FUNCTIONAL MORPHOLOGY OF THE PENIS OF *CELITHEMIS EPONINA* (DRURY) (ANISOPTERA: LIBELLULIDAE)

P.L. MILLER

Department of Zoology, University of Oxford, South Parks Road,
Oxford OX1 3PS, United Kingdom

A description is given of the anatomy of the penis and of associated secondary genital structures. The penis of Anisoptera is not homologous with that of Zygoptera, and it consists of a 4-segmented organ with inflatable structures on the last segment. The hamuli and the anterior frame cooperate to swing the ligula ventrally and this action in turn thrusts the penis posteriorly. The same movement also causes a hamular claw to close, and this is believed to grasp a rim on the female's genital plate during copulation. The penis cannot be withdrawn without the grasp being loosened. Rhythmical movements which appear in stage I of copulation are interpreted to represent activity which packs previously deposited sperm down in the female tract, and it is suggested that sperm removal is less important in this species than the displacement of sperm within the female tract.

INTRODUCTION

Many accounts of the morphology of the secondary genitalia of male dragonflies and damselflies can be found in the literature (references in ASAHINA, 1954; PFAU, 1971; RESTIFO, 1972), but only PFAU (1971) has paid much attention to how the penis functions. Male Odonata have highly specialised secondary genitalia on the ventral surface of the second and third abdominal segments. In Zygoptera the penis is formed by the ligula, a development of the second abdominal sternite; in Anisozygoptera it is formed from the posterior hamuli, and in Anisoptera it is an extension of a zygopteran type of sperm store or vesicle on the ventral side of the third abdominal segment (PFAU, 1971). Thus the penis in the two main sub-orders is not homologous: moreover structural differences suggest that its function

may not be identical in the two.

During copulation some zygopteran species remove sperm deposited by previous males from the female tract before introducing their own, and the zygopteran penis is equipped with a variety of hooks, barbs, whips and proximally pointing bristles in different species which probably help to bring this about (WAAGE, 1979; MILLER & MILLER 1981). It is possible that similar removal of sperm occurs in Anisoptera, and structures on the penis of some species suggest how it may occur (WAAGE, 1982). The penis of *Celithemis eponina* is shown here to possess bristly inflatable structures which might be capable of removing sperm from the female, or alternatively of packing down previously deposited sperm. A mechanism is also described which is specialised to grip the female genitalia during copulation. Some behavioural observations on mating behaviour in this species have already been described (MILLER, 1982), and the tentative conclusion is reached that if sperm displacement occurs it probably involves packing or pushing aside rather than removal.

MATERIAL AND METHODS

Celithemis eponina were collected in southern Florida and preserved in alcohol. Copulating and non-copulating insects have been dissected and examined after rehydration. Scanning electron micrographs were taken with a Philips SEM 500.

The names given to parts of the penis follow the terminology of BORROR, 1942 (cf. also RESTIFO, 1972). Descriptions and diagrams of male genitalia are given viewed laterally with the ventral side uppermost.

RESULTS AND DISCUSSION

The anisopteran penis is a complex jointed organ attached to the ventral side of the third abdominal segment. It comprises four segments the first of which is a large, pear-shaped structure containing separate compartments for sperm storage and for fluid, the latter forming a hydrostatic reservoir (Pfau's Schwellkörper) (Fig. 1A). Segment II is long and cylindrical, and it bears a distal median spur; it is attached to segment I by a weakly elastic hinge which causes it to rest in the nearly fully extended position. The short segment III consists of a pair of hardened sclerites between which there is a medial longitudinal slit which allows sperm to enter from the primary genitalia. It is attached to segment IV (the penis head) by another elastic hinge which normally keeps the head flexed on segments III and II, the inactive penis being partly recessed below the anterior lamina and between the genital lobes (ventral extensions of the tergites of the second abdominal segment).

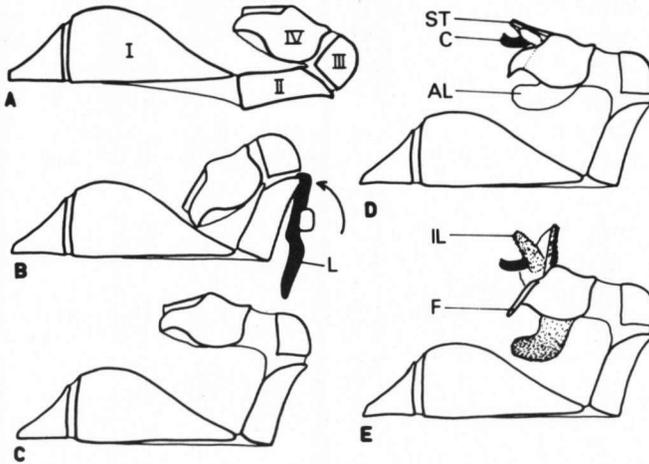Gentle sustained pressure was applied to the first segment by using a pair of

Fig. 1. Diagrams representing the extension and inflation of the penis of *Celithemis eponina* (anterior, to the right; ventral side uppermost): (A) the resting position; — (B) the ligula (L) has engaged on the median spur of the second segment (II) and flexed that segment ventrally; — (C) inflation has caused the penis head (IV) to extend; — (D) further inflation has started to swell structures on the penis head. ST, sperm tube; C, cornua; AL, apical lobe; — (E) the fully inflated head with expanded inner lobes (IL), and with the flanges (F) or lateral lobes swung dorsally and laterally.

forceps closed with a screw clamp. This caused segment IV to extend hydrostatically through about 90° by inflating soft pleated cuticle which spans the joints between segments II, III and IV (Fig. 1C). (At this stage, in vivo, section II is flexed ventrally by the ligula (Fig. 1B), as described below.) Further pressure then caused the head to inflate (Fig. 1D, E). The apical lobe of soft cuticle swung down and expanded. It was seen to be covered in bristles about 18 μm long at the distal end but shorter proximally (Fig. 2). At the same time the sperm tube on the upper side of the head swung upwards and two large hardened hooks (cornua) extended posteriorly (Fig. 1E). Two additional sacs of soft cuticle (inner lobes) were simultaneously inflated and they extended laterally. They were seen to carry a sparse covering of small bristles. More bristles were observed on the soft cuticle surrounding the sperm tube (Fig. 2). The hardened flanges of the penis head (lateral lobes) swung out laterally on an oblique hinge. This movement was caused by the upswing of the sperm tube and was due to a cuticular attachment with the inner margin of each flange: it has been modelled in cut-out cardboard (Fig. 3). When pressure was reduced on segment I, the head structures were first deflated and refolded, and segment IV then folded along II and III.

Segment II remains in an extended position in line with segment I in the resting penis. It is flexed ventrally through 90° (i.e. upwards) in the active penis by the action of the ligula which engages on the median spur. The ligula is caused to swing upwards when its proximal ends are carried inwards by the hamuli: this rotates the ligula about the anterior frame (Figs 1B and 4B) and tilts the distal end outwards. Muscles 11 are responsible for the movement of the hamuli and hence for the elevation of the penis. Pulling these muscles readily mimics the action which is comparable to that in *Orthetrum* (PFAU, 1971).

Contraction of muscles 11 not only causes the penis to be elevated and thrust posteriorly, but it is also responsible for closure of the outer claw-like process of the hamulus on the inner process. Figure 4 explains the action, Figure 4C representing a cardboard model which embodies an hypothesis of how the mechanism is thought to work. Contraction of muscles 11 rotates the hamulus downwards and pulls the anterior frame in the reverse direction. Closure of the claw is brought about through a
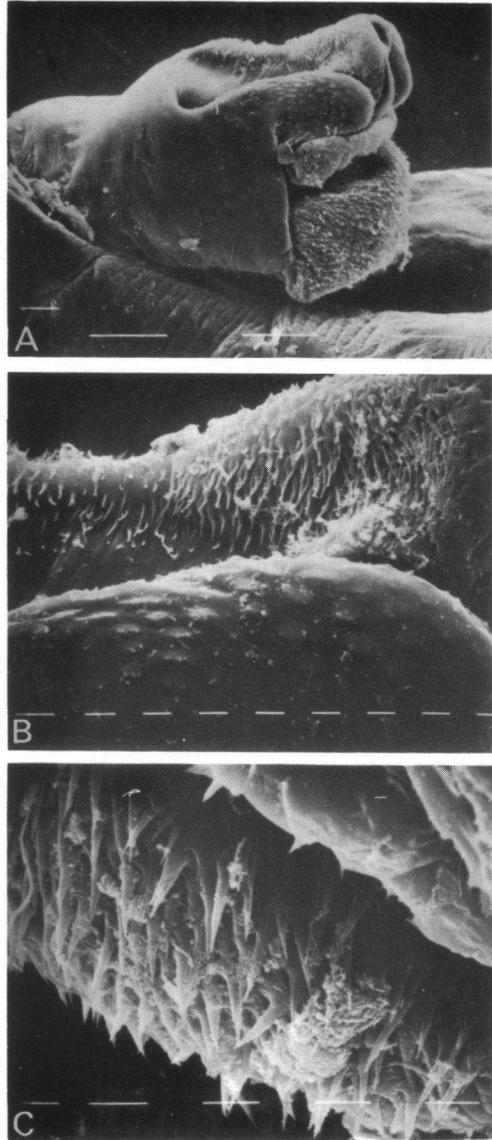

Fig. 2. Scanning electron micrographs of the un-inflated penis head of *Celithemis eponina* (ventral side uppermost; anterior to the left): (A) the whole head (scale bars: 100 μm); — (B) part of the ventral region of the head showing the bristles and processes near the sperm tube. (scale bars: 10 μm); — (C) part of the apical lobe showing the bristles (scale bars: 10 μm).

cuticular strand running between the ventral arm of the frame and a hardened rod which runs along the outer clawlike process. The strand is represented by a thread in the model. As the ventral arm is turned it pulls on the strand causing the claw of the outer process to pitch inwards and to bite on the inner process. The contraction of muscles 11 therefore simultaneously causes the penis to be elevated and thrust posteriorly, and the hamular claw to close. Both movements can be observed when the muscles are pulled.

The significance of the coupling of penis elevation and claw-closing becomes apparent when the female genitalia are examined. The claws can grip a thickened rim or collar which partly surrounds the ventral side of the female genital opening (genital plate). By establishing a firm grip on the female genitalia, the male can thrust the penis posteriorly into the vagina without forcing the genitalia apart (Fig. 5), the more powerful the thrust the tighter being the grip since both depend on the same pair of muscles. However on penis withdrawal which follows relaxation of muscles 11, the grip on the female's collar will be simultaneously loosened, and genital contact may be lost. This stands in contrast to copulation in *Enallagma* where genital contact can probably be maintained after withdrawal of the penis (MILLER & MILLER, 1981).
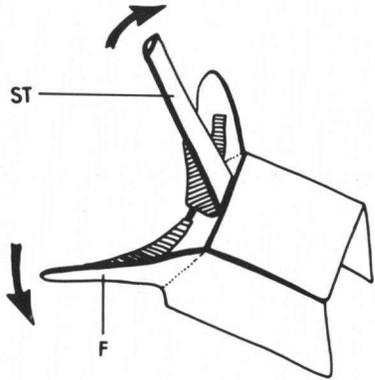

Fig. 3. Model of part of the penis head (segment IV) to show the cuticular tongues which attach the sperm tube (ST) to the flanges (F). As the sperm tube swings ventrally (upwards), it causes the flanges to rotate laterally and dorsally (downwards) on an oblique hinge.

The female genitalia consist of a thick-walled vagina equipped with muscles attached to a dorsal plate: it opens anteriorly into an expanded bursa copulatrix, and ventrally into the oviduct (Fig. 5). Paired spermathecae arise from the anterior part of the bursa and turn posteriorly.

The sequence of actions which it may be suggested occurs at the start of copulation in *Celithemis* is therefore as follows: (a) Pressure is applied to segment I of the penis by internal muscles (muscles 6 and 8 $_{III}$ may be involved in compression and decompression; PFAU, 1971), and segment IV extends on III and II. The ligula is swung ventrally by contraction of muscles 11, elevating the penis by flexing segment II; — (b) At the same time the hamular claws grip the collar, and further contraction of muscles 11 drives the penis into the vagina; — (c) Further pressure is applied to segment I perhaps partly by compression between the male and female abdomens, and the
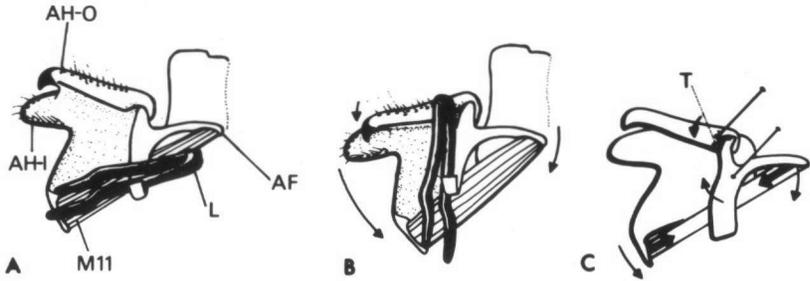
Fig. 4. Diagrams of the right hamulus and the ligula on the second abdominal segment of a male *Celithemis eponina*, viewed from the midline (ventral, upwards; anterior to the right): (A) muscle 11 (M11) is relaxed and the structures are in a rest position; — (B) muscle 11 has contracted and caused the hamular claw to close on the inner process and the ligula to swing ventrally. The ligular movement causes the penis to extend ventrally and posteriorly (not shown here: see Fig. 1B); — (C) a model to show how contraction of M11 rotates the hamulus counterclockwise, and the anterior frame clockwise thus closing the claw. — (AH-O, hamulus-outer process; AH-I, hamulus-inner process; L, ligula; AF, anterior frame; T, thread).

structures on segment IV are inflated and erected. This should not occur before the penis has entered the female. Only segment IV enters the female; segment III rides over the genital plate, and segment II rests in a groove between the two parts of the collar.

The precise location of segment IV in the female tract cannot be gauged from preserved specimens since it may depend partly on activity of muscles in the female which pull on the vagina. However, the shape of the expanded penis head resembles a rough mould of the female tract with the paired inflatable inner lobes corresponding to the spermathecae and the medial lobe to the bursa, or the female tract may be viewed as an ill-fitting glove covering the expanded segment IV whose bristly structures may be able to act on sperm previously deposited in the female.

Sperm expulsion from within segment IV of *Orthetrum* takes place by the action of a special pump in that segment (Ausspritzkammer of Pfau) which is activated by compression and decompression arising from contraction of muscles 6 and 8 $_{III}$ (PFAU, 1971). Compression fills the pump from the sperm store (segment I), while decompression expels sperm through the sperm tube. In *Celithemis* caught in copulation it was noted that mechanical compression of segment I inflated the head and expelled sperm from segment IV, but this may not mimic the natural action correctly.

Stage I of copulation is accompanied by rhythmic raising and lowering of the male's abdomen (MILLER, 1982). Such movements may accompany compression and decompression of segment I of the penis, causing alternate inflation and deflation of structures on segment IV. This activity would be

unlikely to be able to remove previously deposited sperm from the remale tract, but it might pack such sperm down in areas of the bursa and spermathecae remote from the oviduct, and thus render such sperm less likely to fertilise eggs during the ensuing few minutes (WAAGE, 1982). Rhythmic movements in the copulation of *Enallagma* were suggested to represent successive penetrations and withdrawals of the penis (MILLER & MILLER, 1981), but this is probably not the case in *Celithemis* since withdrawal would probably lead to disengagement of the genitalia, as already described. Moreover although spontaneous disengagements of the genitalia during copulation are common in *Enallagma*, they have not been seen in *Celithemis*. Therefore in *Celithemis* the penis is probably withdrawn only at the end of copulation. If such withdrawal removes sperm from the female, many of the male's own sperm might be included. However, it remains possible that the rhythmic movements could move sperm previously deposited by another male from one to another part of the female tract, for example from the bursa into the vagina, from where they might be removed later when the penis was finally withdrawn.
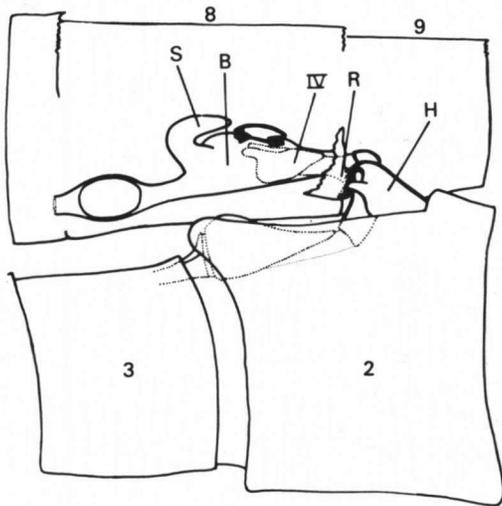

Fig. 5. Conjectural diagram of the positions of male and female genitalia during copulation. The 8th and 9th segments of the female abdomen are shown above, ventral side downwards. — B, bursa; R, genital plate with expanded rim; S, spermatheca. The 2nd and 3rd male segments' are shown below, ventral side uppermost. H, hamulus; IV, penis head. The penis head is placed in a position it was seen to occupy in dissected preserved specimens. In life, contraction of vaginal muscles may permit the head to extend further into the vagina and bursa.

Although the timing of sperm deposition is not known, it may be suggested that it occurs during the apparently inactive. stage II. Thus the function of the inflatable structures on segment IV of the penis is interpreted to be one of pushing down sperm already in the female tract and keeping it away from the vicinity of the oviduct prior to the introduction of the male's own sperm. On a last-in-first-out basis, this would allow the sperm of the copulating male the best chance of fertilising eggs before and during the

ensuing bout of oviposition when the pair remain in tandem. Examples of this type of sperm displacement are common in other insect orders where it has been shown by the use of genetic markers that the last male to mate with a female fertilises most of the eggs laid in the next batch (BOORMAN & PARKER, 1976; SMITH, 1979; WOOL & BERGERSON, 1979).

ACKNOWLEDGEMENTS

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