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Sperm transfer, displacement and precedence in *Ischnura graellsii* (Odonata: Coenagrionidae)

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Summary. Copulation in *Ischnura graellsii* may be divided into three stages, according to the movements and position of the male's abdomen. We measured sperm volumes in males and females interrupted at different phases of copulation in laboratory-reared and field specimens. The results showed that males remove sperm from the female during stage I, and do not transfer sperm until stage II of the copulation. In the field females interrupted during stage I of copulation had less sperm than postcopula females, and the volume of sperm in laboratory females mated once or twice was similar. These results suggest that males can remove most of the sperm during stage I of copulation. Preparations of in-copula specimens showed the horns of the penis (used to remove sperm) inside the bursa copulatrix and the spermatheca. Therefore males can remove sperm from both organs, in contrast to the other *Ischnura* species so far studied, where males can empty only the bursa. The length of these horns is positively correlated with male body length, and there are significant differences in length between the left and right horns of individual males. This suggests great variability in the male's ability to remove sperm. On the other hand, ejaculate volume is positively related to male and female size, and negatively to male age. Males are likely to be able to detect the presence of sperm in females: if the effect of population density and time of start of copulation are taken into account, copulations are longer with mated than with virgin females. Using genetic markers, sperm precedence was studied by rearing the female offspring of 6 females mated with two males of different genotype. In 5 out of 6 crosses, the second male fertilized all the eggs laid by the female in her first clutch. On average, the following clutches were progressively more fertilized by the first male, but there were striking differences between crosses. These differences are probably due to the variability in the amount of sperm transferred and/or removed.

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Introduction

Most female insects store sperm in their reproductive organs and fertilize eggs at the time of egg-laying. When a female has been mated by two or more males, there will be competition among ejaculates to fertilize the eggs. This is known as sperm competition (Parker 1970) and is widespread in both invertebrates (including crustaceans: Diesel 1988; Snedden 1990; arachnids and insects: Smith 1984) and vertebrates (Smith 1984; Møller 1989; Møller and Birkhead 1989). In insects the last male to mate usually achieves an advantage in fertilizing eggs (Gwynne 1984). The mechanism of sperm precedence in most cases is poorly understood, but in the Zygoptera Waage (1979) showed that a male is able to displace most of the sperm stored by the female from previous matings, before transferring his own. Sperm competition is likely to have had a strong influence in the evolution of odonate reproductive behaviour (Waage 1984).

Although sperm competition in insects has usually been studied with genetic markers or sterilised males (Gwynne 1984), in the Odonata the most common method involves measuring sperm volumes in females before, during and after copulation (Waage 1979, 1980, 1982, 1986a, b, 1988; Fincke 1987; Miller 1987b; Watanabe and Adachi 1987; Michiels and Dhondt 1988). Alternative methods are sperm counting (Siva-Jothy 1984, 1987) and the use of irradiated males (Fincke 1984; McVey and Smittle 1984; Michiels and Dhondt 1988; Siva-Jothy and Tsubaki 1989; Wolf et al. 1989). Genetic markers have not previously been used in Odonata, probably due to the absence of good genetic markers and because there are many methodological difficulties in rearing large numbers of larvae from a single cross.

Because *Ischnura* species show several different reproductive behaviours (Cordero 1989), this is a key genus for understanding sperm competition and its relation to mating systems in Zygoptera (Waage 1984). The mechanism of sperm displacement has been studied in *I. ramburi* (Waage 1986a) and *I. elegans* (Miller 1987a,

b), but using only field specimens. In *I. verticalis*, measurements of sperm volumes produced equivocal results (Fincke 1987). Copulatory activity can be divided into three stages in some damselflies (Miller 1987a), including *I. graellsii* (Cordero 1989). In *I. elegans*, these stages involve sperm displacement, sperm transfer and a third stage with no apparent activity (Miller 1987a). By controlling the density in a laboratory population, Cordero (1990a) showed that the long copulation duration (1–5 h) of *I. graellsii* has a guarding function, and that males prolong copulation more with previously mated females than with unmated females, suggesting that displacing sperm requires longer copulations. Females lay eggs alone, in many cases without a previous copulation that day (Cordero, pers. obs.). This behaviour can increase sperm competition if males do not remove all the sperm before transferring their own, because there is more time for mixing of sperm of different males within the female. A study of the change in sperm precedence with time is therefore needed.

This paper presents the results of laboratory experiments designed to study sperm competition in the non-territorial damselfly *Ischnura graellsii* (Rambur). Sperm transfer and sperm displacement were studied by measuring sperm volumes in field-collected specimens and in specimens of known reproductive history in the laboratory. Sperm precedence was studied by using genetic markers. Like other *Ischnura* species, *I. graellsii* has polychromatic females, one phenotype resembling the male (the androchromotypic female, p^a) and two more or less dissimilar phenotypes (the gynochromotypic *infuscans*, p^i, and *aurantiaca*, p^o) (Cordero 1987). This polymorphism is controlled by an autosomal locus with three alelles and a hierarchy of dominance: $p^a > p^i > p^o$ (Cordero 1990b). Males have all possible genotypes but just one phenotype (p^a). All *aurantiaca* females are recessive homozygous (p^op^o), so only this kind of female was used in the experiments.

The aims of this study were to show when males transfer and remove sperm, to see how sperm precedence changes with time, and to investigate the relationship between sperm precedence and the copulation duration.

Methods

Measuring sperm volumes. Sperm transfer and displacement were studied by measuring sperm volumes in individuals preserved at different stages of copulation. We assume that sperm volume is a good estimator of sperm number, because sperm density was very similar in all preparations. A total of 38 females and 27 males were captured in natural populations during 1987 and 1990 in Galicia (NW Spain). Specimens were stored in 70% ethanol at 4° C until dissection. If males remove sperm during copulation, we can expect that the sperm volume in females captured during mating will be smaller than that of females captured after mating (Waage 1979). This method is not very appropriate because the reproductive history of every individual is unknown, as well as the exact moment of copula interruption. Some matings may have been interrupted before sperm removal was appreciable. Furthermore, if no sperm is found in the female, we have no way of distinguishing virgin females from females which have had all sperm from previous matings removed. To avoid this problem, a laboratory experiment was designed. Larvae of *I. graellsii* were collected in April 1990 in a pond (Galicia, NW Spain) and transported to the laboratory. These larvae were maintained in plastic containers filled with water and fed with chironomid larvae and aquatic oligochaetes until adult emergence. Adults were individually marked and measured (body length) to the nearest 0.1 mm a few hours after emergence. Individuals were separated by sex and maintained in insectaries of 50 × 50 × 50 cm. Insectaries were placed in a chamber maintained at 21–23° C, 60–80% humidity and 15 h light: 9 h dark, and fed with adult *Drosophila,* as described in Cordero (1990a, b). Mature virgin females were used in the following experiments. A first group of 24 females was mated once and preserved in 70% ethanol. A second group of 15 females were mated twice on the same ($n=12$) or two consecutive days ($n=3$). This was designed to compare sperm volumes of females after one or two matings without oviposition, and, using once-mated females, to obtain a predictive equation to estimate sperm volume of twice-mated females if males do not remove sperm. For this reason, the following variables were recorded for every mating: duration (total and by stages), number of rocking movements in stage II, number of males in the insectary (density), and time of start of copulation. Most males were used for one mating, but some were used in two to seven matings. This has the disadvantage that not all observations are independent. Nevertheless, in all cases, there was no attempt to induce or prevent a particular male from mating. To obtain matings, females were introduced into the insectary containing males, and the males themselves started the matings. This mimics the conditions of natural populations, where the same male sometimes mates several times. Fortunately, ejaculate volume is not related to the time following a previous mating ($r=0.05$, $P=0.84$), and we have not observed changes in sperm density, so using the same male probably did not affect the results. A third group of 20 females was interrupted during the first mating at different moments in stage I or stage II, to test whether males transfer sperm only during stage II (Miller 1987b). Finally, to study sperm removal, seven females were allowed to mate once and then interrupted at different intervals during stage I of the second copulation.

To measure sperm volumes, specimens were transported to England where the storage organs were dissected out and the sperm mass was compressed to a uniform thickness of 25 μm under a supported coverslip on a slide (Miller 1987b). The sperm area was measured under a microscope at a magnification of 62.5 with an image analyser (Videoplan Kontron). Each mass was measured twice and the mean was used in the analyses. The volume was estimated as the product of the area multiplied by the (25 μm) thickness. With the same procedure five measurements were made of the length of every horn of the penis, when it was possible.

To compare the sperm volumes between groups we used paired *t*-tests, because they are planned comparisons (Steel and Torrie 1985).

Using genetic markers. Adults of *I. graellsii* obtained from the F_3 generation reared to study the inheritance of female polymorphism (Cordero 1990b) were maintained in insectaries as in the preceding experiments. Males of known genotype (p^op^o, p^ap^i and p^ap^a) were placed in separate insectaries, where the *aurantiaca* females (p^op^o) were introduced to mate. Difficulties in synchronizing the mature lifespans of all types of males and *aurantiaca* females, allowed us to obtain only six crosses. To have reciprocal crosses, the same male was used as the first and second mate of different females.

Detailed methods of rearing larvae are in Cordero (1990b). Briefly, damp filter paper was used as an oviposition substrate, eggs then being maintained in plastic containers filled with water until eclosion. Small larvae were fed on *Artemia salina* nauplii, and large larvae on aquatic oligochaetes (*Lumbriculus variegatus*). They were reared separately until the penultimate or last larval instar. Clutches were reared in separate containers, and to increase sample size male larvae were rejected when sex recognition became possible because the polymorphism is expressed in females only. Eggs were counted after eclosion by examining the filter paper

under a binocular microscope. They were scored as fertile or sterile. All eggs wholly or partially hatched and those with dark eye spots were scored as fertile.

These experiments were made with a low density of males (maximum five males/insectary). After 6 months, 359 adult females were obtained from these larvae, with a mean of 60 females per cross, and they were assigned to the first or second male depending on their phenotype. The difference between males in sperm precedence is very unlikely to be due to genetic effects on offspring viability, because in previous work (three laboratory generations) all larval genotypes had the same survival chances (Cordero 1990b). In the analysis, integer variables (x) were transformed as $\sqrt{(x+{}^1/_2)}$ and proportions (y) as arcsine of $\sqrt{y}$ (Steel and Torrie 1985).

Results

Anatomy of genitalia

Miller (1987a) presents a detailed description of the genitalia of male and female *I. elegans*, which is very similar to that of *I. graellsii*. Females have two sperm-storage organs: the bursa copulatrix, at the dorso-anterior end of the vagina, and the spermatheca, joined to the base of the bursa by a narrow duct. In once mated females, the bursa stores 26% of sperm, the same percentage as in *I. elegans* (Miller 1987b).

In the male, the penis is situated in the second abdominal sternite. At the end of the penis there are two horns with distal barbs oriented towards the base of the horn, which are used during mating to remove sperm from the female (Waage 1986a; Miller 1987a). The mean length of both horns was 902.8 μm ($n=19$ males, SE= 10.0 μm) in field collected males and 942.6 μm ($n=35$, SE=7.6 μm) in the laboratory males. In both field and laboratory males, a nested ANOVA indicated highly significant differences ($P<0.001$) in horn length between the two horns of individual males and significant differences ($P<0.05$) between males. The mean difference between the horns of one male was 29.8 ± 6.8 μm ($\pm$SE) in laboratory specimens and 34.1 ± 6.9 μm in field specimens. On the other hand, male body length is correlated with mean horn length in the laboratory specimens ($r=$ 0.34, $P=0.043$, Fig. 1). No data are available on body length for field males.

The length of the spermatheca (including the duct) was measured in 12 females, and gave a mean of 1073.5 μm (SE=62.8 μm), with a range from 518.7 to 1414.4 μm. The range in horn length was 815.4–1010.6 μm; therefore the end of the spermatheca is inaccessible for most males. There are very significant differences among females in spermathecal length ($F=1000.0$, $P<0.0001$), but these differences may be due to the elasticity of the organ, because it is very small in virgin females.

Sperm volumes

Figure 2 shows the sperm volumes stored in males of the different experimental groups. These results indicate that males transfer the whole contents of the vesicle during mating (20 of 25 postcopula males had empty vesicles). As expected, during stage I of copulation, the male does not transfer sperm: none of the 14 virgin females interrupted during stage I had sperm (the interruptions were made in 5 females after 10, 20, 30, 40 and 59 min and in 9 females after 60 min). On the other hand, the sperm volume of laboratory males interrupted during stage I is similar to that of once-mated females ($t=1.56$, $P=0.13$).

Surprisingly, all six virgin females interrupted during stage II had no sperm, although the sperm volume in males is clearly smaller when interrupted in stage II (Fig. 2). Examining the genitalia of these males, sperm masses were found halfway between the vesicle and the end of the penis. It is clear that the interruption (after 1–10 rocking movements, when the average is 20) was too early to allow the sperm mass to travel the more than 4 mm from the vesicle to the end of the penis.

Figure 3 shows photomicrographs of in-copula preparations. It can be seen that one horn from the penis is in the bursa (20/20 specimens), whilst the other is

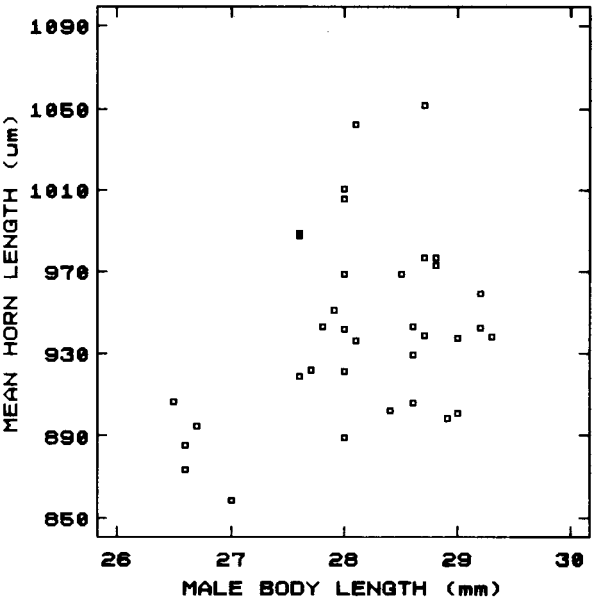

Fig. 1. The relationship between male size and mean length of both horns of the penis in *Ischnura graellsii* ($r=0.34$, $n=35$, $P=0.043$)

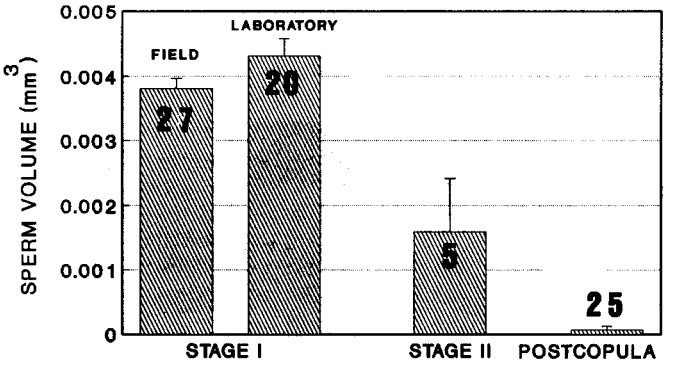

Fig. 2. The volumes of sperm (mean+SE) stored in the seminal vesicle of *I. graellsii* males. *Numbers* are sample size

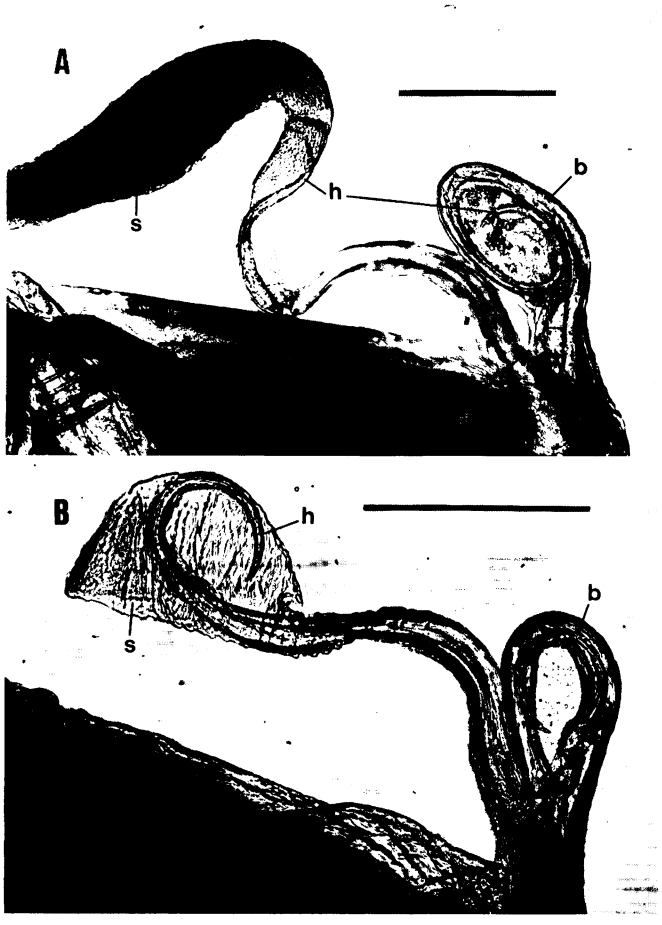

Fig. 3A, B. Photomicrographs of in-copula preparations, interrupted during stage I. **A** Reproductive tract of a previously mated female showing the horns of the penis while removing sperm from the bursa and the spermathecal duct. **B** Virgin female showing one horn inside the spermatheca. *b*, bursa copulatrix; *h*, horn; *s*, spermatheca. *Scale bar* is 0.25 mm

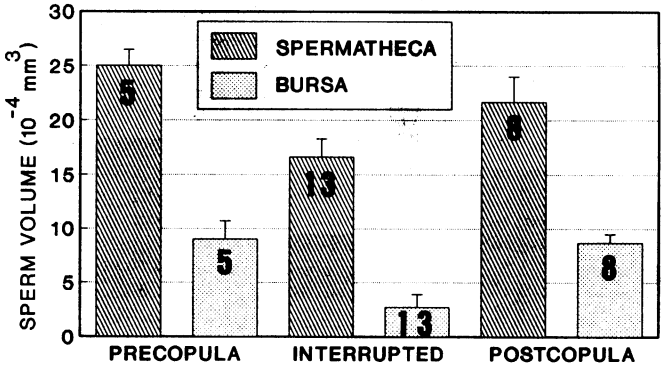

Fig. 4. The volumes of sperm (mean+SE) stored in the bursa and spermatheca of *I. graellsii* females captured in the field. *Numbers* are sample size

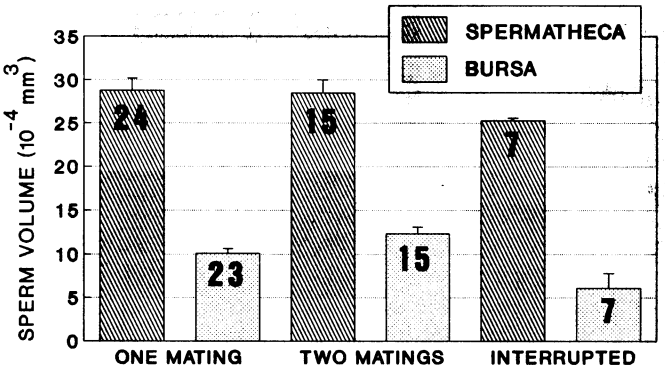

Fig. 5. The volumes of sperm (mean+SE) stored in the bursa and spermatheca of *I. graellsii* females mated once, twice and interrupted during stage I of the second mating in the laboratory. *Numbers* are sample size

in the spermathecal duct (14/20 specimens) and the spermatheca (3/20 specimens). The narrow lumen of the duct probably impedes the horns from entering into the spermatheca more often. This anatomical evidence suggests males are probably able to remove sperm from the spermatheca with the penis horns.

The sperm volume in field collected females shows the expected pattern (Fig. 4). Interrupted females have less sperm than postcopula females ($t=-2.79$, $P=0.012$), and pre and postcopula specimens have similar volumes ($t=0.84$, $P=0.42$). The same results are obtained if bursa and spermatheca volumes are analysed separately (in this analysis six interrupted and six precopula females without sperm were excluded, as well as one postcopula female with an empty bursa and 17% of the average spermathecal volume).

The sperm volume of females mated once or twice in the laboratory (Fig. 5) does not differ ($t=-0.94$, $P=0.35$). This result suggests that the second male removed most of the sperm of the first male, and is not likely to be due to a higher density of sperm in twice-mated females because sperm volume clearly diminishes during stage I (Fig. 5, interrupted females). Spermathecal volume is not significantly different in once- and twice-mated females ($t=0.16$, $P=0.88$), but bursal volume is 23% greater in twice- than in once-mated females ($t=-2.52$, $P=0.016$).

Seven females were interrupted during stage I of the second copulation, after 0.9, 5, 10, 15, 20, 30 and 77 min (Fig. 5). Sperm volume is smaller in these females than in once-mated females ($t=2.10$, $P=0.044$), the difference being significant in the bursa ($t=3.04$, $P=0.005$) but not in the spermatheca ($t=-1.24$, $P=0.23$). We expected that sperm volume in these females would be negatively related to time until interruption, but due the great variability in the volume of sperm transferred by the first male and the small sample size, the correlation is not significant ($r=-0.37$, $P=0.42$). The bursa was empty in the female interrupted after 77 min.

It was not possible to obtain a good regression equation to predict ejaculate size in once-mated females. The variables tested were: male and female age, male and female length, number of males in the insectary, duration of stage I, II and total, number of pumping movements in stage II and inter-mating interval. Nevertheless, ejaculate volume is related to female length ($r=0.51$, $n=23$,

Table 1. Offspring of crosses of *aurantiaca* females of *Ischnura graellsii* with two males of different genotype (in brackets)

Males	Duration (min)		Eggs laid			Offspring of male		
	stage I	stage II	Day[a]	Eggs	% Fertile	1	2	(% ♂2)
Cross 1			2	183	96.7	0	5	100.0
H111 ($p^a p^i$)	33	≈3	3	151	96.7	11	11	50.0
P152 ($p^o p^o$)	212	3.35	5	207	99.0	16	4	20.0
			6	94	97.9	17	2	10.5
			8	211	95.3	20	0	0.0
			9	76	97.4	6	0	0.0
			10	111	99.1	11	0	0.0
			11	92	97.8	3	0	0.0
			12	87	98.9	2	0	0.0
			13	86	93.0	–	–	–
			14	81	98.8	–	–	–
			15	32	93.8	5	0	0.0
			Totals	1411	97.1	91	22	19.5
Cross 2								
H111 ($p^a p^i$)	40	3.58	1	255	98.0	0	26	100.0
U132 ($p^o p^o$)	117	3.72	2	27	100.0	–	–	–
			3	309	97.1	10	8	44.4
			5	224	94.6	18	5	21.7
			Totals	815	96.8	28	39	58.2
Cross 3								
U131 ($p^o p^o$)	84	≈2	2	365	99.2	0	22	100.0
H111 ($p^a p^i$)	71	4.43	3	42	100.0	0	2	100.0
			4	169	99.4	0	9	100.0
			5	114	99.1	0	11	100.0
			6	105	98.1	–	–	–
			7	110	98.2	0	4	100.0
			8	38	100.0	–	–	–
			Totals	943	99.0	0	48	100.0
Cross 4								
P151 ($p^o p^o$)	46	4.00	1	151	91.4	0	5	100.0
H111 ($p^a p^i$)	71	2.57	2	175	99.4	0	5	100.0
			3	101	98.0	0	5	100.0
			5	43	95.3	0	11	100.0
			6	284	82.7	3	6	66.7
			7	63	82.5	5	3	37.5
			Totals	817	90.5	8	35	81.1
Cross 5								
H131 ($p^a p^a$)	121	3.80	1	125	93.6	0	22	100.0
U133 ($p^o p^o$)	285	3.27	2	154	94.2	0	22	100.0
			3	6	83.3	0	2	100.0
			Totals	285	93.7	0	46	100.0
Cross 6								
U133 ($p^o p^o$)	64	3.47	2	272	98.5	2	24	92.3
H131 ($p^a p^a$)	122	2.58	4	145	98.6	1	15	93.8
			6	98	98.0	–	–	–
			Totals	515	98.4	3	39	92.9

[a] Days from second mating

$P=0.014$), male age ($r=-0.36$, $n=33$, $P=0.040$) and male length ($r=0.39$, $n=33$, $P=0.026$). In a stepwise regression using these three variables to predict ejaculate volume, only female length entered and accounted for 26% of the variance. So, most of the variance remains to be explained. On the other hand, the correlation between ejaculate size and female size is not due to size-assortative mating, because there is no correlation between male and female length in more than 300 field pairs so far examined (Cordero, pers. obs.). Taking into account the volume of bursa and spermatheca in separate analyses, it was found that spermathecal volume

is correlated with female length ($r=0.50$, $n=24$, $P=0.014$) and bursal volume with male age ($r=-0.45$, $n=23$, $P=0.033$). Surprisingly, the duration of stage II and the number of rocking movements during this stage are not related to ejaculate size, in contrast to what Siva-Jothy and Tsukabi (1989) found in *Mnais pruinosa*.

Using genetic markers

Table 1 shows the detailed results of all crosses. Males are identified as in Cordero (1990b), using the three first symbols of the code of the parental F_3 female, and the remaining number an ordinal. In 5 out of 6 crosses, the second male fertilized all the eggs in the first clutch. In cross 3, the second male had the advantage for at least 7 days after copula, but this advantage was lost earlier in crosses 1 and 2.

Table 1 shows the relationship between the proportion of the descendents sired by the second male and the [illegible] number of eggs laid by the female. Striking differences are observed among crosses: after 800–900 eggs have been laid, the second male sired 0, 22, 38 and 100% in the four crosses. Male H111 seems to be better able to displace sperm and/or to produce highly competitive sperm than the other males: when this male was the first (crosses 1 and 2) he fertilized at least 50% of eggs in the second clutch, and when he was the second (crosses 3 and 4) all eggs in the four first clutches were sired by him. Crosses 5 and 6 involved the same males, and both seem equally able to displace sperm.

There was no correlation between the duration of stage I in the second mating (when the male displaced sperm) and the proportion of total eggs fertilized ($r=-0.43$, $n=6$, $P=0.391$).

Discussion

In the two species of *Ischnura* previously studied, the last male removes sperm from the bursa copulatrix of the female, but not from the spermatheca (Waage 1986a; Miller 1987b). In *I. graellsii*, however, males are able to remove sperm from both organs. If bursal sperm is the first used to fertilize eggs, the last male should fertilize all the eggs in the first clutch (Waage 1986a; Miller 1987b). The results obtained using genetic markers are in agreement with that hypothesis.

The last-male advantage tends to diminish in subsequent clutches (Table 1), but in crosses 3, 4 and 5, the advantage of the second male was maintained in several clutches. This is probably due to differences in the ability to remove sperm as suggested by the fact that the length of the horns of the penis (used to remove sperm) is highly variable and correlates with male size. Even right and left horns have different lengths. This could be important because sometimes the spermatheca was seen to be oriented to the right side of the female and sometimes to the left. Unfortunately we do not know if this orientation is permanent or whether it can change in the same female; or even if the duct emerges from the bursa to one side or medially, and is then bent to the left or to the right. Thus, some males could be more able than others to displace sperm. Secondly, the sperm volume in mated females interrupted in stage I of the second copulation indicates that removing sperm requires long copulations (the bursa is empty after 77 min but not after 30), while in short copulations males can probably remove only small amounts of sperm. Finally, both bursa and spermatheca are covered by muscular tissue, so females could help or hinder the entry of the horns into the spermatheca, or could even actively expel the sperm. Recently, it has been found that there are significant differences (18%) among males in fertilization success in *Tribolium castaneum*, and that body size correlates with fertilization success (Lewis and Austad 1990). Another factor that could explain the differences between crosses in sperm precedence is that the ejaculate size is variable. The positive correlation between ejaculate size and female size can be explained if males transfer more sperm to larger females (males might be able to estimate female weight during precopula tandem), but this is unlikely because males transfer the whole contents of the vesicle. A second explanation is that spermatheca volume correlates with female size, and in small females the excess ejaculate is expelled by the vagina (some sperm was found in the vagina of one female during this study, and is also common in *Enallagma cyathigerum*, PL Miller per. obs.). That male age correlates negatively with ejaculate volume is not surprising; nor is the positive relationship between male size and ejaculate volume. The only study that has measured the change in sperm precedence in different clutches of one damselfly (*Mnais pruinosa*) showed that the last male sires 100% of the eggs in the first clutch, but this advantage disappeared gradually in later clutches (Siva-Jothy and Tsubaki 1989). In *M. pruinosa* the proportion of eggs sired by the last male showed "erratic" changes with time in some females (Siva-Jothy and Tsubaki 1989), probably due to unequal sperm mixing, although there was a tendency to complete sperm mixing after 6 days.

Matings were made at a variable density (1–21 males/insectary, depending on the availability of specimens). Cordero (1990a) showed that the long duration of the copulations of *I. graellsii* is mainly a method of in-copula guarding to prevent new inseminations of the female before oviposition, and that copulations with mated females were longer than copulations with virgin females, suggesting that sperm displacement requires long matings. The results presented here using females mated twice confirm that suggestion. A stepwise regression with mating duration as the dependent variable and time of start, order (first or second) and density (number of males in the insectary) as independent variables accounts for 77% of the variance, the three independent variables being highly significant and in the predicted direction: time of start, negative, and order and density, positive. This indicates that males appear to be able to adjust their copulation duration according whether their mate is a virgin or not, although the cue males use to do this is still not clear. The same effect of order has been found in the beetle *T. castaneum* (Lewis and Austad 1990). Also, in the spider *Frontinella pyramitela*, males

are able to distinguish between virgin and mated females during the pseudocopula (Suter 1990). In the damselfly *Coenagrion scitulum* males could also be able to detect the presence of sperm in females, because if females are interrupted during stage I of copulation, the next mating of these females is shorter than usual, probably because these females have no or less sperm to remove (Utzeri and Sorce 1988).

The change in last-male precedence with time has important effects on male lifetime reproductive success. Usually (Fincke 1982, but see Fincke 1986; Banks and Thompson 1985; Hafernik and Garrison 1986) it is assumed that mating success gives a good estimate of reproductive success in male coenagrionids. However our results indicate that this is not true if males are unable to control oviposition substrates, since females can lay eggs without remating. This is likely to have been a very important factor in the evolution of long copulations in *Ischnura* species (Cordero 1990a).

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