Odonatologica 16(2): 201-207 *June 1, 1987*

SPERM COMPETITION IN *ISCHNURA ELEGANS* (VANDER LINDEN) (ZYGOPTERA: COENAGRIONIDAE)

P.L. MILLER
Department of Zoology, University of Oxford, South Parks Road
Oxford, OX1 3PS, United Kingdom

Received March 19, 1986 / Accepted July 10, 1986

The capture, preservation and subsequent examination at known times during copulation has shown that most mature females copulate more than once. By marking copulating pairs it was found that some females accepted second males on the same day. Copulations of decapitated males with intact females were relatively short-lasting as also was the case when both sexes had been decapitated. However, copulations of intact males with decapitated females were long-lasting and resembled those of undisturbed pairs. Males therefore apparently control the duration of copulation. Measurements of sperm volumes in copulating females support the hypothesis that copulating males remove all the sperm of rivals from the bursa, but none from the spermatheca. Sperm vesicles of males contain a mean of 0.011 ± 0.0019 mm^3 (n=14) during stage I of copulation. Spermathecae of females after sperm transfer contain a mean of 0.0064 ± 0.0023 mm^3 (n=11), whereas bursae contain a mean of 0.0023 ± 0.0009 mm^3 (n=14). During stage II of copulation, males transfer the whole vesicle contents, which is about 5 times the volume that can be accommodated in the bursa. This apparent anomaly is discussed.

INTRODUCTION

Ischnura spp. sometimes copulate for periods longer than are known in other species of Odonata (KRIEGER & KRIEGER-LOIBL, 1958; PAULSON & CANNINGS, 1980; ROBERTSON, 1985). In a population of *I. elegans* (Vander L.) examined in southern France, copulation commonly lasted for 6-7 h, and I suggested that such long copulations represent a form of mate guarding by the male (MILLER, 1987). This may have benefited males since competition for females in the high-density population was strong, and females did not oviposit until late in the day. I described a method which allowed copulation to be observed closely in pairs whose heads had been crushed and

whose abdomens had been dissected. In this way the movements of the penis within the transparent genital tract of the female could be watched and filmed, and removal of sperm from the bursa by the penis was witnessed (MILLER, 1987). It is important to know more about the extent of sperm competition in this species, and to this end copulating pairs were caught and preserved at recorded times. Sperm masses were dissected out from females and their volumes were measured following the methods of WAAGE (1979, 1982, 1984). The results, presented here, show that females commonly mate more than once, and they suggest that sperm are removed from the bursa but not from the spermatheca by a copulating male, in agreement with what has been found in *I. ramburi* (WAAGE, 1986).

MATERIAL AND METHODS

Ischnura elegans was very abundant at some ponds and recently flooded fields close to a biological research station at La Tour du Valat in the Camargue, southern France (43° 30'N, 04° 30'E), during July and August, 1984 and 1985. Single females and copulating pairs were caught at recorded times; their heads were crushed ("decapitation") and they were immediately preserved in 2% formaldehyde while still in copula. Further copulating pairs were caught, marked on the wings with a permanent--ink writer, and then released.

The terms "sperm volume" and "sperm mass" are used to mean the total mass of sperm and other materials (e.g. seminal fluids) which accompany sperm and may be produced by the male or the female. Sperm volumes were assessed in one of two ways. In the first (cf. WAAGE, 1979, 1984) sperm masses were dissected out from the bursae and spermathecae of females and their lengths and breadths were measured under a binocular microscope with a graticule. Their length x estimated mean breadth was then calculated to give a figure in arbitrary units which could be used to make comparisons between individuals. In the second method, the volumes were measured in mm^3 by compressing sperm masses from females or from the sperm vesicles of males to a uniform thickness of 100 μm under a supported coverslip on a slide. The area of the mass was then accurately determined by drawing it on squared paper under a high-power microscope and from this the volume was calculated. Repeated measurements on the same masses showed good reliability for the cylindrical spermathecal masses and for the kite-shaped masses from sperm vesicles, but bursae gave more variable values probably because parts of the mass were sometimes less than 100 μm thick.

Values are expressed in means $\pm$ standard deviations and significance was estimated using the Mann Whitney U test. The state of maturity of insects was estimated from body coloration (PARR & PALMER, 1971; HAMMOND, 1983).

RESULTS

OCCURRENCE OF MORE THAN ONE COPULATION BY FEMALES

Nine pairs of *I. elegans* were caught in pre-copulatory tandem between 08.00 and 08.30 h (a time when many copulations were starting: sunrise was at 06.29 h on 31st July) and the storage organs of the females were examined. Table I shows that all were found to contain sperm in both storage organs and all had therefore

copulated on a previous day. In a further 20 females caught during stage I of copulation at a time when all the corresponding males had well-filled sperm vesicles, 19 had abundant sperm in their spermathecae, and one contained no sperm. Thus 28 out of 29 copulating females examined contained sperm and had copulated previously.

Forty copulating pairs were then captured between 09:30 and 10:30 h; the males and females were both marked on the wings and then released still in copula. Of these, 2 females were found in copulation with unmarked males later on the same day, 2 were found with unmarked males on the following morning, and one was found in copulation with an unmarked male 48 h later. Thus some females accepted second males on the same or the subsequent day after a mating, but it was not known if the first male had transferred sperm. A further 33 pairs were therefore captured between 09:15 and 10:15 h and the heads of the males were crushed. They were then marked on the wings and released in a region where they could be observed. In some pairs the female flew off towing the male, but most remained settled. Of 15 pairs which were watched, 5 had separated after 78 min and the remainder had done so after 2 h. After separation, the females either perched nearby or flew off while the males dropped to the ground. The copulatory performance was like that of pairs in which the heads of both partners had been crushed (MILLER, 1987). Eighty min after the first pair had been released, a marked female was found in copulation with a new unmarked male. During the ensuing 4 h, 7 marked females were found in copulation with unmarked males, and a further 2 were in tandem. The second copulations persisted for at least an hour and activity appeared to be normal. Thus some females which had copulated with decapitated males accepted second males within a few hours of leaving the first. Sperm transfer by decapitated males was not confirmed, but previous observations have shown that it does occur when both partners have been decapitated (MILLER, 1987).

Thirteen pairs, captured between 10:30 and 10:50 h, were marked on the wings and then released after crushing the heads of the females. Of these, 10 were observed to continue to copulate for at least 150 min, and of these 2 continued for 280 min, after which the females were dropped and the males flew off. Thus intact males copulated with decapitated females for 2-4.5 h, whereas decapitated males copulated with intact females for about 1-1.5 h, as did pairs in which the heads of both had been crushed (MILLER, 1987). This suggests that males control the duration of copulation. In the same population undisturbed pairs were found to copulate for a mean of 324 ± 90 min (S.D., n=13) (MILLER, 1987), whereas Banham (pers. com.) has found a shorter mean in less dense populations in England.

SPERM REMOVAL FROM FEMALES DURING COPULATION

Table I shows the volumes of sperm masses dissected from females which had been caught at the times given. A further 3 solitary females caught between 15:30 and 17:30 h, and one female caught copulating between 10:00 and 10:20 h, contained no sperm and are not included in Table I. The volumes of sperm masses from the spermathecae of females caught before, during and after copulation do not differ significantly. In contrast, sperm masses from the bursae of females caught in stage I of copulation were significantly smaller ($P < 0.005$) than those from pre- or post-copulatory females, or those from females in stage II. As Table I shows, 15 out of 20 (75%) of stage-I females had no detectable sperm in their bursae, whereas 8 out of 10 (80%) stage-II females had well-filled bursae, as did 9 out of 10 (90%) post-copulatory females.

In 12 out of 19 females caught in stage I and preserved in copula, the horns of the penis were found in the bursa, while in the other 7 cases they lay within the vagina. In none were they found within the spermathecal duct.

Table II shows the mean volumes in mm^3 of sperm masses from females' storage organs and from males' sperm vesicles. The males were all caught and preserved in stage I of copulation before they had transferred sperm whereas the females were either in stage II of copulation or they were caught alone late in the day. The Table indicates that the mean figure for sperm vesicles of 0.01 mm^3 is

Table I
The volumes of sperm masses from solitary and copulating females

Time of capture and state of female	Spermathecae (arbitrary units) n	$\bar{x}$	s.d.	no. empty	Bursae (arbitrary units) n	$\bar{x}$	s.d.	no. empty
08:00-08:30 h females in tandem	9	104	34	0	9	49	27	0
10:00-10:20 h in copulation stage I	9	98	15	0	9	0.6	1.3	7
11:00-11:40 h in copulation stage I	11	109	19	0	11	6	13	8
14:00-15:30 h in copulation stage IIB	9	112	30	0	10	28	24	2
15:30-17:30 h solitary females	10	140	51	0	10	29	13	1

Table II
The volumes of sperm masses from males and females

Sex and State	Storage organ	Sperm mass volume (mm^3)			
		n	$\bar{x}$	s.d	range
Males caught and separated in stage I of copulation	Sperm vesicles	14	0.011	0.0019	0.007 -0.015
Females in stage II of copulation, or alone after 15:30 h	Bursae	11	0.0023	0.0009	0.0007-0.0039
ditto	Spermathecae	14	0.0064	0.0023	0.0037-0.011

1.27 times greater than the combined means for spermathecae and bursae (0.0087 mm^3), the difference being significant ($P < 0.05$).

DISCUSSION

MATING FREQUENCY

GRIEVE (1937) showed that female *Ischnura verticalis* are able to lay several batches of fertile eggs over a period of 30 days after a single mating, and FINCKE (1987) has evidence that females in this species may mate only once. In contrast *I. ramburi* females are known to mate several times (ROBERTSON, 1985; WAAGE, 1986). PARR & PALMER (1971) described mating in *I. elegans* as a rare event and it is important to know if females of this species mate more than once. The results described here show that almost all females found in copula had mated on a previous occasion, and that some females were prepared to accept two males on one day.

SPERM COMPETITION

In a previous study the penis of *I. elegans* was seen to remove sperm from the bursa during copulation (MILLER, 1987), and here it has been found that 75% of the stage-I females examined had empty bursae. No change in the volume of the spermathecal mass was detected during copulation and no part of the penis was found to enter the spermathecal duct. However, the high variance and small sample size may have concealed slight changes in the volume of spermathecal sperm. In *I. ramburi,* WAAGE (1986) did find a small increase in volume during copulation and he suggested that the penis horns might drive bursal sperm into the spermatheca. If a small increase or decrease of spermathecal sperm volume did occur in *I. elegans,* it would perhaps depend on the activity of spermathecal

and duct musculature, such as was seen during copulation (MILLER, 1987).

Since apparently only bursal sperm is removed during copulation, a male can replace only about 26% of rivals' sperm within a female. However if females use bursal sperm first, the last male may still be able to fertilise most of the eggs of the next batch. The small proportion of sperm removed contrasts with what is known in some other odonates where most or all the sperm of rivals is removed by copulating males (WAAGE, 1984; McVEY & SMITTLE, 1984), but it is similar to the case of *Orthetrum cancellatum* in which during short copulations by territorial males only about 10-20% of rivals' sperm is removed (SIVA-JOTHY, 1987).

VOLUMES OF SPERM STORED BY MALES AND FEMALES

The male stores 1.27 times more sperm in the sperm vesicle of the secondary genitalia than can be stored in the bursa and spermatheca of a female (Tab. II). Similarly WAAGE (1986) found in *I. ramburi* that males could store more sperm than females could accommodate. However in both species most copulating females had previously copulated and the male replaced only the sperm in the bursa. In *I. elegans* this means that a male may transfer an ejaculate nearly 5 times greater in volume than the capacity of the bursa. This apparent anomaly may be explained in one of several ways:

(1) The male may not transfer the whole contents of the sperm vesicle. Although this is true for some libellulids (MILLER, 1984), 80% of male *I. elegans* examined in stage IIB of copulation had empty sperm vesicles, as did all solitary males examined (MILLER, 1987). Males therefore probably do transfer the whole contents of the vesicle.

(2) The excess ejaculate may remain in the vagina and be passed out later, perhaps when oviposition starts. Preserved solitary females were sometimes found to contain sperm in the vagina, but its origin is unknown: it could have been deposited by the last male, or it could have been ejected by the female perhaps during capture or preservation (thereby giving a reduced estimate of the amount stored).

(3) The sperm in the ejaculate could be diluted considerably by seminal fluid not stored by the female (cf. WAAGE, 1986). The possibility that sperm is more concentrated in the female than in the male is currently being tested by measuring sperm densities in sectioned material.

(4) Measurements from the bursae may be underestimates because sperm at the base of the bursa and in the vicinity of the valve is excluded. However this could not amount to more than 20% of bursal sperm.

Thus the discrepancy between the volume of the male ejaculate transferred and the capacity of the bursa is not explained at present, but sperm concentration and/or the ejection of surplus seem the most likely possibilities.

ACKNOWLEDGEMENT

I am very grateful to Dr J.K. WAAGE for his most helpful comments.

REFERENCES

FINCKE, O.M., 1984. Sperm competition in the damselfly Enallagma hageni: benefits of multiple mating for males and females. *Behav. Ecol. Sociobiol.* 14: 235-240.

FINCKE, O.M., 1987. Sources of variation in lifetime reproductive success in a non-territorial damselfly (Odonata: Coenagrionidae). *In:* T. Clutton Brock, [Ed.]. Reproductive Success. Univ. of Chicago Press. (In press.).

GRIEVE, E., 1937. Studies on the biology of the damselfy Ischnura verticalis Say, with notes on certain parasites. *Entomologia amer.* 17: 121-153.

HAMMOND, C.O., 1983. *The dragonflies of Great Britain and Ireland.* Harley Brooks, Colchester.

KRIEGER, F. & E. KRIEGER-LOIBL, 1958. Beiträge zum Verhalten von Ischnura elegans und Ischnura pumilio (Odonata). *Z. Tierpsychol.* 15: 82-93.

McVEY, M.E. & B.J. SMITTLE, 1984. Sperm precedence in the dragonfly Erythemis simplicicollis. *J. Insect Physiol.* 30: 619-628.

MILLER, P.L., 1984. The structure of the genitalia and the volumes of sperm stored in male and female Nesciothemis farinosa (Foerster) and Orthetrum chrysostigma (Burmeister) (Anisoptera: Libellulidae). *Odonatologica* 13: 415-428.

MILLER, P.L., 1987. An examination of the prolonged copulations of Ischnura elegans (Vander Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 16: 37-56.

PARR, M.J. & M. PALMER, 1971. The sex ratios, mating frequencies and mating expectancies of three coenagriids (Odonata: Zygoptera) in Northern England. *Ent. scand.* 2: 191-204.

PAULSON, D.R. & R.A. CANNINGS, 1980. Distribution, natural history and relationships of Ischnura erratica Calvert (Zygoptera: Coenagrionidae). *Odonatologica* 9: 147-153.

ROBERTSON, H., 1985. Female dimorphism and mating behaviour in a damselfly Ischnura ramburi: females mimicking males. *Anim. Behav.* 33: 805-809.

SIVA-JOTHY, M.T., 1987. Variation in copulation duration and the resultant degree of sperm removal in Orthetrum cancellatum (L.) (Libellulidae: Odonata). *Behav. Ecol. Sociobiol.* 20: 147-151.

WAAGE, J.K., 1979. Dual function of the dragonfly penis: sperm removal and sperm transfer. *Science* 203: 916-918.

WAAGE, J.K., 1982. Sperm displacement by male Lestes vigilax Hagen (Zygoptera: Lestidae). *Odonatologica* 11: 201-209.

WAAGE, J.K., 1984. Sperm competition and the evolution of odonate mating systems. *In:* R.L. Smith, [Ed.]. Sperm competition and the evolution of animal mating systems, pp. 251-290, Academic Press, New York-London.

WAAGE, J.K., 1986. Evidence for widespread sperm displacement ability among Zygoptera (Odonata) and the means for predicting its presence. *Biol. J. Linn. Soc.* 28: 285-300.