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Spermatophore number transferred increases linearly with copulation duration in *Melanoplus differentialis* (Orthoptera: Acrididae)

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Abstract

Melanopline grasshoppers are known to transfer both sperm and nutritive secretions, from the accessory reproductive glands in the form of internal spermatophores, to females during mating. It remains difficult in most species, without extensive destructive sampling, to determine numbers transferred. This study documents that for *Melanoplus differentialis*, remnants of spermatophores, which have already discharged their contents into the spermatheca, can be found associated with the external genitalia after mating. For males mating for the first time and with virgin females, the numbers of spermatophore casings found were significantly positively correlated with copulation duration. Casings were not found on the external genitalia after most matings in *M. femurrubrum*. This study also examined mating behavior in *M. differentialis* and *M. femurrubrum*. In both species, mounting followed relatively simple behavior by the male, such as antenna pointing, indicating that the male was aware of a conspecific's presence. No obvious attempts at courtship were observed beyond preparations to mount.

Key words

Sperm, sperm transfer, mating, *Melanoplus femurrubrum*, courtship, copulation, mating effort

Introduction

Spermatophore transfer in insects, particularly Orthoptera, has been shown to have functions beyond sperm transfer that may enhance a male's probability of reproductive success (Gwynne 1984, Wedell 1991, Simmons *et al.* 1993, Vahed & Gilbert 1996). In addition to their roles in suppressing remating drive in females (Parker & Smith 1975, Hartmann & Loher 1999) and increasing the rate of oviposition (Friedel & Gillott 1976, Lange & Loughton 1985), the spermatophores of Orthoptera provide material incorporated into eggs or female biomass. The benefits to the female of receiving multiple spermatophores have been estimated by following the fate of labeled accessory reproductive gland proteins (Friedal & Gillott 1977) or measuring the number of offspring and/or eggs produced (Pickford & Gillott 1976, Butlin *et al.* 1987). Quantifying this investment directly has proven difficult however, as visualization of the spermatophores transferred has not always been possible.

Melanopline grasshoppers pass multiple spermatophores during copulation, and these are involved in the transfer of nutrients in *Melanoplus sanguinipes* (Fabricius) (Friedel & Gillott 1977). Additionally, protein extracts from the accessory glands that are part of, or accompany, spermatophores, have been reported to act as oviposition stimulants (*ibid.*). A male's chances of paternity are increased if these stimulants make it more likely that a female will oviposit before mating again (Friedel & Gillott 1976, Torres-Vila *et al.* 1997). Males have demonstrated a marked preference to court virgin over mated females (Pickford & Gillott 1972), suggesting that the male's mating effort is not insignificant and is weighed against this probability of paternity.

Courtship behaviors in *M. sanguinipes* (Pickford & Gillott 1972) and *Melanoplus tequestae* Hubbell (Bland 1987) have been reported to be minimal, often involving little more than antennae pointing preceeding mounting. No behavioral evidence has been generated that would suggest female preference for certain males. As do other grasshoppers that have been studied (Uvarov 1966, Otte 1970), *Melanoplus* spp. show considerable intraspecific and interspecific variation in the duration of copulation. Intraspecific variation in copulation duration suggests that males may be varying the numbers of spermatophores they transfer, or are allowed to transfer, to a female. Lacking behavioral or morphological cues, females may judge male quality based on spermatophore quality or quantity. Because spermatophore transfer is internal, obtaining a relative estimate of male investment has been problematic. We report a way to obtain estimates of the number of spermatophores transferred during mating in *Melanoplus differentialis* Thomas and compare this species mating behavior with that of a sympatric species, *Melanoplus femurrubrum* De Geer.

Methods

Animal husbandry.— Late instars and adults of *M. differentialis* and *M. femurrubrum* were collected from Research Farm Unit #1 near North Carolina State University. Nymphs of both species were most commonly found in association with white clover (*Trifolium repens*) and plantain (*Plantago major*) from May through July. Adults were found in high density throughout the farm from July to October, or until

the first frost.

In the laboratory, animals were fed a mixture of wheat, rye, fescue (*Festuca spp.*) and white clover, grown in Styrofoam drinking cups. As an additional nutrient source, ground Purina® Cat Chow, Big Red® rabbit food, wheat germ and whole oat flakes were provided in small petri dishes. All food was provided *ad lib*, to avoid the low growth problems associated with a homogenous diet (Uvarov 1966). Additionally, the provisioning of white clover was found to be essential for the complete development of the reproductive tract; animals raised without it had partially developed ovaries and testes even though external morphology appeared normal (personal observation).

Animals were held under controlled conditions of 14 L: 10 D, 24 °C, 35% RH at the North Carolina State University Animal Research Unit. Additional humidity was provided via daily misting, and additional heat provided by incandescent heat lamps.

Mating behavior.— Field caught, individually marked *M. femurrubrum* were randomly assigned to observation cages, with two males and two females per cage. Individuals were allowed to interact for 2-h observation periods, and video recording used to supplement observer note taking. At the end of each observation period, individuals were returned to common, same-sex cages and were not sampled for at least 48 h.

M. differentialis individuals mate for considerably longer periods and are generally less active than red-legged grasshoppers. Two males and two females were permanently housed in each cage, and observed for 2 h during the afternoon, which often corresponded to peak daily activity.

Spermatophore collection.— Virgin females were introduced to an adult male when both were approximately 7 d past final molt. Food was provided as described above. The pair was held in 20-cm diameter observation cages and surveyed continuously to denote the onset of mating, which occurred quickly within the small cages. At the end of a preset period of copulation or upon termination of mating (the female began flicking her hind legs and kicking at the male, leading to separation), a pair would be sacrificed by being placed in a -20° C freezer.

The mating pair was thawed and separated as needed by pulling the male's cerci out of the female's paraproct. Associated with both male and female genitalia were white masses of ejected spermatophores; these were collected with fine forceps and spread onto a clean slide with the aid of sterile isotonic saline. To aid in visualization of the spermatophores, methylene blue was added as a stain.

Spermatophore counts.— Spermatophore number was estimated through counts by two different observers. Because spermatophores, even when gently separated, were still interwoven, some discrepancy is to be expected. Two counts from each observer were recorded and averaged, allowing for an estimate with error ranges. These values were analyzed and graphed using KyPlot 2.0.

Results

Description of mating behavior.— Both *M. femurrubrum* and *M. differentialis* were available for observation and the account below is based on 75 h of observation. No differences were noted in pair formation in the two species.

Males in both species approached females from the rear, with their antennae pointed towards the abdomen of the female. Antennae pointing served as a signal to observers that a mating may follow, which is when video recording began. Generally, males would halt their approach about 5-8 cm away from the female for a brief interval, from 10-30 s. In 8 of 10 video-recorded matings of *M. femurrubrum*, males would "flick" (Otte 1970) their femurs — the animal lifted its hind legs and, with the tibia flexed up against the femur, raised and lowered them rapidly at an approximate 45-degree angle for an average duration of 0.25 s.

Females either showed no visable response, walked or jumped away, turned to face the signaling male, or flicked. In two instances a female elevated her hind legs and held them in an "L" shape. As in *M. sanguinipes* (Pickford & Gillott 1972), males were far more likely to attempt copulation with passive females. Of 46 mating attempts observed in their entirety, only one involved a male attempting to mount a female with elevated legs.

A copulatory attempt was made by the male leaping on a female. At no time did a male attempt to copulate with a female facing it, and only once did a male try to mount a female with her legs elevated; he was kicked off in 1-2 s. The male's leap usually knocked the pair off balance, and struggling commenced with both animals lying on their sides or backs. Females responded vigorously, kicking at the male and leaping multiple times. Copulation involved the male using his cerci to hook into small gaps in the ventral valves of the female, pulling them down (Fig. 1). A female could avoid this by adopting a "J" pattern: curling her abdomen underneath her while the male twined his abdomen around. This tactic of avoiding genitalia contact with a male was seen in all situations where the female having failed to dislodge a male also did not eventually mate with him.

In all cases, males flicked their femurs rapidly and rhythmically during the struggling for up to several minutes. The flicking was quite pronounced: in one mating, the male flicked 19 times in 10.8 s.

Mating in *M. femurrubrum* in the laboratory lasted up to 2 h, at which point, the male disengaged his genitalia. Mating in *M. differentialis* commonly lasted 8-10 h, but pairs were observed to mate up to 46 h in the laboratory. Dismounting was swift, usually accompanied by the female kicking at the male. Once separated, both animals moved away from one another and did not appear to show any further interaction.

Spermatophore transfer during mating.— Spermatophores were recovered from mating grasshoppers. These were found between the female's valves (Fig. 2), as well as associated with the male genitalia. Spermatophore remnants were found among genitalia in all attempted recoveries in mating *M. differentialis*. Spermatophores were not found in 70%

of *M. femurrubrum* matings where recovery was attempted, even when copulating pairs were sacrificed at predetermined periods after engaging genitalia. If a pair of mating *M. differentialis* was allowed to terminate their copulation naturally, these remnants had to be extracted immediately after the pair separated. One female carried material on her genitalia for 35 min, but most ejected the "ball" of spermatophore casings within 15-20 min of terminating copulation (Figs 3-5).

Spermatophores in *M. differentialis* were essentially tube-shaped (Fig. 3), and averaged 4.82 mm in length. Sperm could be seen inside a spermatophore remnant at 400X (Figs 4, 5), but not in quantity for most of its length: the spermatophore remnant appeared to be an "empty casing". The mass of casings removed from a recently mated female were also bound with an acellular matrix (milky material in Fig. 4), presumably secreted compounds from the male's accessory glands. Cross-sections of spermathecae revealed that the spermatophore during copulation was threaded all the way up the ductus seminalis into the proximal chamber, where sperm could be seen being released and stored (Fig. 6).

The number of spermatophore remnants or casings increased with the time a sacrificed pair (females were virgins) spent in copula (Fig. 7). Note that although a significant linear regression is drawn through the data ($r^2 = 0.775$, $p = <0.001$, Fig. 7), there is some variation in the number of spermatophores transferred per copulation duration. Different males involved in matings interrupted after 5 h of copulation transferred 4, 18 and 28 spermatophores respectively.

Discussion

The courtship behavior patterns observed in *M. femurrubrum* and *M. differentialis* were identical and duplicated those reported for *M. sanguinipes* (Pickford & Gillott 1972) and *M. tequestae* (Bland 1987). Thus, courtship behavior among studied Melanopline grasshoppers shows surprisingly few differences, and males exhibit behavior indicating awareness of nearby females. However, males did not actively court females. Since attempts at mating follow recognition behaviors such as antennae pointing and pivoting to face a nearby individual (typically female, but not always the case), it may be that olfactory cues and lock-and-key fit of genitalia (Eberhard 1985) may provide the majority of barriers to hybridization among *Melanoplus* spp. The most noteworthy element of Melanopline courtship behavior as it compares to other Orthoptera, is that it is silent; if *Melanoplus* spp. are capable of producing sound, it is not audible to human observers (Otte 1970). The motivation for females to accept or reject males, given that they can dislodge them and so discourage copulation, are still unclear.

The number of spermatophore remnants or casings were found to be significantly correlated with time spent mating. Kyl (1938) reported as many as 21 spermatophore fragments could be found following mating in *M. differentialis*, presumably after the longest observed mating of 44 h; in this study, up to 76 spermatophores were counted from a mating of 25 h. Kyl was convinced that the fragments, which could be collected following separation, were but continuations of a single spermatophore. This was refuted by Pickford & Gillott (1971) who, based on the literature and their own unpublished observations, concluded there are two major ways spermatophores are delivered in the Acrididae. Species either deliver multiple, relatively small, spermatophores, each containing a sperm bundle and other material, during a mating or copulation, or species deliver one large spermatophore. During copulation in the latter group, sperm bundles were delivered at intervals, with delivery of other material interspersed between sperm delivery (Johnson & Niedzlek-Feaver 1998).

Guarding of sperm from being displaced by females has been given as an explanation for lengthy copulation in other Orthoptera (Vahed & Gillbert 1996, Simmons & Silva-Jothy 1998, Gwynne 2001). Males will remain on a female's back for some time after copulation (Wicker & Siebt 1985) and in so doing, they appear to be guarding against mating attempts by other males while a female is still receptive. Males in other grasshopper species have also been noted to leave sperm plugs in the females' genital tract, that act as mechanical barriers to subsequent spermatophore transfer by other males (Gregory 1965, Loher & Chandrashekaran 1970, Parker & Smith 1975). As suggested for some species (López-León *et al.* 1993, Johnson & Niedzlek-Feaver 1998), lengthy copulation may be more effective as the male himself acts as a sperm plug. In *M. differentialis*, however, pairs not only maintained genital contact, but males continuously transferred spermatophores for the entire duration of copulation. Following the cessation of copulation, the pair separated and did not show any obvious post-copulatory behaviors. The spermatophore remnants did not persist long after mating, and so were unlikely to serve as a mechanical barrier to further matings.

Brief matings are known to provide sufficient sperm to fertilize multiple egg pods in *Melanoplus* spp. (Kyl 1938, Pickford & Gillott 1976). However, in Orthoptera spermatophore transfer involves delivery of material other than sperm to females. Male grasshoppers have been reported to transfer protein and other products of the male accessory glands, including oviposition stimulants (Friedel & Gillott 1977, Butlin *et al.* 1987, Lange & Loughton 1985). It may be that in *M. differentialis*, by providing spermatophores in excess of what is needed to transfer sufficient sperm, males are also giving the female ample nutrients or secretions which prolong the period females remain unreceptive after the mating, or shorten the period before a female oviposits (Friedel & Gillott 1976, Hartman & Loher 1999). In either case, males benefit by decreasing the probability that females remate before laying eggs.

This first study focused on the number of spermatophores transferred per unit time of copulation and variation among males in size and age used in pairing was kept to a minimum. Males in this study were exposed to similar environmental and nutrient resources once in the laboratory, and males of approximately the same age were introduced to virgin females. Yet males allowed to copulate for the same duration of time did vary in the number of spermatophores transferred, in spite of our attempts to keep

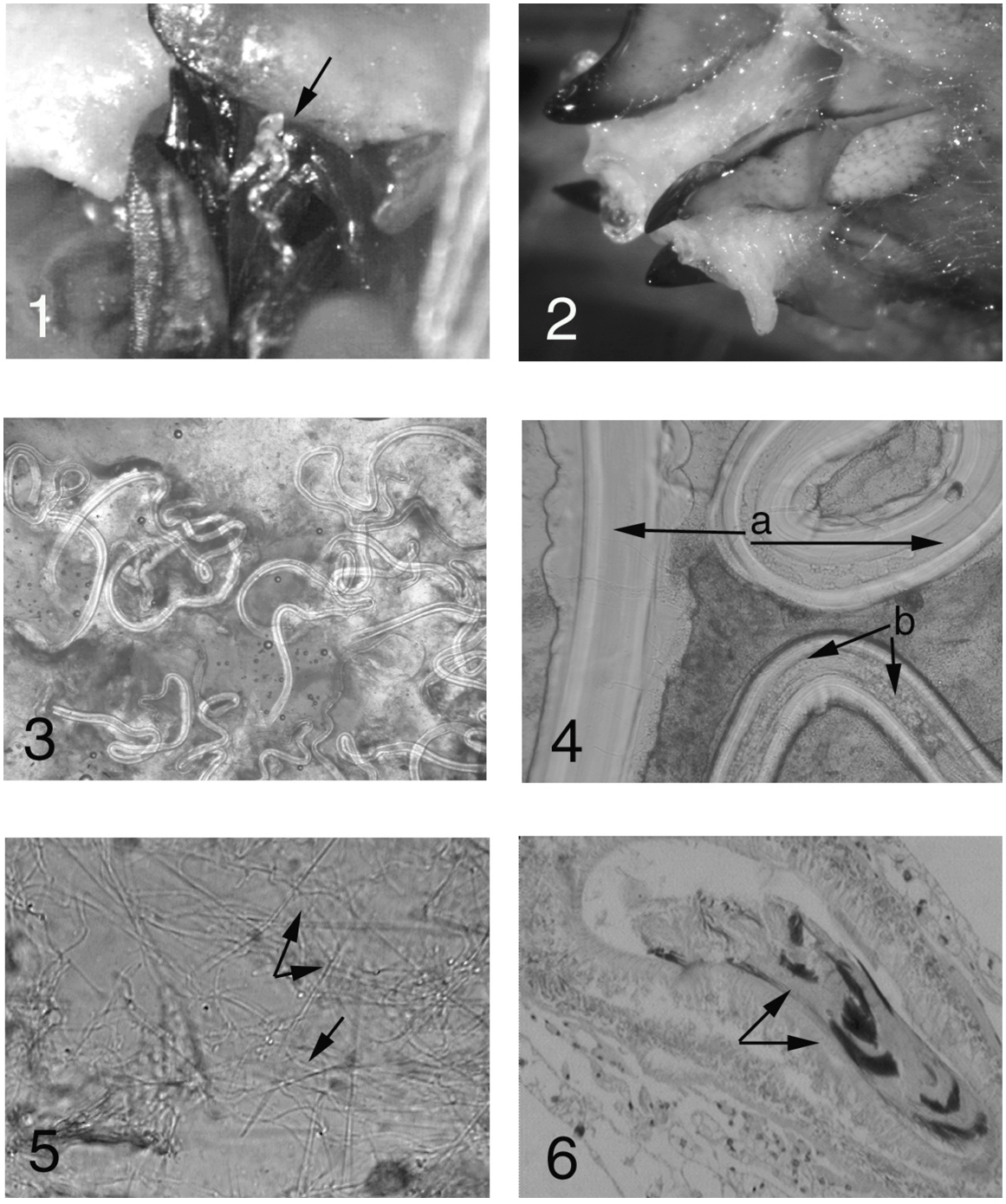

Fig. 1. *M. differentialis in copula.* A closeup showing spermatophores (arrow) being transferred.
Fig. 2. A bundle of spermatophore remnants or casings found between a female *M. differentialis*' valves after a mating was interrupted.
Fig. 3. Casings that have been teased apart from the bundle (15X).
Fig. 4. Magnified portions of spermatophore remnants or casings. Most appear empty (arrows a), although some sperm (arrows b) can be seen within a small portion of any remnant (100X).
Fig. 5. Sperm (arrows) found associated with a portion of a spermatophore remnant or casing (200X). Only a relatively small amount of loose sperm was found associated with spermatophore casings. The thicker rods are believed to be protein fibers that are normally transferred along with sperm in most grasshopper species.
Fig. 6. Cross section of a female's spermatheca (200X). The pair was in copula for 5 h before mating was interrupted. Sperm are transferred in highly compact bundles (arrows) known as spermatodesmes to a female's spermatheca, and these bundles are estimated to contain hundreds of sperm (Pickford & Gillott 1976).

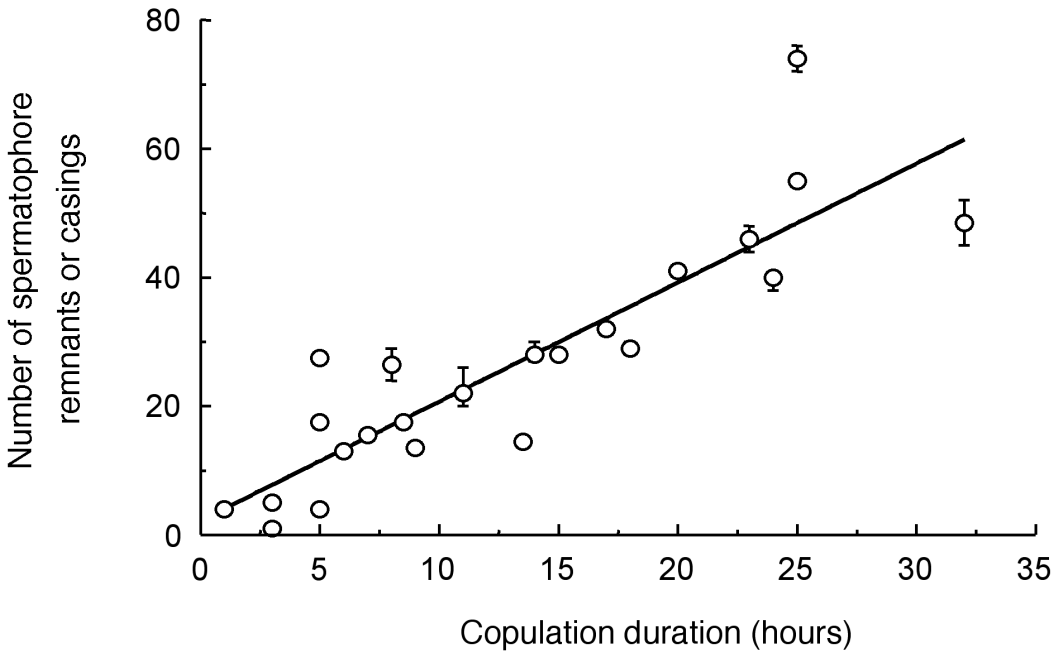

Fig. 7. Linear regression model applied to the number of spermatophores observed transferred during matings of varying duration. Error bars from high and low estimates are displayed where there was discrepancy among observers. r^2=0.775. p=<0.001.

variation to a minimum among males. We suspect that variation was due to a factor harder to control than size or age. Food was provided *ad lib*. to individuals, but we did not monitor closely, variation in time or quality of last feeding. Likewise, parasite load, which may influence overall male condition, and thus relative ability to produce spermatophores, was unknown. Our findings are also in keeping with studies of mating behavior, and studies where spermatophore numbers transferred are reported to vary among individuals and with copulation duration (Cueva Del Castillo 1999, Johnson & Niedzlek-Feaver 1998, Kyl 1938, Otte 1970, Pickford & Gillott 1976, Uvarov 1966). In this study, whether all spermatophores transferred during mating were equivalent in content was also not monitored. However, a histological study is in progress that examines in *M. differentialis*, sperm storage patterns as well as the actual number of sperm packets and spermatophores transferred in copulations of varying duration.

That spermatophore casings can be collected in some species such as *M. differentialis*, following mating should prove fortuitous for studies examining the choices made by males and females in successive matings. Casing number can be used as an indicator of the relative amount a male will "invest" in a particular female, especially if the differences caused by variation in the number of spermatophores transferred by a male is smaller than any differences in number of sperm or nutrients he delivers per spermatophore. The remnants could serve then as a useful tool to judge if males allocate more spermatophores, and so possibly nutrients, to virgin, younger, or larger females. Females likewise may allow larger or heavier males (those potentially capable of transferring more nutrients) to mate longer. Thus, *M. differentialis* can be used as a model system allowing us to discover what differences in investment occur when males and females mate multiply, and more importantly, to identify any potential contributing factors.

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Literature Cited

Bland R.G. 1987. Mating behavior of the grasshopper *Melanoplus tequestae* (Orthoptera: Acrididae). Florida Entomologist 70: 483-487.

Butlin R.K., Woodhatch C.W., Hewitt G.M. 1987. Male spermatophore investment increases female fecundity in a grasshopper. Evolution 41: 221-225.

Cueva del Castillo R., Núñez-Farfán J., Cano-Santana Z. 1999. The role of body size in mating success of *Sphenarium purpurascens* in Central Mexico. Ecological Entomology 24: 146-155.

Eberhard W.G. 1985. Sexual Selection and the Evolution of Animal Genitalia. Harvard University Press, Cambridge, Massachusetts.

Friedel T., Gillott C. 1976. Male accessory gland substance of *Melanoplus sanguinipes*: an oviposition stimulant under the control of the corpus allatum. Journal of Insect Physiology 22: 489-495.

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

Gregory G.E. 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 D.T. 1984. Male mating effort, confidence of paternity and insect sperm competition. Pp.117-149. In: Smith R.L. (Ed.) Sperm competition and the evolution of animal mating systems. Academic Press, London.

Gwynne D.T. 2001. A nuptial banquet and seminal sac: evolution of the spermatophylax meal, pp.122-160. In: Gwynne D.T. Katydids and Bush-Crickets: Reproductive Behavior and Evolution of the Tettigoniidae. Comstock Publishing Associates. Cornell University Press, Ithaca.

Hartman R., Loher W. 1999. Post-mating effects in the grasshopper, *Gomphocerus rufus* L. mediated by the spermatheca. Journal of Comparative Physiology. Series A. 184: 325-332.

Johnson J., Niedzlek-Feaver M. 1998. A histological study on copulation duration, patterns of sperm transfer and organization inside the spermatheca of a grasshopper *Dichromorpha viridis* (Scudder). Journal of Orthoptera Research 7: 139-146.

Kyl G. 1938. A study of copulation and the formation of spermatophores in *Melanoplus differentialis*. Proceedings of the Iowa Acadamy of Science 45: 299-308.

Lange A.B., Loughton B.G. 1985. An oviposition-stimulating factor in the male accessory reproductive gland of the locust, *Locusta migratoria*. General and Comparative Endocrinology 57: 208-215.

Loher W., Chandrashekaran M.K. 1970. Acoustical and sexual behaviour in the grasshopper *Chimarocephala pacifica pacifica* (Oedipodinae). Journal of Experimental and Applied Entomology 13: 71-84.

López-León M.D., Cabrero, J., Pardo M.C., Viseras E., Camacho J.P.M. 1993. Paternity displacement in the grasshopper *Eyprepocnemis plorans*. Heredity 71: 539-545

Otte D. 1970. A comparative study of communication in grasshoppers. Miscellaneous Publications of the Museum of Zoology. University of Michigan. Number 141.

Parker G.A., Smith J.L. 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. 1971. Insemination in the migratory grasshopper, *Melanoplus sanguinipes* (Fabr.). Canadian Journal of Zoology 49: 1583-1588.

Pickford R., Gillott C. 1972. Courtship behavior of the migratory grasshopper, *Melanoplus sanguinipes* (Orthoptera: Acrididae). Canadian Entomologist 104: 715-722.

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.

Simmons L.W., Silva-Jothy, M.J. 1998. Sperm competition in insects: Mechanisms and the potential for selection. Pp. 341-434. In: Birkhead T. T., Moller A.P. (Eds) Sperm Competition and Sexual Selection. Academic Press. London.

Simmons L.W., 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 Series B. 251: 183-186.

Torres-Vila L.M., Stockel J., Rodriguez-Molina M.C. 1997. Physiological factors regulating polyandry in *Lobesia botrana* (Lepidoptera: Tortricidae). Physiological Entomology 22: 387-393.

Uvarov B. 1966. Grasshoppers and locusts: A Handbook of General Acridology. Cambridge University Press, London.

Vahed K., Gilbert F.S. 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.

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

Wickler W., Seibt. U. 1985. Reproductive behaviour in *Zonocerus elegans* (Orthoptera: Pyrgomophidae) with special reference to nuptial gift guarding. Zeitschrift für Tierpsychologie. 69: 203-223.