

FIELD OBSERVATIONS ON COPULATORY BEHAVIOUR IN ZYGOPTERA, WITH AN EXAMINATION OF THE STRUCTURE AND ACTIVITY OF THE MALE GENITALIA

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Copulatory activity is described in *Enallagma cyathigerum*; some observations on reproductive behaviour of *Calopteryx splendens* and some other zygopterans are also included. Copulatory activity is divisible into 3 stages: in stage *I*, which may occupy more than 75% of the time, there are continual rhythmical movements of the abdomen of the male. In stage *II*, the abdominal positions change and a new type of rhythmic activity appears, also due to the male, while in stage *III*, the positions again change but there are no visible movements. Dissection of pairs killed at different stages of copulation suggests that stage *I* may be concerned with sperm removal from the female, while during *II* and *III* sperm is transferred to the female. The female cannot escape from the tandem association but can accept or reject copulation, and she may terminate the activity during stage *I*. Stages *II* and *III* have not been seen to be spontaneously interrupted. — Dissection of the male secondary genitalia and their examination in scanning electronmicrographs shows the penis head to possess a variety of hooks and bristles. These have been seen to hold trapped sperm when pulled from females in copula. In restrained decapitated males, activity of the penis and associated movements of abdominal segments occur spontaneously or can be evoked mechanically. They resemble the activity occurring naturally in stage *I*. Electromyograms have been used to verify the contributions of 3 sets of muscles to the movements.

INTRODUCTION

There is much interest at present in mechanisms of intrasexual selection among insects, and in many species competition between males has been shown to take place after as well as before copulation (BOORMAN & PARKER, 1976; BLUM & BLUM, 1979). Post-copulatory examples include

competition between sperm to fertilise eggs, and the last male to mate with a female before oviposition may fertilise most of her eggs (PARKER, 1970). During oviposition some odonate species remain in tandem and in others the male guards the female while she oviposits (CORBET, 1962, 1980), both activities probably tending to reduce the likelihood of displacement of sperm by other males (WAAGE, 1978, 1979b). WAAGE (1979a) has shown that in *Calopteryx maculata*, the male spends the greater part of copulation removing sperm from the bursa copulatrix and spermatheca of the female, and he deposits his own sperm only towards the end of the activity. Measurements of the total amounts of sperm present in the female before and after copulation, and observations of ejected sperm on the penis and ovipositor, confirm this interpretation.

Many accounts of dragonfly copulation describe little more than its duration, although considerable detail has been given by BUCHHOLTZ (1956) for mating in *Platycnemis pennipes*, while ROWE (1978) has noted that sperm transfer in *Ischnura aurora* occurred only in the last 2 min of a copulatory performance lasting 10-20 min. Copulation in *Enallagma cyathigerum*, is shown here to be divisible into three stages, the first probably occupied by sperm removal and the second and third by sperm deposition. We have tried to relate the behaviour observed in the field to patterns of activity of the penis recorded in the laboratory. Male Odonata are unique among insects in possessing secondary genitalia on the second and third abdominal segments. Before or during copulation, sperm are transferred to a sperm store (sperm or seminal vesicle) from the primary genitalia, from where they are passed into the female during copulation.

MATERIAL AND METHODS

A large population of *Enallagma cyathigerum* (Charp.) was examined at a lake near Oxford on 25 occasions during the period June to mid-August, 1980. A few observations have also been made on *Calopteryx splendens* (Harr.) near Oxford and in the Dordogne, France, as well as on some other zygopterans.

Behaviour was observed with the aid of a pair of field binoculars (King, 10 x 50), mounted on a tripod and fitted with an extension ring so that objects at 1 m could be focussed. Photographs were taken through a 200 mm lens and extension rings, and activity was recorded using a portable tape recorder, verbal records being transcribed later with four tapping keys as an oscillograph. Stereomicrographs were taken using a Philips PSEM 500. Electromyograms were recorded with 30 and 60 μm insulated copper wire, using conventional amplifying, display and recording systems.

OBSERVATIONS

FIELD OBSERVATIONS ON COPULATION

The following account is based on observations and tape recordings of ca. 40 naturally occurring copulating pairs. *E. cyathigerum* appears to be a non-territorial species (NEVILLE, 1959; but cf. also PARR, 1976) and on warm days large numbers of males were found aggregated at various points along the lake shore. Many began to form tandem associations with females before 10.00 hr, but copulation was seldom seen before 11.00 hr. It declined in frequency during the afternoon, but a few copulating pairs were observed up to 18.00 hr. No copulation was observed on overcast days when the temperature was below about 20°C, although many pairs remained in tandem. On sunny days copulations were numerous even when shade temperatures were 14-15°C.

Copulations were seen most commonly between 2 and 5 m from the lake shore and less commonly at the water's edge. Before tandem formation occurred, a male and female faced each other hovering in the air for a few seconds whereupon the male made a rapid pounce, grabbing the female and both then fell to the ground. He then quickly applied his abdominal claspers to her prothorax after which they flew off in tandem to a perch. Alternatively a male might approach a resting female and immediately attempt to form a tandem association. Sperm transfer by the male to his accessory genitalia usually followed tandem formation if conditions for copulation were appropriate, the activity taking about 5 s and resulting in a visibly swollen sperm store.

The male then immediately invited copulation by slowly raising and lowering his wings and at the same time oscillating his abdomen with the female attached. If the female was receptive she flexed her abdomen and brought the tip towards his second segment. She then swung the ventral and inner valves of the ovipositor downwards and made a series of stabbing movements, striking her ovipositor against the sharp, knife-like edge of the ventral side of the shaft of the penis (Fig. 4A). This activity separated the right and left valves so that they slid down on either side of the penis shaft and the tips became lodged into recesses formed by extensions of the lamina batilliformis under the anterior hamuli of the male, a region richly supplied with bristles (cf. PFAU, 1971, and Fig. 4A). The genitalia are therefore apparently locked together both by the penis head slotted between the ventral valves of the ovipositor, and by the ends of the valves fitting into the male's recesses.

Unreceptive females did not flex the abdomen when copulation was invited by the male. Moreover on cool days females were sometimes seen to strike

weakly with the ovipositor and genital contact was usually not established. Males would continue to issue invitations for several minutes before desisting and the pair then remained resting in tandem for long periods.

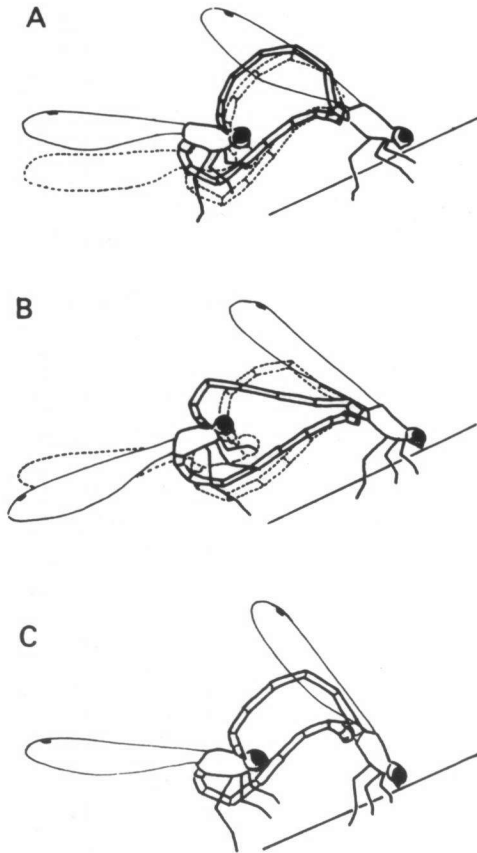


Fig. 1. Copulation in *Enallagma cyathigerum*. Positions of male and female during stage I (A), stage II (B) and stage III (C). The bold outlines indicate the passive or rest positions and the dashed outlines the positions in the active phase. Traced from photos taken in the field.

Immediately after genital contact, copulatory activity commenced. Initially the male's anterior abdominal segments were seen to be straight, as in stage II (cf. below), but within a few seconds they adopted the curved position typical of stage I.

Stage I — The positions of the male and female abdomens in stage I are shown in Figure 1A. The third abdominal segment of the male is flexed dorsally on the second, and this helps to distinguish stage I from III. Rhythmical movements apparently due to the male commenced as soon as the posture had been adopted and continued throughout the stage. In each cycle the male's segment 2 was swung dorsally while segment 3 swung ventrally on 2 as shown in Figures 1A and 2. At the same time the ventral valves of the ovipositor pivoted ventrally allowing the female genital segments to separate from the male's by about 0.5 mm, while remaining securely locked to his by the penis. This, termed the active phase, was maintained for 0.5-2 s and it was followed by a passive phase in which the

segments returned to a rest position and the insects remained immobile. The rhythm commonly started at about 10 cycles min^{-1} and in some pairs accelerated by a reduction of the passive phase to 40-60 min^{-1} . Groups of faster cycles sometimes occurred among slow ones but there was much

individual variation in the details of the activity. Parts of sequences, together with a generalised scheme, are shown in Figure 3. Stage *I* normally lasted 10–20 min, but sometimes up to 40 min. In the middle of this stage the female commonly pulled her genitalia away from the male, leaving his penis extended; the pair might immediately resume copulation or fly in tandem to another perch before doing so.

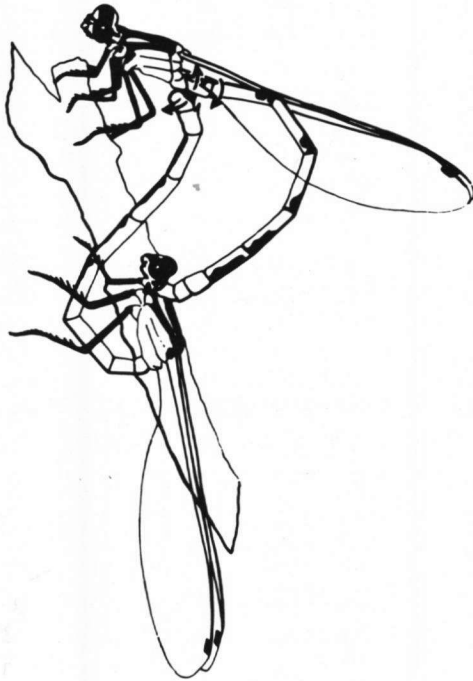


Fig. 2. Copulation in *Enallagma cyathigerum*. The active phase of stage *I* is shown, with arrows indicating the movements of male segments 2 and 3, and of the female terminal segments at the start of the active phase. Traced from photos taken in the field.

If disturbed they usually flew off in the wheel (i.e. copulatory) position but normally disconnected the genitalia in flight to land in tandem on a fresh perch. Both partners beat their wings when they fly in the wheel or tandem positions.

At intervals during stage *I* the male tapped or kicked the terminal segments of the female with one or both hindlegs, particularly at times when the rhythmic movements accelerated, normally contacting her with the base of the tibia. Sometimes he pushed hard against her and occasionally the genitalia disengaged, but this was normally followed immediately by a fresh copulatory invitation. The male intermittently groomed his eyes or one leg with another during stage *I*, and the female also did so but much less frequently.

For most of the time she remained immobile with legs extended and off the ground, sometimes the first pair and rarely also the second adopting the flight posture. Both made occasional head-wagging movements. At the end of stage *I* there was a prolonged and intense active phase (occasionally, 2 or 3 occurred), lasting 10–20 s and exceptionally up to 35 s, followed by a transition to the stage *II* posture.

Stage *II* — In stage *II* the 3rd segment of the male abdomen became flexed ventrally on the second while the more posterior segments were more or less straight and parallel to those of the female, segments 7 and 8 being bent

sharply to allow the female to remain clasped. A new type of rhythmic activity appeared in which the male abruptly elevated the mid-region of the abdomen by flexing the 4-5 joint as shown in Figure 1B. Each elevation lasted less than 0.5 s and was followed by a long passive phase, the cycles occurring at 9-22

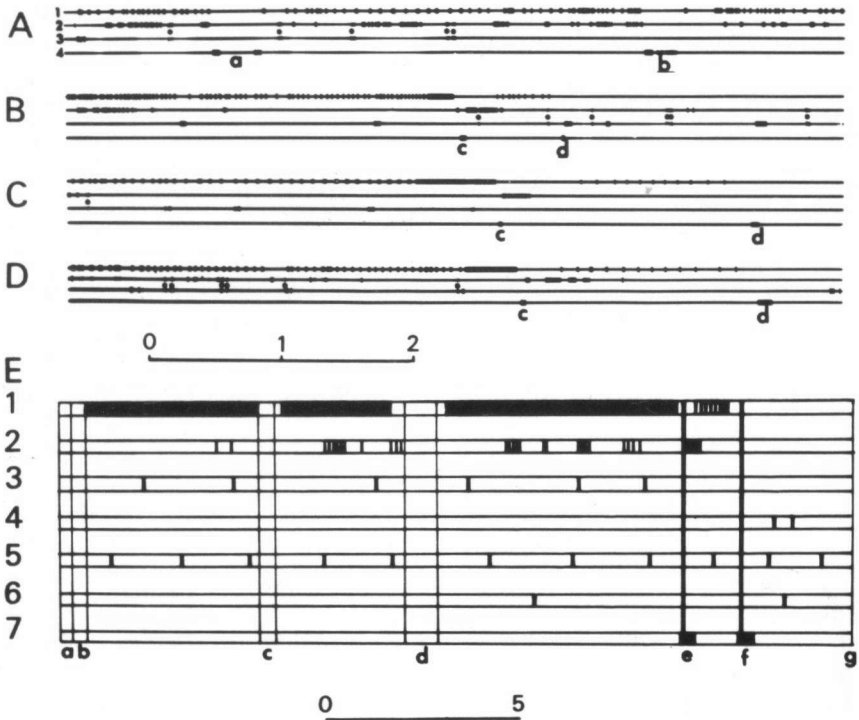


Fig. 3. (A-D) Oscillographs transcribed with 4 tapping keys from tape-recorded field observations of copulation in *Enallagma cyathigerum*. In each, line 1 indicates the occurrence of rhythmical abdominal movements by the male; 2, kicks by the male's hind legs at the terminal segments of the female; 3, grooming by the male; 2+3 (•), wing elevation by both partners in response to an intruding male; and 4, changes of activity: (A) a period of stage I activity with two spontaneous break-offs (a and b). — (B-D) transitions from stages I to II (at c in each), and from stages II to III (at d in each). Note the variable frequency of the stage I rhythm, the sustained active phase which terminates stage I (line 1), and the variability of the duration and rhythmic frequency in stage II. Horizontal scale, 2 min. — (E) a summary of copulatory activity in *E. cyathigerum* based on the tape-recorded field observations: 1, abdominal rhythmic movements; 2, kicks by the male; 3, male grooming; 4, female grooming; 5, wing elevation in response to an intruding male; 6, head wagging in the male; 7, changes of activity or stage. At a, tandem association is formed; b, sperm transferred by male to secondary genitalia; c, spontaneous break-off; d, spontaneous break-off followed by a short flight to another perch; e, transition to stage II; f, transition to stage III. g, genitalia disengage. Horizontal scale, 5 min.

min⁻¹ and the stage lasted altogether 40-120 s in different pairs. No genital movements comparable to those of stage *I* were observed, but the stage was frequently introduced by a period of persistent kicking of the female by the male (Fig. 3). The abdominal positions brought the prominent medial posterior spine on the 8th sternum of the female to rest very close to the male's sperm store where it could possibly play a role by nudging the orifice.

Other types of activity such as grooming have rarely been seen in stage *II*, and no break-offs have been observed. If disturbed the pair flew off, maintaining the wheel position in flight, and instantly resumed stage *II* activity on landing.

Stage *III* — At the end of stage *II* the dorsal elevations ceased and the male abdomen gradually curved upwards into a posture like that of stage *I*, but with the third segment in line with the second (Fig. 1C). This change signalled the start of stage *III* which persisted with little or no observable movement for 200-250 s, and exceptionally for 470 s. Leg kicking was absent, grooming was rare, but in some pairs a weak oscillatory movement of the female segments could be distinguished, possibly caused by ventilatory pumping. If disturbed the pair flew off, maintaining the wheel position in flight, and instantly resumed the stage *III* position on landing.

POST-COPULATORY ACTIVITY

At the end of stage *III*, genital contact was suddenly broken by the female and the pair flew to a fresh perch nearby where they usually rested for several min before flying out over the water. There the female characteristically trailed the tip of her abdomen in the water as they flew in tandem, an activity which might allow her to sample the site for oviposition and would also tend to wash off ejected sperm adhering to her ovipositor. Oviposition then followed with the partners either in tandem at the surface or with the female alone descending below the surface while the male continued to guard her point of submergence, driving off intruding males from the immediate vicinity (cf. ROBERT, 1958).

GENERAL FEATURES OF COPULATORY ACTIVITY

The total duration of copulation was normally 10-20 min, and one uninterrupted performance of 47 min was observed. The duration may be affected by many factors including the number of interruptions and spontaneous break-offs which occur, the age of the participants, their previous copulatory experience on the same day, and their body temperature. In turn body temperature may depend on metabolic activity, air temperature and on incident radiation whose contribution is much affected by postural

orientation (CORBET, 1962; MAY, 1979).

Copulating pairs were frequently visited by single males which caused both male and female to raise their wings without interrupting other activity. The intruding male almost always retired and only one example of a clash in more than a hundred intrusions witnessed has been observed. We have seen no take-over during copulation. Over the water at oviposition sites, however, clashes between tandem pairs and single males were seen commonly but again no take-over was witnessed.

After oviposition under water, females were often seen to struggle at the water surface when no support was available and many were then picked up in the claspers of passing males who would drag them along the water surface to a support, or sometimes lift them bodily from the water.

Apparently only the male can terminate the tandem association with a female. Evidence for this comes from field observations in which the female of a tandem pair was gently pinched, prodded or squeezed by hand producing escape behaviour but no release by the male. Escape behaviour in the male normally caused him to release the female. Even when killed in tandem the female remained clasped for some time by the male. In contrast, copulation is under the control of the female. She may accept or reject the male's invitation to copulate, and forced copulation cannot occur in *Zygoptera*. She can terminate copulation at any stage by straightening the abdomen. The structure of the penis and its muscular system (cf. below) may prevent the male from withdrawing it from between the ovipositor valves, although he may possibly be able to promote disengagement by using his legs. All cases seen where copulation was broken before or at the normal termination appear to have been initiated by the female, possibly sometimes in response to male kicks.

COPULATION IN *CALOPTERYX SPLENDENS*

Six copulations have been witnessed in this species, the duration of each varying between 2 and 4 min. Strong rythmical movements at ca. 1 Hz commenced soon after genital contact was established and they accelerated to 2 Hz later. Similar abdominal positions and stages as those described in *E. cyathigerum* have been identified, the latter in a much accelerated form. WAAGE (1979a) also noted undulatory movements and a final quiescent phase in the copulation of *Calopteryx maculata*.

EXAMINATION OF PAIRS OF *ENALLAGMA CYATHIGERUM* KILLED IN COPULATION

Altogether 38 copulating pairs representing each of the three stages have

been examined either after killing them instantly between the hands or after they were caught in a net. They were dissected and examined under the microscope either fresh or preserved in alcohol. In six instantly killed pairs genital contact was not lost and the relative positions of the organs was determined in them. In four cases the shaft of the penis was fractured on capture, the head remaining in the female.

In stage *I* females, the penis head has been located in the vagina between the vertical cuticular plates, or more anteriorly and dorsally in the bursa copulatrix. The spermatheca in this species takes the form of a single sac lying ventral to the bursa and joined to it by a narrow duct; probably no part of the penis can enter it (cf. ASAHINA, 1954). The penis has also been found in the vagina of stage *II* and stage *III* females. Pulling the penis out caused the head to unfold, its hooks and barbs (cf. below) thereby becoming orientated to point towards the female's posterior (cf. Fig. 4E). On several occasions bundles of sperm were pulled out with the penis, and most stage *I* pairs which became disengaged on capture had sperm on the penis head and between the ovipositor valves, as was found by WAAGE (1979a) in *C. maculata*. In fresh specimens the sperm were found to be active, and each was 75-80 μm long and 2-3 μm in diameter at the slightly broader head end. Fifteen males killed in stage *I* and examined were all found to have filled and swollen sperm stores. Five of eight males killed in stage *II* had at least partly filled stores while in the remaining three they were empty. All stage *III* males examined (11) had empty stores.

Twelve stage *I* females were killed and the state of the bursa and spermatheca was examined. Seven of them (58%) had empty, collapsed bursae but their spermathecae were filled and swollen; in two (16%) both sacs were empty and in another two they were both swollen and filled. Finally in one female the bursa was filled but the spermatheca was shrunk and empty. No systematic attempt has yet been made to kill and examine females at different times during stage *I*, but one interpretation of these results is that the male first empties the bursa of sperm, and the spermatheca is emptied later in stage *I*. The one anomalous female with empty spermatheca and filled bursa cannot be accounted for at present. Only two stage *II* females have been examined and in both the bursa and spermatheca were swollen and filled. Eleven stage *III* females were similarly found all to have well filled bursae and spermathecae. These observations are similar to those of WAAGE (1979a), and are consistent with the hypothesis that sperm is removed from the female during stage *I*, and that the male deposits his own sperm during stage *II* and *III*. However, there is as yet no firm evidence that before these matings the females carried a full sperm load. Examination of five unreceptive females resting in tandem with males showed that four had filled spermathecae and bursas, but one had a filled spermatheca and an empty bursa, suggesting

either that in her, copulation had been terminated during stage *I* or that she had previously used all the sperm in her bursa.

MORPHOLOGY AND MOVEMENTS OF THE PENIS

General descriptions of the secondary genitalia of dragonflies have been given by GEORGE (1929), ASAHINA (1954), PRASAD & SRIVASTAVA (1960), and in most detail by PFAU (1971). Only Pfau has devoted much attention to how the movements of the penis are brought about, but no previous account has analysed muscle action with myograms. We provide some additional morphological information and describe the repertoire of movements which can be evoked.

The penis of *E. cyathigerum* consists of a head and shaft together with supporting cuticular struts, the hamuli and frames, which control its movements (Fig. 4A). The head bears two large terminal hooks and two

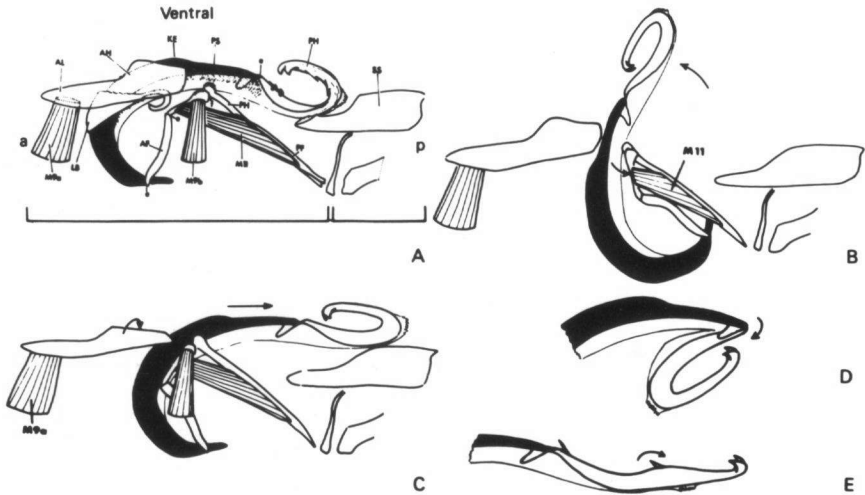


Fig. 4. The structure of the secondary genitalia of male *Enallagma cyathigerum* viewed from the side with anterior (a) to the left, and ventral uppermost: (A) the penis is shown unsheathed from the sperm store (SS) under which it normally lies. AF and PF, anterior and posterior frames; AH and PH, anterior and posterior hamuli; AL anterior lamina; KE, knife-edge on ventral side of penis shaft; LB, lamina batilliformis; PH, penis head, PS, penis shaft; e, elastic cuticular joints; f, flexible cuticular joint; M9a and M9b, tergosternal muscles; M11, longitudinal muscle. — (B) the position of the penis during an anterior-ventral rotation (AVR) resulting from a contraction of M 11. — (C) the position of the penis during a posterior thrust resulting from a contraction of M 9a. — (D) the flexed position of the penis head which probably occurs during passage between the ovipositor valves and entry into the female tract. — (E) the unfolded position of the penis head seen during withdrawal from the female tract.

further projecting folds of cuticle on its inner surface (the surface concealed when the head is folded). The same surface also bears a large number of bristles, each 12-16 μm long and multi-spined at the distal end but tending to be single-spined more proximally (Fig. 5). There is a further group of five stout hooks near the neck. Deformable cuticle on either side of the shaft also bears a large number of backwardly projecting bristles. Withdrawal from the female reproductive tract unfolds the head, and all hooks and bristles are then orientated backwards (i.e. anteriorly) in such a way as to be able to withdraw sperm, as already described (Figs. 4E, 5). On release it springs back to the folded position. The head is joined to the shaft by a second flexible and elastic region which allows it to fold inwards (Fig. 4D), thereby possibly facilitating its passage between the ventral valves of the ovipositor and into and within the female reproductive tract. A region of soft, loose, non-bristly cuticle at the distal extremity of the folded head has been thought to form a hydraulic anchor or glandular cushion within the bursa by becoming inflated with fluid (PFAU, 1971). However, we have not found this region to be inflated in *E. cyathigerum*, and suggest that it may act to ease the passage of the head of the penis into and within the female system, acting like a lubricant. Alternatively if it does become inflated it may assist in sperm transfer into the female (J.K. Waage; pers comm.).

The shaft consists of a stout, curved, cuticular rod bearing along its inner (dorsal) surface a folded membrane which forms a sperm canal. Part of the outer face is formed into a sharp, knife-like edge which act to separate right and left ventral valves of the ovipositor at the start of copulation, allowing the penis to ride between them, as already described (Fig. 4A).

The penis is suspended in a cradle of struts through which its movements are controlled. The inner end of the shaft is attached to the two anterior frames at elastic joints. Each frame itself contains a highly elastic region (*e* in Fig. 4A) which is deformed during posterior thrusts (cf. below) and which aids the return movement. The anterior frames are joined to the posterior hamuli and frames at a further flexible joint. The lamina batilliformis appears to guide the penis shaft and is extended on each side into two coiled structures bearing bristles into which the tips of the ventral valves of the ovipositor pass during copulation. A transparent cuticular tongue (the *Zipfel* of PFAU, 1971) lying between the anterior frames may help to guide sperm from the store into the canal in the penis.

The following types of movement have been observed:

- (1) Release or 'unsheathing' of the penis head from under the sperm store by a dorsal swinging of abdominal segment 3 on 2 which raises the anterior end of the store and clears the head.
- (2) Posteriorly directed thrusts of the unsheathed penis which when vigorous can bring the tip of the head level with the posterior end of the store.

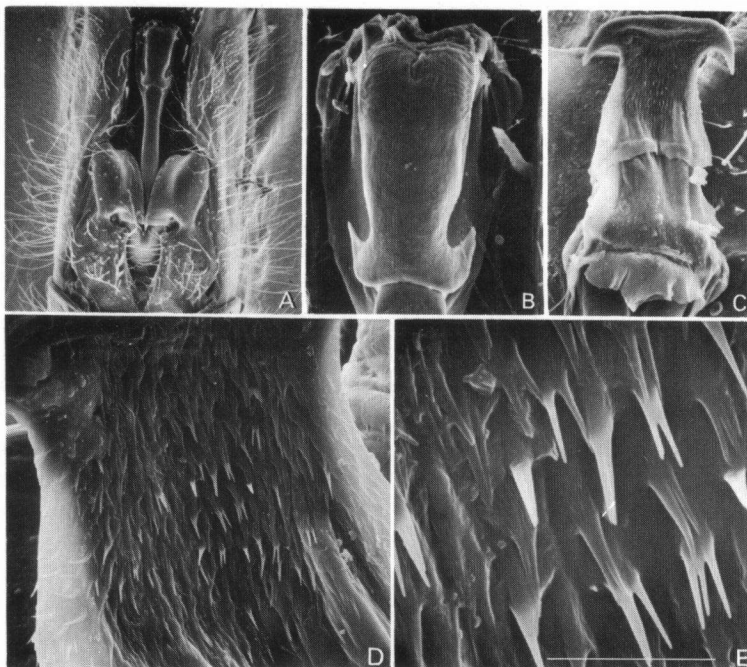


Fig. 5. Scanning electronmicrographs of the secondary genitalia of male *Enallagma cyathigerum*. In each, posterior is upwards: (A) the second abdominal segment viewed ventrally with the penis head unheathed from under the sperm store; — (B) the penis head, ventral view; — (C) the penis head unfolded to reveal its inner surface equipped with backwardly (anteriorly) pointing hooks, cuticular folds and bristles; — (D) inner face of the unfolded penis showing bristles; — (E) single, double- and triple-ended bristles. — Horizontal scale, A, 4 mm; B, and C, 250 μ m; D, 62.5 μ m; E, 16 μ m.

These are produced by contractions of a pair of tergosternal muscles, *9a* in PFAU (1971) (*mdva* in ASAHINA, 1954), which pull the anterior laminae and attached anterior hamuli inwards (i.e. dorsally). The hamuli press against the curved anterior part of the shaft driving it backwards (Fig. 4C). When the muscles relax the return movement is brought about elastically.

- (3) Swinging of the whole shaft and head through 90° about the ventral end of the anterior frame which turns on the posterior hamulus. The head is carried anteriorly and ventrally so that it comes to project downwards between the anterior hamuli. This anterior-ventral rotation (AVR) is brought about by a pair of modified ventral longitudinal muscles which run between the anterior and posterior frames, and are numbered *II* in PFAU (1971) (*ls* in ASAHINA, 1954) (Fig. 4B).

- (4) A smaller dorsal movement of the whole penis and its carriage due to contractions in a further set of tergosternal muscles, *9b* in PFAU (1971) (*mdv* in ASAHINA, 1954).
- (5) Return of the penis under the store, brought about by elevation of the store, as in type 1, possibly aided by contraction of muscles *9b*.

The combination of these types of movement into various patterns is described in the next section.

The structure of the penis and associated cradle is similar in other Zygoptera examined, and the same types of movement have been observed in restrained *Calopteryx splendens*. However, considerable specific differences are found in the structure of the head of the penis. In *C. splendens* for example there are long, curled horns (cf. WAAGE, 1979a), while in *Ischnura elegans* the head is equipped with long unbarbed hooks and sets of stout bristles near the base; in *Coenagrion puella* there are long barbed whips on the tip of the head. All such structures probably aid in sperm removal from the female, and sperm bundles have been found clasped in the hooks of *I. elegans* on withdrawing the penis from the vagina.

ACTIVITY PATTERNS OF THE PENIS

Although it is possible to evoke certain patterns of stereotyped activity of the penis of an unpaired male in the laboratory, their relation to natural copulatory activity, in the absence of stimuli from the female, must remain speculative.

In intact *Enallagma cyathigerum* little spontaneous activity of the penis has been seen. Mechanical stimulation, or contact with female genitalia, seldom produced more than weak dorsally directed contractions. After decapitation, however, the activity threshold was lowered and movements then occurred spontaneously or in response to mechanical stimulation of the ventral region of segment 2. Injection of locust Ringer into the thorax was also found to increase activity of the penis. Contact with female genitalia was no more effective than artificial mechanical stimulation of the secondary genital region, and the application of the male claspers to the female prothorax was without effect. Activity consisted of a stereotyped sequence in which the penis was initially released from under the sperm store; it then made a strong AVR followed by a powerful thrust posteriorly. Alternatively an AVR might be followed by a series of smaller thrusts. The cycle might occur once or be repeated many times at regular intervals without further stimulation. Either an AVR or a posterior thrust could occur alone, but there was a strong tendency for AVRs to be followed by thrusts suggesting some form of neural coupling of the two movements. AVRs were always accompanied by a ventral swinging of segment 3 on 2 as occurs in stage 1. Impeding the posterior thrust

mechanically caused the production of a short series of rapid thrusts, implying feedback control from receptors.

Activity of the penis in intact *Calopteryx splendens* was much easier to evoke, and often occurred spontaneously in tethered males. Activity consisted of very strong AVRs followed by powerful posterior thrusts. Sometimes a sequence of weaker thrusts followed an AVR as in *E. cyathigerum*. In decapitated males, spontaneous activity might persist for several minutes and mechanical stimulation gave rise to long sequences of activity. Sometimes very fast sequences of short thrusts (ca 3 s⁻¹) alternated with flips of the penis head ventrally before the next long AVR intervened. Interestingly, teneral males of both species exhibited little or no activity of the penis under similar conditions.

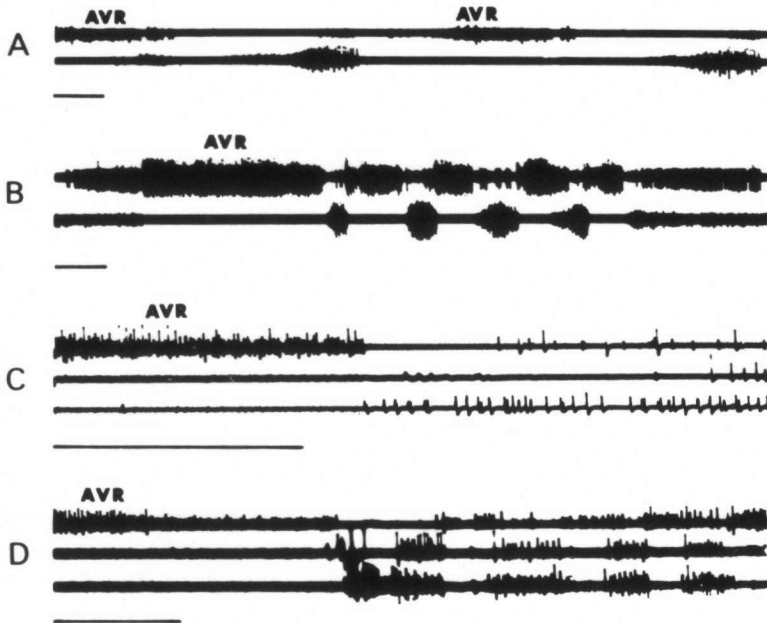


Fig. 6. Electromyograms from restrained male damselflies during spontaneous or mechanically-evoked penis movements (AVR, anterior-ventral rotations of the penis): (A) records from muscles 11 (upper trace) and 9a (lower trace) of *Enallagma cyathigerum*. (there is an alternation of AVRs (M 11) with posterior thrusts (M 9a)); — (B) records from the homologous muscles in *Calopteryx splendens* (a long AVR is followed by a series of short alternating bursts in M 11 and M 9a which produce a series of quick ventral flips of the penis head with intervening brief posterior thrusts); — (C-D) records from M 11 (top trace), M 9b (middle trace) and M 9a (bottom trace) in *C. splendens* (in C, an AVR is followed by a slow posterior thrust; in D, an AVR is followed by a series of short posterior thrusts and alternating head flips; M 9b shows activity in phase with M 9a and the posterior thrusts). — Horizontal scales: A, C and D, 1 s; B, 5 s.

ELECTROMYOGRAMS RECORDED DURING SEQUENCES OF ACTIVITY

Recordings have been made from males of *E. cyathigerum* and *C. splendens* (Fig. 6). Pairs of wires were inserted into each of muscles 9a, 9b and 11 in a male restrained on a plasticene block. AVRs were found to be produced by high-frequency bursts in several units supplying m 11, and in *C. splendens* they sometimes persisted for up to 10 s (Fig. 6 B-D). Lower-frequency bursts with one or two units contributing accounted for contractions in m 9a which produced posterior thrusts. In *E. cyathigerum* bursts in the two muscles tended to alternate without overlap and sometimes with 1 s between the termination of one and the start of the other. M 11 and 9a were seldom seen to fire synchronously and they normally acted as antagonists. In *C. splendens* the sequences of brief thrusts and rapid ventral flips of the penis head were produced by a fast alternation of short bursts in m 11 and 9a (Fig. 6D). Bursts occurred in m 9b more or less synchronously with those in m 9a, but sometimes starting later. Contractions of m 9b may prevent the shaft from riding ventrally during posterior thrusts, but the contribution of this muscle is not fully understood.

The stereotyped nature of the activity and the strong coupling of AVRs to subsequent posterior thrusts suggest that this sequence is a normal component of copulatory activity. Ventral swings of segment 3 on 2 accompany AVRs, and the similar segmental movement which helps to define the active phase of stage *I* may therefore also be accompanied by an AVR. This is discussed further below.

DISCUSSION

BUCHHOLTZ (1956) described the occurrence of rhythmic movements in the copulation of *Platynemis pennipes* in all except the final few minutes, and similar movements have been noted in other zygopteran species (CORBET, 1962; ROBERT 1958; WAAGE, 1979a). Copulation lasts for relatively long and variable periods in several aeshnids and zygopterans, and perhaps the record is 340 min observed in a pair of *Ischnura elegans* (CORBET, 1962). In contrast, some libellulids copulate very rapidly, the activity lasting an average of only 3 s in *Platthemis lydia* (JACOBS, 1955). Sperm removal or displacement may be prevalent in species with prolonged copulatory behaviour, but other factors also affect its duration and no detailed study of libellulid copulation has yet been made. Clearly less than 1.5 min is adequate for male *Calopteryx maculata* to remove most of the sperm from females (WAAGE, 1979a).

In *E. cyathigerum* the occurrence of a filled and swollen sperm store in stage *I* males and the absence of sperm from the bursa and spermatheca of at

least some stage *I* females suggest that sperm are removed from the female during this stage, although we have not been able to confirm that early-morning females carried full sperm loads. Similarly WAAGE (1979a) thought that the undulatory activity of copulating *C. maculata* was connected with sperm removal. The activity of the penis evoked in males in the laboratory may represent stage *I* activity, although probably somewhat altered and at a lower frequency. The predictability of the activity and its association with ventral swings of segment 3 on 2 support this interpretation. The active phase of stage *I* coincides with a small displacement of the female genital segments away from those of the male, which could possibly be produced by an AVR of the penis as it was pulled out of the vagina and rotated ventrally and outwards to deposit extracted sperm on the valves of the ovipositor, a region where abundant sperm have been found. The ensuing posterior thrust would re-introduce the penis into the vagina and carry it forward into the bursa where it has been located in some dissected stage *I* females. Other activity comparable for example to the stage *II* rhythm has not been seen in the laboratory.

In most non-mating tandem females, both the spermatheca and the large sac-like bursa were filled and swollen with sperm, implying that the damselflies had mated previously. However, 58% of stage *I* females dissected were found to have the bursa collapsed and empty although the spermatheca was still swollen and full of sperm, 16% had both sacs full, and 16% had both empty. Since no record was made of the time in stage *I* at which the pairs were caught several interpretations are possible, one being that the bursa is emptied early and the spermatheca late in stage *I*. In several pairs of mating *C. maculata*, WAAGE (1979a and pers. comm.) has found the curled penis horn inserted into the T-shaped spermatheca. However, in *E. cyathigerum* the penis has no comparable structure which could enter the narrow spermatheca duct, and it may be suggested that, if the bursa is emptied, the spermatheca is stimulated to contract slowly and eject the sperm within it. If this did not occur readily, sperm stored there would be at a disadvantage compared to sperm in the bursa.

A prolonged stage *I* in *E. cyathigerum* may thus ensure the removal of all sperm. However, we have observed copulation in *Platycnemis latipes*, *Cercion lindeni*, and *Coenagrion scitulum* (all in France) to be similarly prolonged (e.g. 53 min in *C. scitulum*, with sperm transfer to the secondary genitalia occurring only after 44 min). All these species have been found to possess horns or whip-like processes on the penis head comparable to those of *C. maculata* and possibly able to enter the spermatheca. Prolonged copulations in some species are not therefore necessarily to be explained by the lack of structures which can enter the spermatheca.

During stages *II* and *III*, the sperm store is compressed and emptied, and

sperm then pass into the female and enter the bursa and spermatheca. The examination of pairs killed late in copulation supports this interpretation. The sperm store is compressed between segments 3 of the male and 8 and 9 of the female, aided probably by the rhythmic movements of stage *II*. Sperm are not enclosed in a spermatophore in the sperm store, and experimentally applied compression can force sperm out of the orifice. The medial spine at the posterior end of the female 8th sternum may also assist in liberating sperm, but such a spine does not occur in other European zygoterans. During stages *II* and *III* the penis remains inserted in the vagina and its hollowed dorsal surface forms a conduit along which sperm enter the female. The mechanism of their transport is unclear, but the stage *II* rhythmical movements may assist. Ventilatory pumping has been seen to draw sperm into the vagina in the laboratory, but whether this contributes normally is unknown.

The evidence discussed earlier suggests that while males alone can initiate and terminate the tandem association, females can choose to accept or reject copulation, and can terminate it before completion. Observations in the field have shown that stage *I* is frequently broken off by the female violently withdrawing her abdomen. Copulation may be resumed shortly after such interruptions, or after a change of perch. Disturbed or captured pairs also readily disengage the genitalia if in stage *I*. In contrast, spontaneous disengagement has not been seen in stage *II* or *III*, and disturbed or captured pairs may remain in the copulatory (wheel) position. Females apparently show a much stronger commitment to the completion of copulation after stage *I*, possibly because by that time they have been deprived of most sperm. Males which find females to be consistently unreceptive finally abandon the tandem association and free them.

One cost to the male of sperm removal is the unavoidable delay before he can introduce his own sperm and the consequent risk of losing the female before this has been achieved. Although sperm removal may not benefit the female, she may gain subsequently by being guarded during oviposition, which WAAGE (1979b) has suggested is advantageous to ovipositing female *C. maculata*. A further cost of sperm ejection in a territorial species may be that prolonged copulation increase the risk of losing a territory to another male. The performance is strikingly more rapid in the territorial *C. maculata* than in the apparently non-territorial *E. cyathigerum*.

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