

**MALE'S ABILITY OF SPERM DISPLACEMENT DURING  
PROLONGED COPULATIONS IN  
*ISCHNURA SENEGALENSIS* (RAMBUR)  
(ZYGOPTERA: COENAGRIONIDAE)**

K. SAWADA

Ohori High School, 1-12-1, Ropponmatsu, Chuou-ku, Fukuoka, 810  
Japan

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Sperm displacement was studied during extremely long copulations in this sp., in order to estimate the ♂ ability of sperm removal and insemination. Copulations began early in the morning and continued until late noon, and thereafter females oviposited alone, without ♂-guard. In the field, copulation lasted  $395.3 \pm 11.6$  min (s.e., n=12), and stage I, during which sperm in the ♀ sperm storage organs was removed, occupied almost all of the copulation duration. However, sperm in a bursa copulatrix was mostly removed within 1 h after the initiation of copulation, and the bursal sperm volume remained low for several hours. Sperm volume in the spermatheca, which was almost of equal volume to the bursal sperm in precopula, did not significantly change during copulation. Stage II occupied by insemination into the bursa copulatrix, lasted only about 1 min prior to the termination of copulation. The process of sperm displacement, specially the ♂ ability to displace sperm during the extremely long stage I, is discussed.

**INTRODUCTION**

In most insects, eggs are fertilized at oviposition with stored sperm. Therefore, if females mate more than once, competition between the sperm from two or more males occurs for the fertilization of the eggs (PARKER, 1970). WAAGE (1979) was the first to discover that the males of *Calopteryx maculata* before insemination removed from the female the sperm from previous matings. If females use the last male's sperm in egg-fertilization, his sperm precedence is very high.

Sperm displacement has been studied in several species of Odonata (WAAGE, 1979, 1982, 1984, 1986a, 1986b; MILLER & MILLER, 1981; MILLER, 1982a, 1982b, 1984, 1987a, 1987b; FINCKE, 1984; SIVA-JOTHY, 1987, 1988; MICHIELS

& DHONDT, 1988; SIVA-JOTHY & TSUBAKI, 1989; CORDERO & MILLER, 1992). The degree of sperm displacement is different in different species. Males of *Calopteryx maculata* remove 88-100% of previously by the female stored sperm (WAAGE, 1979), while males of *Lestes vigilax* displace 40-50% of the rival's sperm (WAAGE, 1982). The variation of copulation duration in the same species may be associated with a difference of sperm removal. In *Orthetrum cancellatum* long copulations by satellite males ( $894 \pm 142$  s) resulted in almost 100% sperm removal, but the shorter copulations by territorial males ( $21 \pm 13.5$  s) resulted in only 10-15% sperm removal (SIVA-JOTHY, 1987). However, in *Mnais p. pruinosa*, it was shown by irradiated male techniques that the last male to mate had almost 100% sperm precedence immediately after copulation, regardless of the duration of copulation (SIVA-JOTHY & TSUBAKI, 1989).

Several *Ischnura* species copulate for longer (more than 30 min) than other odonate species examined (ROBERTSON, 1985; HAFERNIK & GARRISON, 1986; FINCKE, 1987; MILLER, 1987a; CORDERO, 1989; NARAOKA, 1994). Sperm displacement in long copulations has been studied in *I. ramburi* (WAAGE, 1986a), *I. elegans* (MILLER, 1987b), *I. graellsii* (CORDERO & MILLER, 1992) and *I. asiatica* (NARAOKA, 1994). In *I. elegans*, the removal of bursal sperm seems to occur early after the initiation of copulation (MILLER, 1987b). However, the detailed process of sperm removal and insemination during the long copulation is unknown. Nor is it known why the *Ischnura* males appear to need a longer duration to remove the rival's sperm and to inseminate than other dragonflies. I describe the mating behaviour of *I. senegalensis* in Japan, and confirm the detailed process of sperm displacement during long copulations.

## METHODS

The field observations were made at the moats in Heiwadai Park in Fukuoka City, Japan ( $33^{\circ}35'N$   $130^{\circ}22'E$ ), mainly in spring and autumn, from 1988 to 1992. Mature adults were marked with a number on the wings, using a permanent marker pen, and their behaviour was recorded.

In *I. senegalensis*, copulation consists of three stages. In stage I, the male of a pair continues the slow abdominal flexions for a long time after the initiation of copulation. In stage II, the male begins to flex his abdomen rapidly. Finally, in stage III, the male stops the abdominal flexions until the termination of copulation. To observe the positions of the penis in the female genitalia, 15 copulating pairs during stage I were frozen with liquid nitrogen and the female genitalia were dissected out under a stereomicroscope.

To examine sperm displacement, I collected copulating pairs during precopula-tandem and in each stage ( $n = 80$ ). Some copulating pairs during stage I were interrupted after every 30 minutes from the initiation of the copulation, and were fixed in 70% ethanol ( $n = 45$ ). As the duration of stage II was very short (see below), some copulating pairs during stage II were caught after every three flexions from the initiation of the stage ( $n = 25$ ). The female sperm storage organs were dissected out and the sperm mass volume was measured under a stereomicroscope with an eyepiece graticule. As the form of the sperm mass in the spermatheca was like an ellipsoid, its volume was estimated from the value of  $3/4 \times 3.14 \times abc$  (a, b, or c indicated each radius of the three dimensions). As the form of the sperm mass in the bursa copulatrix was like a thin plate, its volume was estimated at the value of base area  $\times$

mean breadth (base area was determined by the number of a grid under a grid eyepiece graticule). The estimated value of their volume was expressed in means  $\pm$  standard error.

## RESULTS

### DAILY ACTIVITY AND COPULATION BEHAVIOUR

At the moats in Heiwadai Park, adult individuals were observed at the water edge at a high density at sunrise. When a male encountered a mature female early in the morning, he grasped her thorax with his legs and then clasped her thorax with his anal appendages (precopula-tandem). After sperm translocation to his seminal vesicle, he copulated with her. After the initiation of the copulation, the slow abdominal flexions of the male were observed during stage I (Fig. 1). If a copulating pair was disturbed by other males, the pair repelled them by widely spreading their wings. After stage I, the rapid abdominal flexions of males were observed during stage II (Fig. 1). Finally, males stopped abdominal flexions and remained in the

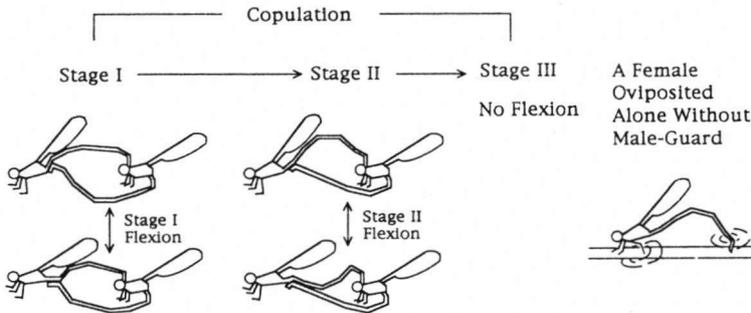


Fig. 1. Phases of reproductive behaviour in *Ischnura senegalensis*.

copulation position during stage III.

Early in June, sunrise was at about 05:10 h. However, the first copulation was observed at about 06:30-08:00 h and at an air temperature of 14.0-17.1°C. The total duration of copulation was  $395.3 \pm 11.6$  min (s.e.,  $n=12$ ), and was longer than in any other of the examined *Ischnura* species (cf. ROBERTSON, 1985; HAFERNIK & GARRISON, 1986; FINCKE, 1987; MILLER, 1987a; CORDERO, 1989; NARAOKA, 1994). Stage I occupied almost all of copulation,  $370.9 \pm 11.5$  min (s.e.,  $n=13$ ), while the duration of stage II was  $51.5 \pm 2.6$  s (s.e.,  $n=11$ ), and the number of flexions during stage II was  $11.7 \pm 1.0$  (s.e.,  $n=14$ ). Stage III lasted  $28.6 \pm 2.5$  min (s.e.,  $n=11$ ). The copulations continued through the morning, and usually terminated between 12:00 and 14:00 h. After copulation, the female ate small insects among the vegetation or on the water surface. She repelled males using the wing-flutter display and oviposited alone, unattended by the male. Adult individu-

als gathered at the water edge in the evening, and perched on the vegetation. No copulations were observed in the evening. Consequently, females mated at the most only once per day, in the morning.

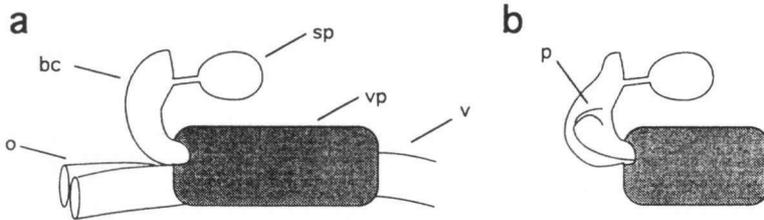


Fig. 2. *Ischnura senegalensis*: (a) lateral view of the female sperm storage organs [o=oviducts; - bc=bursa copulatrix; - sp=spermatheca; - vp=vaginal plate; - v=vagina]; - (b) ditto, during copulation [p=penis horns].

#### GENITALIA

A diagrammatic lateral view of female sperm storage organs is shown in Figure 2. They consist of a bursa copulatrix, shaped like a thin plate, and an ellipsoid spermatheca, joined to the bursa copulatrix via a narrow spermathecal duct (Fig. 2a). The spermathecal duct arises from near the apex of the bursa, rather than towards its base. It is similar to that in *I. ramburi* (WAAGE, 1986a) and *I. verticalis* (WAAGE, 1984), but not like in *I. elegans* (MILLER, 1987a). The terminal part of the complex penis (ligula), with two attached coiled horns, is similar to that in *I. elegans* (MILLER, 1987a).

In 14 out of 15 samples of copulating pairs which were frozen with liquid nitrogen, the horns of the penis could

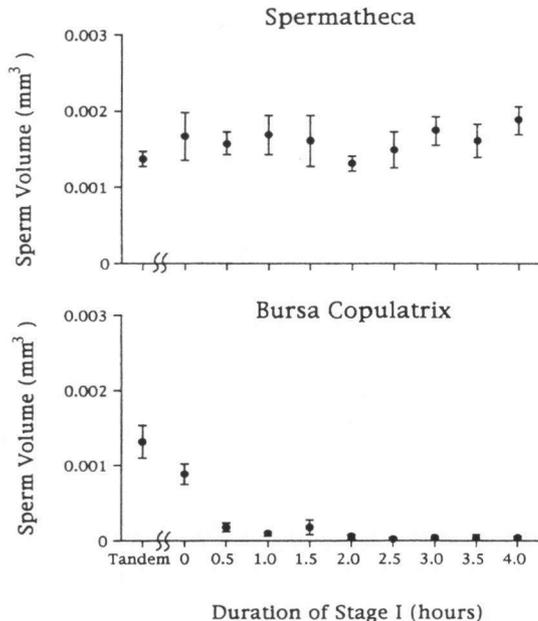


Fig. 3. The sperm volume change in the spermatheca (above) and in the bursa copulatrix (below) during precopula-tandem and stage I. - [0 point in X axis shows the initiation of stage I; - Mean  $\pm$  S.E., each sample size is five].

be seen only in the bursa copulatrix (Fig. 2b). However, in one sample, the horns of the penis could be seen both in the bursa copulatrix and in the spermathecal duct.

#### MEASUREMENT OF SPERM DISPLACEMENT

The sperm volume in the bursa copulatrix quickly decreased within the first hour of copulation, and it remained small throughout stage I (Fig. 3). Twenty-seven out of 35 (77%) females, whose stage I duration was from 1 to 4 hours, had no sperm in the bursae. While 9 out of 10 females, whose stage I duration was from zero (the initiation) to 30 minutes, had sperm in the bursae (only a single female, whose stage I duration was 30 minutes, had no sperm in the bursa). All males during stage I ( $n=13$ ) had a considerable volume of sperm in their seminal vesicles. Males, thus, removed almost all of the bursal sperm within one hour after the initiation, and did not inseminate during stage I.

At the initiation of stage II (0 point in X axis of Fig. 4), sperm volume in the bursa copulatrix was almost zero. However, the bursal sperm volume then rapidly increased during stage II. The number of flexions during stage II was highly correlated with the bursal sperm volume ( $r^2=0.63$ ,  $P=0.001$ ,  $n=25$ ) (Fig. 4). No males at the termination of stage II ( $n=7$ ) had any sperm in their seminal vesicles. Males would inseminate into the bursa copulatrix by means of the flexions during stage II within about a minute.

The sperm volume in the spermatheca did not significantly change from precopula-tandem to stage III ( $F=0.687$ ,  $P > 0.7$ ,  $d.f.=15$ , ANOVA) (Figs 3, 4). During stage III, it was not significantly different in the spermatheca and bursa copulatrix (Mann-Whitney's

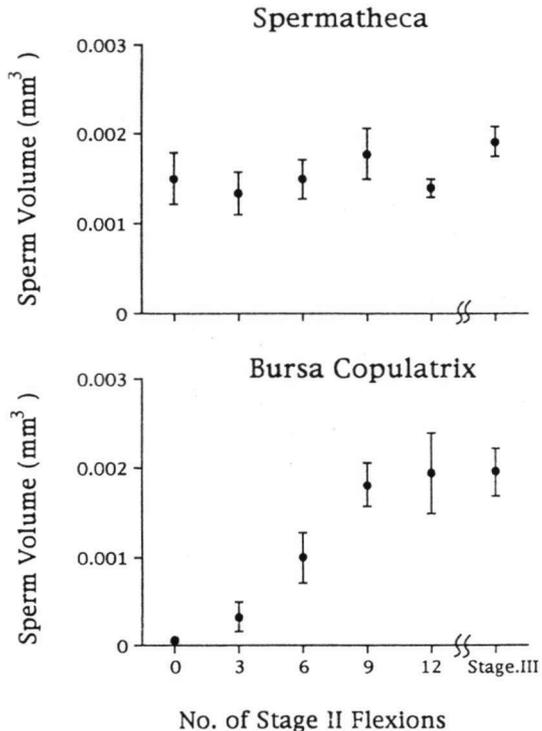


Fig. 4. The sperm volume change in the spermatheca (above) and in the bursa copulatrix (below) during stage II and stage III. - [0 point in X axis shows the initiation of stage II. - The number in X axis shows the times of flexions during stage II. - Means  $\pm$  S.E., each sample size is five].

*U*-test,  $U = -0.105$ ,  $P > 0.8$ ) (Fig. 4). It appears that the spermathecal sperm from previous copulations was almost equal in volume to the bursal sperm from the last copulation. Therefore, males of *I. senegalensis* are able to remove about 50% of the female's previously stored sperm.

## DISCUSSION

The copulation during of about 6 hours in *I. senegalensis* may be one of the longest in the Odonata examined. However, it has been shown that the required duration for a male to remove the bursal sperm is about one hour, and that required to inseminate is about one minute. Therefore, copulation duration is considerably longer than is needed for sperm displacement, although much of the previously stored sperm remains in the spermatheca. Did males displace the spermathecal sperm during stage I or not?

CORDERO & MILLER (1992) discovered that in *I. graellsii*, the penis horn reaches the end of the spermatheca in a virgin female, and a male can remove any volume of the spermathecal sperm in a mated female. Also in *I. asiatica*, the removal of the spermathecal sperm during copulation was studied (NARAOKA, 1994). In *I. senegalensis* the penis horns were once observed in the spermathecal duct, and males may be able to remove some spermathecal sperm. But it would be difficult for the male to remove much of the spermathecal sperm through the very narrow duct in the *Ischnura* species.

WAAGE (1986a) found in *I. ramburi* a small increase in spermathecal volume during copulation, and he suggested that the penis horns pushed sperm into the spermatheca (repositioning), but no sperm volume increase was observed in *I. senegalensis*. However, in order to avoid "sperm flow" from the spermatheca to the bursa copulatrix, males may pack sperm into the back of the spermatheca.

If removal and packing occurred for several hours, there might be a relationship between copulation duration and sperm precedence. Such a relationship has been investigated in *Mnais p. pruinosa* (SIVA-JOTHY & TSUBAKI, 1989), *Leucorrhinia intacta* (WOLF et al., 1989) and *Sympetrum danae* (MICHIELS, 1992), but not in any *Ischnura* species. By using the irradiated male technique, the tentative significance of the extremely prolonged duration of stage I for sperm precedence could be examined.

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