

## SPERM TRANSFER PROCESS IN THE NON-TERRITORIAL *ISCHNURA ASIATICA* (BRAUER) DURING COPULATION (ZYGOPTERA: COENAGRIONIDAE)<sup>1</sup>

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According to the movements of the ♂ abdomen, the copulation process in *I. asiatica* is divided into 3 stages (I, II and III). The mean duration of each stage was  $75.8 \pm 8.8$  min,  $6.4 \pm 0.3$  min and  $15.8 \pm 0.9$  min for stage I, II and III, respectively (S.E.). No sperm transfer was found during stage I. The prolonged duration in stage I was related to the time of onset of copulation. Sperm was transferred into the bursa copulatrix during stage II. Although stage III was a phase without apparent abdominal movement, the sperm transfer was continued, following the sperm migration from the bursa copulatrix to the spermatheca. Immediately after copulation termination, the estimated number of sperm was  $64,500 \pm 4,425$  in the bursa copulatrix and  $43,143 \pm 6,397$  in the spermatheca (S.E.). The role of each stage in copulation will be discussed from the viewpoint of sperm competition.

### INTRODUCTION

Sperm competition has resulted in the evolution of several male morphological and behavioral traits (PARKER, 1970). In Odonata, WAAGE (1979) demonstrated that *Calopteryx maculata* males use their specialized secondary genitalia to remove the sperm of rival males stored in the female before transferring their own sperm. Sperm removal is an efficient way to gain a high fertilization success for the male because the stored sperm is withdrawn from the female sperm storage organs. This mechanism has been repeatedly demonstrated in many zygopteran species (e.g., MILLER, 1987b; SIVA-JOTHY & TSUBAKI, 1989).

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Studies of sperm competition in Odonata based on sperm volume but not on the number of spermatozoa have been reported (CÓRDOBA-AGUILAR et al., 2003). Because odonate sperm tend to clump into a dense, interwoven mass (WAAGE, 1979), it has been assumed that sperm number was homologous to sperm volume. However, little about the relationship between spermatozoa number and sperm volume has been clarified (REINHARDT, 2005). To gain more information about sperm competition, studies of the spermatozoa dynamics in the female during copulation and the total number of spermatozoa transferred in a single copulation are needed.

To clarify the sperm transfer process in *Ischnura asiatica*, we allowed virgin males and females to copulate in the laboratory. An experiment in which copulation was interrupted was conducted, and the number of spermatozoa transferred into the females' sperm storage organs was counted.

#### MATERIAL AND METHODS

Each larva that was captured in the field was kept individually in a bottle ( $\phi$  3.5cm, height 5.8cm) and fed with live *Tubifex* and brine shrimps. A twig 10cm in length was placed against the inner wall of the bottle for the final instar larva as a support for emergence.

All imagos were kept in a cage (40×40×50cm) with wooden frames covered by nylon mesh (1×1mm). They were fed on *Drosophila* spp. cultured.

We introduced each pair of virgin sexually matured males and females into a cage (30×30×30cm) with wooden frames covered by nylon mesh (1×1mm) in the morning (05:00-11:00), which is a time of day when many copulations in the fields were observed (NARAOKA, 1989). The cage was placed by a window with direct sunlight. According to the process of zygopteran copulation which has been divided into three stages by the male's abdominal movement (MILLER & MILLER, 1981), we divided the copulation of *I. asiatica* into three stages. In stage I, the males depressed and stretched the first and second abdominal segments. In stage II, the males thrust in the third abdominal segment. Stage III was a phase without apparent movement of the abdomen of either sex. After that, the pair separated.

The copulating pairs were interrupted 10 and 30 minutes from the onset of copulation, 0, 1, 2, 3 and 4 minutes from the onset of stage II, and 0, 2, 4, 6 and 8 minutes from the onset of stage III. The total number of spermatozoa transferred in a single copulation was also examined for five pairs.

Each female was decapitated and the abdomen dissected to detach the bursa copulatrix and the spermatheca. The number of spermatozoa was estimated according to a method described by SIVA-JOTHY (1987). That is, the bursa copulatrix and spermatheca were separately placed into a tissue-homogenizer containing a given volume of saline (stage I: 0.3ml, stage II: 0.5ml, stage III: 1ml) and ruptured with about 10 strokes of a pestle. The spermatozoa number was counted in a given volume by a blood-haemocytometer more than five times from the same sample, disregarding the volume of the bursa copulatrix and spermatheca, because they were much smaller than the volume of the saline.

#### RESULTS

Immediately after the encounter of both sexes in a cage, each female showed the mate refusal display, even though each female was virgin. The females opened their wings, raised and ventrally curled their abdomen when perching, or curled

their abdomen ventrally on the wing. After repeated approaches by the males, however, most females finally accepted copulation. Consequently, a total of 50 pairs successfully copulated.

The duration of stage I was  $75.8 \pm 8.8$  min ( $n = 45$ , S.E.), varying with the time of day in which copulation began (Fig. 1). The longest duration of stage I was 188.5 min and the shortest was 20.1 min. The duration of stage I was negatively correlated with the time of day. Thus, when copulation began in the late morning, stage I was of shorter duration. The time of termination of stage I was estimated by the regression curve, indicating that stage I would last until around 08:30 or 09:00.

The frequency of male abdominal movements in stage I was  $513 \pm 186.3$  (S.E.,  $n = 7$ ). Both the bursa copulatrix and spermatheca of the females during stage I was still flat, and no spermatozoa were found. Therefore, no sperm transferred during stage I.

The duration of stage II was  $6.4 \pm 0.3$  min ( $n = 30$ , S.E.). The abdominal movement of the males during stage II became gradually slower compared to that of stage I. The frequency of abdominal movement was  $24.4 \pm 5.6$  ( $n = 7$ , S.E.). Figure 2 shows that the number of spermatozoa increased in both the bursa copulatrix and spermatheca. The number of spermatozoa estimated to have been transferred into the bursa copulatrix and spermatheca during stage II was  $39,333 \pm 5,306$  (S.E.,  $n = 3$ ) and  $17,600 \pm 15,673$  (S.E.,  $n = 3$ ) respectively. Therefore, a male transferred about 47,000 spermatozoa in stage II.

The duration of stage III was  $15.8 \pm 0.9$  min ( $n = 12$ , S.E.). The estimated number of spermatozoa increased in both the bursa copulatrix and spermatheca (Fig. 2). Just after copulation termination, the total estimated number of spermatozoa was  $64,500 \pm 4,425$  (S.E.,  $n = 4$ ) in the former and  $43,143 \pm 6,397$  (S.E.,  $n = 4$ ) in the latter, indicating that about 110,000 spermatozoa were transferred by males in a single copulation.

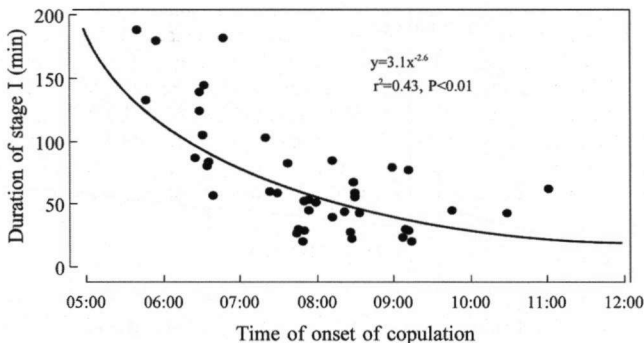


Fig. 1. The relationship between the duration of stage I and time of day in which copulation began. A regression curve was calculated by defining 00:00 as  $x = 0$ , 24:00 as  $x = 1$ .

## DISCUSSION

Although many zygopteran species spend less than one hour in copulation (CORBET, 1999), ROBERTSON (1985) and MILLER (1987a) reported that *Ischnura* species spend several hours copulating. PARKER (1970) stated that prolonged copulation might be the consequence of sexual selection favouring male adaptations to avoid sperm competition. Prolonged copulation could cause the male body to act as a mating plug that prevents the female from remating before oviposition (ALCOCK, 1994), as well as enabling the removal of the sperm of rival males (SIVA-JOTHY, 1987). In the present study, since all females used were virgin, the males of *I. asiatica* did not require a long copulation in which to remove sperm. Therefore, the prolonged copulation of *I. asiatica* is likely to be mate guarding, as in case of *I. graellsii* (CORDERO, 1990) and *I. senegalensis* (SAWADA, 1999). Because most copulations of *I. asiatica* terminated around 08:30 to 09:00, females might not accept copulation after 09:00, though several females accepted copulation after 9:00 in the laboratory.

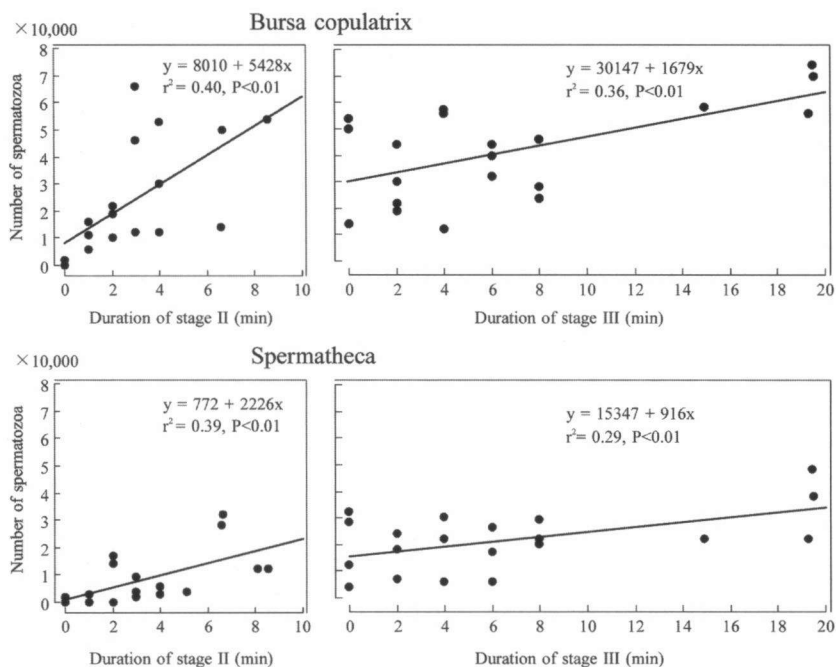


Fig. 2. Change in the number of spermatozoa stored in the bursa copulatrix and spermatheca during stage II and stage III.

Many odonate males remove the sperm of rival males stored in the female using abdominal movements before transferring their own sperm (e.g., WAAGE, 1979). Sperm volume reduction during stage I has been reported in *I. elegans* (MILLER, 1987b). SAWADA (1995) found sperm removal in *I. senegalensis* within 30 min after the onset of copulation. In *I. asiatica*, depressions and stretches of second and third abdominal segments of males were observed in stage I. In addition, at the end of the male genitalia in *Ischnura* species, there are two horn-like appendages, which are used to remove sperm (WAAGE, 1984). Sperm removal might occur during stage I in experienced females of *I. asiatica*.

An increase in sperm volume during stage II was reported in many *Ischnura* species (e.g., SAWADA, 1995). In the present study, stage II was a sperm transfer stage. Since the duration of stage II is 2–8 minutes in many *Ischnura* species (e.g., CORDERO, 1989; ROBERTSON, 1985), the duration of stage II and the form of abdominal movements in the males in *I. asiatica* were similar to that of other *Ischnura* species (e.g., MILLER, 1987a).

In *I. asiatica*, sperm transfer was continued during stage III, suggesting that stage III is also a sperm transfer stage, though there were no abdominal movements. Sperm transfer was previously believed to occur by the abdominal movement of males (SIVA-JOTHY & TSUBAKI, 1989). However, CORDERO & MILLER (1992) reported that both the duration and frequencies of the abdominal movements of males during stage II were not related to sperm volume in *I. graellsii*. A mechanism for sperm transfer without abdominal movements might exist in *I. graellsii* as well as *I. asiatica*.

A male of *I. asiatica* transferred about 110,000 spermatozoa to a female in a single copulation. There have been several studies on the spermatozoa number that are transferred in a single copulation. In many *Ischnura* species, the sperm transferred during a single copulation are sufficient to fertilize a female's lifetime reproduction (FINCKE, 1987; CORDERO, 1990; SIROT & BROCKMANN, 2001). In fact, 110,000 spermatozoa that transferred in a single copulation in *I. asiatica* could compare with 2,900 eggs loaded by a female (WATANABE & MATSU'URA, 2006).

Males remove sperm only in the bursa copulatrix using horn-like appendages on the genitalia of *I. senegalensis* and *I. elegans* (MILLER, 1987a; SAWADA, 1995). Since the spermatheca is bound to the bursa copulatrix by a long, narrow spermathecal duct in *I. asiatica*, males might be able to remove sperm in the bursa copulatrix but not in the spermatheca. Therefore, sperm competition will occur on 40% of spermatozoa transferred in the spermatheca, though NARAOKA (1994) has reported, by examining the change in the volume of the bursa copulatrix and spermatheca in the fields, that 61% of sperm in the bursa copulatrix and 71% in the spermatheca were removed.

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