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AN EXAMINATION OF THE PROLONGED COPULATIONS OF *ISCHNURA ELEGANS* (VANDER LINDEN) (ZYGOPTERA: COENAGRIONIDAE)

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Copulatory activity of *I. elegans* is described in a high-density population in the south of France. Copulations lasted for 324 ± 90 min (s.d.) with stage I occupying 239 ± 112 min, and stage II, 85 ± 48 min. The characteristic movements of stages I and II are described. They differ from those in other zygopterans by being frequently interrupted by extended inactive pauses which account for the long duration of copulation. Long copulation may allow males to guard females until they are prepared to oviposit after 16:00 h. Close-up observations of copulations were made possible by crushing the heads of captured pairs: This allowed females to be dissected without interrupting copulation, and video films of the penis movements within the transparent genital tract of the female were made. Sperm removal from the bursa and the subsequent transfer of sperm from the male were witnessed. Movements of the penis were correlated with those of the second and third abdominal segments of the male. Ablation experiments showed that continuation of copulation depended only on the maintenance of contact between the ovipositor blades and recesses in the male, probably containing sensory bristles. Reflex responses of vaginal and spermathecal muscles were brought about by stimulation of vaginal campaniform sensilla by the penis. Pairs with crushed heads performed copulations without pauses and lasting about 1 h, as did tethered intact pairs. Similar short copulations also sometimes occur in the wild.

INTRODUCTION

Prolonged copulation is a common feature of the reproductive behaviour of many insects. It may allow males to transfer more sperm and other materials to females, to displace the sperm of rivals more effectively, or to retain and guard a female until she is ready to oviposit (PARKER, 1970; WALKER, 1980;

SILLÉN-TULLBERG, 1981; THORNHILL & ALCOCK, 1983; SIVINSKI, 1983; WAAGE, 1984). *Ischnura elegans* may sometimes copulate for 340 min (KRIEGER & KRIEGER-LOIBL, 1958), while *I. ramburi* does so for up to 400 min and for a mean of 202 min (s.d. ± min) (ROBERTSON, 1985), periods longer than are known in other odonates.

I. elegans is known to continue to copulate after capture (PFAU, 1971), and this makes possible close observation and filming of the movements as well as manipulation of both partners. This advantage has been utilised in the present study in an attempt to explain the long duration of copulation in this species.

Copulatory activity is known to continue in decapitated male mantids; likewise the isolated abdominal ganglia of male cockroaches generate motor patterns which are probably copulatory in nature (ROEDER et al., 1960). I describe here a means of decapitating male and female *I. elegans* while in copula, a procedure which allows them to be manipulated and dissected without interrupting the copulatory activity. It has permitted the direct observation of sperm removal and sperm transfer and it promises to provide valuable information about the mechanisms involved, as well as about the sensory and motor control of copulatory movements. Sperm removal is now well known in several odonate species (WAAGE, 1979, 1982, 1984, 1986a, 1986b; McVEY & SMITTLE, 1984; FINCKE, 1984), but there is still a lack of information about the precise mechanisms involved.

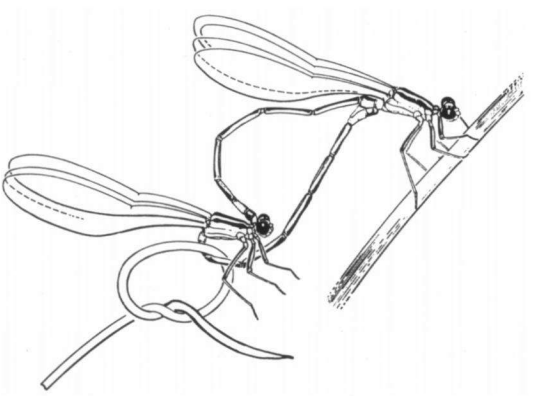

Fig. 1. A pair of copulating *Ischnura elegans* tethered by knotting a *Juncus* stem between their abdomens.

MATERIAL AND METHODS

Ischnura elegans were examined at several sites close to the biological research station at La Tour du Valat in the Camargue (4°38'E, 43°35'N), southern France, during July 1984 and August 1985. The species was exceptionally abundant among vegetation in fields flooded for experimental purposes and previously used for rice growing, and numerous copulations could be observed there. In nearby unflooded fields of lucerne some copulating pairs were also found along the margins, but copulation was not otherwise observed away from water. Except for small numbers of *I. pumilio*, no other zygopteran was present.

Undisturbed copulating pairs were observed through a close-focus monocular and activity was

recorded on a pocket tape recorder. It was also filmed with a Panasonic WVP 200E portable video camera fitted with a 300 mm lens (35 mm format) and extension rings to enable copulating pairs to be filmed at a distance. Pairs were tethered by threading a *Juncus* stem or a wire through them and then knotting it (Fig. 1), and they were then brought to a central observation site. They sometimes struggled initially but usually persisted with copulation. Copulating pairs were also caught in a net, individually marked on the wings with patterns of coloured dots using permanent-ink felt pens, and then released. This could easily be done without breaking copulation.

Further copulating pairs were caught by net and their heads were then crushed ("decapitation"), after which they could be handled and dissected without interrupting copulation. Examinations and dissections of pairs in copula were carried out in a Volkswagen van parked beside the site and fitted with work table, binocular microscope, video camera, tripod and spot lamps. This enabled examinations and close-up video recordings to be made, using a 55 mm micro lens and extension rings, within one minute of capture. Copulating females were dissected from the dorsal side on a plasticene block and Ringer was added periodically. Under these conditions copulation persisted for over one hour. Video records were analysed by using the freeze-frame and slow-motion facility and drawings were made on transparencies laid on the monitor screen. Voice records were analysed with the help of an oscilloscope and press-button keys. Shade temperatures in the middle hours of the day were 28-32° C, while the temperature in the van was normally 30-32° C.

For microscopical examination, female genitalia were placed in Bouin's fixative, serially sectioned at 10μm and stained in Masson's trichrome stain. Scanning electron microscopy was carried out with a Philips PSEM 500.

DAILY ACTIVITY OF *ISCHNURA ELEGANS*

Observations on reproductive behaviour were made at the flooded fields throughout several days early in August. Teneral and mature individuals of both sexes were present at the site and they roosted at night on vegetation emergent from the water or on that which grew nearby. Sunrise was at about 06:30 h EST and by 07:20 h the first individuals had begun to flutter and to fly to the tops of the vegetation when still in the shade and at air temperatures of 16-20° C. From 07:30 to 07:45 h, males made longer flights inspecting vegetation and flying with the tips of their abdomens turned downwards (hockey-stick position), apparently searching for females. They grasped perched conspecifics and formed tandem linkages with them. Most often these were with other males since males outnumbered females considerably, but such homosexual tandems lasted only a few seconds (cf. ROBERTSON, 1985). Heterosexual tandems in contrast usually lasted a few minutes on sunny mornings before copulation started and for up to an hour when it was misty. The first copulations were observed at about 07:50 h and by 08:30 h in the sun they had become very numerous with as many as 2-3 copulating pairs m^{-2} in some parts of the field and an overall density of 0.13 m^{-2}. No feeding was observed to precede early mating, but males which failed to find a mate continued to search intermittently during the morning, sometimes approaching tandem pairs, and occasionally feeding.

In *Enallagma cyathigerum* and some other Coenagrionidae, copulation can be divided into three stages (MILLER & MILLER, 1981). In the first and longest,

there are fast jerking movements by the male's anterior abdominal segments during which sperm are removed from the female. In the second brief stage, much slower rocking movements appear and sperm are transferred to the female; finally in a third stage no visible movement occurs. Movements characteristic of stages I and II have been identified in copulating *I. elegans* but they are commonly interspersed with long inactive pauses within each stage.

By 09:00 h most mature females present were in copula, but a few copulations were seen to start later in the morning perhaps as new females arrived. Surveys were made of the stage of copulation reached at particular times along 10 m of bank at the edge of the flooded field. The stages were identified by the characteristic abdominal positions and movements (see below). In Table I it is shown that most copulating pairs were in stage I in the morning and had reached stage II by 14:00 h.

Table I

The occurrence of different copulatory stages among *I. elegans* surveyed at intervals along 10 m of bank beside a flooded field in the Camargue on 7th August 1985

Stage	*Time*				
	9-10:00	11-12:00	14-15:00	15-16:00	16-17:00 hr
I	15	11	2	1	0
II	1	1	25	10	1
Tandem	1	2	7	3	1

During 3 days 40 copulating pairs were captured, marked on the wings and then released at either 09:30 or 10:00 h in one of two well-defined areas. Fifteen pairs were watched until copulation had been completed. Taking 09:00 h as the starting time for copulation (several had probably started earlier) the duration of copulation was 324 ± 90 min (s.d., n = 13). Stage I lasted 239 ± 112 min (n = 15) and stage II, 85 ± 48 min (n = 13). Figure 2A shows records of 7 marked pairs all at one site, Figure 2B shows shorter records from additional unmarked pairs at the same site, and Figure 2C shows the state of further pairs nearby; all are approximately in phase. Thus copulations lasting 5-6 h were common in this population and the longest recorded copulation lasted 456 min.

During copulation some pairs made several changes of perch, possibly in response to temperature or wind, but others did not move. Copulating pairs in flight attracted single males which grappled with them and sometimes succeeded in breaking the copulatory wheel, but no successful take-over was witnessed. Those pairs in which the tandem linkage but not genital contact had been broken, and others which rested in tandem, also attracted attention from single males but settled copulating pairs were seldom seen to be approached.

At the end of copulation, pairs perched in tandem for periods ranging from 4 to 68 minutes; after this they separated and sometimes continued to perch alone in the

same region. From 16:00 h to dusk (sunset at about 21:05 h EST), many individuals were observed to feed and oviposit. No oviposition was observed before 16:00 h in agreement with earlier observations (ROBERT, 1958; KRIEGER & KRIEGER-LOIBL, 1958; HEYMER, 1967) and, in 1985, only one copulation was observed after 17:00 h. Females oviposited alone without male guarding and they were aggressive towards intruding males or females, attacking and sometimes clashing with them with the abdomen in the hockey-stick position. They flew at intruders, accelerating rapidly with a flight similar to that used in the capture of settled prey (cf. BICK, 1966; JURZITZA, 1970). In this way females reserved an oviposition site temporarily for their own exclusive use.

Most odonate species break off copulation when captured by net. *I. elegans* is unusual in its commitment to copulation after capture (PINHEY, 1969; PFAU, 1971). All of a sample of 15 pairs netted in stage I persisted with copulation after capture, but 5 of them broke the tandem linkage. Only 3 out of a further 15 pairs netted in stage II persisted with copulation after capture, the remainder separating in the net. Thus the commitment to copulation is greater in stage I than in stage II. Those stage I pairs which broke the tandem connection but maintained genital contact persisted with copulation without reforming the tandem. Occasional undisturbed pairs have also been seen to be in copulation without tandem linkage. Thus although tandem linkage probably provides information to female Zygoptera about the species identity of males (ROBERTSON & PATERSON, 1982) and is necessary for the initiation of copulation in all species, it is not needed for its continuation. The breakage of the tandem is presumably a male decision, whereas disengagement of the genitalia may be a female or a joint decision.

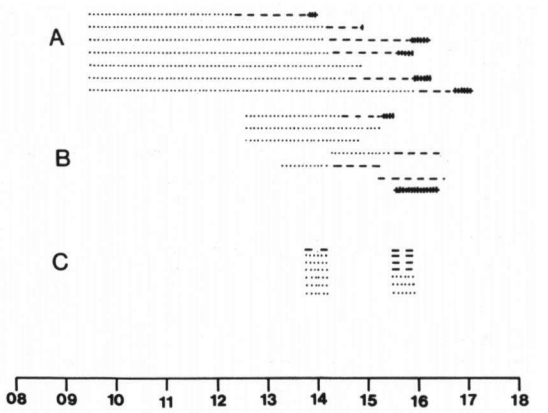

Fig. 2. The duration of copulations of *Ischnura elegans* at flooded fields in southern France: (A) 7 marked pairs observed continually from 09:30 h until separation; — (B) 7 unmarked pairs observed continually but for shorter periods at the same site; — (C) 7 unmarked pairs observed intermittently at the same site. — (......, stage I; ———, stage II; ++++++++++, post-copulatory tandem. H, time in hours).

ANATOMY OF THE GENITALIA
MALE

The secondary genitalia of male Zygoptera (Fig. 3) consist of a sperm vesicle formed on the 3rd abdominal sternite, and a complex penis (ligula) formed from the 2nd abdominal sternite and divided into a long rigid shaft and a flexible head. The penis is moved by muscles which act on the anterior and posterior frames and on the anterior lamina (Fig. 3). The sperm vesicle retains sperm translocated there from the primary genitalia shortly before copulation (ASAHINA, 1954; PFAU, 1971; MILLER & MILLER, 1981).

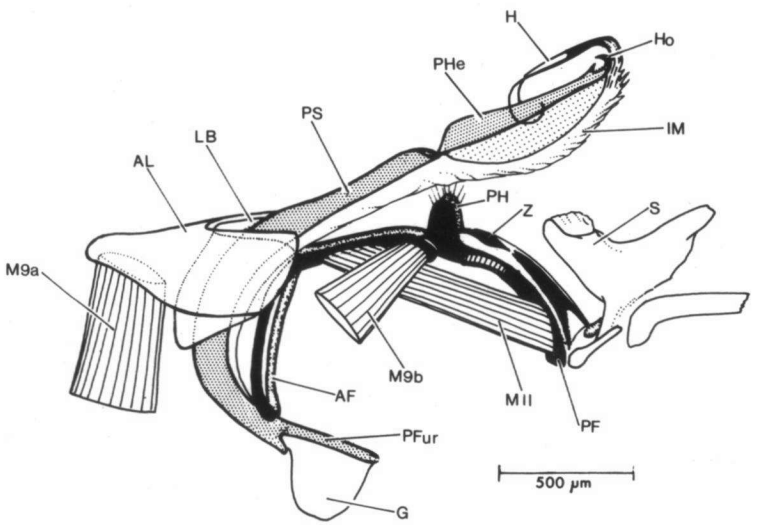

Fig. 3. Diagram of the penis of *Ischnura elegans* showing the surrounding cuticular structures and muscles which move the penis. AF, anterior frame; AL, anterior lamina; G, glandular sac; H, horns of penis; Ho, hooks of penis; IM, inflatable membrane; LB, lamina batilliformis; M, muscle; PF, posterior frame; PFur, processus furculiformis; PH, posterior hamule; PHe, penis head; PS, penis shaft; S, sperm vesicle; Z, zipfel.

In *I. elegans* the penis head terminates in a small flap of cuticle normally folded back along the ventral ("upper") side, to which are attached two coiled horns bearing a few distal barbs, each horn being about 3-5 µm wide distally and 6-700 µm long (Figs 4, 5). The flap tilts upwards when pressure is applied to the membrane, as indicated in Fig. 5C. The horns and flap are resilient and they snap back along the head after being released. A pair of rigid hooks occurs near the base of the flap, and there are about 7 stout bristles on each side protruding laterally close to a small sclerite in the neck region of the penis.

The dorsal side of the penis bears a large and deeply furrowed, pliant membrane which extends back to the base of the shaft and ends in the processus furculiformis on the dorsal side of which there is a glandular sac (Fig. 3). The space enclosed within the sac communicates with the liquid in the furrowed membrane, the whole being termed the Schwellkörper by PFAU (1971). The cells of the glandular sac are thought to be able to secrete a liquid into the internal cavity, thereby inflating the membrane and extending the crumpled distal portion (Fig. 5A). Such inflation occurs after the start of copulation, as described below. The possession of a separate fluid system allows the penis to remain inflated during copulation unaffected by changes in haemolymph pressure. The membrane system of the penis of *I. elegans*, and the extent of its inflation, are greater than in any other zygopteran so far examined (Miller, P.L., unpublished).

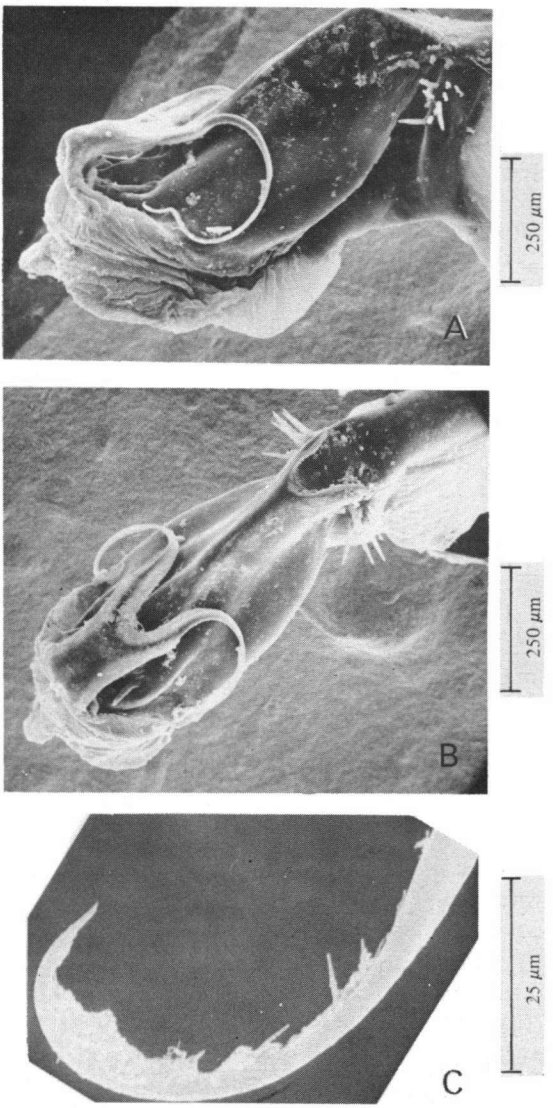

Fig. 4. Scanning electron-micrographs of the penis of *Ischnura elegans:* (A) top view; — (B) side view; (C) tip of one horn.

FEMALE

The vagina comprises a short pouch-like posterior part and an anterior region

bounded by two flat cuticular plates lying in the dorso-ventral plane (Fig. 6A). The plates are hinged along the mid-dorsal line and are joined ventrally by a region of thick infolded and rubbery cuticle which allows them to separate but causes them to snap back on release. The plates can be pulled apart by muscles running between them and the body wall, or they can be forced apart from within. Each plate bears 18-20 campaniform sensilla which are scattered along the mid-line and have been identified in scanning electronmicrographs (Fig. 6A). The sensilla send their axons to the 8th abdominal ganglion in the anterior lateral nerve.

Anteriorly the vagina leads through a complex valve into a pair of oviducts, and dorsally into the bursa and spermatheca where sperm are stored. The spermatheca is joined to the base of the bursa by a narrow muscular duct, about 600 μm long and very much convoluted; it has a lumen 12-19 μm wide when flattened. The walls of the bursa and spermatheca have intrinsic muscle fibres, and further fibres run from the spermatheca to the dorsal side of the vagina.

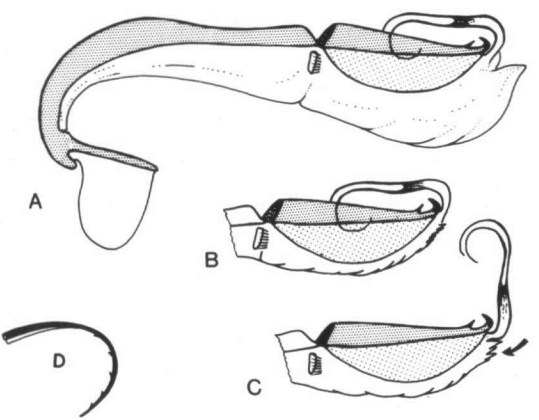

Fig. 5. Penis of *Ischnura elegans:* (A) the membrane is shown inflated as occurs during copulation; — (B) membrane deflated — (C) elevation of the horns by applying pressure to the distal end (arrow); — (D) the distal part of a horn showing the barbs.

The valve, formed of transparent resilient cuticle and staining green in Masson's trichrome stain, is attached ventrally and forms an ovoid structure blocking the vaginal passage anteriorly (Fig. 6B). It has anterior and posterior lips which allow an egg to pass from the oviduct to the vagina but prevent its return. Access from the vagina to the bursa is blocked by a thick rib of cuticle which presses down on the dorsal side of the ovoid valve (Fig. 6C). By inserting a micro-syringe into the vagina it was possible to force a dilute solution of methylene blue into the bursa which became inflated. Closure of the rib against the valve prevented the escape of the fluid, but strong pressure applied by forceps to the bursa, or gentle opening of the valve with a pin, allowed small puffs of blue solution to enter the vagina. Thus the valve and rib normally provide a tight seal between the bursa and vagina, but it can be opened from the vagina. A similar valve has been found in all Zygoptera so far examined (Miller, P.L., unpublished).

Spermathecal muscle shows much activity in dissected living females, twitching and contracting continually in some individuals, while bursal muscle is less active. The movements of a mounted needle or of a penis artificially introduced into the vagina caused strong reflex contractions of vaginal muscles in some preparations, which pulled the whole tract posteriorly; simultaneous spermathecal contractions caused that organ to become C-shaped. When the anterior lateral nerves of the 8th abdominal ganglion were cut, these responses ceased although slower spontaneous contractions in the spermatheca persisted. Spermathecal activity therefore seems to be partly endogenous, perhaps arising myogenically (cf. T.A. MILLER, 1975), and partly due to the activation of receptors in the vagina. The duct of an isolated spermatheca was seen under the microscope to be active and to be able to drive sperm peristaltically in either direction.

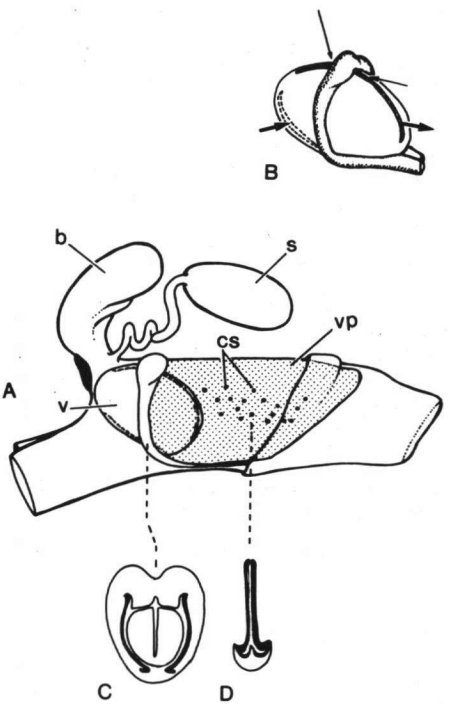

Fig. 6. The genital tract of female *Ischnura elegans:* (A) view from the left, dorsal upwards; — (B) the ovoid valve which lies between the vagina and the oviducts (thick arrows indicate the route of an egg into the vagina. Thin arrows indicate sperm entry into or exit from the vagina); — (C) section through the valve region of the vagina; — (D) section through the vagina at the level of the plates (b: bursa; — cs: campaniform sensilla; — s: spermatheca; — v: valve; — vp: vaginal plates).

The following sequence of events at fertilisation can be postulated (Fig. 7). An egg is forced into the vagina through the anterior and posterior lips of the ovoid valve by contractions of the oviduct assisted by a muscle which pulls the vagina anteriorly; simultaneous contractions of vaginal muscles pull open the plates. The plates are then allowed to close posteriorly, forcing the egg anteriorly: its pointed end rides dorsally on the ovoid valve and opens the entry from the bursa. This allows the release of sperm driven proximally by spermathecal and bursal contractions which in turn result from the excitation of the vaginal campaniform sensilla by the egg. After making contact with the sperm, the egg is ejected by closure of the plates at the anterior end and by contraction of muscles which pull the vagina posteriorly.

COPULATORY ACTIVITY

In undisturbed copulating pairs in the field, the activities characteristic of stages I and II were periodically interrupted by long inactive periods which greatly extended the duration of copulation. The same movements and sequences of stages occurred in tethered or decapitated and dissected pairs as in undisturbed pairs, but in the former copulation lasted only about one hour because there was no period of inactivity. Dissections of females enabled the movements of the penis to be correlated with segmental flexions, and they verified that the female contributed no rhythmic movement other than those of ventilation and of vaginal and storage-organ muscles (see below). The following description is based on video and voice recordings of undisturbed pairs, and on pairs examined under the microscope, intact or after decapitation and dissection.

Initiation. — After variable intervals of tandem perching the copulatory wheel position was assumed. Manipulaton of the penis has shown that it can readily be inserted into the genital opening of a female in both *I. elegans* and *Enallagma cyathigerum* after separating the ovipositor blades and without the need to fold the head, contrary to what was thought previously (MILLER & MILLER, 1981).

Stage I. — The male alternately depressed then straightened the first two abdominal segments for the first few minutes of stage I, doing so at 30-50 min^{-1} without interruption (Fig. 8A). This type of movement was then replaced by a rhythmical lifting and lowering of these segments (Figs 8B, 10) which persisted either intermittently in undisturbed pairs, or continually in tethered or decapitated pairs throughout the remainder of stage I. In tethered or decapitated pairs this stage lasted for a mean of 16 ± 10.3 min (s.d.; n =8) after capture (time of start not known), and in a further pair, decapitated 1 min after the start of copulation, for a total of 36 min. The frequency of movements (excluding pauses) ranged from 12-17 min^{-1} at 20° C to 33-40 min^{-1} at 32° C. In undisturbed pairs, sometimes only 2 or 3 cycles occurred before the onset of the next long pause, and some pauses lasted for as much as 123 min.

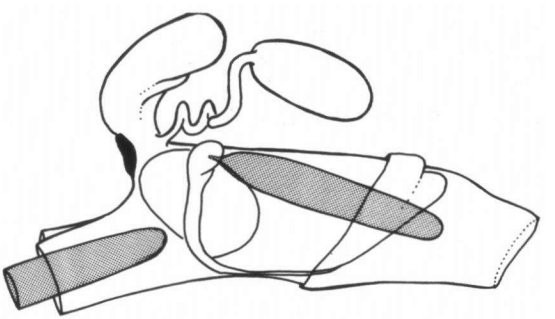

Fig. 7. The genital tract of a female *Ischnura elegans* showing the postulated positions of an egg before and during fertilisation.

In dissected females the penis could be seen to make anteriorly directed thrusts within the genital tract corresponding to the lowering of segment 2 of the male. The penis withdrew partly just after segment 2 was lifted, and the magnitude of the excursions of the penis within the female's tract corresponded to the amplitude of the segmental flexions. In undissected females the size of penis movements could be gauged from the extent of the inward bending of the anterior lamina, a movement responsible for forcing the penis posteriorly (PFAU, 1971, MILLER & MILLER, 1981).

In dissected females the flap and horns of the penis could be seen to be within the bursa for much of stage I. Small retractions of the penis pulled the penis head posteriorly in the vagina, uncurling the horns and partly withdrawing them from the bursa (Fig. 9A). The ensuing thrust then pushed the horns back into the bursa where they again coiled round through 180-360° (Fig. 9B). Large retractions of the penis pulled the penis head back through 700-1000 μm into the posterior pouch of the vagina, while the uncoiled horns lay between the vaginal plates (Fig. 9C). Throughout copulation the penis was never totally withdrawn from the genital tract of the female, and no part of the penis was seen to enter the spermathecal duct at any stage. Similarly in 19 females captured and preserved in copula, the horns were commonly found in the bursa but never within the spermathecal duct (MILLER, 1987).

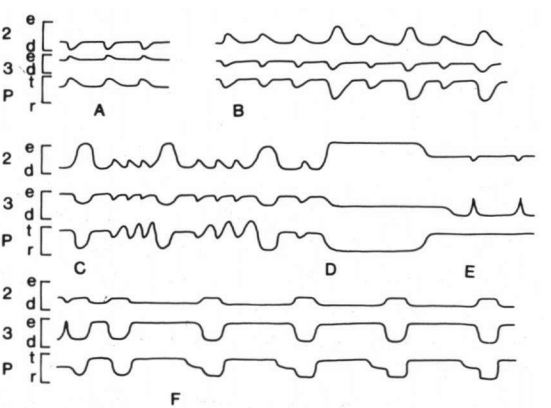

Fig. 8. Schema of the correlated movements of the second (2) and third (3) abdominal segments of a male *Ischnura elegans* and of the penis (P) during episodes of copulation: (A, B, and C) early, middle and late times during stage I; — (D) transition; — (E) stage IIA; — (F) stage IIB. Further explanation in the text. (e: elevation; — d: depression; — t: thrust; — r: retraction).

Towards the end of stage I, the amplitude of some flexions of segment 2 increased to 15-20°, corresponding to more extensive penis thrusts and retractions. At first one or two small penis retractions alternating with weaker thrusts preceded each more extensive and sustained retraction (Fig. 8B); later, up to 5 or 6 small retractions alternating with increasingly extensive thrusts again preceded each large retraction (Fig. 8C). Electromyograms, taken from muscle 11 of a single decapitated *Calopteryx splendens* and described previously (MILLER & MILLER, 1981), showed a pattern of 4-5 small motor bursts

followed by a prolonged burst. Muscle 11 rotates the anterior frame on the posterior frame pulling the penis anteriorly in doing so, and it probably contributes to the retraction (PFAU, 1971). The pattern recorded in *Calopteryx* closely resembled that of the penis movements seen in *I. elegans*. The strong retractions were seen to pull clumps of sperm on the penis hooks from the bursa and they may have accumulated in the posterior vaginal pouch until the end of copulation.

Dissection of a copulating female allowed the bursa, spermatheca and most of the vagina to be removed, leaving the penis inserted through the genital opening; the unrestricted movements of the penis could then be observed. Strong retractions were now seen to be accompanied by a rotation of the whole penis through 80-90° so that it stood pointing dorsally within the female. Such movements, termed anterior-ventral rotations (AVRs), have previously been described in decapitated male *Calopteryx splendens* and *Enallagma cyathigerum* when penis activity was induced (MILLER & MILLER, 1981). The rotation of the penis is restricted in intact females but it may lift the vagina dorsally and push the female away slightly during retraction. With each thrust of the penis the segments of the partners were pulled together by the combined actions of muscles 9a and 9b (Fig. 3; PFAU, 1971).

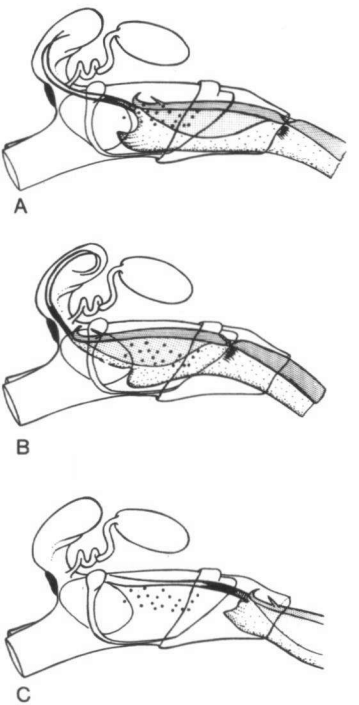

Fig. 9. The observed positions of the penis in the female genital tract during copulation of *Ischnura elegans:* (A) partial retraction of the penis leaving the horns still in the bursa; — (B) a thrust of the penis which causes the horns to coil round in the bursa; — (C) strong retraction of the penis which results in the horns being pulled back between the vaginal plates.

Throughout stage I the posterior margin of the female's sternite 8, which bears a prominent spine, remained above and anterior to the sperm vesicle (Fig. 10A, B), and it was sometimes pushed down onto the anterior end of the vesicle forcing the vesicle contents posteriorly. Inspection of the vesicle in 20 males caught and preserved during copulation showed that it remained filled with sperm throughout stage I.

Transition. — Stage I was terminated by a strong elevation of the male's segment 2 and retraction of the penis, both being maintained for about 16 s (exceptionally for 40 s in decapitated insects) (Fig. 8D). The penis head remained in the posterior pouch of the vagina. Segments 1 and 2

were then slowly lowered and the penis was forced anteriorly so that its head again lay between the vaginal plates. After a further 10-12 s, segment 3 of the male was flexed downwards through about 20° and stage IIA commenced (Fig. 8E).

Stage IIA. — In this stage a series of swift elevations of segment 3 (i.e. dorsal flexions), each followed by a rapid depression and altogether taking about 400 ms, occurred at intervals which gradually increased from 6 to 10-16 s (Fig. 8E). Stage IIA lasted 2-4 min and there were up to 20 cycles of movement throughout which the penis remained in the vagina without movement.

Stage IIB. — The segmental movements of this stage were similar to those of IIA (Fig. 10C, D), but the elevations of segment 3 were extended in duration while the depressions were shortened (Fig. 8F), and the frequency declined to about 1 cycle min^{-1}. The penis was again active, thrusting anteriorly within the

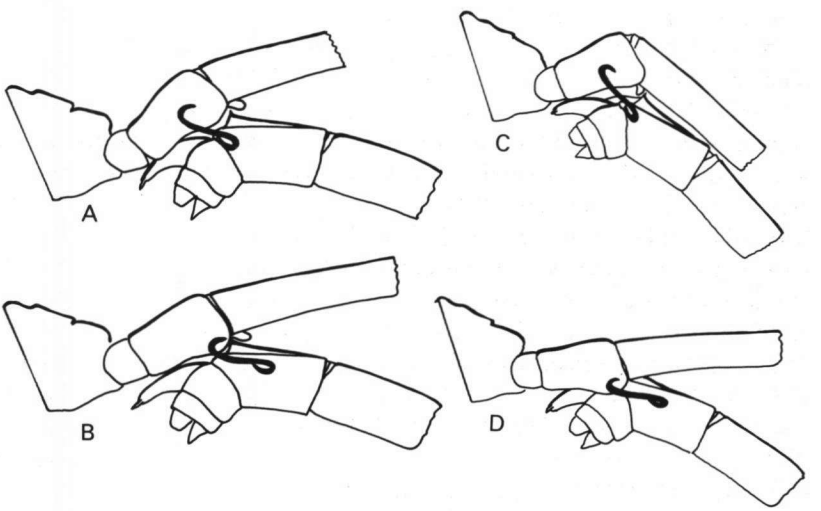

Fig. 10. The correlated positions of the second and third segments of the male and of the penis during copulation of *Ischnura elegans:* (A) stage I, retraction of the penis; — (B) stage I, thrust of the penis; — (C) stage IIB, retraction of the penis; — (D) stage IIB, thrust of the penis.

female with each elevation of segment 3 (which coincided with a small depression of segment 2) while it was retracted slowly and in two stages during the depression of segment 3 (elevation of segment 2). In dissected females most retractions of the penis were seen to be accompanied by AVRs as in stage I. Table II compares the duration of the phases of stage II in undisturbed, tethered and dissected pairs and shows them to be similar.

In tethered or decapitated pairs, long pauses did not occur in stage IIB and the durations of the stage in 5 decapitated pairs were 15, 27, 55, 57 and 72 min,

Table II

The duration of phases in Stage IIB copulatory activity in *Ischnura elegans* under various conditions. (Each horizontal line is from a single insect)

State	Elevation of segment 3 (seconds)			Depression of segment 3 (seconds)			Cycle duration (seconds)
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	
Undisturbed	15	44	19	20	9	3.5	53
Tethered	12	21	7.6	11	10.2	2.6	31
Decapitated	4	37	2.4	4	22.5	2.9	59
Decapitated	7	49	2.2	7	15	0.8	64
Decapitated	5	37	1.3	6	14.5	1.1	51
Decapitated & dissected	8	32.5	2.4	9	15	1.4	47

termination being signalled by the disengagement of the genitalia. In a 6th pair disengagement was attempted after 55 min but it failed and the pair then recommenced stage IIA followed by a further long bout of IIB.

In stage IIB, the partners separated slightly as segment 3 was depressed and the sperm vesicle was forced anteriorly allowing the female's 8th sternite to fit behind it. The ensuing elevation of segment 3 and thrust of the penis pulled the segments together, compressing the vesicle and expelling sperm from it (Fig. 10C, D). The furrowed membrane of the penis was seen to ride over the opening of the vesicle, carrying sperm into a sperm canal formed between the penis membrane and the female's genital opening. The zipfel (Fig. 3), a small flap of cuticle which lies below the penis and probably acts as a piston helping to drive sperm into the female's opening (PFAU, 1971), was seen to be rotated into the sperm canal with each thrust of the penis. Inspection of the vesicles of 10 males preserved in stage IIB showed 8 to be empty and 2 to be partly filled.

The stored sperm of previous males, after being removed from the bursa, was accumulated along the ventral ("upper") side of the penis and possibly also in the posterior vaginal pouch. It was removed, clasped between the hooks and the folded horns, only when the penis was finally withdrawn at the end of copulation.

Stage III. — In decapitated pairs no stage III or postcopulatory tandem was discernible and separation occurred at the end of stage IIB.

PENIS INFLATION

By observing the start of copulation in the field in 5 pairs and then pulling each pair apart at known intervals, it was found that inflation had commenced 5 min after the start of copulation, had reached about 50% after 10-15 min, and was completed after 15-20 min. At the end of copulation penis deflation was found to take over an hour. This conveniently allows males which have recently copulated

in the field to be identified. Inflation of the membrane is probably important for the transfer of sperm from the male into the genital tract of the female, an activity associated with stage II. Since stage I lasts for at least 30 min, and commonly much longer, the time needed for inflation is not disadvantageous. In other Zygoptera examined in which copulation is of shorter duration, the penis inflates to a much smaller extent or not at all.

THE CONTROL OF COPULATORY ACTIVITY

Copulatory activity by males was largely unaffected when females were dissected and even when most of the vagina with the bursa and spermatheca was removed. When this was done in stage I, the normal sequence of stages followed with the usual timing. Thus the duration of stage I and the transition to stages IIA and IIB were not dependent on information about the sperm contents of the female's storage organs. Activity which brings about the removal of rivals' sperm from females therefore seems to depend on a stereotyped programme of action. Indeed there is no information at present about possible sensory structures on the odonate penis, although the male genitalia of some other orders bear sensilla (e.g. ARIKAWA & AOKI, 1984).

The posterior frame supports the posterior hamules (Fig. 3) which project ventrally and bear many bristles: they make contact with the female during thrusts of the penis. When they were cut off from males in stages I or II there was a cessation of copulatory movements for a few seconds, but they were then resumed; the bristles are not therefore sensory structures essential for the continuation of copulation.

In 5 dissected pairs the ovipositor blades (i.e. anterior 2 pairs of gonapophyses) were pulled out from under the lamina batilliformis (Fig. 3) during stage I or II and this caused copulation to cease either immediately or after the completion of a further cycle of activity. In a 6th instance a male continued to make copulatory movements after being separated from the female. Thus copulation normally seems to depend on the presence of the ovipositor blades in pockets close to the lamina, where many possibly sensory bristles can be seen; some copulatory movements however can be induced in solitary decapitated males (MILLER & MILLER, 1981).

In a decapitated copulating male, the removal of the distal part of the penis or of the abdomen posterior to segment 2 did not interrupt the performance. The thoracic ganglia (possibly only the metathoracic) and the first abdominal ganglion therefore provide the minimal neural requirements for copulatory movements by the male.

In dissected copulating females the vaginal muscles and those in the bursa and spermatheca were seen to be active. In some females each retraction of the penis excited a strong vaginal contraction which pulled it posteriorly, and there were

also synchronised spermathecal contractions similar to those described above in non-copulating females. These responses ceased after section of both anterior lateral nerves from the 8th abdominal ganglion, but weaker unsynchronised activity persisted in the bursa and spermatheca.

DISCUSSION

SPERM MOVEMENTS WITHIN FEMALES

DEWITZ (1886) originally suggested that contractions of the musculature of the female reproductive tract were responsible for sperm movement in insects, and evidence supporting this view has been obtained from *Rhodnius* (DAVEY, 1958, 1985), bees (RUTTNER, 1956), *Anthonomus* (VILLAVASO, 1975), *Schistocerca* (OKELO, 1979) and *Plodia* (LUM et al., 1981: see RETNAKARAN & PERCY, 1985). However sperm motility may also contribute to or alone be responsible for sperm migration in a few insects (e.g. mosquitos, JONES & WHEELER, 1965). The high density of sperm commonly found in the spermatheca and its duct in *I. elegans* caused the sperm tails to become mechanically coupled (i.e. their waves were in phase) and may have prevented the migration of individual sperms. Peristaltic contractions of the spermathecal duct muscles were seen to be able to drive sperm masses in either direction, and mechanical pressure applied to a spermatheca was sometimes succesful in forcing sperm into the bursa. Sperm are therefore probably moved within the female system of *I. elegans* mainly by the contractions of intrinsic wall muscles.

The horns of the penis of *I. elegans* have not been seen to enter the spermathecal duct nor have they been found there in specimens preserved in copulation. However in *I. ramburi,* WAAGE (1986b) has found a horn in the duct, while in *I. pumilio* the duct is much broader and could readily accommodate a horn (pers. obs:). In these species therefore the horns may help to extract sperm from or push it into the spermathecae.

Experimental stimulation of the vaginal plates which contain campaniform sensilla sometimes produced strong reflex contractions of vaginal and spermathecal muscles. Such a response may also be elicited by an egg in the vagina and cause the expulsion of sperm from the spermatheca during fertilisation. A similar mechanism has been suggested to operate in boll weevils (VILLAVASO, 1975), locusts (OKELO, 1979), and *Spodoptera* (ETMAN & HOOPER, 1979). The movements of a penis in the vagina could excite the same reflex during copulation, as observations have suggested, and help a male to remove sperm from storage organs which were otherwise inaccessible. However examinations of the sperm volumes in spermathecae of copulating female *I. elegans* (MILLER, 1987) and *I. ramburi* (WAAGE, 1986b) do not suggest that sperm is removed from those organs during copulation. The spermathecal contractions seen in *I. elegans*

may therefore fail to expel sperm, possibly because the duct is closed or because continual peristaltic movements of the duct drive sperm back into the spermatheca. It may be noted that VILLAVASO (1975) found that sperm displacement in boll weevils was reduced from 65 to 21% after section of the spermathecal nerve, suggesting that a comparable reflex did release sperm in that species.

THE STAGES OF COPULATION

Stage I has been identified in several Coenagrionidae and it normally occupies most of copulation. In *I. elegans* the male's vesicle remained filled during stage I as the sperm of rivals was moved from the bursa to the vagina. The strong retractions which the penis made late in stage I and the subsequent sustained retraction during the transitional phase may have caused sperm to accumulate in the posterior vaginal pouch. The consequent closure of the vaginal plates may also have helped to expel sperm posteriorly. The rocking movements of stage IIA probably expelled fresh sperm from the male's vesicle, while the subsequent IIB activity with renewed thrusts of the penis may have carried that sperm into the vagina and then into the bursa. The juxtaposition of the folded membrane of the penis to the tip of the vesicle and the female's genital opening all combine to form a canal along which sperm are impelled by compression of the vesicle and by piston-like movements of the zipfel (PFAU, 1971).

THE DURATION OF COPULATION

Some *Ischnura* spp. copulate for longer than any other odonate so far described. In *I. erratica,* for example, copulation lasts 62-84 min (PAULSON & CANNINGS, 1980), in *I. ramburi* 202 ± 114 min (s.d., n =11) (ROBERTSON, 1985), and in *I. pumilio* probably a similarly long time (Miller, P.L., unpublished). However the copulations of *I. elegans* lasting 324 ± 90 min exceed those of other *Ischnura* spp.

Long copulations in some insects may allow males to transfer more sperm or other materials to females (RIEMANN et al., 1967; LEW & BALL, 1980; SILLÉN-TULLBERG, 1981; THORNHILL & ALCOCK, 1983; GRANT, 1983), but this is not known to apply to dragonflies. Alternatively prolonged copulations may allow males to displace more of the sperm of rivals (PARKER, 1970), as occurs in *Orthetrum cancellatum* (SIVA-JOTHY, 1984, 1986). However in some other odonates most of the rivals' sperm contained in a female can be removed in relatively short copulations (WÅAGE, 1979, 1982, 1986a; FINCKE, 1984; McVEY & SMITTLE, 1984) and even in *O.cancellatum* most sperm are removed in the first two minutes. Moreover, in *I. elegans* much of the time in extended copulations is spent without visible movement, and the removal of bursal sperm seems to occur early in stage I (MILLER, 1987).

A third explanation for extended copulations is that a male is able to retain and

guard a female for long periods and prevent take-overs by other males. In *I. elegans* many males obtained females early and retained them in copula until late in the day when they were prepared to oviposit. This seems an appropriate strategy when males are abundant and competition for females is strong. The copulatory behaviour of *I. elegans* at low densities is not known, but one observation on a solitary pair made in England at a pond on 8th June showed that in a mid-day copulation, stage I lasted 36 min, stage II 30 min, and stage III 4 min. The copulation was performed without a cessation of rhythmic movements until stage III and it resembled the copulations of tethered or decapitated pairs. Extended copulations at high density in the Camargue may therefore represent a form of in-copula guarding whereby a male guards a female until she is ready to oviposit. The same suggestion has been made for *I. ramburi* (ROBERTSON, 1985). The synchronization of oviposition by most females in the evening may represent a means of reducing predation.

Perched copulating pairs were rarely approached or harassed by single males. In contrast pairs in tandem were more frequently attacked and they were sometimes separated. Females in copulation may therefore be difficult to displace, and this may explain the persistence of copulation by pairs captured in the net. Most other species of which I have experience separate on capture, but one exception is *Aeshna mixta* copulating at low temperatures. Thus prolonged copulation seems to represent a strategy for guarding females until they are prepared to oviposit, and its considerable cost to males (reduced opportunities for further mating and loss of feeding time) are presumably offset by the increased chances of paternity. Females, like males, also pay the cost of being unable to feed.

Even in the accelerated copulations of tethered or decapitated pairs, stage IIB lasted between 15 and 72 min, whereas in the normal copulations of *Enallagma cyathigerum* it takes only 1-2 min (MILLER & MILLER, 1981), in *Erythromma viridulum,* a few tens of seconds, and in *Calopteryx splendens,* it is not distinguishable (pers. obs.). One explanation for the unusually long stage II of *I. elegans* may be that sperm take time to enter the spermatheca through its narrow duct. Since sperm can readily be removed by a copulating male from the bursa but not from the spermatheca, a male may retain hold of a female until some of his sperm are in the spermatheca. The presence of a long, narrow and tortuous duct leading to the spermatheca suggests that the female has evolved a mechanism which makes it difficult for the male to remove sperm from that organ (cf. WALKER, 1980). Another consequence may by a reduction of the amount of mixing between bursal and spermathecal sperm. Species in which males have thin barbed processes on the penis and females have long, narrow spermathecal ducts (*Ischnura* spp., some *Coenagrion* spp.) occur also in the Libellulidae (e.g. *Orthetrum* spp., *Pantala flavescens;* MILLER, 1984). Such features suggest a conflict of interests between males which attempt to extract rivals' sperm and females which try to conserve some of their stored sperm.

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