J. Orthoptera Research 7, Dec. 1998: pp. 139-146

A histological study on copulation duration, patterns of sperm transfer and organization inside the spermatheca of a grasshopper, *Dichromorpha viridis* (Scudder)

J. A. Johnson and M. Niedzlek-Feaver

The Department of Zoology, North Carolina State University, Box 7617, Raleigh, NC 27695-7617

Abstract

The mechanism of sperm transfer and sperm organization inside the spermatheca was investigated in *Dichromorpha viridis* (Scudder). Spermathecae from single- and multiple- mated females whose copulations were interrupted at various prescribed time intervals, were examined histologically. Sperm organization inside the spermatheca from 24 to 120 hours after copulation had terminated was also investigated.

The first observation of sperm inside the spermatheca did not occur until 30 minutes had passed into copulation. The majority of sperm transferred into the spermatheca were in the form of sperm bundles, or spermatodesmes, and the rate at which sperm bundles were transferred appeared to decrease after 13 hours into copulation (average mating duration in the laboratory was 28 hours). The occurrence of abundant sperm with sperm bundles was observed only in females that had mated before and less than 9 hours into copulation, and so believed to be from previous matings. As mating continued, less and less individual sperm was observed, and by 15 hours into copulation only sperm bundles were observed inside the spermatheca. The interrupted multiple matings indicate that at least some sperm bundles remain inside the spermatheca while individual sperm were removed by some mechanism, possibly sperm flushing by an excess of seminal fluid provided by the mating male.

Some sperm bundles were shown to keep their bundle integrity for at least 7 days after copulation had terminated. The sperm bundles must disassociate into individual sperm prior to fertilizing the female's eggs; therefore, it may be the female that provides the mechanism or chemical stimulus to initiate sperm bundle disassociation prior to oviposition and/or a multiple mating.

The results of this investigation implicate sperm competition, perhaps mediated by female choice, as a primary reason for lengthy copulations in *D. viridis*. Males may also act as mechanical plugs by remaining *in copula* for an extended duration after a sufficient amount of sperm has been transferred, or males may be overcompensating the process of sperm removal by transferring an excess of seminal fluid to "flush-out" any sperm present from previous matings.

Introduction

In many animals, sperm competition has been a major selective force in the evolution of mating strategies (Parker 1970, Smith 1984, Birkhead and Moller 1992). Sperm competition occurs when two or more males inseminate a single female during a reproductive cycle and compete to fertilize her eggs (Parker 1970). In the grasshoppers (Acrididae), sperm precedence studies suggest sperm competition, because the two males that mate with a female often do not contribute equally to paternity, and sperm competition is the logical explanation (Bella *et al.* 1992, Hewitt *et al.* 1989, Hunter-Jones 1960, Parker and Smith 1975, Gwynne 1984, Walker 1980, Longo *et al.* 1993, Lopez-Leon *et al.* 1993). Competition among males to fertilize a female's eggs leads to antagonistic mechanisms, where a given male either reduces the effectiveness of subsequent matings to protect his sperm or gains precedence in paternity over stored sperm (Parker 1984). The particular adaptations which develop depend on various factors such as the presence and form of sperm storage (*i.e.*, spermathecae), sperm longevity, multiple mating opportunities, and energetic costs associated with reproduction in both sexes (Parker 1970, Smith 1984).

Beyond general descriptions and repertoires of mating behavior, little work on mating strategies has been done on grasshoppers. The few detailed studies to date treat those species that are territorial or have complex pair formation behavior (Greenfield and Shelly 1985, Niedzlek-Feaver 1995, Otte and Joern 1975, Shelly *et al.* 1987, Shelly and Greenfield 1985, Steinberg and Willey 1983, Wicker and Siebt 1985, Willey and Willey 1969, Otte 1972). This study treats a species, *Dicrhomorpha viridis* (Scudder), in which pair formation, according to Otte (1970), is simple. Although acoustical signals do not precede mating in the field, they are used by copulating males presumably to thwart would be intruders. Males have not been observed to fight except in attempts by one male to dislodge a copulating male. The behavioral data indicate that it is the copulation itself that is the primary social concern. Given preliminary data that copulation duration is highly variable although generally lengthy, this species becomes a choice candidate for studies directed at assessing the significance of "cryptic" female choice (choice exerted after pair formation) and/or male sperm competition in shaping the mating system. The planned approach involved monitoring the sperm transfer histologically, quantifying nutrient transfer via the use of radioactive isotopes and using DNA markers to assess paternity when females mate more than once. This paper treats the results of the histological investigation as a possible explanation into the mechanism of sperm transfer and in doing so partially explains the lengthiness and variability of copulation duration.

Methods

Field: Two censuses were conducted daily, initiated at 1100 and 1400 hours, with mating pairs caged and examined at 1700 and 900 (and on weekdays at 2000) to determine if pairs had separated. After separation, individuals were marked, weighed and photographed. Femur, tegmen, and overall length were also measured. In addition, every two weeks, 50 individuals that did not mate that day were weighed, and femur and overall length determined. Between censuses, behavior of marked males was monitored. There was no indication that interacting individuals were disturbed by an observer's presence, and males in some cases approached and attempted to mate with females who were feeding on the observer's clothing. A tape recorder was used to record the observer's descriptions of pair formation.

Histology

Single and Multiple Interrupted Matings

Either teneral or last instar females and males were collected in the field and maintained in the laboratory until controlled matings could be conducted. Females were housed separately from males, and individuals were marked for identification as soon as they molted into adults. Both single and multiple matings were conducted. Females were isolated from males for at least seven days prior to copulation. Between 6 and 10 females were placed in the male cage and allowed to form pairs. Once a pair had formed, they were removed and placed in a smaller cage to prevent takeover by another male. The females assigned to single matings were allowed to mate for a prescribed time interval. The females assigned to multiple matings were allowed to mate to completion on the first mating and then reintroduced to the male cage two to five days after the first mating for a second copulation. No oviposition occurred during the experimental period between first and second matings, and, therefore, loss of ejaculates through utilization for fertilization can be excluded. In both groups, copulations were interrupted at a prescribed time interval by placing the copulating individuals in a -70°C freezer. The spermathecae were dissected from the female, fixed in Carnoy's fixative for three hours, dehydrated with an ethanol series, and embedded in paraffin. Transverse serial sections were cut (7 μ) and stained with Feulgen's stain which is specific for DNA (Boone and Drijver 1986) and, therefore, allowed easy recognition of sperm within the spermathecae. By comparing sequential sections so that individual sperm bundles could be identified, the locations and number of sperm bundles transferred to the female spermatheca during interrupted matings could be obtained. These assessments represent minimum estimates of sperm bundles present as, despite efforts to the contrary, a few sections were probably lost as ribbons were cut or transferred to slides. Also, it was sometimes difficult to tell where one sperm bundle ended and another began. We erred on the side of conservatism and only tallied two sperm bundles at the exact same location if one appeared in that location two or more sections after the other had disappeared. Two sperm bundles that happened to lie on top of one another and were located closer to each other than two sections (14 μ) would be counted as one bundle.

Sperm Organization x Hours After Copulation Finished

Individuals were allowed to mate to completion and then males were removed from the individual pair cages. The females were then placed in a -70 °C freezer at a prescribed time (24, 48, 72, 96, and 120 hours) (n=2 for each) after copulation had finished. No oviposition occurred after copulation had finished and within the prescribed time period. Therefore, loss of ejaculates through utilization for fertilization can be excluded. The spermathecae were prepared for histological examination as described above.

Sperm Organization with Regard to Oviposition

Adult females (n=8) were collected from the field and placed immediately in individual cages with oviposition sand cups for seven days. The females were then placed in a -70°C freezer and the presence of any egg pods in the sand cups noted. The spermathecae were prepared for histological examination as described above.

Scanning Electron Microscopy

Virgin mature females were dissected in Hoyle's solution, and the spermathecae were cut open using a very sharp blade under a dissection microscope. The individual pieces were fixed in 3% glutaraldehyde (pH 7.4, phosphate-buffered saline) at 4°C for 24 hours. Secondary fixation in osmium tetroxide (2%) was followed by an ethanol dehydration series and critical point drying. Samples were mounted on stubs, sputter-coated with gold-palladium, and examined using a Joel T300 scanning electron microscope.

Results

Field Behavior

No sign of any behavior that could functionally be categorized as "courtship" was observed in the field. Two to three males could be observed simultaneously for periods of 60-120 minutes. Males spent most of the day perched on vegetation, 10 - 20 cm above the ground. About 10% (29) of the 278 males whose behavior was monitored moved out of the vicinity during an observation period. In 26 cases, this occurred after a female moved near (within about 30 cm) but then moved off in another direction without orienting to the nearby male. Females who moved to within about 30 cm of a male were approached, although females were not pursued if they moved away. There were only five instances in which two males approached the same female at about the same time. In all cases, the second male to mount appeared to be trying to dislodge the first male in order to mount the female. In four cases both males dismounted before engaging genitalia. Only in one case did the second male succeed in displacing the first male to mount the female.

In general, males who encountered copulating pairs while moving through the habitat oriented toward, but did not approach the mating pair. Only in two instances did males who encountered mating pairs try to displace the first male. In one of those instances the second male was successful. In 8 instances (of 36 observations) males who were encountered, "stridulated", as described by Otte (1970). Hind femora were vibrated rapidly against the tegmina producing audible sound.

Copulation Duration

In the field, a mating was defined as taking place if the pair engaged genitalia. Mean copulation durations for pairs included in the census was 13.2 hr. Copulation duration, however, was highly variable, lasting from less than 5 min to 72 hr.

In the laboratory, mating was also defined as taking place if the pair engaged genitalia. However, in the laboratory, because densities were high in mating cages, "accidental pairings" were common. Feeding females bumped into males who then turned toward them and attempted to mount. To avoid an estimate of copulation duration that would be biased towards accidental encounters not common in nature, copulatory attempts lasting less than 15 min were not scored. The mean copulation duration among pairs who were allowed to mate to completion was 28.5 hr with a minimum of 17 hr and a maximum of 55 hr.

Histological Survey

The sperm storage organ, or spermatheca, of *D. viridis* consists of a small chamber, the preapical diverticulum or distal chamber(dc), and a long duct, the ductus seminalis (ds) (Fig. 1). The ductus seminalis connects the spermatheca to the genital chamber where fertilization occurs as the eggs pass from the oviducts into the genital chamber (Davey 1985). The external appearance and shape of the spermatheca of *D. viridis* appears very similar to that of *Locusta migratoria* (L.)compared to other Acrididae spermathecae (Dirsh 1957, Gregory 1965). Gregory (1965) provides an excellent anatomical survey of the spermatheca, spermatophore formation, and sperm transfer in *L. migratoria*. Gregory describes the ductus seminalis as expanding into a vestibule prior to its connection with the preapical diverticulum and apical diverticulum. *Dicromorpha viridis* also possesses an apical diverticulum (ad) which is in the form of a small protuberance (Fig. 1). Here we will call the vestibule the proximal chamber (pc) due to the presence of small hair-like structures on the inside wall similar to the preapical diverticulum (Fig. 2).

Single Interrupted Matings with Virgins

The first observation of sperm in the spermatheca did not occur until after copulation had passed approximately 30 min in duration. During copulation the spermatophore of the male extends the length of the ductus seminalis and into the preapical diverticulum. Sperm is transferred from the male to the female inside a single spermatophore. The majority of the sperm transferred were in the form of sperm bundles, or spermatodesmes, with the spermatozoa being attached at their heads by a hyaline cap. A few individual sperm were also transferred along with the sperm bundles (Fig. 3). The sperm were released into the spermatheca, and the number of sperm bundles inside the spermatheca gradually increased with copulation duration (Fig. 4). In 1 hr into copulation, as many as five bundles were observed in the spermathecae, and after 3 hr into copulation, as many as 14 bundles were observed in the spermathecae. However, individual variation occurred. For example, in one two hour mating, no sperm were transferred, while in another of the same duration, five bundles were transferred.

The rate at which sperm bundles were transferred appeared to decrease after 13 hr (fig 4). For example, a mating for 5 hr yielded 26 bundles, a mating for 10 hr 47 bundles, but a mating for 20 hr only 44 bundles. In all matings interrupted before 6 hr, most sperm bundles were found in the distal chamber (98% of all sperm bundles found). After that, equal numbers were found in the distal chamber and the proximal chamber. For example, in the mating interrupted at 13 hr into copulation, 47 bundles were found. Twenty four bundles were located in the proximal chamber and 23 bundles were located in the distal chamber. Similarly, for a mating of 19 hr into copulation, 20 bundles were found in the proximal chamber and 25 in the distal chamber.

Multiple Sequential Matings

Both sperm bundles and individual sperm were found inside the spermatheca at early mating times in females that mated twice (Fig. 6). The number of sperm bundles remained high throughout copulation. The numbers found for any one copulation duration vary among individuals. For example, after one mating for 18 hr there were 56 sperm bundles found in the spermathecae; in another, only 11 sperm bundles were found in the spermathecae (Fig. 4). Generally the numbers of sperm bundles found after 4 hr or less into copulation in a female that has mated previously are higher than the numbers found in virgins.

The number of individual sperm present decreased as copulation progressed. From 1 to 4 hours, individual sperm were found throughout the spermatheca (Fig. 6a). Yet at 9 hr, only a few individual sperm were observed inside the spermatheca, and most were present in the proximal chamber of the spermatheca and inside the ductus seminalis. At 15 hr into copulation, only sperm bundles and no individual sperm were observed inside the spermatheca. A few individual sperm were observed inside the spermatheca in 18 and 20 hr into copulation, and their occurrence can be possibly noted as early fragments from the sperm bundles. In some sections where matings were terminated before 6 hr, individual sperm were observed in the "neck" between the distal and proximal chambers, apparently moving from one chamber to the other (Fig. 6). One interpretation that is consistent with these data is that sperm bundles lose their integrity and break down, and the resulting individual sperm move or are moved inside the proximal chamber and eventually out of the spermatheca.

Larger numbers of sperm bundles were found earlier in the spermathecae of doubly mated females than singly mated females for the same copulation duration . For example, in copulations for one hour, 13 sperm bundles were found in the previously mated female, only five in the virgin female (fig. 4). After about 12 hr into copulation, there appears to be fewer sperm bundles found in females who have mated previously than in virgin females.

Where sperm bundles were located inside the spermatheca varied among individuals. After 12 hr into copulation, 21 bundles were found in the distal chamber and eight in the proximal chamber of one individual. Yet after 15 hr into copulation, 23 bundles were found in the proximal chamber and seven in the distal chamber of another individual. Almost equal numbers were observed in both chambers in six individuals, more in the proximal chamber (at least 2x as much) in four individuals and more in the distal chamber of

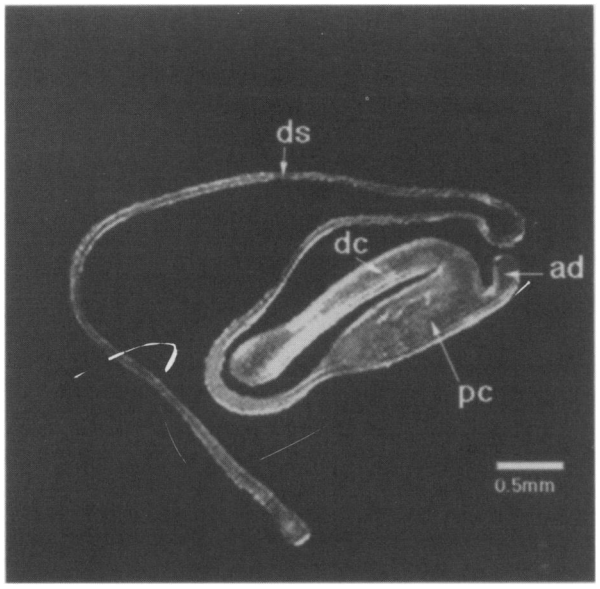

Fig. 1. Spermatheca of *Dichromorpha viridis*. (*pc*=proximal chamber or vestibule, *dc*=distal chamber or preapical diverticulum, *ad*=apical diverticulum, *ds*=ductus seminalis).

six individuals.

Sperm Organization x Hours After Copulation Finished

Histological cross sections of spermathecae were obtained from females who had been isolated 24, 48, 72, 96 and 120 hr (n=2 for each) after copulation had terminated. Sperm bundles were only found within the distal chamber of the spermathecae, with one exception. Sperm bundles were found in both distal and proximal chambers in those females that were isolated for 24 hr after copulation ended. Individual sperm were present in both the distal and proximal chambers of the spermathecae in all isolated females.

Sperm Organization with Regard to Oviposition

Adult females were held in individual cages and allowed to oviposit in sand cups for up to seven days in the laboratory. In all females, individual sperm were found in both chambers of the spermathecae, and when found sperm bundles were only observed in the distal chamber. The number of sperm bundles appeared to decrease with the number of egg pods laid. Three females did not oviposit. Thirty sperm bundles were found in two of these females, none in the third. Three females oviposited one egg pod, and two of the three had sperm bundles (n=10 and 13 bundles). Two females oviposited two egg pods; only one of these females had three sperm bundles. More data will have to be collected to determine how many pods a female typically lays before remating, whether sperm bundles do decrease with egg pods laid and what occurs if females do remate between ovipositions.

Discussion

Insects in particular have evolved a variety of mating strategies. Unlike the sperm of many animals, insect sperm can remain viable for the duration of a female's life span (Page and Metcalf 1982, Star 1984, Taber and Blum 1960). Most female insects possess a spermatheca, which can take

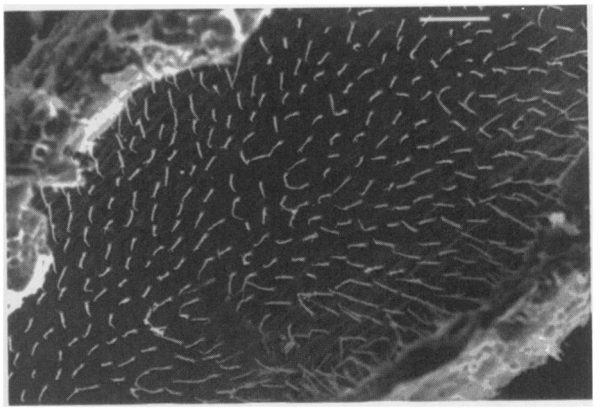

Fig. 2. Scanning electron micrograph showing the inside of the spermatheca. Note the small hair-like structures. The scale bar represents 23.5 μm.

a variety of sizes and shapes (Dirsh 1957, Walker 1980, Smith 1984). In females that have mated multiple times, the spermatheca is the site wherein sperm from the most recent insemination compete with sperm from previous matings. The ability to store sperm prior to fertilization coupled with prolonged sperm viability within the spermatheca may be one reason why sperm competition is so common in the insects. Evidence for competition comes from studies that show that when females mate with two males, the males do not contribute equally in paternity. Some insects show fertilization precedence for sperm from the initial mating (Gwynne 1988, Simmons *et al.* 1994, Bella *et al.* 1992) and others, as in the majority of studied cases, show sperm from the last insemination partially or completely displacing previously stored sperm to fertilize the female's eggs (Gwynne 1984, Hunter-Jones 1960, Walker 1980, Parker 1970, Birkhead and Hunter 1990, Longo *et al.* 1993, Lopez-Leon *et al.* 1993).

In general, copulation times vary among grasshoppers (Otte 1970, Reide 1987, Uvarov 1977). Many species transfer enough sperm to fertilize an egg pod in less than one hour. Yet there are species such as *Dichromorpha viridis* that typically mate for a day in the laboratory. On average, five bundles of sperm are transferred per hour. Previous studies estimate at least 256 sperm per bundle (Longo *et al.* 1993, Pickford and Gillott 1976). An hour or two of copulation should result in enough sperm to fertilize an egg pod of approximately 26 eggs (although Pickford and Gillott (1976) showed a decrease in hatchability with decreased copulation duration especially in the last pods a female lays). Obviously, other factors besides adequate sperm deposition must play a role in determining copulation duration in *D. viridis*.

Previous studies have proposed sperm competition among the reasons for lengthy copulations in grasshoppers. Gregory (1965) proposed that *L. migratoria* mated for an extended duration for a number of reasons other than the need to transfer most of the sperm into the spermatheca. He suggested that it took time to form a complex spermatophore that could act by blocking the deposition of other spermatophores. Parker and Smith (1975) suggested that one function of the lengthy copulation in this same species *Locusta migratoria*, was to achieve sperm precedence in fer-

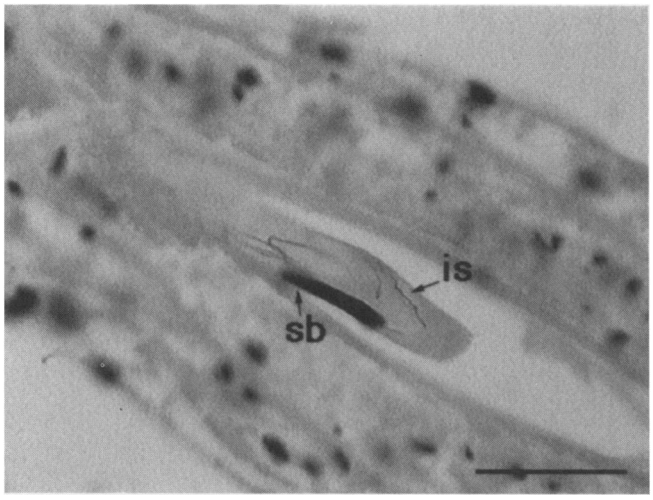

Fig. 3. Cross-section of ductus seminalis (*ds*) showing spermatophore (*sp*) with sperm bundle and individual sperm (*is*) present; 1-2 hour single copulation. 40x.

tilization. Others also have more recently proposed that a lengthy copulation duration is an adaptation to reduce sperm competition (Eberhard and Cordero 1995, Gwynne 1984, Thornhill 1984). Proposed mechanisms include the male remaining in genital contact, or staying mounted on top of the female, to act like a mechanical plug. Or the male may transfer substances within the seminal fluid which act to change a female's receptivity to other males, or stimulate immediate oviposition. The results of this study implicate sperm competition, perhaps mediated by female choice, as a primary reason for lengthy copulations in *Dichromorpha viridis*. Histological evidence suggests that males may not only act as living plugs but replace, at least in part, previously deposited sperm.

Sperm competition appears to start as soon as the pair engages genitalia. Histological slides of spermathecae demonstrate large numbers of loose or individual sperm only during the initial stages of multiple matings. Individual sperm appear in both distal and proximal chambers, and then all appear to move to the proximal chamber before disappearing from the spermatheca. Yet in single matings, sperm does not appear to loose its bundle integrity throughout copulation, and if any chamber is preferred for receiving sperm early in mating, it is the distal chamber. It appears in multiple matings that sperm bundles disassociate so individual sperm can be removed by some mechanism, possibly sperm flushing by an excess of seminal fluid by the second mating male (Gwynne 1984, Parker 1970). Or the female may allow the male to transfer some chemical during mating that causes such disassociation. Dragonflies are the noted insect example for mechanical removal of previous males' sperm by a copulating male, although males in some species of bushcrickets also remove a competitors' sperm before depositing their own (Ono *et al.* 1989, von Helversen and von Helversen 1991, Waage 1979). This type of sperm competition is rarer than stratification (Birkhead and Hunter 1990, Sakaluk 1985) where the sperm of males that contribute earlier are placed by new contributions of sperm to the back of the spermatheca and so probably the back of the "line" leading to insemination.

The sperm bundles also disassociate into individual sperm

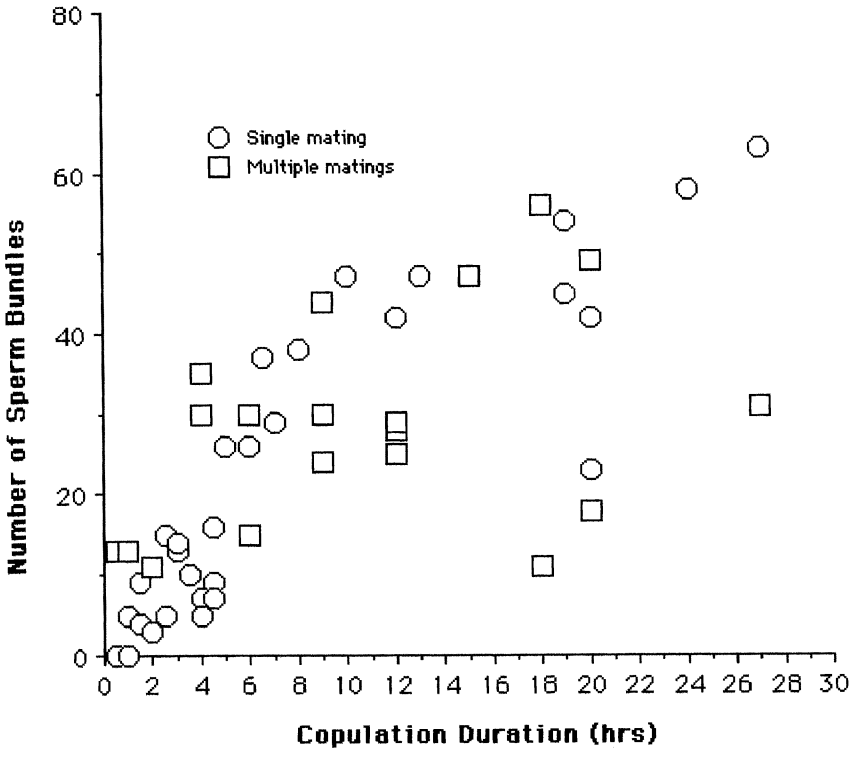

Fig. 4. The number of sperm bundles transferred in interrupted matings for prescribed copulation duration.

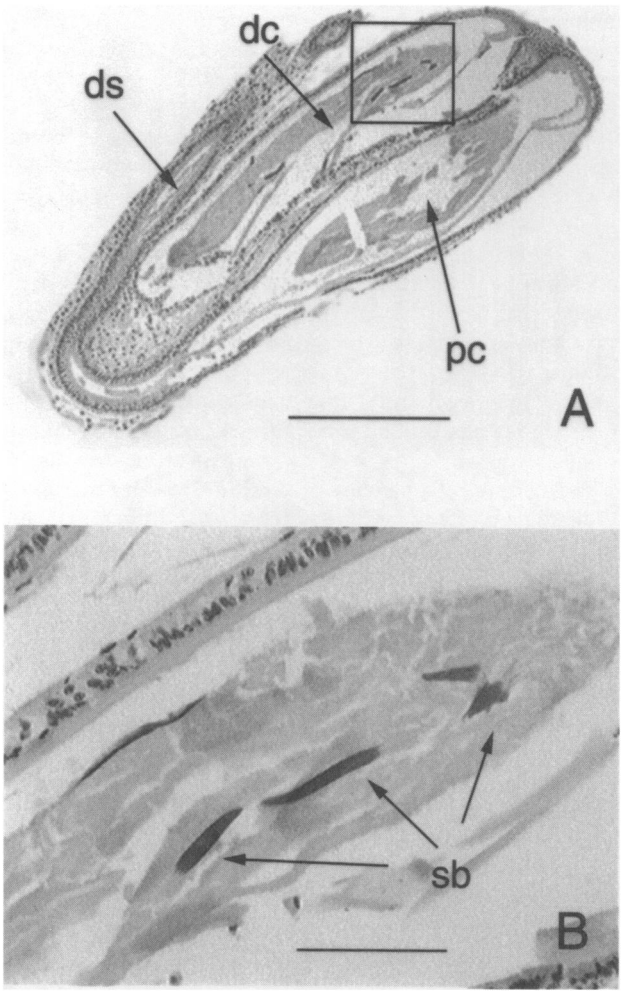

Fig. 5. a. Spermatheca cross-section with sperm bundles in distal chamber (*dc*), or preapical diverticulum; 1.5 hour single copulation (*pc*=proximal chamber, *ds*=ductus seminalis). **b.** 20x sperm bundles (*sb*).

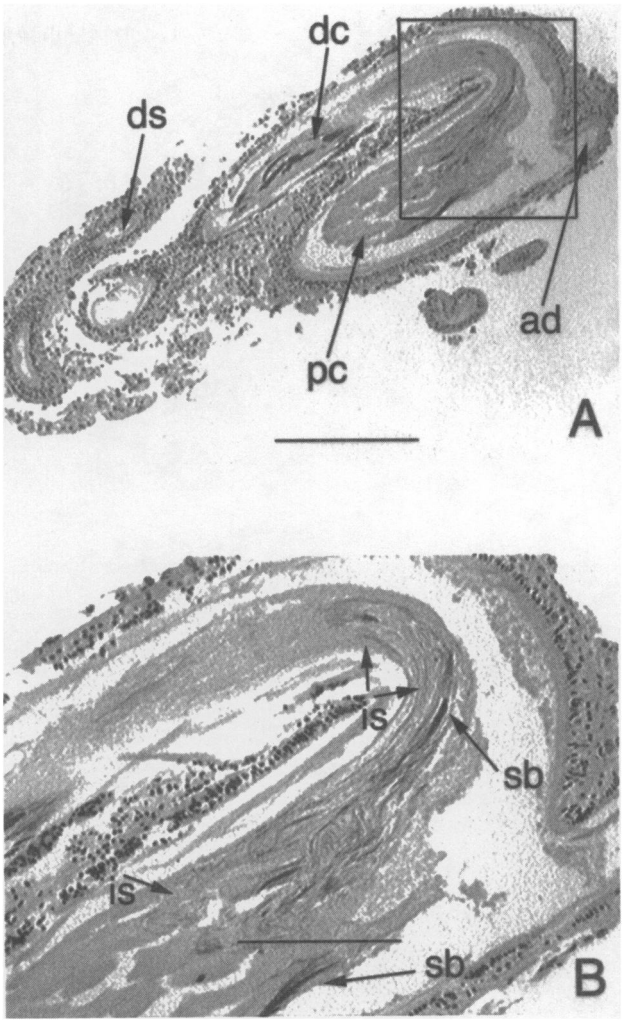

Fig. 6. a. Spermatheca cross-section with sperm bundles and individual sperm present in both proximal (*pc*) and distal chambers (*dc*); 4 hour multiple copulation (*ad*=apical diverticulum, *ds*=ductus seminalis). **b.** 10x at proximal-distal chamber connection (*sb*=sperm bundles, *is*=individual sperm).

before they fertilize eggs. Individual sperm were found in ovipositing females, and the number of sperm bundles appeared to decrease with the number of eggs pods laid. As prior to oviposition, during a second mating selective early disassociation of bundles may be initiated by the female. A mechanism in which females choose cryptically (or after copulation begins) would better explain the variation in number and location of sperm bundles found among females that had previously mated than male replacement of sperm (Eberhard and Cordero 1995, Knowlton and Greenwell 1984). Females have been noted to influence sperm deposition, storage, and use in insects (Lopez-Leon *et al.* 1993, Knowlton and Greenwell 1984, Ward 1993). Male characteristics also have been shown to influence the effectiveness of sperm removal (Simmons and Parker 1992). Which sex plays the more important role in *D. viridis* in controlling sperm displacement may be revealed by future studies using molecular markers.

It seems likely that not all of the previous sperm is removed during the second mating. Some sperm from the first mating, if still in the form of sperm bundles, appears to remain inside the spermatheca while the individual sperm disappears. This probably explain why there were more sperm bundles inside the spermatheca in early multiple matings as compared to corresponding early single matings in this study.

Also, it appears that the distal chamber of the spermatheca in *Dichromorpha viridis* functions as the primary storage facility for the sperm bundles. Early in the single matings, more sperm bundles were found in the distal chamber. Also, 48 hours after copulation terminated, sperm bundles were only found in the distal chamber. Interestingly enough, the inside of the spermatheca of *D. viridis* is scattered with small hair-like structures pointing into the lumen of the proximal and distal chambers. The functions of these hair-like structures have not been investigated, and only a few references mention their presence in spermathecae (Ahmed and Gillott 1982, Gregory 1965, Richards and Richards 1979). Gregory

(1965) states that the "fine cuticular spines that project inwards from the walls of the vestibule" in *Locusta migratoria* function possibly by rupturing the thin wall of the spermatophore to allow the sperm bundles to migrate within the spermatheca. Unfortunately, that is all that was written on the cuticular spines, and no pictures were provided to compare with *D. viridis*. It is possible that the hair-like structures could function by rupturing the spermatophore, but why are the spines also located throughout the distal chamber in *D. viridis* where the spermatophore does not reach? We suspect that the hair-like structures function in preventing sperm bundle removal during copulation, but allow individual sperm to be displaced or flushed out by the excess seminal fluid provided by the second mating. This would help explain why we observed the removal of individual sperm in multiple matings late in copulation duration without removal of sperm bundles. More research is needed to help determine a possible function of the small hair-like structures inside the spermatheca and whether or not similarities exist among other insects concerning the internal morphology of spermathecae.

It has been observed that mating pairs of *D. viridis* can remain in genital contact for up to 72 hr in the field, and on average copulate around 13.2 hr. Results show that the rate of sperm transfer appears to decrease after 13 hr into copulation. We suspect that many copulations of more than this duration function more to insure mate guarding against future matings than to transfer needed sperm, or as already proposed, flush out (with or without female "help") a previous male's contribution. In some grasshoppers males will remain on a female's back for some time after copulation (Wicker and Siebt 1985). Such contact has been hypothesized to be one way a male can guard against mating attempts and avoid sperm competition while a female is still receptive (Parker 1970). Males in other grasshopper species have been noted to leave sperm plugs in the females' genital tracts that act as mechanical barriers to subsequent spermatophore deposition by other males (Gregory 1965, Loher and Chandrashekaran 1979, Parker and Smith 1975). As suggested for *Eyprepocnemis plorans* (Charpentier) (Lopez-Leon *et al.*1993), lengthy copulations in *D. viridis* may be more effective as the male himself acts as a sperm plug.

Guarding of sperm from being displaced by females has been given as a explanation for lengthy copulation in other Orthoptera (see refs in Vahed 1996). Guarding of sperm has also been given as a possible explanation for large protein investments observed in various orthopteran species (Sakaluk 1986, Simmons *et al.* 1993, Vahed and Gilbert 1977, Wedell 1993). A grasshopper was the first insect in which male accessory gland products were demonstrated to pass to the female during mating (Friedel and Gillott 1977). It will be interesting to see if *Dichromorpha viridis* males, as also reported for *Chorthippus brunneus (Thunberg)* (Butlin *et al* 1987), transfer protein(s) during mating and if they continue this transfer during lengthy copulations after the rate of sperm transfer decreases. Vahed (1996) has observed in katydids that lengthy copulations are correlated with the disappearance of protein transfer or decreases in the amount of protein transferred. He suggests that in katydids. and it would be interesting to know if also in grasshoppers, lengthy copulation appears to take the place of "feeding" a female in guarding sperm.

Acknowledgments

We would like to thank Sarah Liebling for her assistance in collecting individuals for the laboratory investigations. Thanks are also due to Betty Black and Erica Kosal. This work has been supported by the North Carolina Agricultural Research Service.

Literature Cited

Ahmed I, Gillott C. 1982. The spermatheca of *Melanoplus sanguinipes* (Fabr.) II. Ultrastructure. International Journal of Inverterbrate Reproduction. 4: 297-309.

Bella JL, Butlin RK, Ferris C, Hewitt GM. 1992. Asymmetrical homogamy and unequal sex ratio from reciprocal mating-order crosses between *Chorthippus parallelus* subspecies. Heredity. 68: 345-352.

Birkhead TR, Hunter FF. 1990. Mechanisms of sperm competition. Trends in Ecology and Evolution. 5: 48-52.

Birkhead TR, Moller AP. 1992. Sperm Competition in Birds: evolutionary causes and consequences. Academic Press. London.

Boone ME, Drijver JS. 1986. Routine Cytological Staining Techniques. MacMillan Education Ltd, London.

Butlin RW, Woodhatch CW, Hewitt, GM. 1987. Male spermatophore investment increases female fecundity in a grasshopper. Evolution. 41: 221-228.

Davey KG. 1985. The female reproductive tract. Pp.15-36. IN: Kerkut GA, Gilbert LI (eds), Comprehensive Insect Physiology, Biochemistry, and Pharmacology (Vol. 1), Embryogenesis and Reproduction. Pergamon Press. Oxford, England.

Dirsh VM. 1957. The spermatheca as a taxonomic character in Acridoidea (Orthoptera). Transactions of the Royal Entomological Society of London . 32: 107-114.

Eberhard WG, Cordero C. 1995. Sexual selection by cryptic female choice on male seminal products - a new bridge between sexual selection and reproductive physiology. Trends in Ecology and Evolution. 10: 10-15.

Friedel T, Gillott C. 1977. Contribution of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. Journal of Insect Physiology. 23: 145-151.

Greenfield MD, Shelly TE. 1985. Alternative mating strategies in a desert grasshopper: evidence for density dependence. Animal Behavior 33: 1192-1210.

Gregory GE. 1965. The formation and fate of the spermatophore in the African migratory locust, *Locusta migratoria migratorioides*. Reiche and Fairmaire. Transactions of the Royal Entomological Society of London. 117: 33-66.

Gwynne DT. 1984. Male mating effort, confidence of paternity, and insect sperm competition. Pp. 117-144. IN: Smith RL (ed), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. Orlando, Florida.

Gwynne DT. 1988. Courtship feeding in katydids benefits the mating male's offspring. Behavioral Ecology and Sociobiology. 23: 373-377.

von Helversen D, von Helversen O. 1991. Pre-mating sperm removal in the bushcricket *Metaplastes ornatus* Ramme 1931 (Orthoptera, Tettigonoidea, Phaneropteridae). Behavioral Ecology and Sociobiology. 28: 391-396.

Hewitt G M , Mason P, Nichols R A. 1989. Sperm precedence and homogamy across a hybrid zone in the alpine grasshopper *Podisma pedestris*. Heredity 62: 343-353.

Hunter-Jones P. 1960. Fertilization of eggs of the desert locust by spermatozoa from successive copulations. *Nature*. 185: 336.

Knowlton N, Greenwell SR. 1984. Male sperm competition avoidance mechanisms: the influence of female interests. Pp. 62-83 IN:

Smith RL (ed), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. Orlando, Florida.

Loher W, Chandrashekaran MK. 1970. Acoustical and sexual behavior in the grasshopper *Chimarocephala pacifica pacifica* (Oedipodinae).Experimental and Applied Entomology. 13: 71-84.

Longo G, Sottile L, Viscuso R, Giuffrida A, Privitera R. 1993. Ultrastructural changes in sperm in *Eyprepocnemis plorans* (Charpentier) (Orthoptera: Acrididae) during storage of gametes in female genital tract. Invertebrate Reproduction and Development. 24: 1-6.

Lopez-Leon MD, Cabrero J, Pardo MC, Viseras E, Camacho JPM. 1993. Paternity displacement in the grasshopper *Eyprepocnemis plorans*. Heredity 71: 539-545.

Niedzlek-Feaver M. 1995. Crepitation, pair formation, and female choice in *Chortophaga viridifasciata* (DeGeer) (Orthoptera: Acrididae). Journal of Orthoptera Research. 4: 131-142.

Ono T, Siva-Jothy MT, Kat A. 1989 Removal and subsequent ingestion of rival's semen during copulation in a tree cricket. Physiological Entomology. 14: 195-202.

Otte D. 1970. A comparative study of communication in grasshoppers. Miscellaneous Publications of the Museum of Zoology. University of Michigan. No. 141.

Otte D. 1972. Simple versus elaborate behavior in *Syrbula*. Behaviour 42: 291-322.

Otte D, Joern A. 1975. Insect territoriality and its evolution: population studies of desert grasshoppers on creosote bushes. Journal of Animal Ecology 44: 29-54.

Page RE, Metcalf RA. 1982. Multiple matings, sperm utilization, and social evolution. American Naturalist. 119: 263-281.

Parker GA. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Review 45: 525-567.

Parker GA. 1984. Sperm competition and the evolution of animal mating strategies. Pp. 2-55. IN: Smith RL (ed), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. Orlando, Florida.

Parker GA, Smith JL. 1975. Sperm competition and the evolution of the precopulatory passive phase behavior in *Locusta migratoria migratorioides*. Journal of Entomology Series A. General Entomology. 49: 155-171.

Pickford R, Gillott C. 1976. Effect of varied copulatory periods of *Melanoplus sanguinipes* (Orthoptera: Acrididae) females on egg hatchability and hatchling sex ratios. Canadian Entomologist. 108: 331-335.

Richards AG, Richards PA. 1979. The cuticular protuberances of insects. International Journal of Insect Morphology and Embryology 8: 143-157.

Riede K. 1987. A comparative study of mating behaviour in some neotropical grasshoppers (Acrididae). Ethology. 76: 265-296.

Sakaluk SK. 1985. Spermatophore size and its role in the reproductive behavior of the cricket *Gryllodes supplicans* (Orthoptera: Gryllidae). Canadian Journal of Zoololgy. 63: 1652-1656.

Sakaluk SK. 1986. Sperm competition and the evolution of nuptial feeding behavior in the cricket, *Gryllodes supplicans* (Walker). Evolution. 40: 584-593.

Shelly TE, Greenfield MD. 1985. Alternative mating strategies in a desert grasshopper: a transitional analysis. Animal Behaviour. 33: 1211-1222.

Shelly TE, Greenfield MD, Downum KR. 1987. Variation in host plant quality: influences on the mating system of a desert grasshopper. Animal Behaviour. 35: 1200-1209.

Simmons LW, Parker GA. 1992. Individual variation in sperm competition success of yellow dung flies, *Scatophaga stercoraria*. Evolution. 46: 366-375.

Simmons LW, Craig M, Llorens T, Schinzig M, Hosken D. 1993. Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. Proceedings of the Royal Society of London. B 251: 183-186.

Simmons LW, Llorens T, Schinzig M, Hosken D, Craig M. 1994. Sperm competition selects for male choice and protandry in the bushcricket, *Requena verticalis* (Orthoptera: Tettigoniidae). Aminimal Behaviour. 47: 117-122.

Smith RL, ed. 1984. Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. Orlando, Florida.

Starr CK. 1984. Sperm competition, kinship, and sociality in the aculeate Hymenoptera. Pp. 428-459. IN: Smith RL (ed), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. Orlando, Florida.

Steinberg J, Willey R. 1983. The mating system of *Trimerotropis maritima* (Acrididae: Oedipodinae). Pp. 285-304. IN: Gwynne DT, Morris GK (eds), Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects. Westview Press. Boulder, CO.

Taber S, Blum MS. 1960. The preservation of honey bee semen. Science. 131: 1734-1735.

Thornhill R. 1984. Alternative hypotheses for traits believed to have evolved by sperm competition. Pp. 151-176. IN: Smith RL (ed), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. Orlando, Florida.

Uvarov B. 1977. Grasshoppers and locusts, A handbook of general Acridology. (Volume 2): Behavior, ecology, biogeography, population dynamics. University Press, Cambridge.

Vahed K. 1996. Prolonged copulation in oak bushcrickets (Tettigoniidae: Meconematinae: *Meconema thalassinum and M. meridionale*). *Journal of Orthoptera Research* 5: 199-204.

Vahed K, Gilbert FS. 1996. Differences across taxa in nuptial gift size correlate with differences in sperm number and ejaculate volume in bushcrickets (Orthoptera: Tettigoniidae). Proceedings of the Royal Society of London. Series B. 263: 1257-1265.

Waage JK. 1979. Dual function of the damselfly penis: sperm removal and transfer. Science. 203: 916-918.

Walker WF. 1980. Sperm utilization strategies in nonsocial insects. American Naturalist. 15: 780-799.

Ward P I. 1993. Females influence sperm storage and use in the yellow dung fly *Scathophaga stercoraria (L.)*. Behavioral Ecology and Sociobiology. 32: 313-319

Wedell N. 1991. Sperm competition selects for nuptial feeding in a bushcricket. Evolution 45: 1975-1978.

Wicker W, Siebt U. 1985. Reproductive behavior in *Zonocerus elegans* (Orthoptera: Pyrgomophidae) with special reference to nuptial gift guarding. Zeitschrift fur Tierpsychologie. 69: 203-233.

Willey RB, Willey RL. 1969. Visual and acoustical social displays by the grasshopper *Arphia conspersa* (Orthoptera: Acrididae). Psyche. 76: 280-305.