

**SPERM PRECEDENCE IN THE DAMSELFLY *ISCHNURA SENEGALENSIS* (RAMBUR): IS PROLONGED COPULATION ADVANTAGEOUS TO SPERM PRECEDENCE ?
(ZYGOPTERA: COENAGRIONIDAE)**

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To understand the relationship between copulation duration and sperm precedence in *I. senegalensis*, which mate for several hours, laboratory experiments and field observation were conducted. By irradiated male techniques, P2 value (sperm precedence of the last male to mate) was measured. P2 value was almost 100 % until 2 days after copulations regardless of the copulation duration. The interval between copulations in a ♀ was about 2.3 days in the field. It is suggested that the last ♂ to mate gains advantages in sperm precedence regardless of the copulation duration in the field. And complete sperm mixing (the point when the P2 value was 50 %) occurred 6 days after copulation.

INTRODUCTION

Sperm competition occurs in many animals, when a single female mates with two or more males and their sperm compete to fertilize her eggs (PARKER, 1970). Therefore, sperm competition is a potentially powerful form of sexual selection. Sperm displacement, one important mechanism for sperm competition, has been documented in some odonate species in which last male sperm precedence is typical (FINCKE, 1984; McVEY & SMITTLE, 1984; MICHIELS & DHONDT, 1988; SIVA-JOTHY & TSUBAKI, 1989; MICHIELS, 1992; CORDERO & MILLER, 1992). In *Ischnura senegalensis* which mate for several hours, however, males removed most sperm in the bursa copulatrix within one hour after the initiation of copulation (SAWADA, 1995).

In Odonata, a relationship between copulation duration and sperm precedence

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has been investigated in *Mnais p. pruinosa* (SIVA-JOTHY & TSUBAKI, 1989), *Leucorrhinia intacta* (WOLF et al., 1989), and *Sympetrum danae* (MICHIELS, 1992). In *M. 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). In *L. intacta*, longer copulation (more than 300 s) lead to essentially 100% sperm precedence, presumably because more rival sperm can be removed (WOLF et al., 1989). In *Ischnura* species, CORDERO & MILLER (1992) showed last-male sperm precedence by using a genetic mark in *I. graellsii*. However, in *Ischnura* species, the detailed relationship between copulation duration and sperm precedence is unknown. Do males of *Ischnura* species need a longer copula duration to gain advantage in sperm precedence by more displacement of a rival's sperm? I conducted this study by irradiated male techniques and measured P2.

Last-male sperm precedence decreases over time because sperm eventually mixes within females (SIVA-JOTHY & TSUBAKI, 1989; MICHIELS, 1992). Therefore, intermating intervals of females can affect sperm precedence in the field. How much can the last male secure sperm precedence until the next copulation of his mate? In the field, I measured the interval between copulations of females, and the issue is discussed.

METHODS

SPERM DISPLACEMENT – To examine sperm displacement, copulations with virgin females were conducted in a laboratory in 1992. Colour of heteromorph female's thorax changed from orange (teneral colour) to brown (mature colour). The females of teneral colour in a natural population were captured and the sperm storage organs (a bursa copulatrix and a spermatheca) were dissected out. All females (N=15) had empty sperm storage organs, so teneral females were regarded as virgin females. The teneral females in a natural population were maintained in insectaries of 35 × 60 × 35 cm, and fed with adult *Drosophila*. The mature virgin females were mated once or twice and fixed in 70% ethanol. Sperm mass volume was measured under a stereo microscope with an eyepiece graticule. As the form of the spermathecal sperm mass was like an ellipsoid, its volume was estimated from the formula $\frac{3}{4} \times 3.14 \times abc$ (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 as the value of base area × mean breadth (base area was determined by the number of a grid under a grid eyepiece graticule).

IRRADIATED MALE TECHNIQUES – To examine sperm precedence, mature males in a natural population were irradiated by 14.0 Kilo-rad gamma ray from a ⁶⁰Co source. Virgin females mated either with a normal male or an irradiated male. She then laid eggs on damp filter paper. In the first case, eye spots in most of the eggs were visible after 10 days from oviposition. Eye spots were not visible in eggs laid by females mated with irradiated males.

I denoted copulations with the normal males as N, and those with the irradiated males as I. After NI or IN mating with virgin females, each female was maintained in a polypropylene case of 7.5 × 9.0 × 4.5 cm with damp filter paper as an oviposition substrate. The filter paper was changed daily, and was kept with water in the laboratory. Fertilized eggs were defined by the eye spots visible after 10 days from oviposition. P2 value was determined following the method devised by BOORMAN & PARKER (1976).

FIELD OBSERVATIONS – To examine intermating intervals of females, a relatively isolated population was studied at the moat in Heiwadai Park in Fukuoka city, Japan (33°35'N 130°22'E). The area of the sixth moat was 25m × 30m, and 1km away from the other moats. Most of copulations were observed on shore. Teneral females were captured around the moat and were marked individually on the wing. When a marked female was found, the individual number and behaviour were recorded. This mark-release method was carried out every day for a month of September in 1993.

RESULTS

SPERM DISPLACEMENT

Figure 1 shows sperm volume change in virgin females in four categories; those that have copulated once, those interrupted after one hour and after two hours of the initiation of the second copulation, and those that copulated twice. After the first copulation, high volume sperm was found both in the bursa copulatrix and the spermatheca. The total (both the bursa copulatrix and the spermatheca) sperm volume was not significantly changed between the first copulation and the second copulation (Mann-Whitney's U -test, $U = -0.43$, $P > 0.6$).

The slow abdominal flexions (stage I) had been observed after the initiation of copulations. The volume of bursal sperm was low after 1 hour after the initiation of the second copulation. However, the spermathecal sperm was not significantly changed from the first copulation to the second copulation ($F = 0.73$, $P > 0.5$, $d.f. = 3$, ANOVA). SAWADA (1995) showed that

males removed only bursal sperm with the slow abdominal flexions (stage I) within 1 hour after the initiation of copulations in the field. Therefore, males would normally displace bursal sperm during copulations both in insectaries and in the field.

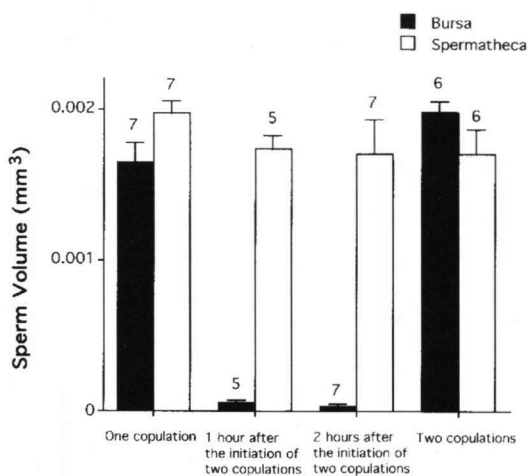


Fig.1. Sperm volume change in virgin females in four categories; those that have copulated once, those interrupted after one hour and after two hours of the initiation of the second copulation, and those that copulated twice. – [Means \pm S.E., sample sizes are shown above their bars.]

SPERM PRECEDENCE

Figure 2 shows change of the percentage of fertile eggs laid by virgin females

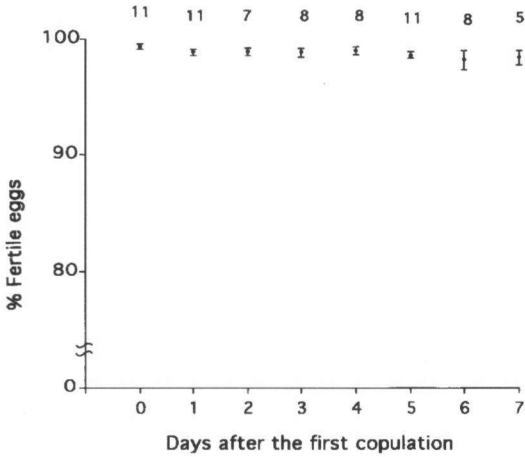


Fig.2. Change of fertilized rate in eggs laid by the virgin females which mated only once with the normal male. - [Means \pm S.E., sample sizes are shown above their bars.]

mated once with the normal male. The fertilization was almost 100% until 7 days after the copulation. Therefore, the rate of fertilization with the sperm from normal males was $98.8 \pm 0.1\%$ (\pm S.E., N=69). On the other hand, fertilization success with the sperm from the irradiated males was $1.0 \pm 0.4\%$ (\pm S.E., N=16).

Figure 3 shows the change of P2 after NI (first normal males, second irradiated males), IN (first irradiated males, second normal males), and total (NI + IN) as the function of days post mating.

Paternity expectations did not differ between NI and IN ($F=0.59$, $P>0.7$, $d.f.=6$, 2-factor ANOVA). Therefore, it appeared that sperm motility of irradiated males was not affected. On the day after the second copulation (0 point in X axis), P2 was almost 1, indicating that last-male sperm precedence was very high. P2 gradually decreased indicating sperm mixing within females. On the six days after the second copulation, P2 was almost 0.5, indicating that complete sperm mixing had occurred.

INTERMATING INTERVALS OF FEMALES

Table I
Comparison of intermating interval (days, mean \pm S.E.) between heteromorph and andromorph. U refers to Mann-Whitney's U-test

Heteromorph	Andromorph	U	P
2.24 \pm 0.13 (N=203)	2.65 \pm 0.29 (N=43)	-1.16	>0.1

Table I shows intermating intervals of females whose copulations were observed in the field. Intermating intervals were not significantly different between heteromorph and andromorph females. Mean of intermating intervals

was 2.3 ± 0.1 days (\pm S.E., N=246).

RELATIONSHIP BETWEEN COPULATION DURATION AND P2

I regarded P2 averaged over all clutches laid by an individual female 2 days after the copulation as "short term" sperm precedence. Figure 4 shows the relationship

between the duration of stage I in the second copulation and "short term" sperm precedence. The duration of stage I varied from 92 to 412 min, however, "short term" sperm precedence was almost 100% ($98.6 \pm 0.9\%$, \pm S.E., $N=14$) regardless of stage I duration.

On the other hand, I regarded P2 averaged over all clutches laid by an individual female 6 days after the copulation as "long term" sperm precedence. Figure 5 shows the relationship between the duration of stage I in the second copulation and "long term" sperm precedence. "Long term" sperm precedence had a wide range (0.5-1.0), and was not significantly correlated with the duration of stage I (simple regression, $r^2 = 0.18$, $P=0.15$, $N=13$).

DISCUSSION

MECHANISM OF SPERM COMPETITION

In several species of Odonata, there is last-male sperm precedence immediately after copulation (FINCKE, 1984; McVEY & SMITTLE, 1984; SIVA-JOTHY & TSUBAKI, 1989;

MICHIELS, 1992; CORDERO & MILLER, 1992). In some of these species, the last-male advantage is lost afterwards by sperm mixing (McVEY & SMITTLE, 1984; SIVA-JOTHY & TSUBAKI, 1989; MICHIELS, 1992; CORDERO & MILLER, 1992). In *Sympetrum danae*, NUYTS & MICHIELS (1996) found that P2 increased with the time between copulation and oviposition, and suggested that

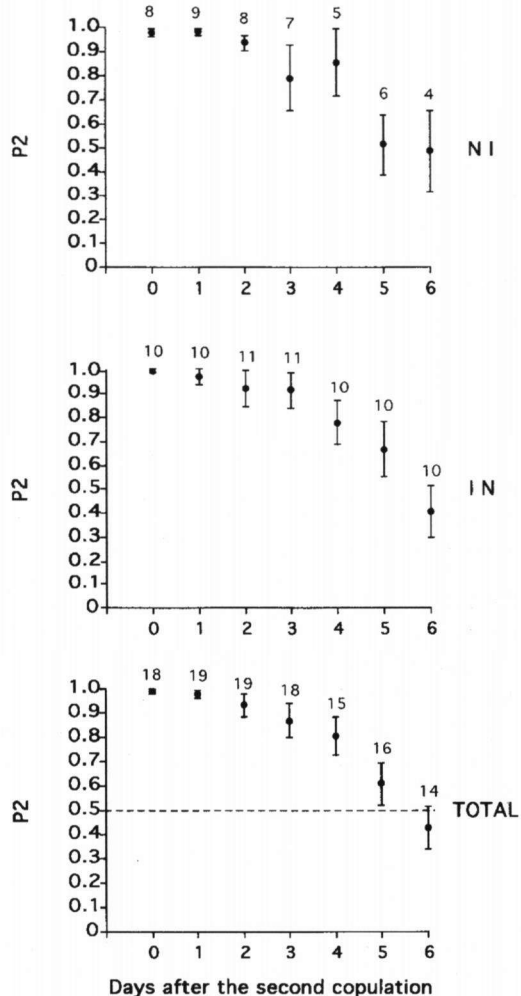


Fig.3. The relationship between days after the double copulation by means of the virgin females and P2. N and I indicate copulations with the normal males and those with the irradiated males. - [Means \pm S.E., sample sizes are shown above their means.]

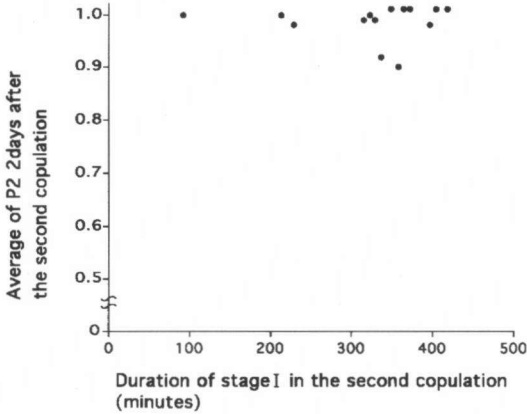


Fig. 4. The relationship between duration of stage I in the second copulation and P2 averaged over all clutches laid by an individual female 2 days after the copulation.

the first male initially had a positional advantage. In *I. senegalensis*, the narrow spermathecal duct arises from near the apex of the bursa copulatrix, rather than towards its base (SAWADA, 1995). Therefore, the bursal sperm from the last male will have a positional advantage for egg fertilization. In contrast, *I. elegans* (MILLER, 1987), *I. graellsii* (CORDEIRO & MILLER, 1992), and *I. asiatica* (NARAOKA, 1994), the narrow spermathecal duct arises from near the base of the bursa copulatrix. In these two types of *Ischnura* species, there is a possibility that the pattern of change of sperm precedence is different, i.e. in the former sperm mixing in sperm storage organs will be relatively slow, whereas in the latter it will be relatively quick.

RELATIONSHIP BETWEEN PROLONGED COPULATION AND SPERM PRECEDENCE

Is prolonged copulation advantageous to males of *I. senegalensis* in the field in terms of likely sperm precedence? The mean of the intermating interval in a female was about 2.3 days in the field. Therefore, the female to mate would fertilize her eggs by using stored sperm for about 2 days after the copulation. And also, "short term" sperm precedence of the last male was almost 100 % regardless of the copulation duration. Therefore, it was suggested that the last male to mate was advantageous to sperm precedence regardless

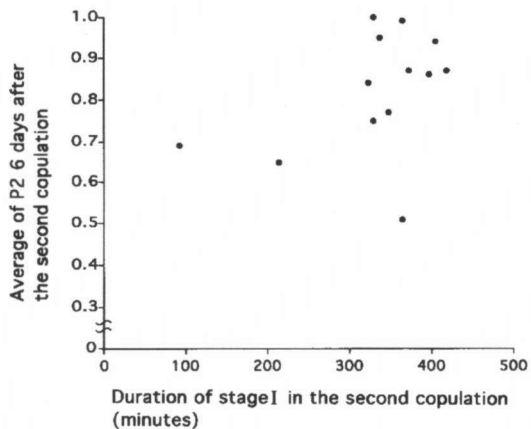


Fig. 5. The relationship between duration of stage I in the second copulation and P2 averaged over all clutches laid by an individual female 6 days after the copulation.

of the copulation duration in the field. At that time, prolonged copulations may have the function of mate-guarding to prevent from additional copulations of the female (ROBERTSON, 1985). In the future, the possibility of mate-guarding should be examined.

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REFERENCES

- BOORMAN, E. & G. A. PARKER, 1976. Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol. Ent.* 1: 145-155.
- CORDERO, A. & P. L. MILLER, 1992. Sperm transfer, displacement and precedence in *Ischnura graellsii* (Odonata: Coenagrionidae). *Behav. Ecol. Sociobiol.* 30: 261-267.
- FINCKE, O. M., 1984. Sperm competition in the damselfly *Enallagma hageni* Walsh (Odonata: Coenagrionidae): benefits of multiple mating to males and females. *Behav. Ecol. Sociobiol.* 14: 235-240.
- McVEY, M. E. & B. J. SMITTLE, 1984. Sperm precedence in the dragonfly *Erythemis simplicicollis*. *J. Insect Physiol.* 30: 619-628.
- MICHIELS, N. K., 1992. Consequences and adaptive significance of variation in copulation duration in the dragonfly *Sympetrum danae*. *Behav. Ecol. Sociobiol.* 29: 429-435.
- MICHIELS, N. K. & A. A. DHONDT, 1988. Direct and indirect estimates of sperm precedence and displacement in the dragonfly *Sympetrum danae* (Odonata: Libellulidae). *Behav. Ecol. Sociobiol.* 23: 257-263.
- MILLER, P. L., 1987. An examination of the prolonged copulations of *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 16: 37-56.
- NARAOKA, H., 1994. Diurnal rhythm of the damselfly, *Ischnura asiatica* Brauer (Coenagrionidae, Odonata) (2) Sperm displacement. *Gekkan-Mushi* 279: 18-21. [Jap.]
- NUYTS, E. & N. K. MICHIELS, 1996. High last-male sperm precedence despite unfavourable positioning of sperm in the bursa copulatrix of *Sympetrum danae* (Sulzer) (Anisoptera: Libellulidae). *Odonatologica* 25: 79-82.
- PARKER, G. A., 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* 45: 525-567.
- ROBERTSON, H. M., 1985. Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: females mimicking males. *Anim. Behav.* 33: 805-809.
- SAWADA, K., 1995. Male's ability of sperm displacement during prolonged copulations in *Ischnura senegalensis* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica* 24: 237-244.
- SIVA-JOTHY, M. T. & Y. TSUBAKI, 1989. Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae). 1. Alternative mate-securing tactics and sperm precedence. *Behav. Ecol. Sociobiol.* 24: 39-45.
- WOLF, L. L., E. C. WALTZ, K. WAKELEY & D. KLOCKOWSKI, 1989. Copulation duration and sperm competition in white-faced dragonflies (*Leucorrhinia intacta*; Odonata: Libellulidae). *Behav. Ecol. Sociobiol.* 24: 63-68.