment. At this stage it is presumably not long enough to enter the bursa copulatrix. When expanding, both cornua may "hook" through the bursa communis and bursa copulatrix into the spermathecae, thus fixing the penis firmly and probably pulling the whole genital complex slightly backward. Only the medial process would enter the bursa copulatrix, while the apical and lateral lobes would remain in the anterior vaginal pouch. The narrowness of the vagina prevents complete lateral unfolding of the lateral lobes: they probably remain more or less parallel with the apical lobe and may have a widening effect on the vagina.

In the spermathecae, sperm removal may be accomplished by the diluting effect of fresh and perhaps more liquid sperm which is ejected at the base of the cornua. Movements of the latter may result in a continous up and down movement of the gradually changing mixture of old and new sperm along the cornual grooves. The surplus then may be pressed away via the dorsal side of the

fourth penis segment.

In the bursa copulatrix, the expansion of the inner lobes might push the surrounding sperm down through the bursa communis into the widened vagina. Back and forth movements of the spined, apical lobe would result in the gradual displacement of sperm from the anterior vaginal pouch to the ventral side of the 3rd penis segment and finally out of the female's genital tract.

Rhythmical movements of the 2nd and 3rd abdominal segments by the male may lead to alternating expansion and contraction of the inner lobes on one hand and ejaculation on the other. As fresh sperm is ejected on the very top of the penis, the "removal zone" is separated from the "ejaculation zone", preventing excessive removal of own sperm.

The proposed mechanism assumes that removal and ejaculation occur throughout copulation. It seems to contradict our previous suggestion of a two-stage copulation with removal before ejaculation, as found when measuring sperm volumes in interrupted copulating pairs (MICHIELS & DHONDT, 1988). However, the latter finding may be understood if most rival sperm that will be removed during a whole copulation is "under control" within 5 minutes. This would mean that it is partly displaced or attached to the penis in such a way that it will be removed when the copulation is interrupted. At that time, ejaculation has not lasted long enough to be measurable or even has not started due to the initial resting pause. However, it may be premature to compare two independent findings based on indirect and artificially obtained data on a complex process such as a odonate copulation.

## CONCLUSIONS

According to the classification made by SIVA-JOTHY (1984) of libellulid genitalia, the female genital complex of *S. danae* can be assigned to female type 1, being characterized by separate, narrow spermathecae. The *danae* penis corresponds with male type 2, identified by two cornua on the medial process. The agreement with *S. depressiusculum* is striking (SIVA-JOTHY, 1984), suggesting a similar system of sperm displacement in both species. In *S. rubicundulum,* however, genital morphology is completely different and suggests a different mechanism of sperm manipulation (WAAGE, 1984).

Sperm removal in both spermathecae and bursa copulatrix may be achieved by rhythmical movements and alternated inflation and deflation of the structures on the medial process. Removal and ejaculation seem to occur simultaneously instead of being restricted to 2 distinct parts of the copulation. This contrasts with findings in Zygoptera, where ejaculation follows removal (WAAGE, 1979; MILLER, 1981, 1987) and a 2-stage copulation was previously suggested in other libellulids by MILLER (1981, 1984), SIVA-JOTHY (1987a) and MICHIELS & DHONDT (1988). In the latter case, however, this may also have been a

consequence of the artificial process of copula interruption, which overestimated the apparent sperm removal in the first part of the copulation.

## ACKNOWLEDGEMENTS

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