ABSTRACT

HINN, JERALD C. Male and female mating strategies as they relate to the spermatheca in *Melanoplus bivittatus* (Orthoptera: Acrididae). (Under the direction of Marianne Niedzlek-Feaver)

Grasshoppers in the genus *Melanoplus* are known to transfer accessory reproductive gland proteins during mating, and these have been demonstrated to be incorporated in eggs. In this study, the mating strategies of males and females are explored as they relate to spermatophore transfer as a male-controlled resource. Three trials in which a female was caged with two males demonstrate that heavy males are more successful ($\alpha > .042$) in initially mating with a female. Females do not appear to discriminate between males over the course of their lifetime, however. Males were shown to mate preferentially with virgin females and with females who have recently oviposited.

From histological preparation of the spermathecae from interrupted matings, sperm can be found in all three of the chambers by five hours of mating, but in different forms. Sperm bundles remain intact in the long apical chamber, but are increasingly more degraded over time in the distal chamber. By 8.5 hours, half of the sperm bundles in the distal chamber are completely disassociated, and loose sperm becomes increasingly scarce as time progresses. Melanoplines do not oviposit immediately after mating, delaying an average of 4.7 days from mating to oviposition. This would suggest that a part of a male’s sperm contribution, like the accessory gland proteins, is being used for nourishment. The role of sperm from secondary matings is discussed.
MALE AND FEMALE MATING STRATEGIES AS THEY RELATE TO THE SPERMATHECA IN *MELANOPPLUS BIVITTATUS* (ORTHOPTERA: ACRIDIDAE)

BY

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A thesis submitted in partial fulfillment of the requirements for the degree of

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Approved by _____________________________________________________
Chairperson of Supervisory Committee

____________________________________________________
____________________________________________________
Biography

Jerald Hinn was born in Alexandria, Virginia on June 12th, 1974. He attended school there until 1992, where he graduated from Thomas Jefferson High School for Science and Technology. He then attended Virginia Polytechnical Institute and State University in Blacksburg, Virginia, graduating in 1996 with a Bachelor of Science degree in biology. After that, Jerry went on to begin his graduate work at North Carolina State University, where he served as a teaching assistant for both the Zoology department and Biological Sciences Interdepartmental Program. World domination is one step closer…
Acknowledgments

I would like to thank my major advisor, Dr. Marianne Niedzlek-Feaver for her generous contribution of time, resources, and above all, patience, as I developed not only a research project but my personal awareness as a scientist. I would also like to thank Dr. Blanche Haning for her deep commitment to my growth as an instructor and for her friendship. To my friends and family who have remained by my side despite not really knowing what I have been doing with myself these past few years, I appreciate your good natured ribbing and fond wishes. I would also like to thank my committee members, Dr. Coby Schal and Dr. Betty Black, for their time and interest in my work. Lastly, I would like to thank those few grasshoppers who did not spit on me or kick me during my work with them; your proud sacrifice should be an example to all your brethren.
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INTRODUCTION

The question of mate choice and its importance in the natural history of species has been long debated. Wallace (1890) made the first case for sexual selection, arguing that females could be choosing mates with attractive markings. Male dominated systems, such as Bighorn sheep (Hogg 1984) or Elephant seals (Haley 1994), are extreme examples of systems where females exhibit little choice. Females generally gain no benefit from matings; males offer no other resource than guarding the harem from other males. Conversely, black widows (Breene and Sweet 1985) and praying mantids (Kynaston et al. 1994) are both well described systems suggesting that males have little choice, at least on the subject of a second mating, if the female is hungry. In the latter systems, males can potentially contribute all of their own fitness to a single mating effort.

Melanopline grasshoppers (Orthoptera: Acrididae, subfamily Melanoplinae) fall between these extremes, but males still make an investment in reproduction, in the form of a nuptial gift. As described in the scorpionfly, Hylobittacus apicalis (Thornhill 1976), females choose males based on both size and palatability of captured insect prey, which they consume during copulation. Male katydids in the genus Ephippiger produce a specialized spermatophore that may mass up to thirty percent of his weight; females consume the external portion while sperm is passed by the internal portion (Batten 1992). Nutrients have been shown to be passed in both lepidopterans (Boggs and Gilbert 1979) and orthopterans; radiolabeled proteins from the male accessory reproductive
glands (ARGs) were found in the female's hemolymph within 24 hours of mating (Friedel and Gillott 1976) in *Melanoplus sanguinipes*. Other studies have suggested that some of these proteins may stimulate oviposition, even of unfertilized eggs (Pickford *et al.* 1969).

In mating systems where resources are exchanged for reproduction (essentially a female controlled resource), male and female interactions are strongly influenced by the type of controlled resource. Where the resource is confined to a territory, such as a breeding site or a patchy distribution of food, males would be expected to guard such a territory from interloping males and unreceptive females. Male/male interactions would tend to be marked by aggressive displays, and male/female interactions would show the female being “coy,” choosing among several offers of resource providers before settling for one of them. Grasshoppers found in open meadows do not appear to need to control territories; their saltatory movement prevents a male from forming a harem or easily preventing other animals from invading a particular region of the environment, and both feeding and breeding sites would appear to be copiously abundant to all animals. The main resource males control, besides sperm, is their nutrient gift to the females, exchanged during reproduction. Females may be choosing males based on potential to contribute proteins both to her fitness and the production of fertilized eggs.

Such potential should be advertised to females, much as crickets and birds advertise their status to potential mates by singing (Ridley 1995). Because Melanoplines do not stridulate, such advertisement should be visual. Females in
both *Chortophaga viridifaciata* (Feaver 1995) and *Schistocerca americana* (Kosal and Niedzlek-Feaver 1997) choose males based on size and mass, preferentially mating with heavier and larger males. Larger males may have more stored proteins, and thus would be able to contribute a larger gift to the females, and thusly to the male’s offspring should his sperm be used to fertilize eggs. This may not be assured, however.

Insect females have a complex storage organ known as the spermatheca, capable of storing enough sperm to fertilize a lifetime’s worth of eggs. For some insects, this is not a long span of time, but hymenopteran queens can live for years, laying all the eggs needed to support a colony from just one mating (Wilson 1974). Further studies with *M. sanguinipes* have shown that even a brief mating (5-15 minutes, far less than the usual 45 minutes) can provide enough sperm for multiple, non-parthenogenic egg pods (Pickford and Gillott 1976). Sperm must be kept viable, and it is suspected that glands within the spermatheca may help nourish or preserve sperm while it is stored (Clements and Potter 1967, Davey and Webster 1967).

Because of this storage capacity, males may not be assured of paternity if they are the last to mate before oviposition (Parker 1984). Females may use sperm from a previous mating and utilize the nutrient gift, making the proteins less a contribution to the male’s potential offspring, but instead more of a mating effort (Pardo *et al.* 1994). Males may opt for other strategies to increase their reproductive fitness, such as mate guarding, fertilization plugs, or removing sperm from previous matings. The latter is known to happen in dragonflies.
(Waage 1984) and in bushcrickets (Helversen and Helversen 1991). Histological preparations of _Dicromorpha viridis_ have shown that in a second mating, loose sperm from the first mating are being removed, possibly by the actions of the second male (Johnson 1998). Thus males may increase their chance of paternity by both indirectly competing with other males via sperm competition, and by encouraging females to oviposit after a mating by supplying enough oviposition stimulant during protein transfer.

Because of the potential for cryptic mate choice, with both male and female mechanisms for influencing sperm precedence, simple behavioral observation alone cannot explain the mating strategies of these animals. This study attempts to elucidate the mating adaptations in Melanopline grasshoppers from a behavioral and anatomical view. It is from this evidence that direction in mate choice, if any, can be discerned.
MATERIALS AND METHODS

Collection and Husbandry

Late instars and adults of both *Melanoplus femurrubrum* (red-legged grasshoppers) and *M. bivittatus* (two-striped grasshoppers) were collected from Research Farm Unit #1 (North Carolina State University, Raleigh, NC). 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. Both red-legged grasshoppers and two-striped grasshoppers were sympatric in the field, although *M. bivittatus* was far more commonly encountered in taller grasses. This may be biased towards ease of capture in tall grasses, as they tend to stand out more based on their far larger size. Neither species is capable of sustained flight above wing assisted jumping, and large numbers could be captured during their peak abundance.

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. Even freshly caught animals adapted very quickly to the diet, showing a marked preference towards oat flakes and cat food. All food was fed *ad libitum*, partially to avoid the low growth problems associated with a monophagous diet (Uvarov 1966), but mostly to avoid problems with cannibalism, coprophagy, and poor
reproductive output. Based on experience, *M. femurrubrum* do not develop fully matured ovaries when raised in the absence of clover, and corpses are quickly attacked. This could enhance the spread of gregarine and sarcodine parasites, *Gregarina polymorpha* and *Malameba locustae* particularly. Animals were caged in plastic terrariums with sand on the bottom, to absorb pooled water and keep frass from concentrating. Cages were soaked in a 20% bleach and soap solution to reduce the risk of parasite transmission.

Animals were held in sex-segregated cages to prevent unsupervised mating. To distinguish between individuals, animals were weighed and measured using digital calipers and digital balance able to read to 0.001g. Animals were marked with Testor paint on the pronotum and assigned a number and a data card.

Despite the provision of sand cups for the purpose, mated females tended to oviposit in grass cups. Thus, egg pods were separated as much as possible from soil and roots, and placed in damp, microwave sterilized sand. Egg cups were dampened and then sealed in plastic bags. Egg pods were allowed to incubate under heat lamps until hatching was observed. Efforts to break diapause in long-overdue egg pods, such as cold shock and darkness, were not successful. Nymphs were collected and reared under the same conditions as adults.

**Behavioral Observation**

To determine patterns in mate choice, females were presented with different males throughout the course of mating trials. In *M. femurrubrum,*
animals mate for one to three hours (averaging at 89 minutes), whereas in *M. bivittatus*, matings have been seen to last up to 46 hours in cage situations, but such durations are extreme, generally mating around eight to ten hours. Because of this, cages with mixed males and females of *M. bivittatus* could be left unsupervised for longer without risking missed matings, whereas *M. femurrubrum* had to be monitored during all interactions.

**M. bivittatus Mate Choice**

Because of their mating duration of eight to ten hours, observation tanks containing two-striped grasshoppers did not need to be continuously monitored. Males and females could be housed together without risk of missing a high number of matings. Because of this, random sampling was not used in favor of colony tanks, where interaction in a population of males and females could be studied.

Two forms of binary choice trials were used, fixed binary choice (both sexes represented by two individuals) and fixed binary female choice (one female, two males). Individuals used for the former were randomly assigned to a cage (of seven), and then held together for the life of the animals. Fixed binary female choice trials (21 cages with one female and two males) were not randomly assigned, but were chosen based on individual color phenotype; still, differences in size and mass of the males could be correlated to likelihood of mating. In both cases, males and females were replaced when they died unless they had been observed to mate. Animal measurements were used as a basis for
comparison in Wilcoxon Rank Sum tests to discern patterns of mating choice. Oviposition was recorded and attributed to animals if possible.

Spermatophore Counts and Histology

For visualization of sperm transfer during mating and to discern the structure and function of the spermatheca, virgin animals were allowed to mate for known lengths of time. At the end of this time, animals were sacrificed by being placed in a – 70°C freezer. The mating pair was then separated by pulling the male’s cerci out of the female’s paraproct. Within the valvulae of the female were white spermatophore bundles, these were removed and teased apart onto slides with dissecting pins, for later counting.

The spermatheca was removed and fixed in Carnoy’s solution. Before being embedded in paraffin, the fatty coat surrounding the spermatheca was removed, revealing the structure of the spermatheca. Each spermatheca was photographed and then embedded in TissuePrep wax for histology. Cross sections were taken at 7 μm, and stained using hematoxylin and eosin Y following Humason’s guidelines (Presnell and Schreibman 1997). Using clay reconstructions of the original photographed spermatheca, cross sections of the spermatheca chambers could be attributed to the whole structure, and a time sequence of sperm distribution was achieved.
RESULTS

Mating Behavior

*Melanoplus femurrubrum*

The mating behavior of *M. femurrubrum* strongly matches the described courtship patterns of *M. sanguinipes* (Pickford and Gillott, 1972) and *M. tequestae* (Bland, 1987). 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 noise, it is not audible to human observers.

From over seventy-five logged observational hours, male red-legged grasshoppers approach 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 two or three inches away from the female for a brief interval, from 10-30 seconds. In 80% of recorded matings, males would “flick” (Otte 1970) their femurs — the animal lifts its saltatory legs and, with the tibia folded up against the femur, raises and lowers them rapidly at an approximate 45 degree angle for an average duration of 0.25s ± .01. Because the third pair of legs is elevated, the abdomen rests against the substrate, perhaps transmitting a pulse to nearby animals. Mechanoreceptive setae, or brustia (Uvavov 1966) on the ventral surface of the genitalia are found in large numbers in both *Melanoplus spp.* as illustrated in figure 1.
Figure 1: Brustia, or mechanoreceptive setae found on both female (top) and male (bottom) Melanoplines. These hairs may help transmit and receive vibratory pulses.
Following the vibratory pulse, females either do not respond visibly (very commonly), begin to walk or jump away, turn to face the signaling male (uncommon), or pulse back (rare; only documented three times). A female may also elevate her saltatory legs and hold them in an “L” shape, as demonstrated in figure 2, which was only documented twice.

Figure 2: A female elevates her legs at a nearby male (middle) who had just approached.

A copulatory attempt is made by the male leaping atop the 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 1.2s. The force of the male’s spring often knocks the pair off balance, and in all but one filmed incident, struggling commences with both animals lying on their sides or backs. Females respond vigorously, kicking at the male and leaping multiple times. Copulation in Melanoplines involves the male using his cerci to hook into
small gaps in the ventral valves of the female and pull them down (Figure 3). If the struggling fails to dislodge the male with the initial struggling efforts, copulation may be prevented by keeping the valves away from the male. Females can adapt a “J” pattern by curling her abdomen underneath her while the male twines his abdomen around. This tactic of avoiding genitalia is seen in all situations where the female does not successfully force a male to disengage but does not mate.

![Figure 3: M. bivittatus in copula (10X). The cerci (A) have pulled open the lower valves of the female, allowing spermatophores (B) to be transferred.](image)

In all cases, males flick their femurs rapidly and rhythmically during the struggling for up to several minutes until the pair right themselves and go
dormant. The flicking is quite pronounced; in one mating, the male flicked 19 times in 10.8 seconds. One male was documented to bite at the female’s pronotum during her struggling; this did not result in a successful mating. Older *M. femurrubrum* females show heavy damage to the wings near the pronotum, possibly suggesting that “pterophagy” is a common occurrence in aborted matings. Males also show signs of wing damage as they age, possibly due to the same reason: Males will attempt to mount other males. In one population study, 8 of 22 documented rejected mating efforts were between two males. Naturally, such encounters cannot resolve with a successful genitalia “lock,” perhaps accounting for the wing damage. It is not known why males attempt this, but in all cases, the mating took place in close proximity to females (in one case, the assaulted male showed precopulatory signals towards a nearby female right before the second male leaped atop him), suggesting more a case of bad aim than open aggression.

Mating in *M. femurrubrum* lasts up to 2 ½ hours, at which point, the male disengages his genitalia. Mating can be halted by other animals walking onto a mating pair (in two filmed cases, a second male interrupts mating by attempting to copulate with the mating pair) or by handling the pair. Dismounting is swift, usually accompanied by the female kicking at the male. Once separate, both animals move away from one another and do not appear to show any further interaction.

These behaviors are represented in an ethogram as seen in table 1.
Table 1: Male and female behavioral patterns exhibited during observation of mating attempts in *Melanoplus spp.* Females demonstrate various behavioral responses to male approach, but it is the lack of any obvious response (sessile) that leads to successful copulation.

*Melanoplus bivittatus*

Copulation behavior for the two-striped grasshopper does not differ sharply from that of red-legged grasshoppers. All of the behavior patterns described above can be witnessed in *M. bivittatus*, although perhaps not with the same commonality. Mating was often observed to commence following nothing more indicative than antennae pointing.

Copulation in these animals lasts far longer than in the smaller melanopline, lasting up to 46 hours in the laboratory. More commonly, however, mating length averages around nine hours. Despite the length of the copulation, males do not disengage to feed, nor do they remain atop the female without
engaging her genitalia; spermatophore transfer seems to continue for as long as the pair remains *in copula*, as will be described later.

**Mate Choice and Mating History in *M. bivittatus***

Males in both paired sex (two males and females) and paired male (two males, one female) were given constant access to the female or females in their cage, and mate choice was recorded from daily observations. Animals were weighed and measured, and females were caged with males of different sizes and weights (as demonstrated on Table 1). Based on a comparison of males with whom females mated versus the other male in the colony tank, a Wilcoxon Rank-Sum test was performed on the data, to determine factors for male mating success such as weight, length, or symmetry (Table 2).
Table 2: Averages, standard deviations, and variance in male weight and body length in the three experimental populations.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Weight</th>
<th>Body length</th>
<th># Males</th>
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<tr>
<td></td>
<td>Avg</td>
<td>σ</td>
<td>σ²</td>
</tr>
<tr>
<td>1</td>
<td>1.274</td>
<td>.1302</td>
<td>.0158</td>
</tr>
<tr>
<td>2</td>
<td>1.118</td>
<td>.1451</td>
<td>.0193</td>
</tr>
<tr>
<td>3</td>
<td>0.991</td>
<td>.2204</td>
<td>.0475</td>
</tr>
</tbody>
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Table 2: Statistical tests of male success. Only in the shortest trial was there any strong (α < .05) statistical significance to male success.

<table>
<thead>
<tr>
<th>N</th>
<th>Weight</th>
<th>Body length</th>
<th>Leg asymmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W+</td>
<td>p</td>
<td>W+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>W+</td>
</tr>
<tr>
<td>Trial</td>
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<td>1</td>
<td>34</td>
<td>403</td>
<td>.0726</td>
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<tr>
<td>2</td>
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<td>.0020</td>
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<tr>
<td>3</td>
<td>48</td>
<td>622</td>
<td>.7312</td>
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</tbody>
</table>

The Wilcoxon Rank-Sum test does not establish any statistically significant reason to reject the null hypothesis, that of random mating, for either the first or third trial. Intriguingly, the second trial does give a very low α with which to reject the null hypothesis. One explanation for this may involve the
length of the trial; the second group died out suddenly after fourteen days, thus not giving a representation of mate choice over the life of the animals. To test whether or not the first mating recorded showed any significant bias, both body length and weight were analyzed for all three groups (leg asymmetry was not considered, as it had no discrepancy among trials) as in Table 3.

Table 3: Statistical tests of male success in a female's initial mating. While the third trial does not support this, weight appears to be a significant factor in mate choice.

<table>
<thead>
<tr>
<th>N</th>
<th>Weight</th>
<th>Body length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W+</td>
<td>p</td>
</tr>
<tr>
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<td>11</td>
<td>.0420</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>.0391</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>.6282</td>
</tr>
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</table>

At any $\alpha > .042$, the first two trials suggest that heavy males are more likely to mate with a female in her first mating in the laboratory. Body length is not strongly preferred. It should be noted that the males in the third experimental group were both smaller and lighter, on average, than the other two groups of males. Also, the first and second experimental groups were field caught animals, thus it cannot be said that the matings observed in the cages comprised the whole of the female's mating activity.
Males did not mate frequently during the trials despite having continuous access. Females would delay for up to 21 days before mating again. Most (N=45) rematings occurred within 4 days following the initiation of the previous one, but 17 matings were initiated after 7 days of delay or more (Figure 4).
Figure 4: Intervals between matings vary widely, with some females not remating for as long as 21 days. Most females (75%) appear to remate within 4 days of her last copulation.
Males attempted to mate with virginal females (figure 5) soon after the females were introduced into the experimental cages. 22 virgin females were mated within 2 days of introduction to the males, whereas 14 field caught adults were mating within the same span of time (n=26, 21 respectively). It should be noted that field caught animals may have a diverse range of mating history and oviposition states. This may correlate to the wide range of mating delays in field caught animals, whereas 85% of known virgins were mated quickly in the confines of a cage. Two field caught animals remained unmated for the first nine days of their trial, at which point both oviposited (neither were recorded to mate after this).

Of note, when oviposition was observed by newly introduced field animals, mating occurred quickly thereafter. Of the few (n=6) instances where oviposition was observed during the trials, half of the females were seen to be mating later that day, the other half was mating the following day.

**Variability in Egg Pod Hatching**

It was noticed that if nymphs were removed from bagged sand cups and the egg pod allowed to incubate longer, the pod could give rise to more nymphs. This was observed in both *Melanoplus spp.*, with the average time delay among pods demonstrated to show sporadic hatching (N=18) being 4.88 days. Seven of the eighteen pods yielded nymphs on at least three different intervals, and the average of total hatching intervals, from when the first viable nymphs were seen to hatch to the last hatching event, was 7.75 days. Both intervals show a high
standard deviation (Hatching intervals had a standard deviation of 3.80, total hatching interval had a standard deviation of 4.34). These results are demonstrated in Figures 6 – 7.
Figure 6: Egg pods can show multiple discrete hatching events. The most common interval is 2 days, but one hatching interval was shown to be 12 days.

Figure 7: The total duration in which egg pods produce viable nymphs also shows high variation. Total hatching time could encompass up to four discrete hatches over a period of up to 15 days.
Spermatophore Transfer

*Melanoplus* spp. pass multiple spermatophores during mating, and their role in nutrient transfer is well studied (see Friedel and Gillott 1977), but not their rate of transfer. Spermatophores were recovered from mating grasshoppers that had been interrupted (either by sacrifice or simply handling a mating pair). These were found between the female’s valves (See figure 8).

Figure 8: A cluster of spermatophore casings found between a female *M. bivittatus*’ valves following mechanical separation (10X).

Spermatophore remnants were also found associated with the male’s genitalia, which was also examined in collection.

It should be noted that these remnants must be extracted immediately after mating is terminated, and the odds of their successful removal decrease
sharply with time. While spermatophores were successfully recovered in all instances with two-striped grasshoppers, spermatophores were not found in 70% of red-legged grasshopper matings, even in matings which had been halted by interrupting the animals.

Spermatophores in *M. bivittatus* are essentially tube-shaped (figure 9), and reach an average of 4.82 mm in length. Sperm can be seen inside of spermatophores at 400x, but not in great numbers, as spermatophores found outside of the female have presumably already delivered their payload. Cross-sections of spermathecae reveal that the spermatophore threads all the way up the ductus seminalis into the proximal chamber, where sperm can be seen being released (figure 10).

![Figure 9: Spermatophore remnants of *M. bivittatus* (40x).](image)
The rate at which spermatophores are transferred in *M. bivittatus* appears to be linear, suggesting that time spent *in copula* is productive for sperm and nutrient transfer, and not simply mate guarding. Because the tendency for spermatophores to be intertwined makes them difficult to count, two counts were made by two observers. Where appropriate, error ranges are displayed in Figure 11.

Figure 10: Sperm (A) seen being released from a spermatophore (B) inside the ductus seminalis, which connects the spermatheca to the genitalia (400x)
Figure 11: Spermatophore transfer as a factor of time in *Melanoplus bivittatus*. Error ranges are given where individual counts vary. Two regressions have been performed on the data; A represents all the data, where B only includes matings from 1 to 15 hours. This was done to ensure that longer matings did not highly influence the trend in the data. Both regressions show a highly linear relationship between spermatophore transfer and time.

**Structure and Histology of the Spermatheca**

The spermatheca of *M. bivittatus* is composed of four distinct parts: an elongated entry tube (ductus seminalis, following the terminology used by Johnson 1998 to describe *Dicromorpha viridis*), the proximal chamber, which serves as a juncture point for the spermathecae divisions, and two blind
chambers attached to the proximal chamber. One of the chambers, the distal
chamber, is by far the most noticeable chamber due to its width, and this
chamber is attached to the proximal chamber by means of a fairly narrow
passage emerging from the center of the proximal chamber. The other chamber
is not notably wide except for at its end, which is bulbous, but is both long and
highly convoluted. In *D. viridis*, the analogue for this chamber is a nub-shaped
apical diverticulum, to reflect this, this chamber will be referred to as the apical
chamber in *M. bivittatus*. As the name implies, the apical chamber extends from
the end of the proximal chamber furthest from the end bearing the ductus
seminalis. The apical chamber is also notably more muscular than the rest of the
spermatheca, particularly at the bulb end. The spermatheca and its terminology
is demonstrated in Figure 12; a chart of lengths and widths follows in Table 4.
Figure 12: Spermatheca of *M. bivittatus*, showing the four major divisions at 40x (*ds*=ductus seminalis; *pc*=proximal chamber; *dc*=distal chamber; *ac*=apical chamber).
Table 4: A comparison of estimated weights and lengths of the chambers of the spermatheca in *M. bivittatus*

<table>
<thead>
<tr>
<th></th>
<th>Proximal chamber</th>
<th>Distal chamber</th>
<th>Apical chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width</td>
<td>.15-.2 mm</td>
<td>.27-.45 mm (at widest point)</td>
<td>.05-.2 mm (at bulb end)</td>
</tr>
<tr>
<td>Length</td>
<td>.8 mm</td>
<td>1.75 mm</td>
<td>3.3 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.3 mm connecting tube)</td>
<td></td>
</tr>
</tbody>
</table>

The chambers of the spermatheca are not apparent *in vivo*; the organ is covered in a viscous matrix of fat body and trachae, which envelope the organ into a small bean-shape.

In cross section, the spermatheca chambers have a hollow lumen, surrounded first by a layer of mucosa, and that is surrounded by a layer of ectathelia (Kaulenas 1992). In the apical chamber, that ectathelia shows a higher amount of muscle cells than seen in the other regions. Interspersed between the muscle layer are small flask-shaped cells, which appear to be Type-3 cells. Their function is not fully known, but are assumed to be useful in either preserving or nourishing sperm (Clements and Potter 1967). Other workers have demonstrated that these glands have a dense amount of rough endoplasmic reticuli and golgi bodies, suggesting a secretory function (Kaulenas 1992, Ahmed and Gillott 1982b). These glands can be seen in Figure 13, and are found in great quantity throughout the spermatheca.
Figure 13: A cross section of the apical chamber, showing the different tissue layers. The contents of the lumen are sperm. Arrows denote the position of the gland cells found throughout the spermatheca.

Structure and contents of the spermatheca during mating

The main function of the spermatheca, of course, is to receive and store sperm from matings. To discern the progress of sperm as it is transferred into the spermatheca via spermatophores, virgin animals were allowed to mate for a known length of time, and were then sacrificed. Ten spermathecae were able to be analyzed with certainty as to the identity of each chamber, and these came from matings ranging from 1 to 26 hours.

As can be seen in Figure 14, spermatophores can be seen threading up the ductus seminalis and into the proximal chamber, where the sperm is released in well-defined bundles. These bundles of sperm primarily remain in the proximal chamber during all mating durations, but can be seen in both the apical and distal chambers within five hours of the initiation of mating, in equal numbers. By 8.5
hours, not only can sperm be found in abundance throughout the proximal and distal chambers, but also is found at the bulbous end of the apical chamber.

Figure 14: A spermatophore in the proximal chamber (200x). Note the darker blue sperm bundles.

Sperm bundles, despite their even distribution, do not share the same appearance in the different chambers. Sperm in the apical chamber looks similar to that found in the proximal chamber, save that it has been loosened from the spermatophore matrix. While still in bundles, much of the sperm found in the apical chamber has been inserted into the mucosa in a “lawn” pattern, as seen in Figure 15. In contrast, sperm is not often found in intact bundles in the distal arm. While the bundle can be estimated from the proximity of nearby sperm clusters, as mating proceeds, less sperm is actually found in bundles, but instead
is loose, often showing individual spermatozoa in the lumen. Additionally, the lumen of the distal chamber is very pronounced, showing a haze of fibers in which individual spermatozoa can be seen (Figure 16). By 8.5 hours, estimated bundles in the distal chamber were half of that in the apical chamber, and these bundles were surrounded by a matrix of spermatophore contents and loose sperm.

Snodgrass, in his description of the spermatheca, refers to a spermathecal duct with well developed muscle tissue which could be used to pump sperm out of the spermatheca for use in fertilizing eggs (Snodgrass 1935). If this apical chamber shares homology with such a duct, its function has been modified for sperm storage.

Figure 15: A sperm bundle, or “lawn,” seen embedded into the mucosa of the apical chamber (1000x).
Interestingly, in matings exceeding the usual mean length of 8-10 hours, sperm shows a sharply skewed distribution. In a ten-hour and twenty-six hour mating, no sperm bundles were seen in either the distal or apical chamber, but were found in heavy abundance in the proximal chamber. Alternately, a twenty-two hour mating showed three bundles in the apical chamber, a matrix of loose sperm in the distal chamber, and thirteen bundles in the proximal chamber. It would appear that individual females can have varying rates of sperm processing speeds; where sperm can be found throughout the spermathecae as early as five hours in some females, it can also be found highly concentrated right where it is deposited as late as twenty-six hours into mating.

It is also noteworthy to mention that in longer matings only, the lumen stained a deep magenta. While first suspected to be an artifact, the lumens in

Figure 16: Sperm splitting out of a sperm bundle in the distal chamber (1000x).
both the apical and distal chamber were often vividly stained for those studied matings that exceeded 10 hours. This may be a protein-carbohydrate complex; histochemical studies in *Rhodnius spp.* demonstrate a similar phenomenon when using protein and polysaccharide sensitive staining techniques. (Davey and Webster 1967).
DISCUSSION

From the behavioral description of mating in *M. femurrubrum* and its behaviorally similar relative, *M. bivittatus*, it would appear on the outset that mating in Melanoplines is dominated by male activity, with little participation on part of the female. However, females are capable of successfully displacing males or otherwise thwarting them from copulating through a variety of behavioral means, ranging from changing body orientation and posture to struggling and kicking. Females may not, however, always engage in apparent rejection patterns because of costs to fitness – the vigorous struggling that commences following the copulatory leap may well attract the attention of predators. There is also the strong possibility that females have little to lose from secondary matings, and may gain fitness from the protein contributed by a male, or from the sperm itself.

From the behavioral data, it is clear that weight influences the chance of a male successfully mating with a female that has been introduced into a cage. After this initial mating, which occurs rapidly for virgin females, matings are uncommon and do not show any particular bias towards one male or another. This suggests that males are not competing for recently-mated females, and in fact only appear to resume mating after the female they are caged with oviposits. In essence, male competition for mates is a scramble for virgin and recently-oviposited females, and mating with females between those states may be unproductive for males.
In many orthopteran mating systems, nutrient transfer and sperm transfer are inextricably linked in the same package, that of the spermatophore. The transfer of multiple spermatophores in melanoplines may well insure that sperm will be transferred in any mating attempt where a spermatophore is passed, but it also means that whenever sperm is passed, nutrients must be passed alongside them. In essence, sperm transfer has a cost involved to it beyond the metabolic cost of producing the sperm, and if this cost is high enough, a male should refrain from liberal matings. This has been seen in a cage situation; males do not mate with females *ad libitum*.

It is known from other melanoplines that males mate preferentially with virgin females, and that has been demonstrated again with *M. bivittatus*. In these animals, antennae pointing always precedes mating; quite possibly antennae pointing enables males to investigate the reproductive status of a female. It has been noted that antennectomy reduces male mating vigor (Uvarov 1966); it is possible that the antennae pointing behavior witnessed so frequently in *Melanoplus spp.* is a way of assessing female condition. Males point their antennae directly at the abdomen of a nearby female, and, in some cases, touch it directly. This system is not perfect, as seen from numerous male-male copulatory attempts, but is a possible avenue for pheromone investigation.

The spermatheca of *M. bivittatus* sharply differs in both shape and chamber function from that of *D. viridis*. In the latter species, the apical diverticulum apparently directs sperm bundles into the distal chamber, but does
not itself store sperm. Instead, sperm storage is found only in the distal chamber. In contrast, it is the apical chamber that stores sperm in *M. bivittatus*; sperm bundles sent to the distal chamber become rarefied until little evidence of the number of bundles that entered the chamber can be seen. What is left of those bundles is loose sperm and a dense matrix of fibers, for lack of a better description, with no trace of the pink-hued spermatophore casing that entered the spermathecae with the sperm. Densely bundled sperm with some trace of spermatophore casing can be found at least partway up the apical chamber at five hours.

In order to be used for the purpose of fertilization, sperm must be split out from their bundles. However, with the presence of a male passing spermatophores down the ductus seminalis, it seems unlikely that a female will attempt to move sperm up the duct and use the sperm to fertilize eggs during a mating. As shown by the “lawn” of sperm embedded into the mucosa of the apical chamber, sperm does not need to be split apart from its bundles to be stored. The only other reason sperm may be vanishing from the distal chamber during mating is that it is being digested.

It is known that melanoplines can absorb proteins transferred during mating, and the absence of the spermatophore matrix from the distal chamber at all observations suggests that it is quickly absorbed. One hypothesis for the function of the flask-shaped type-3 cells is that they may serve less to nourish or protect sperm, but instead to nourish the female and her eggs by participating in either enzymatic degradation of the spermatophore and its contents, or by
providing a means to absorb the proteins transferred into the spermatheca during mating. In this study, virgin females who were fed *ad libitum* show a tendency to digest up to 50% of the sperm that had been moved out of the proximal chamber. Females who may have sperm stored in the apical chamber may not use the sperm from secondary matings, but instead may digest it entirely.

Using sperm as a nutrient source is not unheard of in insects; hemocyl insemination in bedbugs is presumed to nourish the female. Among lepidopterans, some of the sperm transferred is apryene or anucleate, and these are also suggested to be used for female nourishment (Boggs 1995).

To demonstrate this, of course, more investigation would have to be done. The fate of sperm stored in the apical chamber needs to be better understood; females sacrificed in increments after mating and certainly after ovipositing would give a better indication as to how much sperm was left to fertilize another egg pod. Additionally, it must be demonstrated that secondary matings do not have the ability to flush out sperm from the prior one. If males cannot reduce sperm numbers in the spermatheca substantially, this may well mean that the cost to males for mating with an inseminated female may not only involve the proteins transferred during mating, but the cost of the sperm digested by the distal chamber.
SUMMARY

From this study, it is evident that mating behavior in melanoplines is strongly influenced by the role of the spermatheca. Due to its sperm storage properties, a female may collect enough sperm in an initial mating to last through adulthood, and only demonstrates mate preference in this initial mating for heavy males. Part of the sperm from that initial mating appears to be digested in one of the chambers of the spermatheca, an area that in a related acridid, is used for sperm storage. Males may therefore seek to maximize their reproductive investment by seeking out virgin and freshly-oviposited females to ensure that the sperm is not shunted to the distal chamber to be digested.
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