SPERM UTILIZATION PATTERNS

IN

GRYLLUS INTEGER (ORTHOPTERA: GRYLLIDAE)

Vickie Lynn Backus B.A.

Department of Biological Sciences

submitted in partial fulfillment of the requirements
for the degree of Masters of Science

BROCK UNIVERSITY, St. Catharines, Ontario

@ November, 1984.
Sperm competition is the competition for fertilizations between ejaculates, within a female, following multiple mating. There are four sperm utilization or precedence patterns: first male precedence, where the first male to mate fertilizes most of the eggs laid by a female; last male precedence, where the last male to mate fertilizes most of the eggs laid by a female; "all-or-none" pattern, where sperm from either male fertilizes all the eggs laid by a female but which male's sperm that is used is random; or sperm mixing, where sperm from each male is used equally in fertilizing eggs laid by a female. Intermediate utilization patterns are also possible. Sperm competition occurs in a wide variety of insect species as well as other animals. This study was undertaken to study sperm competition in the field cricket, *Gryllus integer*. Four experiments were conducted: a radiation and sterilization experiment, a diapause experiment, and 2 competition experiments. It was found that 7,000 rad of gamma radiation sterilized adult *G. integer* males. There was no diapause in the laboratory in *G. integer* eggs. In the first competition experiment, three groups of females were used: females mated with a normal male, then with a second normal male (NN group); females mated with a normal male, and then with a sterile male (NR group); and females mated with a sterile male, and then with a normal male (RN group). The results obtained from this experiment showed that the mean proportion of eggs hatched was significantly different between 3 groups of females, with the proportion hatched much greater in the NN group than in either the NR or RN groups. The pattern for
the proportion of eggs hatched following a double mating most closely resembled a pattern expected if sperm mixing is occurring. Results obtained in the replicate competition experiment showed that the mean proportion of eggs hatched for the females in the NR group was significantly lower than the proportion hatched in the other two groups. This also supports a model of sperm mixing as a precedence pattern. Values calculated following Boorman and Parker (1976), for the proportion of eggs fertilized by the second male to mate following a double mating, were 0.57 in competition experiment 1 and 0.62 in the replicate. These values indicate that sperm mixing occurs in G. integer.
ACKNOWLEDGEMENTS

I would like to acknowledge the help and support of Dr. William Cade, for his assistance, advice, and support throughout the preparation of this thesis. I would also like to thank my supervisory committee, Dr. A. Houston and Dr. D. Ursino for their time, advice, and help. L. McGowan, W. French, K. Dixon, J. Chardine and S. Sakaluk provided advice and encouragement throughout this study. L. McGowen and D. Kozlovic proof-read this manuscript. Acknowledgement must be made to the following people for technical support: Dr. E Cherniak and the Dept. of Chemistry for use of the Gammacell; Dr. A Croy for help in determining radiation doses; N. Fuller for the photography; J. Chardine for his statistical and computer expertise; J. Siderius for sonagraping male calling song; E. Basalyga for providing me with sonagrophs of G. integer calling song. Most of all, thanks go to my family, especially William and Dorothy Backus, for their continuing support throughout my academic career. Support for this work was provided a the Natural Sciences and Engineering Research Council grant to Dr. W. H. Cade.
TABLE OF CONTENTS

Abstract..................................................................................3
Acknowledgements.....................................................................5
Introduction...............................................................................11
Literature Review......................................................................14  
Sperm Competition.................................................................14  
Methods of Studying Sperm Competition...............................15
Examples of Sperm Competition.............................................19
Factors Influencing the Occurrence of Sperm Competition.....48
Consequences of Sperm Competition........................................64
Mating Behaviour in Field Crickets.......................................76
Mating Behaviour in *Gryllus integer*.................................86
Specific Behaviour Indicating that Sperm Competition May Occur..................................................89
Materials and Methods..........................................................93
Results...................................................................................106
Radiation and Sterilization Experiment.................................106
Diapause Experiment..............................................................107
Competition Experiment 1....................................................107
Competition Experiment 2....................................................111
Discussion..............................................................................123
Sperm Utilization Patterns in *Gryllus integer*.....................123
Sperm Competition as a Selection Pressure..........................136
Summary and Conclusions.....................................................141
Literature Cited......................................................................145
<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix I</td>
<td>Radiation Biology</td>
<td>160</td>
</tr>
<tr>
<td>Appendix II</td>
<td>Formula for $\overline{P}$, and a Sample Calculation</td>
<td>166</td>
</tr>
<tr>
<td>Appendix III</td>
<td>Relationship Between the Species, Gryllus rubens and G. integer</td>
<td>170</td>
</tr>
<tr>
<td>Appendix IV</td>
<td>Life History Data, Competition Experiment 1</td>
<td>173</td>
</tr>
<tr>
<td>Appendix V</td>
<td>Calculation of $\overline{P}$ for Data Obtained in $\frac{1}{2}$ Competition Experiment 1</td>
<td>183</td>
</tr>
<tr>
<td>Appendix VI</td>
<td>Life History Data, Competition Experiment 2</td>
<td>184</td>
</tr>
<tr>
<td>Appendix VII</td>
<td>Productivity and Proportion of Eggs Hatched for the In Progress (IP) Dishes, Competition</td>
<td>196</td>
</tr>
<tr>
<td>Appendix VIII</td>
<td>Calculation of $\overline{P}$ for Data Obtained in $\frac{1}{2}$ Competition Experiment 2</td>
<td>201</td>
</tr>
<tr>
<td>Appendix IX</td>
<td>The Use of 4 Matings in the Competition Experiments</td>
<td>202</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1  Females insects that are known to mate multiply.....50
Table 2  Productivity (nymphs + unhatched eggs) of females mated with R males or mated with N males in the radiation and sterilization experiment.............108
Table 3  Proportion of eggs hatched following mating with an R male, or following mating with an N male in the radiation and sterilization experiment.....109
Table 4  Between group productivity (nymphs + unhatched eggs) in competition experiment 1..............................112
Table 5  Between group differences in proportion of eggs hatched in competition experiment 1.........................113
Table 6  Within group differences in productivity for oviposition dishes 1, 2, and 3 in competition experiment 2.............................................116
Table 7  Within group differences in proportion of eggs hatched in oviposition dishes 1, 2, and 3 in competition experiment 2..........................117
Table 8  Between group differences in productivity for dishes 1, 2, and 3 in competition experiment 2.....118
Table 9  Between group total productivity in competition experiment 2..........................................................120
Table 10 Between group differences in proportion hatched for 3 treatment groups for dishes 1, 2, and 3 in competition experiment 2..........................121
Table 11 Between group aggregate proportion hatched in competition experiment 2.................................122
| Figure 1 | A Flowchart Showing the Mating Pattern Followed for Females in Competition Experiment 1..............102 |
| Figure 2 | A Flowchart Showing the Mating Pattern Followed for Females in Competition Experiment 2..............104 |
| Figure 3 | Mean Nymph Emergence per Day Following First Nymph Emergence in the Diapause Experiment..........................110 |
| Figure 4 | Dissected Female *Gryllus integer*, Showing the Spermatheca..................................................135 |
Always be sure who your mother is,
for you can never be sure of your father.

(D. Backus)
INTRODUCTION

In females of species that mate multiply, store sperm between matings, show efficient sperm usage patterns, and keep sperm viable for long periods of storage, the possibility exists for an overlap of ejaculates within the sperm storage organ. This is sperm competition, or the competition between different ejaculates for fertilization of a female's eggs (Parker, 1970a). Sperm competition results in 4 possible precedence or sperm utilization patterns; first male precedence where the first male to mate fertilizes the majority of the eggs laid by that female; last male precedence where the last male to mate fertilizes the majority of the eggs laid by that female; "all-or-none" pattern where sperm from either male fertilizes the all of the eggs laid by a female, but which male's sperm used is random; and sperm mixing where the sperm from each male to mate is used equally in fertilizing eggs. It is also possible for an individual to show a sperm utilization pattern that is intermediate between sperm mixing and first male precedence or sperm mixing and last male precedence.

Hybrid crosses, genetic markers, electrophoretic techniques, or sterile males can be used to determine paternity following 2 or more matings. Once paternity is determined for offspring the sperm utilization pattern can be determined.

Insects fulfill the pre-adaptations that are necessary for sperm competition to evolve, thus most sperm competition studies have used insects as study animals. Insects are suited for these studies because females mate multiply, store viable sperm for long periods of
time in a sperm storage organ (the spermatheca). Finally, sperm utilization patterns in insects are such that sperm from different matings can overlap in the sperm storage organ.

Sperm competition is thought to be a selection pressure leading to the evolution of male behaviour that functions to displace previously stored sperm from the female's sperm storage organ, or to prevent displacement of sperm. The first of these counter-adaptations to sperm competition involves physical displacement of sperm from within the spermatheca. Counter-adaptations that may function to prevent displacement of one male's sperm by another male's sperm include; mate plugs deposited by the male in the genital opening of the female that function to prevent subsequent copulations by that female; male induced refractory periods, such that females do not respond to male courtship attempts following an initial mating; and, mate guarding following copulation, such that males can not gain access to mated females.

Female field crickets, including *G. integer*, mate several times prior to oviposition, and sperm are stored in a viable condition within a spermatheca. Therefore it seems likely that ejaculates overlap, and that sperm competition occurs in field crickets. Also male field crickets show post-copulatory mate guarding, and this may function to monopolize females and prevent them from remating with another male (Alexander, 1961), thus maximizing the number of eggs fertilized by preventing females from copulating with more than 1 male.

This study was designed to determine the sperm utilization pattern shown by the field cricket *G. integer* using the sterile male
technique. Four experiments were performed and will be reported on here, a radiation and sterilization experiment, a diapause experiment, competition experiment 1, and a replicate of competition experiment 1.
SPERM COMPETITION

Sperm competition is the competition between the ejaculates from two or more mates for the fertilization of ova within a single female (Parker, 1970a). Sperm competition can also occur between the sperm within a single ejaculate (Sivinski, 1980; In Press), but discussion of this process is beyond the scope of this work. Sperm precedence refers to the order in which sperm from the different ejaculates is used or is the sperm utilization pattern. There are two major patterns of precedence, first male sperm priority, defined as when the first male to mate fertilizes most of the eggs laid by the female, and last male sperm precedence which occurs when the last male to mate fertilizes most of the eggs laid by a female (Parker, 1970a; W.F. Walker, 1980). Two other patterns can occur. One is sperm mixing, where neither male's ejaculate is favoured. The other pattern is an "all-or-none" phenomenon, where one male's sperm is used exclusively to fertilize all eggs laid by the female, however, some females use sperm from the first male to mate and others use sperm from the second (W.F. Walker, 1980).

In this section I will review the techniques used to study sperm competition and determine sperm utilization patterns in animals, the literature concerning examples of sperm competition for various orders of insects, and in animals other then insects, and then discuss possible determinants of sperm utilization patterns in animals. Finally I will discuss the consequences for an animal in terms of the evolution of behaviour as a result of a particular sperm utilization
Methods of Studying Sperm Competition.

There are 4 techniques that are commonly used to determine sperm utilization or precedence patterns in insects. These involve the use of hybrids or sterile backcross hybrids, genetic markers, electrophoresis and, chemo or irradiation sterilized males.

a) Hybrids

Hybridization experiments involve crossing females of species A with males of a closely related species B. In cases where the eggs from a hybrid cross are inviable, females of species A are mated with a male of species A, and then with a male of species B. First male precedence patterns would be characterized by a high proportion of eggs hatched following the double mating. Second male precedence patterns would mean that the female would lay inviable eggs and that none would hatch. Precedence can be determined as a function of the percentage of viable eggs.

A variation of this technique is used when a female of species A is mated with a male of species B thus producing sterile hybrid offspring. When the sterile male hybrids are backcrossed to a female of species A, inviable eggs are produced. Precedence would be determined by mating a female of species A, first with a male of species A and then with a hybrid male.

Hybrid studies are not reliable in all cases because the barrier to gene flow (failure to produce offspring) may be pre-zygotic. That is, the male's sperm may be unable to fertilize eggs (Harrison, 1983). One pre-zygotic barrier to hybridization may
be that the female of species A will not respond to the courtship display of the males of species B (Thornhill and Alcock, 1983). Another pre-zygotic mating barrier is a structural difference in the genitalia of the two species involved, making it impossible for the male to inseminate the female (Alexander and Otte, 1967). In these cases the pre-zygotic barriers mean that all eggs laid are due to the mating to the conspecific male, and precedence can not be determined.

b) Genetic Markers

Another technique used to study sperm utilization patterns is to use a recessive genetic marker in order to determine parentage. A female who is homozygous recessive for a trait is mated with a male who is homozygous recessive and then to a male who is homozygous dominant for the trait. Offspring exhibiting the dominant trait were due to fertilizations obtained by the second male, while offspring resembling the mother were due to fertilizations obtained by the first.

For genetic markers to be effective in determining paternity the trait must be inherited in a simple Mendelian fashion. It is often hard to find traits that are inherited in this manner. As well, each genotype of sperm must be equally competitive, and this in not always the case (Holmes, 1974; Prout and Bundgarrd, 1977; Wilkes, 1966).

Studies with the parasitic wasp *Dahlbominous fuscipennis* (Hymenoptera: Eulophidae), have shown that following inter-mating intervals of less then one day the proportion of daughters sired by the first male to mate depends on the genotype of the sperm. (In Hymenoptera only the eggs that develop into females are fertilized.) If virgin females homozygous for the recessive marker carmine are
mated first to carmine males and then to wild-type males, 75% of the daughters are the result of fertilizations obtained by the first male. In reciprocal matings, a carmine female mated first to a wild-type male and then a carmine male, only 24% of the daughters are the result of fertilizations obtained by the first male. Thus, sperm from males of the carmine genotype are able to obtain more fertilizations than sperm from a male with the wild-type genotype (Wilkes, 1966).

In the parasitic wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae), sperm from males that are recessive for the eye colour "oyster" are used preferentially over sperm from either scarlet-eyed males or wild-type males (Holmes, 1964).

Experiments conducted with 3 stocks of *D. melanogaster*, 2 with a recessive colour marker and 1 wild-type stock, showed that there are clear differences between males of different genotypes in ability to displace sperm from prior matings and obtain fertilizations (Prout and Bundgaard, 1977). Genetic differences may lead to differential sperm motility which would give one male's sperm a better chance of reaching ova than the other male's sperm (W.F. Walker, 1980). As well, the genotype of the sperm may be able to influence its phenotype so that different sperm morphs, linked to different sperm genotypes, are able to compete more effectively then other sperm morphs and genotypes (Sivinski, In Press).

c) Gel Electrophoresis

This technique is similar to using the homozygous recessive markers to determine paternity in that it involves the use of electrophoresis to determine the genotypes of the offspring. Males and females with different genotypes are found and the females are
mated with one male of each genotype. Paternity of the offspring is established by comparing their enzyme separation patterns with their parents'.

d) Sterile Male Technique.

The most common way to study sperm competition in insects is to damage one male's sperm so that it induces a dominant lethality in the embryos. If used to fertilize eggs, the sperm from these males will halt egg development and render the eggs inviable. The sperm is damaged by exposing the male to sublethal doses of chemosterilant or radiation in order to induce chromosome abnormalities in the sperm. Information on how radiation acts to damage sperm, and details on the methods used to calculate the length of time necessary to expose an animal to a given dose of radiation are presented in Appendix I.

When the sterile male technique is used to study sperm utilization patterns matings occur in a reciprocal fashion, that is, a normal female mated first to a normal male and then to a sterile male (a NR mating) and a normal female mated to a sterile male and then to a normal male (a RN mating). Precedence patterns are revealed by comparing the proportion of eggs hatched between the two experimental groups. If first male precedence occurs then one would expect a greater proportion of eggs hatched in the NR group and a lower proportion of eggs hatched in the RN group. Second male precedence would give opposite results, a greater proportion of eggs hatched in the RN group then in the NR group.

In NR or RN matings a small proportion of the eggs that do not hatch are due to the natural infertility of the species. In order to account for natural infertility Boorman and Parker (1976) developed a
formula to determine the proportion \( P \) of eggs fertilized by an R male following a double (RN or NR) mating. This formula is

\[
P = \left( \frac{1 - x}{p} \right) + \left( z \cdot \frac{1 - p}{p} \right)
\]

when \( P \) = the proportion of eggs fertilized by the R male following a double mating, \( x \) = the proportion of eggs that hatch following an RN or NR mating, \( p \) = the fertility of a female following an N mating, and \( z \) = fertility of a female following an R mating.

This formula can be used to calculate \( P \) or the proportion of eggs fertilized by the second male in a double mating. In an NR mating the second male to mate is the R male and therefore \( P^2 = P^R \). In an RN mating pattern the second male to mate is the N male, therefore the proportion of eggs fertilized by the second male is \( 1 - P \). When reciprocal matings are performed \( P^2 \) can be determined.

This is the proportion of offspring following a double mating that are due to fertilizations by the second male in both mating patterns. A \( P = 1.0 \) shows complete second male precedence. If \( P^2 = 0.0 \) then complete first male precedence is occurring, and if \( P^2 = 0.5 \) then sperm mixing is occurring. Intermediate values are also possible, for example if \( P^2 = .80 \) then 80% of the progeny following a double mating are due to fertilizations obtained by the second male. More information concerning Boorman and Parker's formula, including its assumptions and a sample calculation are presented in Appendix II.

Examples of Sperm Competition.

In this section I will review the literature concerning studies of sperm competition in a wide variety of insects and other animals.
a) Diptera

A great number of the sperm competition studies have used species belonging to the order Diptera. Bryan (1968) used a sterile hybrid backcross technique and found first male sperm precedence in the mosquito, *Anopheles gambiae* (Diptera: Culicidae). Crosses made with *A. gambiae* males and *A. melas* females produce sterile hybrids. Female *A. gambiae* were crossed first with fertile *A. gambiae* males and then with hybrid males, and 54 out of 56 females tested laid fertile eggs. When the reciprocal crosses were made in which *A. gambiae* females mated first to the hybrid males and then to fertile males, all eggs laid were sterile. Goma (1963) used a genetic marker of resistance to the insecticide Dieldren in *A. gambiae* and found that progeny batches were never of mixed parentage. Rather they would all be from the first male or the second, the "all-or-none" phenomenon.

Drosophila also show considerable variation in sperm utilization patterns. Lefevre and Jonsson (1962) used the genetic marker technique and determined that the second male to mate with a female tends to fertilize more eggs than the first male. In these experiments female *D. melanogaster* (Diptera: Drosophilidae), from a mutant stock with the phenotype of vermilion eyes and forked scute bristles were mated with either wild-type *D. melanogaster* males or males that were mutant for the same sex-linked recessive traits. When mutant females were mated with wild-type males all daughters were wild-type phenotype. When the same strain of females were mated with mutant males the daughters were vermilion-eyed and had forked scute bristles. Females were twice mated, first to a wild-type male and then to a mutant male. As well reciprocal matings were performed. In
both cases the second male to mate fertilized more female offspring than the first male to mate, however, the proportion of eggs fertilized by the first male was lower when the second male had a wild-type genotype than when the second male had a mutant genotype. Henneberry et al. (1967), used the sterile male technique to test sperm precedence patterns in D. melanogaster. Males were sterilized with 16 krad of gamma radiation at 3 days of adult age. Untreated females were mated once with either a normal (N) or a sterile (R) male and then isolated for 5 days. On the fifth day post-mating females were remated either with a sterile or normal male. Females mated in an NN pattern showed 70 - 75% fertility following the second mating. Females mated in the NR pattern showed 6 - 8% fertility, while females mated in the RN pattern showed 53 - 56% fertility following the second mating. The authors concluded that sperm mixing occurred with a bias favouring the second male. Boorman and Parker (1976) used these data and obtained a $P_2$ value of 0.82, indicating that 82% of the progeny were due to the second male to mate. Boorman and Parker (1976) also used the sterile male technique on D. melanogaster and found $P_2$ values that were between 0.83 - 0.99, with $P_2$ increasing as a function of time between matings.

Prout and Bundgaard (1977) used the genetic marker technique to study sperm competition and displacement in D. melanogaster. Females that were double recessive for the marker brown;scarlet, which gives a phenotype of white eyes, were mated with either stock wild-type males or males recessive for the brown marker. Matings were accomplished by placing 100 females and 100 males into a flask for 2 hrs. Females were then placed individually into vials containing oviposition media.
Following 36 hrs, the females that had oviposited were placed into a new vial containing 3 males of the opposite marker and were given 24 hrs in which to mate. The females were transferred into new oviposition vials for 24 hrs and then were changed into new vials daily. Analysis of the progeny production on a day by day basis showed that although there was some degree of individual variation, the second male tended to fertilize most offspring following mating, that sperm are partitioned in some way and differentially used, and that sperm displacement was occurring. Triple matings of brown;scarlet females with brown, wild-type and brown;scarlet males showed that generally the last male to mate fertilized most of the subsequent offspring. However, the genotype of the male was important, with sperm from brown;scarlet males more effective in displacing brown sperm, and brown sperm more effective in displacing wild-type sperm.

Gromko and Pyle (1978) used the sex-linked genetic marker forked, giving a phenotype of forked scute bristles in D. melanogaster, to test precedence patterns. Female D. melanogaster that were homozygous recessive for the gene forked were mated with either mutant or wild-type males and allowed to oviposit overnight. The next day they were transferred to fresh vials and allowed access to males of the opposite genotype for 2 hrs. Any females remating were no longer given the opportunity to mate, but were transferred into fresh vials. Females not remating were given access to males for 2 hrs every day until they remated. It was found that following remating most offspring produced were due to fertilization by the second male to mate, females remated when, on the average about 78% of the total female progeny had been produced, and 16% of a male's initial sperm load was
displaced when the female remated. There was no difference in displacement associated with male genotype. Gromko and Pyle concluded that the large proportion of fertilizations due to the second male to mate was a function of sperm displacement and not sperm precedence.

Gromko and Pyle (1978) attributed the difference between their data and those obtained by Lefevre and Jonsson (1962) and Prout and Bundgaard (1977) to both strain differences and the effect of confining males and females for 24 - 48 hrs (the technique used by Lefevre and Jonsson, 1962 and Prout and Bundgaard, 1977) versus a 2 hr confinement. Male-female interaction of short duration leads to a low incidence of remating (Manning, 1962). The amount of time elapsed between remating would affect the amount of sperm remaining in the female's spermatheca. The longer amount of time between matings observed in the Gromko and Pyle (1978) study suggests there would be less sperm remaining within the spermatheca and a lower probability of precedence being observed.

Dobzhansky and Pavlovsky (1967) used a marker of various gene arrangements on the third salivary chromosome to test for precedence patterns in D. pseudoobscura. Three markers were used, standard, arrowhead, and chiricahua. Females that were homozygous for a marker were placed with 60 males (20 of each type) and left for 5 days to mate. Following this the females were removed and allowed to oviposit for 5 days. Then each female was placed in a separate vial with 6 males (2 of each type) for 5 days, at the end of which females were again allowed 5 days oviposition. Eggs laid following the second mating were fertilized with different sperm than those laid following
the initial mating. However, there was overlap in the type of progeny produced so that some sperm from the first male to mate was being used.

The recessive genetic marker of orange eyes was used to test utilization patterns in D. pseudoobscura. Virgin mutant females were placed with 2 males, either mutant or wild-type, and were observed for 30 min. Females were removed and allowed to oviposit for 3 days. Following the oviposition period the females were placed with 2 males, of either the same genotype or of the opposite genotype from their first mate, for 1 hr. Females were observed continuously and those that did not mate were given an additional opportunity to remate on day 6 following the initial mating. The second male displaced 68 - 90% of the sperm from the first mating as measured by the eye colour of the offspring hatched following the second mating. Wild-type males displaced more sperm, 98% versus the 68% that mutant males displaced, but this difference was not significant (Pruxan-Hotchkiss et al., 1981).

The irradiated male technique was used to determine the pattern of sperm precedence in the dung fly, Scatophaga stercoraria (Diptera: Anthomyidae). Male dung flies were sterilized with 10 krad of gamma radiation. Gravid virgin females were divided into 2 groups. The first group of females was mated with an irradiated male and then with a normal male (an RN pattern), while the second group was mated in the reverse or NR pattern. Eggs laid by a female mated in the RN sequence showed that 92% of the viable eggs were fertilized by the second male. Eggs laid by a female in the NR sequence showed 70% fertilized by the second male. It appears that on average 81% of
the viable eggs laid after a double mating are fertilized by the second male to mate in *S. stercoraria.* (Parker, 1970b)

Myers *et al.*, (1976) used the sterile male technique to demonstrate that there was incomplete second male sperm precedence in the apple maggot, *Rhagoletis pomonella* (Diptera: Tephritidae). Sterilization was accomplished by exposing the pupae to 3 krad of radiation from a Cesium-137 source. All treated males that eclosed within 24 hr of treatment were used in competition experiments. Three groups were used in these experiments, an RN group, an NR group and an NN group (a normal female mated with two normal males). All individuals were 12 days old at the time of first mating and 2 days were allowed between matings, however, oviposition was not allowed to occur until after the second mating. Females were allowed to oviposit onto an apple and were changed to a new oviposition site daily for a total of 9 oviposition periods. Fertility rates were found to be the following; for females in the NR group 22%, for females in the RN group 66%, and for females in the NN group 85%. Using the published data and Boorman and Parker's (1976) formula, I calculated $P$ value of 0.76 for *R. pomonella*. This means that 76% of the fertilizations occurring after double mating were due to the second male to mate.

Female melon flies, *Dacus cucurbitae* (Diptera: Tephritidae) show a precedence pattern of sperm mixing. Females mated first to an R male and then to an N male, without an intervening oviposition period, showed 71% hatch rate, while eggs laid by females mated in an NR pattern showed 43% fertility (Teruya and Isobe, 1982). Using these data and Boorman and Parker's (1976) formula I calculated a $P$ value
of 0.68, or 68% of the fertilizations were due to the second male to mate.

Studies with the olive fruit fly, *Dacus oleae* (Diptera: Tephritidae), using the irradiated male technique have shown that almost complete sperm mixing occurs (Cavalloro and Delrio, 1974). The fertility rate for eggs laid by a female following an RN pattern of matings was 45%, while the fertility rate following an NR pattern was 42%. Using these data and Boorman and Parker's (1976) formula I calculated $P$ of 0.52, almost complete sperm mixing.

A study conducted with the mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), showed a pattern of sperm mixing, tending toward second male precedence (Katiyar and Ramirez, 1970). Again, the irradiated male technique was used, however females were allowed a 1 week oviposition period between matings. Fertility rates for eggs laid by a female following an N mating were 94%, falling to 44% after a second mating with an R male. In the reciprocal mating pattern the fertility rate rose from 1.0% following the R mating, to 61% after remating with a normal male. Using Boorman and Parker's formula and these data I calculated a $P$ of 0.60, that is sperm mixing tending toward second male precedence. The trend toward second male precedence may have been due to sperm depletion within the females following a week of oviposition.

Curtis (1968) used the irradiated male technique and determined that 75% of the fertilizations in the Tsetse fly, *Glossina austeni* (Diptera: Muscidae), belonged to the first male. This low $P$ value of 0.25 (Boorman and Parker, 1976) indicated that 25% of the fertilizations belong to the second male to mate. This may be due to
the exclusion of the second male's sperm. That is, the second male may not transfer a full load of sperm to the female since twice mated females used sperm from both males when fertilizing eggs.

Linley (1975) used the sterile male technique to determine if sperm competition occurs in the midge, Culicoides melleus (Diptera: Ceratopogonidae). Sperm from the second male fertilized approximately 35% of the offspring produced by a doubly mated female. This is proportional to the amount of sperm contributed to the female by the second male. Linley concluded that mixing of the two males' sperm occurs in the spermatheca. I calculated a value of 0.44 for $P_2$, using Boorman and Parker's (1976) formula. This value indicates almost complete mixing.

b) Coleoptera

Schlager (1960) used the recessive genetic marker of black body colour and found that with the introduction of the second male's sperm the production of offspring from the first male ceased in the darkling beetle, Tribolium castaneum (Coleoptera: Terebrionidae). The first male's sperm would be used again when the second male's sperm was depleted. Boorman and Parker (1976) assigned a $P_2$ value of 1.0 for T. castaneum females immediately after the second mating and noted that $P_2$ decreased as the time from the second mating increased.

Wool and Bergerson (1979) used three genetic markers, paddle or mutant antennae, pearl or white eyes, and black body and two wild-type strains to test precedence patterns in T. castaneum. Mutant females were fertilized by males of the same strain, then were separated and housed individually with a wild-type male for 6 days. Following this 6 day period each female was again placed with a mutant
male that was the same strain as herself. Pairs were transferred every three days into fresh oviposition media. No effort was made to control for the number of matings within or between pairs. More offspring were fertilized by sperm deposited by the second male to mate than were fertilized by the sperm from the first male to mate. In a majority of pairs there were some offspring carrying the markers from the first male to mate with the female. This may indicate some sperm mixing within the spermatheca, or may have been due to eggs laid prior to mating with the second male.

Vardell and Brower (1978) used the semi-dominant marker of McGill black body colour to test T. confusum precedence patterns. The McGill black body colour is semi-dominant: when wild-type individuals are crossed with homozygous black individuals the offspring are bronze. Females of either genotype were placed in a container for 1 week with a male of either the same or the opposite genotype to the female. At the end of the first week the females were placed with a male of the opposite genotype to the first male. The body colour of the progeny rapidly changed showing that sperm from the second male was taking precedence over sperm from the first male. At no time however was precedence complete, sperm from the first male continued to fertilize low numbers of eggs, thus some mixing occurred. Again there was no control over the number of matings between a male and a female.

A 2% solution of Apholate can be used as a dip to sterilize male boll weevils, Anthonomus grandis (Coleoptera: Curculionidae). Gilliland and Davich (1966) mated female A. grandis to either sterile or untreated males and found that when the number of matings is controlled, the last male to mate with the female fertilizes more eggs
than the first male. Boorman and Parker (1976) calculated a $P$ value of 0.80 or 80% of the eggs are fertilized by the second male to mate with a female. Bartlett et al., (1968) used the genetic marker of white eyes in the boll weevil, *A. grandis*, and found that the amount of precedence for the second male ranged from 10% of the fertilizations to 90% of the fertilizations, depending on the amount of time between inseminations. When time between inseminations was less then 3 days most fertilizations were by the first male to mate, however, with an inter-mating interval of 3 days or more sperm from the second male to mate predominated.

Electrophoretic techniques were used by Huettal et al., (1976) to determine the sperm precedence in the plum curculio, *Conotrachelus nenuphor* (Coleoptera: Curculionidae). It was found that approximately 86% the progeny were fertilized by the second male. In many cases the sperm precedence by the second male was complete, and the authors hypothesised that the fertilizations due to the first male resulted from inadequate second matings.

c) Hymenoptera

Taber (1955) used the genetic marker of cordovan colour in the honey bee, *Apis mellifera* (Hymenoptera: Apidae), and reported that sperm from multiple matings did not mix in the queen's spermatheca. Queen *A. mellifera* were artificially inseminated with sperm from either a normal male or a cordovan coloured male and 2 days later was inseminated with sperm from a male of the opposite type. Females that were inseminated first with normal sperm and then with mutant or cordovan sperm had female offspring that were 31 - 72% normal for
colour, with the average being 56.5% normal. Females inseminated with sperm from a mutant male and then with sperm from a normal male had female offspring that ranged from 29 - 75% normal, with the average being 49.7% normal. Taber concluded that the sperm do not mix within the spermatheca but rather that they clump. However, the data do not support this contention. While there is a high degree of variability concerning the proportion of fertilized eggs laid per day showing one colour over another, indicating that sperm of one type may be "bundled together" in the spermatheca, there is no trend day by day for sperm of one male to be favoured over sperm from the other. As well, these data were gathered on females that had been artificially inseminated, and therefore conditions may not have resembled those that occur in the wild.

Page and Metcalf (1982) used electrophoretic techniques to determine the sperm utilization pattern in naturally mated *Apis mellifera* queens. The population surveyed were polymorphic for the malate dehydrogenase (Mdh) locus with 3 detectable morphs present in the population. Drones laid by each queen, resulting from unfertilized eggs, were examined by electrophoresis in order to determine the queens' genotype. Workers were assayed once a week for 11 weeks to determine if females had mated multiply and if the sperm utilization pattern changed over time. For the 12 queens studied, 8 used sperm from at least 2 males and 5 used sperm from at least 3 males. There was no change in the phenotypic frequency of offspring over the 11 weeks of the study. Therefore a sperm utilization pattern of sperm mixing seems to occur in *A. mellifera*.

In the parasitic wasp *Dahlbominus fuscipennis* (Hymenoptera: *
Eulophidae), the amount of second male precedence depends on the time between matings, and on the genetic strain of the male (Wilkes, 1966). When the inter-mating interval is greater than 1 day, approximately 70% of the daughters produced are sired by the first male. When the inter-mating interval is shorter than 1 day, genotype of the male sperm determines which male sires more daughters.

d) Hemiptera

Economopoules and Gordon (1972) used both the sterile male technique and genetic markers to study precedence in the milkweed bug, Oncopeltus fasciatus (Hemiptera: Lygaeidae). Females that were homozygous recessive for the marker white body were mated with either a white body or wild-type male for 6 days, then placed with a male of the opposite type for 6 days. The number of matings was not controlled for. In parallel studies white body female O. fasciatus were placed with either a white bodied male or a wild-type male that had been chemo-sterilized. In both cases, mating caused a loss of previously stored sperm, and the second male to mate fertilized most of the eggs laid. Females that had mated with wild-type males and then with castrated males, that is, males that had their testes and seminal vesicles removed, did not show any sperm displacement. This could have been caused by injury to the semen transfer mechanism of the male by the surgery, or because the accessory glands that were still remaining did not contribute to the volume of seminal fluid.

Harwalker and Rahalker (1973) used the sterile male technique to study the red cotton bug, Dysdercus koenigii (Hemiptera: Pyrrhocoridae). They found that the sperm from the second male to mate tends to be used preferentially to the first male's sperm.
their data and Boorman and Parker's formula (1976), I calculated a $P^2$ value of 0.69.

In the giant water bug, *Abedus herberti* (Hemiptera: Belostomatidae), Smith (1979) found that the second male to mate fertilizes approximately 99.7% of the eggs. Female *A. herberti* retain motile and viable sperm for up to 5 months. Females lay their eggs on the male's back and male brooding is necessary for successful egg hatch. The timing and duration of copulation and oviposition are male controlled and male *A. herberti* copulate with an ovipositing female in a cyclic fashion; the female lays 3 eggs and then the pair copulates. By mating a virgin wild-type female to a male with a dominant genetic marker of striped and then to a wild-type male, Smith determined that 99.7% of the fertilizations were obtained by the last male to mate. It is hypothesised that multiple copulations push the sperm from prior copulations back into a blind end of the spermatheca.

e) Lepidoptera

All possible precedence patterns occur in members of the order Lepidoptera. Retnakaran (1971) used the sterile male technique with the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), and found first male sperm precedence. Later work by Retnakaran (1974) that used a recessive genetic marker of red eye color in *C. fumiferana* yielded less clear results. Red-eyed *C. fumiferana* were mated, first with a red-eyed male and then, either 1 or 2 days later, with a black-eyed (wild-type) male. If the second mating occurred within 24 hrs of the first the offspring would be either all red-eyed, all black-eyed or mixed red and black-eyed, indicating that they developed from eggs fertilized by the first
male's sperm, the second male's sperm or from a mixture of sperm from both males. If the second mating followed the first by a 2 day period the offspring were all red-eyed, indicating first male precedence. Retnakaran (1974) hypothesized that it takes at least 24 hrs for sperm from the first mating to get into the seminal receptacle from the spermatophore located in the female's bursa copulatrix. If a mating occurs before the sperm from the first male are into the seminal receptacle, the new spermatophore pushes the first one away from its position in the bursa copulatrix and the sperm from the second male will fill the seminal receptacle and thus will be available for fertilizations. When the second mating follows the first by a 2 day period, sperm from the first mating will have filled the seminal receptacle and sperm from the second mating will not be able to enter. Therefore, first male precedence occurs.

Other species of Lepidoptera show second male sperm priority. Labine (1966) used the sterile male technique in order to determine precedence in the checkerspot butterfly, *Euphydryas editha* (Lepidoptera: Nymphalidae). When females were mated to normal males immediately following mating with a sterile male, 1 female laid eggs that were 99% fertile, while the 2 other females laid eggs that showed very low (1 - 21%) fertility. Therefore it seems that when matings follow quickly sperm competition follows an "all-or-none" pattern, however the sample size was low (N = 3) and therefore the data were preliminary.

Female *E. editha* who were mated first with either a sterile or normal male, allowed to oviposit for several days, and then mated to a male of the opposite type showed a hatching pattern consistent with
second male precedence. All females mated in the NR pattern (except
one where the first spermatophore blocked the bursa copulatrix so that
the spermatophore could not be inserted properly) showed very low
rates of hatch. Of the females mated in the RN pattern, 2 showed
clear second male precedence, 1 did not show any change in hatching
pattern following the second mating, and 1 showed an increase from 5%
fertility to 60% fertility following the second mating. In the case
of these last 2 females, these results could be due to low natural
fertility (Labine, 1966). Boorman and Parker (1976) calculated a $P$
value of 1.0, or complete second male precedence in *E. editha*.

Brower (1975) used the recessive trait of melanism as a genetic
marker to test the pattern of precedence in *Plodia interpunctella*
(Lepidoptera: Pyralidae), the Indian meal moth. Individuals with the
recessive melanic genotype have black wings. Melanic females were
mated with either a melanic or normal male and then with a male of the
opposite type. There was time allowed in between matings for the
females to oviposit. When the order of mating was melanic female
mated first with a normal male and then with a melanic male there was
an average of 3.0 normal offspring and 133.7 melanic offspring
oviposited following the second mating. When the mating sequence was
reversed, with the first male being melanic and the second male having
normal wing colour, there was an average of 6.9 melanic offspring and
114.2 normal offspring. Thus the sperm from a second mating take
precedence over sperm from the first in *P. interpunctella*. Brower
hypothesised that sperm from the second mating physically displaced
the sperm from the first mating to the back of the spermatheca and
that the sperm used for fertilizations are those closest to the
spermathecal opening.

North and Holt (1968) used the recessive genetic marker of yellow eyes to test precedence patterns in the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). When a yellow-eyed female was mated first with a wild-type male and then with a yellow-eyed male, 86% of the progeny had yellow eyes. Following the reciprocal mating, a yellow-eyed female with a yellow-eyed male followed by a wild-type male, 98% of the progeny were normal eye colour. Therefore, second male precedence occurs in *T. ni*.

Etman and Hooper (1979) sterilized male armyworms, *Spodoptera litura* (Lepidoptera: Noctuidae), and found second male sperm precedence following both NR and RN matings. They also found that in twice-mated females, 50% had sperm in their spermatheca immediately following the second copulation, but 30 to 40 min following the second copulation no female showed stored sperm in the spermatheca. Sperm was again detected in the spermatheca at 1 hr after the second copulation. In a single mating it takes 45 - 60 min before sperm are detectable in the spermatheca. Therefore it is possible that the second mating leads to a physiological response in the female to expel sperm already present in the spermatheca before the second male's sperm reach the spermatheca.

The tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae), has been shown to have an "all-or-none" pattern (Flint and Kressin, 1968), or a second male precedence pattern of displacement (Pair *et al.*, 1977). If displacement mechanisms, such as the ones operating in *Spodoptera litura* (Etman and Hooper, 1979) also operate in *H. virescens*, these contrary results from different researchers can
be explained. If the time between the second mating and egg laying is very short, or the second mating is defective in sperm transfer, the first male's sperm will be used to fertilize eggs. If neither of these problems occur, the second male's sperm can displace the first male's sperm and will be used to fertilize the eggs.

Two other Lepidoptera show the "all-or-nothing" type of precedence. These are the ermine moth, *Atteva puncella* (Lepidoptera: Yponomeutidae) (Taylor, 1967), and the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Snow et al., 1970). It is unknown whether a mechanism of sperm displacement similar to the one operating in *Spodoptera litura* (Etman and Hooper, 1979), that is, emptying and refilling of the spermatheca as a physiological response to mating, operates in these species.

f) Odonata

The mechanism of sperm competition in the damselfly, *Calopteryx maculata* (Odonata: Calopterygidae), was documented by Waage (1979), in field and laboratory observations. The mean volume of sperm within the female is not different if the female mates once or twice. Females that were captured in copula, dissected and examined in the lab had little or no sperm present, while females captured while flying in tandem with males prior to copulation, or after copulation has occurred have approximately the same amount of sperm stored in their spermatheca. Observations of copulating pairs showed that a male inserts his penis into the vagina of the female, but the sperm vesicle is not in position to pass on sperm. While in this state the male makes undulating movements with the penis. In the final second of copulation the male stops these movements and the sperm vesicle is
moved into contact with the sperm channel of the penis. Waage (1979) interpreted the undulating movement of the penis as involved in sperm removal. Sperm from prior matings is physically removed from the female and the male's own sperm is transferred to an empty spermatheca. Thus second male sperm precedence is likely.

Male damselflies, *Lestes vigilax* (Odonata: Lestidae), also remove and reposition sperm within the female spermatheca. Indirect evidence of sperm displacement is obtained by sampling females in the field from 1 of 3 contexts, precopula, interrupted copula, and postcopula, and measuring the average sperm volume carried by the females. Females captured in precopula, that is before the copulatory position occurs, or females captured as they came to the water, showed that the females contained sperm from prior matings. Females collected in copula but before sperm transfer show significantly lower volumes of sperm in their spermatheca than precopula females. Females collected postcopula, but prior to oviposition contained amounts of sperm that were significantly greater than females captured in copula, but were not significantly higher or lower than females capture at the precopula stage. Examination of pairs collected in copula suggested that the male both withdraws sperm and compacts and repositions sperm within the female reproductive tract (Waage, 1982).

Fincke (1984) used the irradiated male technique with the damselfly, *Enallagma bagoni* (Odonata: Coenagrionidae), and found that the last male to mate fertilized approximately 80% of the eggs laid in the first clutch after mating. Dissections of females collected before, during and after copula showed that males remove 87% of the sperm stored within the female.
Other species of damselflies that are thought to displace sperm are: *Calopteryx dimidiata* (Odonata: Calopterygidae), *Argia fumipennis violacea* (Odonata: Coenagrionidae), *Enallagma cyathigerum* (Odonata: Coenagrionidae), and *Ischnura verticalis* (Odonata: Coenagrionidae) (Waage, In Press).

McVey and Smittle (1984) used the irradiated male technique to determine precedence in the dragonfly, *Erythemis simpliciollis* (Odonata: Libellulidae). It was found that the last male to mate with a female fertilizes 99.5% of the eggs laid within 5 - 8 min of mating. Eggs laid 24 - 48 hr after mating however are fertilized by sperm from more than 1 male, with the last male fertilization 75% of the eggs laid 1 day post-mating and 66% of the eggs laid 2 days post-mating. There is a 57% change in spermathecal volume from pre-to interrupted copula females, and the same degree of change from interrupted to postcopula females, indicating that some physical displacement of the sperm occurs (Waage, In Press).

Another species of dragonfly that is thought to show sperm displacement is: *Celithemis elisa* (Odonata: Libellulidae). In addition, *Sympetrum rubicundulum* (Odonata: Libellulidae), is thought to repostion sperm within the female's reproductive tract (Waage, In Press).

**g) Orthoptera**

Nabours (1927) demonstrated last male precedence in the grouse locust, *Paratettix texanus* (Orthoptera: Tettigidae). A genetic marker study, using several different contrasting colour patterns and normal recessive patterns, was used to show that the last male to mate fertilizes most of the eggs laid by the female.
Hunter-Jones (1960) used albanism as a genetic marker to study the order of sperm precedence in the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Albino females were placed with albino or wild-type males for 24 hrs. Females were then allowed to oviposit 2 egg cases, and were placed with a male of the opposite type for 24 hrs. The number of matings was not controlled. Almost all eggs that hatched following the female's mating with the second male were fertilized the second male. Boorman and Parker (1976) calculated a $P$ of 1.0 in *S. gregaria*.

The sterile male technique was used by Parker and Smith (1975) to study the locust, *Locusta migratoria migratorioides* (Orthoptera: Acrídidae). Two oviposition routines were followed yielding 2 precedence values. In 1 oviposition routine, female *L. migratoria* were mated first with either a sterile (R) male or a normal (N) male and were allowed to oviposit 1 egg case. Females were then mated with a male of the opposite type, allowed to oviposit 1 egg case, and mated to normal males and allowed to oviposit between each mating until 3 more egg cases were obtained. Under this routine, approximately 90% of the eggs laid following the second mating were fertilized by the second male. Eggs laid following the third, fourth, and fifth matings (all with normal males) showed high rates of hatching and, therefore, the eggs were probably fertilized by the last male to mate prior to oviposition. This yielded a $P$ value of 0.86 (Boorman and Parker, 1976).

In the second oviposition routine *L. migratoria* females were mated with either an N or R male and then immediately remated to a male of the opposite type. Following the appearance of the first egg
case females were either remated to an N male or were not mated again. Females mated in an NR pattern with no subsequent mating showed a percent hatch ranging from 45 - 60%. Females mated in an NR pattern with subsequent mating showed approximately 45% hatch from the first egg case laid, and a recovery to approximately 90% fertility in the cases laid following remating. Females mated in the RN pattern without a subsequent mating showed low average proportion of eggs hatched (fertility) ranging from approximately 20 - 30%. Females mated in the RN pattern with subsequent matings to an N male showed low fertility for the first egg cases laid, approximately 20%, but high fertility rates of 90% in the egg cases laid following the matings with the normal male. It seems that 62% of the fertilizations to occur following a double mating without an intervening oviposition period are due to the first male's sperm (Parker and Smith, 1975). Parker and Smith stated that the differences in precedence values obtained under the 2 routines may be because in the second routine (mating without an intervening oviposition period) the first spermatophore tube may block the spermathecal duct and act to prevent further effective copulations. When the female oviposits this tube is removed and future copulations can be effective in storing sperm in the spermatheca.

Cochran (1979) used a recessive genetic marker of rose eye colour to test for precedence in the german cockroach, Blattella germanica (Orthoptera: Blattellidae). Two mating regimes were used in this study. In the first mating routine a mutant female was placed in a container with a mutant male and the pair were observed until mating occurred. Immediately after mating the mutant male was removed and
replaced by 2 - 4 wild-type males. Data were obtained by recording
the eye colour of offspring emerging from the first egg case produced
by the female. In 48 of 49 egg cases examined, all progeny showed the
mutant rose eye colour, such that, when rapid remating occurs the
first male to mate fertilizes most offspring. This experimental
design, however, failed to control for any effects due to male
genotype in the mating sequence. The second mating routine used by
Cochran did control for male genotype.

In the second mating routine a virgin male *B. germanica* of either
genotype was added to a container with a virgin mutant female. This
pair was maintained until the female produced her first egg case. When
the first egg case was produced the male was removed and replaced by a
male of the opposite genotype. Data were obtained by observing the
progeny produced for all egg cases laid by the female. Of the fertile
matings that produced 2 egg cases, 80% of the progeny were fertilized
by the first male to mate with the female. In many cases, however,
this could be because the females did not remate in between periods of
egg case production. Data obtained from the females who were known to
have remated between production of egg cases showed that sperm from
subsequent matings gradually replaced sperm from the first
insemination. Therefore, it seems that *B. germanica* shows first male
sperm priority (Cochran, 1979).

Three studies have been attempted in order to demonstrate sperm
competition in crickets and to determine the order of precedence.
Sakaluk (Unpub MS) used the irradiated male technique to determine the
sperm utilization pattern in the decorated cricket, *Gryllodes
supplicans* (Orthoptera: Gryllidae). Male *G. supplicans* were exposed
to 20 krad gamma radiation as adults. The proportion of eggs hatched following an RN mating was 0.34, while the proportion of eggs hatched following and NR mating was 0.54. A $P$ of 0.38 was found, indicating that sperm mixing with a slight advantage to the first male to mate is occurring in G. supplicans.

Sakaluk (1980), used electrophoretic techniques to determine the order of precedence for the field cricket, Gryllus integer (Orthoptera: Gryllidae). Only 2 of the enzymes surveyed, esterase and amylase, showed significant variation and no mating combinations of appropriate types were found.

Bidochka (1981) used a pattern of conspecific and heterospecific matings in order to test for sperm precedence in the cricket Gryllus veletis (Orthoptera: Gryllidae). When G. veletis females are mated with G. pennsylvanicus (Orthoptera: Gryllidae) males infertile eggs are produced. A pattern of conspecific (G. veletis to G. veletis) and heterospecific (G. veletis to G. pennsylvanicus) matings were used. Female G. veletis crickets were mated conspecifically, allowed to oviposit, mated heterospecifically, allowed to oviposit, and again mated heterospecifically. Nymphs were mouth aspirated and counted and unhatched eggs were counted. No difference was detected between the percentage of eggs hatching before or after one of two heterospecific matings. Thus sperm precedence was not determined (Bidochka, 1981).

h) Animals other than insects.

There are some sperm competition studies that have been attempted with animals other than insects, and these are reviewed here. As well, the overlap of ejaculates following multiple mating is thought to occur in a wide variety of animals, and these suspected instances
of sperm competition will also be reviewed here.

Austad (1982) determined the sperm utilization pattern in the bowl and doily spider, *Frontinella pyramitela* (Araneidae: Linyphiidae), using the sterile male technique, and found almost complete first male sperm priority (Austad, 1982). Using the formula for $P$ (Boorman and Parker, 1976), I calculated a value of $P$ of 0.003.

Martyniuk and Jaenike (1982) used electrophoresis and wild-caught copulating pairs of the spider, *Prolinyphia marginata* (Araneae: Linyphiidae) to determine if females mate multiply and to determine the sperm utilization pattern for polygamous females. Pairs of spiders were collected in copula and copulation was allowed to be completed in the laboratory. Following copulation, males were removed from the females and were stored for subsequent electrophoretic analysis. Females were allowed to produce egg cases and the egg cases were maintained until spiderlings emerged. Following offspring emergence, females, males, and their offspring, were subjected to electrophoretic analysis to determine differences at the phosphoglucomutase (Pgm) locus. At least half the females surveyed had mated with a different male prior to the observed copulation. When multiple mating was detectable, most offspring were fertilized by the penultimate male to mate. Thus *P. marginata* also shows first male precedence.

Vollrath (1980) sterilized male spiders, *Nephila clavipes* (Araneae: Argiopidae), in order to determine precedence using the irradiated male technique. Each female *N. clavipes* was placed on a web with a sterilized male for either 2 or 7 days. Following this
time period the sterilized male was removed and replaced with a normal male for 7 days. Egg cases were collected and spiderling emergence was monitored. The results showed that first males tend to fertilize more eggs than second males following a double mating, however male size and length of time spent on the web with the female are important factors; larger males and males that spend longer periods of time on the web may achieve more fertilizations. This experiment however did not use a reciprocal NR mating, therefore some of the advantage to larger N males may be due to low competitiveness of R sperm.

Jackson (1980) sterilized male salticid spiders, Phidippus johnsoni (Araeae: Salticidae) by exposing them to either 10 or 30 krad X radiation. Females were first mated with a sterile male and then with a normal male, allowed to oviposit and spiderling emergence was monitored. In 55% of the females tested no offspring emerged following RN matings. This indicates that first male precedence is occurring in half the females tested. Partial displacement of the R males' sperm occurred in 18% of the females tested, while total displacement of the R males' sperm occurred in 27% of the females tested. These results are confounded because sterile males copulated for a shorter mean duration then normal males did. Females mating with normal males showing short copula durations fail to deposit fertile eggs 50% of the time. Shorter than average copula duration may account for these females showing second male precedence, the first male did not inseminate the female and thus sperm competition was not occurring. In order to test this hypothesis reciprocal matings should be performed.

Sperm precedence was tested in the mold mite, Tyrophagus
putrescentiae (Acari: Acaridae), using the irradiated male technique. The percent fertility following the second mating in both the RN and NR mating patterns was over 90% for all doses of radiation tested (60, 80, and 100 krad). Therefore it was impossible to determine a precedence pattern using these data for T. putrescentiae (Ignatowicz et al., 1983).

Sperm competition and priority have been studied in two types of pillbugs or sow bugs (Isopoda; Oniscoidea). Sassman (1978) used electrophoretic techniques to examine precedence patterns in the Isopod, Porcellio scaber. He found that in the wild there are frequent multiple matings, and each male contributes gametes equally to the brood. Mating does not induce any short term or long term changes in the usage of the sperm mix, that is, equal sperm mixing occurs throughout the female's reproductive life with no changes due to time since mating.

Johnson (1982) used a recessive marker to examine the possibility of sperm competition in the Isopod, Venezillo evergladensis. The first male fertilizes a greater proportion of the first and second broods than the second male. The proportion decreases so that the third brood produced since the second mating has a 50:50 proportion of first and second male paternity. Johnson concluded that there is significant sperm mixing and no competition between ejaculates. These results could be due to experimental design as the number of matings between males and females was not controlled.

Sperm storage and multiple inseminations are likely to occur in many birds. Extra-pair copulations are likely to occur when the female is fertilizing eggs and, therefore would result in sperm
competition (Hatch, 1983; McKinney et al., In Press). Warren and Kilpatrick (1929) found that the chicken, Gallus gallus, will store sperm for up to a month. By using a recessive genetic marker of colour it was demonstrated that when a male rooster is replaced by a different male the sperm used to fertilize eggs is that of the second male. Second male precedence is thought to occur because the flagellum of the sperm is lost during the first days existence in the oviduct of the female. Therefore, fresh sperm have an advantage over stored sperm (Warren and Kilpatrick, 1929).

The sex-linked, recessive, marker of dwarfism was used to determine the sperm utilization pattern in the domestic chicken. Chickens were artificially inseminated with equal amounts of the 2 sperm types, with a 4 hr period between inseminations. Between 71 - 83% of the offspring were fertilized by the second male to mate, indicating that there is a large advantage to the second male in chickens (Compton et al., 1978). As the period between inseminations was so short, it is unlikely that the advantage to the second male is due to senesence of the sperm belonging to the first male.

Female mallards, Anas platyrhynchos, can store sperm for up to 10 days. Multiple matings occur between a female and her mate. As well, males will copulate with females who are not their mate and these "forced copulations" can result in fertilized eggs (Burn et al., 1980). Cheng et al. (1983) used genetic markers and artificial insemination to test for sperm precedence in A. platyrhynchos. They found that when the inseminations occurred simultaneously, or were separated by 1 - 3 hrs the proportion of progeny belonging to each male was equal. If the inseminations occurred 6 hrs apart, however,
70% of the progeny were fathered by the second male.

Gowaty and Karlin (1984) used electrophoretic techniques to examine the esterase - 2 and nucleoside phosphorulase loci in the mountain bluebird, *Sialia sialis*. They surveyed 16 complete families, as determined by the male and female tending a nest site and nestlings from the nest, and found that 25% of the families showed evidence of multiple parentage for nestlings. As nothing was known concerning the identity of males attempting extra-pair copulations, precedence values could not be determined.

Another animal which is likely to show sperm competition is the common garter snake, *Thamnophis sirtalis*. Gibson and Falls (1975) examined litters of snakes showing a recessive trait, melanism, and found evidence of multiple male parentage. It is not known if the multiple matings are due to separate fall and spring matings or sequential spring matings. Both long term sperm storage, which is storage of sperm from fall matings, and short time sperm storage, which is storage of sperm from spring matings, occur in the same female (Halpert et al., 1982).

Electrophoresis has demonstrated that multiple paternity occurs in 78% of the litters surveyed of the Belding's ground squirrel, *Spermophilus beldingi*. Females are receptive for an average of 4.7 hrs on 1 afternoon per year and will mate with 1 - 5 males during that period. Intrabrood paternal representation was found to be unequal, but precedence values could not be assigned as the order of mating by the males could not be determined (Hanken and Sherman, 1981).

Sperm utilization patterns have been determined for a number of rodent species. Dewsbury and Baumgardner (1981) used the genetic
markers of albino and wild-type coat colours in the prairie vole, *Microtus ochrogaster*, and found a significant advantage to the last male to mate. Similar experiments with deermice, *Peromyscus maniculatus*, used the genetic markers of brown, wide-banded agouti, blonde, and wild-type coat colours, but found no significant effect of mating order on litter composition.

Dewsbury (In Press) reviewed the literature concerning sperm competition experiments in rodents and reported that sperm competition studies in rodents may be confounded by differences between strains in the ability of sperm to fertilize eggs. Experiments that tested for differential fertilizing abilities of different strains, as well as for the precedence pattern shown, indicate that the golden hamster, *Mesocricetus auratus*, showed second male precedence with no effect of strain, while the Norway rat, *Rattus norvegicus*, showed sperm mixing.

Factors Influencing the Occurrence of Sperm Competition.

There are 4 preadaptations that are necessary for an animal to have before sperm competition can occur within a female. These are, the female must mate several times before eggs are fertilized, sperm is stored within the female, the sperm remain viable during storage, the female shows efficient sperm utilization patterns, that is, the female uses sperm in such a way that sperm from previous matings will still be present in the spermatheca when the female remates (Parker, 1970a). If a female shows these preadaptations she will contain sperm from more than 1 male at a time and ejaculate competition may occur. The most important preadaptation for the occurrence of sperm competition is multiple mating.
In sexually reproducing species, mating behaviour may be expensive in terms of time and energy to individuals (Daly, 1978). To females the cost of mating can be reduced if she mates only once and can store sufficient sperm to fertilize all subsequent offspring. Despite increased costs attached to repeated mating behaviour, many insects mate more than once. In this section I review some examples of insects that mate more than once, and discuss the benefits of multiple mating to females.

a) Examples of Female Insects that Mate Multiply.

Table 1 is a partial listing of species in which female insects are known to mate multiply. These examples will be discussed individually in the following section concerning the benefits of multiple mating to females. (For a more extensive review see Parker, 1970a; Kirkendell, 1977.)

b) Benefits to Females who Mate Multiply.

Multiple mating by females may be selected for because it increases a female's fitness by: i) compensating for inadequate first matings or decreased fertility over time; ii) the female gains nutrition from multiple matings; iii) increasing genetic diversity of offspring; iv) females gain access to a needed resource other than food or more energy is required to avoid copulation than to copulate (Kirkendall, 1977). Studies supporting each potential adaptation are reviewed here.

i) Increased Fertility/Compensation for Inadequate Matings.

For many insects more than 1 insemination may be necessary to fill the female sperm storage organ, the spermatheca. Wilkes (1966) noted that it takes up to 10 inseminations to fill the spermatheca in
TABLE 1. Female insects that are known to mate multiply.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diptera</strong></td>
<td></td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Pyle and Gromko, 1978</td>
</tr>
<tr>
<td></td>
<td>Griffiths <em>et al.</em>, 1982</td>
</tr>
<tr>
<td></td>
<td>Boorman and Parker, 1976</td>
</tr>
<tr>
<td><em>D. psuedoobscura</em></td>
<td>Cobbs, 1977</td>
</tr>
<tr>
<td><em>Rhamphomyia aigrita</em></td>
<td>Downs, 1970</td>
</tr>
<tr>
<td><em>Anopheles gambiae</em></td>
<td>Goma, 1963</td>
</tr>
<tr>
<td><em>A. quadrimaculatus</em></td>
<td>French and Kitzmiller, 1962</td>
</tr>
<tr>
<td><em>Glossina palpalis</em></td>
<td>Curtis, 1968</td>
</tr>
<tr>
<td><em>Ceratitis capitata</em></td>
<td>Katiyar and Ramirez, 1970</td>
</tr>
<tr>
<td><em>Scatophaga stercoraria</em></td>
<td>Parker, 1970b</td>
</tr>
<tr>
<td><strong>Hymenoptera</strong></td>
<td></td>
</tr>
<tr>
<td><em>Dahlbominus fascipennis</em></td>
<td>Wilkes, 1966</td>
</tr>
<tr>
<td><em>Apis mellifera</em></td>
<td>Taber, 1955</td>
</tr>
<tr>
<td><em>Anthidium maculosum</em></td>
<td>Alcock <em>et al.</em>, 1977</td>
</tr>
<tr>
<td><strong>Hemiptera</strong></td>
<td></td>
</tr>
<tr>
<td><em>Oncopeltus fasciatus</em></td>
<td>Gordon and Bandal, 1967</td>
</tr>
<tr>
<td></td>
<td>Econompoules and Gordon, 1972</td>
</tr>
<tr>
<td><em>Dysdercus koengli</em></td>
<td>Harwalker and Rahalker, 1973</td>
</tr>
<tr>
<td><em>Abedus herberti</em></td>
<td>Smith, 1979</td>
</tr>
<tr>
<td><strong>Lepidoptera</strong></td>
<td></td>
</tr>
<tr>
<td><em>Atteva punctella</em></td>
<td>Taylor, 1977</td>
</tr>
<tr>
<td><em>Spodoptera frugiperda</em></td>
<td>Snow <em>et al.</em>, 1970</td>
</tr>
<tr>
<td><em>Plodia interpunctella</em></td>
<td>Brower, 1975</td>
</tr>
<tr>
<td><em>Heliconius hecale</em></td>
<td>Boggs and Gilbert, 1979</td>
</tr>
<tr>
<td><em>Euphydryas editha</em></td>
<td>Labine, 1964</td>
</tr>
<tr>
<td><em>Choristoneura fumiferana</em></td>
<td>Retnakaran, 1974</td>
</tr>
<tr>
<td><strong>Orthoptera</strong></td>
<td></td>
</tr>
<tr>
<td><em>Teleogryllus commodus</em></td>
<td>Loher and Edson, 1973</td>
</tr>
<tr>
<td><em>Gryllus integer</em></td>
<td>Saka(luk and Cade, 1980</td>
</tr>
<tr>
<td><em>Acheta domesticus</em></td>
<td>Saka(luk and Cade, 1980</td>
</tr>
<tr>
<td><em>Paratettix texanus</em></td>
<td>Nabours, 1968</td>
</tr>
<tr>
<td><em>Schistocerca gregaria</em></td>
<td>Hunter-Jones, 1960</td>
</tr>
<tr>
<td>SPECIES</td>
<td>REFERENCE</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Coleoptera</td>
<td></td>
</tr>
<tr>
<td><em>Tribolium castaneum</em></td>
<td>Schlager, 1960</td>
</tr>
<tr>
<td><em>Anthonomus grandis</em></td>
<td>Bartlett <em>et al.</em>, 1968</td>
</tr>
<tr>
<td><em>Conotrachelus nenuphar</em></td>
<td>Huettel <em>et al.</em>, 1976</td>
</tr>
<tr>
<td>Odonata</td>
<td></td>
</tr>
<tr>
<td><em>Calopteryx maculata</em></td>
<td>Waage, 1979</td>
</tr>
<tr>
<td>Mecoptera</td>
<td></td>
</tr>
<tr>
<td><em>Hylobitticus apicalis</em></td>
<td>Thornhill, 1976</td>
</tr>
</tbody>
</table>
the wasp, *Dahlbominus fuscipennis*. Taber (1955) stated that the queen honey bee, *Apis mellifera*, requires insemination by 6 - 7 males in order for her spermatheca to be filled. Studies have shown that more individual sperm, measured using a haemacytometer, were found in the spermatheca of a queen honey bee following multiple inseminations with a small amount of sperm than following 1 insemination with the same total volume of sperm (Bolton and Harbo, 1982).

In the milkweed bug, *Oncopeltus fasciatus*, multiple matings may be necessary either because few sperm are transferred at each insemination or because sperm remain viable for a short period of time. Stimulation received during mating, possibly hormonal, is necessary to maintain the activity of the corpora allata, a gland producing hormones controlling yolk deposition, and is therefore necessary to produce eggs (Gordon and Bandal, 1967). More than 1 mating per day is common for both male and female milkweed bugs (Economopoulous and Gordon, 1972).

Multiple mating is common in *Drosophila melanogaster* (Pyle and Gromko, 1978; Griffiths *et al.*, 1982). Pyle and Gromko (1978) found that in the laboratory 84% of females remated with 1 other male and 23% remated twice. Females remate while they still contain sperm from previous males. Doubly-mated females produce more progeny than singly-mated females and show greater fertility and fecundity than singly-mated females, thus remating may compensate for depleted sperm. Griffiths *et al.* (1982) showed that a model allowing for 21% of the female population to be doubly-mated gives the best fit for the distribution of the Adh genotype of progeny actually found in a natural population of *D. melanogaster*. 
Cobbs (1977) tested for multiple mating in field captured Drosophila pseudoobscura females with an electrophoretic survey of the esterase - 5 (est-5) locus. It was determined that 55% of females showed a detectable double or triple paternity for their offspring. He noted that this value may be low because females showing unipaternity may have mated multiply and exhausted the previous male's sperm.

Levine et al. (1980) examined larvae obtained from a population of field captured D. pseudoobscura using markers of different gene arrangements on the third chromosome, and found that 43% of the females carried sperm from 2 or more males.

Laboratory studies with D. pseudoobscura females revealed 72% of the females remated within 6 days of the first mating, and that twice mated females had significantly more offspring than once mated females (Pruzan-Hotchkiss et al., 1981).

The female ermine moth, Atteva punctella, is also known to mate multiply. Taylor (1967) found that females with depleted sperm supplies or females that who had been involved in copulations resulting in no eggs or inviable eggs being laid, remated more frequently than females who had been involved in fertile matings.

Snow et al. (1970) reported that 24% of singly-mated females of the fall army worm, Spodoptera frugiperda, did not oviposit. Many of the females that laid eggs showed poor fecundity and low fertility. Only 7% of the doubly-mated females failed to oviposit.

In crickets, mating induces an increase in oviposition rates. In Teleogryllus commodus (Orthoptera: Gryllidae), mating induces a substantial increase in the number of eggs laid (Loher, 1981; Loher
and Edson, 1973). Loher and Edson (1973) postulated a "mating factor" that is transferred from the male to the female to account for the increase in egg production. Multiple mating may maintain these levels and allow the female to lay a maximum number of eggs.

Sakaluk and Cade (1980) reported that female *Acheta domesticus* (Orthoptera: Gryllidae), which mated twice produce significantly more offspring than singly-mated females. In singly-mated *A. domesticus*, 13% of the females fail to reproduce.

A similar pattern of results were found in the field cricket *Gryllus integer*. Between 24 - 50% of singly-mated female *G. integer* did not produce offspring whereas 2 - 13% of twice mated females did not produce offspring (Salaluk and Cade, 1980; 1983). Thus for crickets multiple matings may compensate for inadequate first matings.

ii) Nutrition and Multiple Mating.

Gaining nutrition from males is an important activity for some female insects. For example, male scorpionflies, *Hylobittacus apicalis* (Mecoptera; Bittacidae), provide females with nuptial prey offerings of recently captured arthropods. The females feed on the prey items during copulation. The nuptial prey offerings may be enough food to sustain females and allow maximal fecundity. The feeding of the female by the male also decreases the likelihood that the female will hunt for food and therefore she will be less likely to fall prey to spiders. Female scorpionflies are known to mate multiply (Thornhill, 1976).

Boggs and Gilbert (1979) used radiotracer experiments to determine the fate of *C* labelled spermatophore contents in female butterflies, *Heliconius hecale* (Lepidoptera: Heliconiidae). Males
were fed with C and mated to unlabelled females. Radioactivity was detected in fertilized eggs, the unfertilized eggs that remained in the female, and eventually, throughout the female. These data show that substances other than sperm are being transferred at mating via the spermatophore, and that they are important to both egg production.

Radiotracer experiments with C labelled sperm from male bean weevils, *Acanthoscelidus obtectus* (Coleoptera: Bruchidae), showed that substances transferred during mating become detectable in the haemolymph of females 24 - 48 hrs after matings. The function of these components has not yet been identified but they may supply energy factors for egg formation (Huignard, 1983).

Experiments with *Drosophila mojoavensis* have shown that males contribute nutrients to oocytes and to female somatic tissue. Male *D. mojoavensis* and *D. melanogaster* were labelled by culturing eggs and larvae on a medium containing H amino acids. The labelled males were mated to unlabelled females of the same species. At 24 hrs following mating significant amounts of radioactivity were detected in the head, thorax, abdomen, reproductive tract, and eggs of the mated *D. mojoavensis* females, but not in the mated *D. melanogaster* females (Markow and Ankney, 1984).

Male bushcrickets, *Requena verticalis* (Orthoptera: Tettigoniidae) feed their mate a spermatophylax, a protein body attached to the spermatophore ampulla, during mating. Experiments varying the number of spermatophylaxes consumed by a singly-mated female, while maintaining each female on a low protein diet, showed that the number of eggs laid by a female and the weight of those eggs increases with the number of spermatophylaxes eaten (Gwynne, 1984). Male *R.*
14 C labelled protein hydrolysate showed detectable levels of 14 C throughout the body, but especially in the reproductive glands (testes and accessory glands). Females mated with these males had detectable levels of 14 C present in the spermatheca and in unfertilized eggs stored in the ovaries. The 14 C present in the ovaries could not be due to the presence of labelled sperm, but rather was due to the nutrition obtained from feeding on the labelled spermatophylyax (Bowen et al., 1984). Therefore in R. verticalis male derived nutrients may be an important part of female reproductive success.

The male katydid, Conocephalus nigropleurum (Orthoptera: Tettigoniidae), also transfers a spermatophylax to the female during mating. Protein from the spermatophylax is incorporated into both hatched and unhatched eggs. Male derived proteins may be important to egg production and female reproductive success (Gwynne, 1982).

In the cricket, Plebeio Gryllus guttiventris (Orthoptera: Gryllidae), starving females who are artificially inseminated with a spermatophore show increased survival and decreased egg production when compared to a control group of starving virgins. This is attributed to the oocyte resorption, leading to decreased egg production but longer life. Normal females who have been artificially inseminated do not exhibit longer life and show increased egg production compared to normal virgins (Bentur and Mathad, 1975). Thus under normal conditions it seems that nutrition is not gained by the female P. guttiventris from matings.

Downes (1970) noted that nuptial feeding occurs in four species of danceflies, Rhamphomyia nigrita, R. filicauda, R. hoeli, and R.
ursinella (Diptera: Empididae). Females of each of these species are known to mate multiply and may be obtaining a small amount of nutrition from the nuptial prey feedings.

iii) Increased Genetic Diversity and Multiple Mating.

Multiple mating may confer an advantage to the female because it allows for an increase in the genetic diversity of the resulting offspring. Sassman (1978) noted that the increase in variation that results from multiple matings compensates for the genetic variation lost due to inbreeding. Multiple mating also acts to decrease the chance of mating with a full sibling, therefore acts to increase the genetic variation in all subsequent generations (Johnson, 1982).

Richmond and Ehrman (1974) theorized that multiple paternity within a single batch of eggs may benefit the offspring if among offspring competition for resources occurs. If the offspring from a female have different genetic backgrounds, and environmental conditions change, some of the offspring may be able to compete better for resources than the others, and thus show a higher survival rate than their siblings. This hypothesis however rests on the assumption that sperm mixing occurs, that is, multiple mating leads to multiple paternity within a brood.

Taylor (1967) noted that multiple matings do not necessarily imply multiple paternity, especially if remating functions to correct for inadequate first matings. Also, in species that show a high degree of precedence, remating will not increase genetic diversity because one male's sperm fertilizes all eggs laid by a female.

Williams (1975) noted that even within a completely monogamous mating system, mating with very few males would give a female's
offspring the maximum obtainable genetic diversity. Therefore the costs in terms of time and energy to the female to engage in multiple mating may not be balanced by the small gain in increased genetic diversity.

iv) Acquisition of Male Controlled Resources, and the Costs of Avoiding Matings.

Parker (1970a) stated that females will not engage in extra matings unless the copula duration is less then the time it takes to reject additional courtship. Alcock et al. (1977) demonstrated this for the megachild bee, *Anthidium maculosum* (Hymenoptera: Megachilidae). In this species males acquire territories at patches of flowers that the females feed on. Females mate multiply while at these patches because it may be more adaptive to copulate passively for 30 sec, and more importantly gain access to feeding patches, than to try to evade or repel the males.

There are resources other than food held by males that females may need in order to reproduce successfully. Male dragonflies and damselflies (Odonata) hold territories containing one or more oviposition sites. Females arrive at oviposition sites with large sperm reserves. Males court females entering their territories and mate with them. Females attempting to oviposit without mating are intercepted by males. Therefore, by remating, females gain access to oviposition sites (Waage, 1983; In Press).

Multiple matings that occur because it takes less energy to copulate than to avoid copulation should occur if, copula duration is short (Parker, 1970a), males hold a resource that females need and the female must mate in order to obtain access to that resource, or if
males are larger and more aggressive than females (Alcock et al. 1977).

c) Factors Other than Multiple Mating

Multiple mating is a major factor in driving the evolution of sperm competition, however it is not sufficient to explain the evolution of different precedence patterns following multiple mating. Complete mixing of ejaculates could occur and does in some species. For example: the olive fruit fly, Dacus oleae, (Cavalloro and Delrio, 1974) and the mediterranean fruit fly, Ceratitis capitata, (Katiyar and Ramirez, 1970). However, as has been reviewed, many more species show precedence patterns of one form or another.

Parker (1970b) hypothesized that sperm competition evolved as a response to male-male competition as males attempt to maximize the number of eggs fertilized and not the number of females mated, or the number of eggs laid per female. Males can maximize their chances of fertilizing eggs by evolving ways of insuring that their sperm is used to fertilize eggs. These counter-adaptations are discussed later.

Waage (1983) and Knowlton and Greenwall (In Press) noted that while sperm competition is a form of male-male competition, it is controlled by females. The necessary preadaptations (Parker, 1970a) for sperm competition to occur must have evolved in females first. In this context many authors hypothesize that sperm competition and sperm precedence patterns are examples of post-mating female choice (Lloyd, 1979; Waage, 1983). Lloyd (1979) stated that females may mate with any male to insure that they get a mate and then may become choosy. If the female remates with a "better" male she may be able to manipulate stored sperm so that the sperm from the best male
fertilizes her eggs.

W.F. Walker (1980) postulated that sperm precedence patterns evolved in response to the sperm utilization strategies of females. He rejected Parker's (1970b) argument that sperm displacement evolved at a value that gives males a maximum total number of fertilizations. Rather, displacement values are determined by the female and how she chooses to mate and manipulate her stored sperm, in other words, the female may sacrifice laying a large number of fertile eggs in order to lay a smaller number of eggs fertilized by a "better" male's sperm.

Gwynne (In Press) stated that sperm competition evolved as the result of the female's efforts to increase male parental investment in her offspring. The sex with less parental investment will compete for matings and that the sex with a greater parental investment will discriminate in mate selection (Trivers, 1972). In insects most parental investment involves the cost of producing sperm or ova, with little post-zygotic care. Gwynne (In Press) expanded Trivers' concept to include mating effort (ME) because large expenditures in ME can decrease chances for finding and mating with multiple mates. Females tend to be a limiting resource for males and thus females will be more choosy. Female choosiness will increase the amount of male ME necessary to mate, and thus will decrease the number of matings a male can obtain. As the necessary amount of ME is increased, the number of matings an individual can obtain decreases, and the male's need for paternity assurance should increase. Thus, sperm utilization patterns have evolved as a way to assure paternity. An example of this may have occurred in the giant water bug, Abedus herberti. Female *Abedus herberti* lay their eggs on the backs of males. Brooding by the male
is necessary if the eggs are to hatch successfully. Female giant water bugs have increased the amount of paternal investment that the males must provide. Males may have responded to the increase in paternal investment by increasing the amount of paternity assurance shown, by requiring multiple copulations and evolving strong second male precedence (Smith, 1980)

d) Factors Influencing the Sperm Utilization Pattern

Several factors influence which type of precedence operates in a species. One factor that has been reviewed is genotypic differences between the two males expressing themselves as differential fertilizing ability of the male's sperm. This is known to occur in Hymenoptera (see Homes, 1974; Wilkes, 1966), in Drosophila (Prout and Bundgaard, 1977), and rodents (Dewsbury, In Press), and may occur in other animals as well.

Another factor that may determine the precedence pattern shown by a species is the shape of the spermatheca. W.F. Walker (1980) stressed the shape of the spermatheca as the most important factor in determining the pattern of precedence. Female insects with an elongate or tubular spermatheca tend to show second male sperm precedence, whereas female insects with an ovoid or spheroid spermatheca tend to show first male sperm priority.

There are exceptions, however, to the rule that females with elongate spermatheca tend to show second male precedence, while females with ovoid spermatheca tend to show first male sperm precedence. Tribolum castaneum has a long tubular spermatheca and shows second male precedence, as expected (Schalager, 1960; Wool and Bergerson, 1979). Tribolum confusum has a bulbous spermatheca,
however, it also shows second male precedence (Vardell and Brower, 1978). Therefore, it seems that spermathecal shape does not influence the sperm utilization pattern in Tribolum sp.

Austad (In Press) noted that first male sperm priority is common in spiders. Spiders show two types of spermathecal morphology, the first, known as "cul-de-sac" which has sperm entering and exiting the spermatheca by the same opening. The other, known as "conduit" morphology, has an opening for the sperm to enter and second opening for sperm to exit. The "cul-de-sac" morphology is thought to be associated with first male sperm precedence. However, there have not been enough studies of sperm competition in spiders in order to test this hypothesis.

Another factor that can influence the sperm utilization pattern shown in a species is the sexual experience of males. Holmes (1974) found that if both males to mate with a female were virgins, 2% of the daughters produced could be attributed to the second male to mate in the wasp, Nasonia vitripennis. If both males were sexually experienced, 65% of the female offspring could be attributed to the second male to mate.

Vollrath (1980) found that both size of males, and the length of time males stayed on the female's web, influenced the precedence pattern shown by the spider, Nephila clavipes. When the second male was larger and stayed on the web longer then the first male he fertilized more eggs than if he was the same size and stayed on the web the same amount of time or was larger and stayed on the web the same amount of time. Larger males have longer conductors or sperm insertion organs, therefore, the advantage to larger males may be
because larger males can insert sperm deeper into the spermatheca where it may be closer to the exit.

The method of sperm transfer may be responsible for the confused precedence pattern found in the honey bee, *A. mellifera*. Taber's (1955) data showed that on any given day, sperm from 1 male may fertilize most of the eggs laid by a doubly-mated queen, but over several days sperm from both males is used randomly. Wilkes (1965) reported that sperm from the wasp, *Dahlbominus fuscipennis*, is passed to the female in bundles. The bundles are ejaculated into the female in front of the sperm duct, where contractions of the ovaries pull the sperm bundles into the spermatheca capsule. Inside the spermatheca capsule the sperm move around slowly. The process is similar in *A. mellifera*. If the sperm bundles break up slowly, sperm may be mixing within the spermatheca, but sperm from 1 male may remain bundled so that on any given day it is used to fertilize eggs (Page *et al.*, 1984).

Gromko *et al.* (In Press) reviewed the processes of sperm transfer and displacement in *Drosophila sp.* and noted that these processes are complex. Factors other than male genotype that may affect precedence values are: the temperature of the male during development (but see Johnsen and Zarrow, 1971), the temperature of the female during oviposition, the interaction between males and females due to strain differences, and male age and the effects of age on hormone and seminal fluid levels.

Once a sperm utilization pattern has evolved in a species, it can be viewed as a selection pressure driving the evolution of counter-adaptations to that precedence pattern. Counter-adaptations
can function to help a male to displace or take priority over sperm from previous matings, and/or may function to prevent his sperm from being displaced by future males to mate with a female (Parker, In Press).

Consequences of Sperm Competition.

Male insects have evolved a number of counter-adaptations which apparently function to insure that their sperm is used to fertilize eggs. Possible counter-adaptations to sperm competition include, mate plugs, male induced refractory periods, mate guarding, and the ability to displace sperm from within the spermatheca. (Parker, 1970a; but see Thornhill, In Press).

a) Mate Plugs and Physical Barriers

Mate plugs are physical barriers to future inseminations placed by the male in the female's genital tract, usually the vagina or bursa copulatrix. Mate plugs occur in a wide variety of insects. In the checkerspot butterfly, *Euphydryas editha*, for example, the external genital openings of the female are sealed with a viscous material secreted by accessory glands in the male reproductive system. Once this viscous material has hardened, males are unable to insert a spermatophore in the bursa copulatrix and thus remating is not possible (Labine, 1964). Taylor (1967) reported that the presence of the spermatophore in the bursa copulatrix of the female ermine moth, *Atteva punctella*, may play a role in the inhibition of receptivity in this insect. The spermatophore may plug the bursa and make future inseminations difficult.

The role of mate plugs is uncertain in the members of the order
Lepidoptera as a whole. Ehrlich and Ehrlich (1978) dissected 1,670 female Lepidoptera, represently 303 species, and examined them for the presence or absence of mate plugs and the presence or absence of multiple mating as determine by multiple spermatophores in the bursa copulatrix. The mean spermatophore count for species showing mate plugs was 1.3 spermatophore per female, while the mean spermatophore count for species showing no evidence of mate plugging was 1.25 spermatophores per female. Thus it is not clear whether mate plugs actually prevent multiple mating by females in all species of Lepidoptera that plugging occurs in.

Parker and Smith (1975) noted that the male grasshopper, Locusta migratoria migratoriodies, leaves part of the spermatophore tube in the spermathecal duct after mating, and speculated that the tube is a mate plug which blocks displacement of sperm by subsequent mates. The tube is ejected by the female as she lays her eggs.

Male fireflies, Pierptyx valida (Coleoptera: Lampyridae), possess a genital pocket and the genitalia are located in this pocket. When the female places her genitalia in this pocket an abdominal clamp grips it. One function of the clamp is to manipulate the female so a hard, rubbery sperm plug can be positioned. This plug is thought to be the remains of the spermatophore and remains in place for the life of the female (Wing et al., 1983).

Copulatory plugs are also found in the spider, Phidippus johnsoni. Examination of females before and after remating opportunities showed that mating plugs prevented copulation in approximately 30% of the females (Jackson, 1980).

Several species of garter snake, Thamnophis sirtalis, T. butleri,
and *T. radix* have copulatory plugs. Male *T. radix* will not court a female with an intact seminal plug (Ross and Crews, 1977), and it is possible that male *T. sirtalis* and will also not court females with intact copulatory plugs. Multiple paternity does occur in *T. sirtalis* (Gibson and Falls, 1975). The copulatory plug in *T. sirtalis* may serve two functions. Movements made by the female to remove the plug tends to force sperm into the oviduct where it is stored. Also the copulatory plug may delay another mating insuring that sperm from the first male to mate reached the sperm storage organs (Devine, 1975).

Copulatory plugs are also found in deer mice, *Peromyscus maniculatus*, prairie voles, *Microtus ochrogaster*, rats, *Rattus norvegicus*, and golden hamsters, *Mesocricetus auratus*. The copulatory plug in these species do not prevent multiple matings by females, nor do they hinder successful insemination by the second male to mate (Dewsbury and Baumgardner, 1981). However, a feature of male mating behaviour in each of these species is a series of multiple intromissions without ejaculation, followed by an intromission with ejaculation. The multiple intromissions may function to remove copulatory plugs and allow the second male to copulate successfully (Dewsbury, pers. comm.).

In some insects, the genitalia may act as a physical barrier to repeated copulations. Following mating some Drosophila females exhibit an insemination reaction in which the vagina begins to swell and enlarge. Female *Drosophila sp.* were mated and then dissected at different intervals following mating. Within 4 min of copulation the swelling began and reached a maximum size (approximately 3 - 4 times
greater than its size in the virgin female) at 1 hr after mating. Within 25 - 30 min from copulation the swelling vagina turns opaque. At 2 hr after mating the vagina begins to clear and is completely clear and normal sized at 8 hr after mating. This insemination reaction occurs in many species of Drosophila, including D. mojavensis and D. funebris, but does not occur in D. melanogaster or D. pseudoobscura (Patterson, 1946). The function of the insemination reaction, and its role in sperm competition is not known, but the swollen vagina may be a barrier to future matings.

b) Non-Receptive Periods

Many species of insects show female refractory or non-receptive periods following mating which are the result of substances passed from the male to the female. During these periods females will not respond to courting males or will actively discourage mating attempts by males. Female mosquitos, Aedes aegypti, for example, enter a refractory period after copulating and multiple inseminations occur only when several males copulate with a female in a short period of time. The refractory period is induced by an agent produced in the male accessory glands and is passed to the female via the seminal fluid. The sterilizing effect of this agent lasts the female's lifetime, but requires several hours after mating to take effect (Craig, 1967). Later studies have identified this substance as matrone (Williams and Hagan, 1977).

Baumann (1974) detected Paragonial Substance - 1 (PS-1) in the accessory glands of male Drosophila funebris. The effect of PS-1 is to decrease the sexual receptivity. Virgin females injected with PS-1 show decreased receptivity to males. A similar decrease in
receptivity is noticed in mated D. funebris females (Baumann, 1974; Manning, 1967), and is thought to be due to the transfer of PS - 1 from the male to the female (Baumann, 1974).

Male D. melanogaster court decapitiated virgins more often and with greater persistence than they court decapitated mated females. When given the choice between a virgin female and a recently mated female, males will court the virgin female first. Therefore it seems that male D. melanogaster detect a difference between virgin females and inseminated females. The difference is thought to be mediated by pheromones (Gromko et al., In Press).

Mating in the olive fruit fly, Dacus oleae, also leads to a female non-receptive period. The refractory period is not permanent and its length varies widely. The refractory period is induced by mating, but is not related to the number of sperm passed from male to female, as males that have been rendered aspermic following exposure to irradiation and multiple matings, produce the refractory period as well as intact males (Cavalloro and Delrio, 1974).

Female scorpionflies, Hylobittacus apicalis, show a 3 - 4 hr refractory period after mating. The refractory period is related to copulation duration. When copula duration is less then 15 min, 72 out of 73 females tested would remate within 0.5 hrs of the first copulation. When copula duration was 20, 23, or 23+ min (normal copulation durations), females showed a 3 - 4 hr refractory period. A substance produced by the male accessory glands and transferred to the female during the last 5 min of copulation induces the female refractory period. This substance not only induces the refractory period but also triggers an increase in oviposition rates. The sperm
precedence pattern is unknown for H. apicalis, but if this insect shows second male precedence the refractory period will insure that the last male to mate fertilizes most of the eggs laid (Thornhill, 1976).

The wasp, Nasonia vitripennis, shows a female non-receptive period that lasts for several days after mating (Holmes, 1974). As well, all eggs laid during the first 24 hr following mating are not fertilized and develop into males. Fertilization rates increase following the initial 24 hr post-mating period and female offspring will be produced. The change in fertilization rates is a function of time; if oviposition is delayed for 24 hr after mating both male and female offspring are produced. Van dem Assem and Feuth-De Bruijn (1977) postulated that either a mate plug or some chemical stimulation functions to keep sperm in the spermatheca. Precedence patterns in this wasp are mixed, with the genotype of the male and his prior sexual experience important in determining which male's sperm is used to fertilize eggs (Holmes, 1974). A female refractory period would insure that there is little competition with another male's sperm, thus insuring fertilizations.

Huettel et al. (1976) found a mean refractory period in the plum curculio, Conotrachelus nenuphar, of 2.2 weeks with a range of 1 - 8 weeks. C. nenuphar shows second male precedence and the refractory period helps to insure that no other male will mate with the female and take precedence in fertilizing eggs.

In some cases the male is able to induce not a refractory period where the female does not respond to courtship advances, but a period where she actively avoids male advances. In the checkerspot
butterfly, *Euphydryas editha*, before the mate plug has hardened, the female exhibits behavioural barriers to males attempting to mate. The behaviour consists of the female kicking the male and flying away from him. This active resistance occurs even if the hardened plug is removed, and it appears to be induced by the distention of the bursa copulatrix by the spermatophore. If the nerves to the bursa are severed so that the female does not receive stimulation as a result of the presence of the spermatophore, rematings occur readily (Labine, 1964).

In the midge, *Culicoides melius*, a mated female will vigorously kick the abdomen and genitalia of a second male attempting to mate. The kicking behaviour is thought to be induced by pheromones passed from the male to the female (Linley, 1975). Linley and Hinds (1975) mated male *C. melius* to virgin females, singly-mated females, doubly-mated females, and doubly-mated females with their legs removed so that they could not respond to the male by kicking. Following completion of the mating sequence the females were dissected and the number of sperm in the spermatheca were counted. Males mating with singly mated-females transferred 85% of a full load of sperm, while males mated with doubly-mated females with legs intact, transferred 79% of a full load. Males mated with doubly-mated females with no legs transferred a full load of sperm, as did males mated to virgin females. Therefore the kicking response reduces the number of sperm transferred. Since sperm mixing occurs in *C. melius*, and the number of fertilizations obtained may depend on the proportion of sperm stored within the females' spermatheca, attempts by the males to prevent additional matings may result in little or no competition for
fertilizations.

Male *Heliconius erato* butterflies transfer an antiaphrodisiac pheromone to females at mating. This pheromone causes a species-specific odor in females, and males will not mate with females possessing this odor (Gilbert, 1976). The sperm utilization pattern for *H. erato* is not known, but based on the existence of an antiaphrodisic pheromone, one would expect the precedence pattern to be second male precedence.

c) Mate Guarding and Passive Phases

Another way that males limit sperm competition is to guard the female after mating to prevent other males from mating with her. Mate guarding can take on several forms. In some cases the male and female remain in physical contact while the female oviposits. In the dung fly, *Scatophaga stercoraria*, the male remains mounted on the female after copulation has ceased and raises his abdomen so that the female may oviposit. By remaining mounted the male can defend the mated female from other mating attempts and, since the female shows no refractory period, he defends his sperm from competition from other ejaculates (Parker, 1970c).

Waage (1973) reported mate guarding during oviposition for the female damselfly, *Calopteryx maculata*. Males hold oviposition sites as territories and the female generally must copulate with the male in order to oviposit at his site. Since the male uses his penis to displace sperm from the spermatheca (Waage, 1979), the last male to mate fertilizes all eggs laid. By requiring copulations prior to oviposition males insure that their sperm fertilizes all the eggs laid in his territory (Waage, 1973). Mate guarding during copulations
insures that other males do not invade the territory and mate with ovipositing females. Post-copulatory mate guarding is common in Odonates, and all Odonates known to displace sperm show tandem oviposition or mate guarding (Waage, In Press).

Similar behaviour is observed in the megachild bee, Anthidium maculosum (Alcock et al., 1977). Males establish territories around feeding areas and copulate with the female in that territory. Between copulations males escort females, flying within a few centimeters of the recently mated female and repelling intruding males. This guarding behaviour could serve to decrease the chance of a female remating with a different male and assure paternity for the resident male.

As has been discussed previously, giant water bugs, Abedus herberti, exhibit multiple bouts of copulation interspersed with oviposition periods. Male brooding is a significant investment for the male with the high costs in terms of, decreased ability to obtain prey, decreased ability to obtain copulations, and an increase in the male's chances of being preyed upon. These costs mean that paternity assurance is important to the male giant water bug and multiple copulations function to assure the paternity of each group of eggs laid (Smith, 1980).

The male stick insect, Diapheromera veliei (Orthoptera: Phasmatidae), uses a cercal clamp to grasp the female while coupling. The cercal clamp gives males control over coupling duration. Coupling lasts 3 - 156 hrs in captive pairs with up to 9 intromissions occurring during coupling. The intromission periods occupy approximately 60% of the coupling duration. Females with attached
males are capable of ovipositing. Experiments conducted with D. veliezi have shown that extended matings are probably not a form of male defense against predators. Since copula duration increases with increased male density in the lab, long copula durations may act to insure fertilizations (Sivinski, 1983).

When first male sperm precedence occurs it would benefit the male to find an immature female and guard her until she is able to mate. First male precedence occurs in the spider, *Frontinella pyramitela* (Austad, 1982). Male *F. pyramitela* remain on the web with a soon to mature female and depart the web after mating, thus assuring fertilizations (Austad, In Press).

Parker (1974) has used sperm competition to determine the theoretical duration of mate guarding for a species. The time invested in post-copulatory guarding will be determined when the amount of gain due to guarding is greater than the amount of gain due to withdrawal from guarding that female and searching for another female (Parker, 1974). Insects with a high population density and high values of $P$ should show a longer duration of mate guarding than insects with low $P$ values and low densities.

d) Displacement of Sperm

The preceding counter-adaptations reduce the likelihood of sperm competition occurring by preventing females from remating. Another way counter-adaptations can function is to minimize the effect of sperm already in the spermatheca. The most successful way to minimize the effect of stored sperm is to remove the other sperm from the competition, that is, displace the sperm out of the female's spermatheca.
Lefevre and Jonsson (1962) found that more sperm is passed by the male to the female than is stored in *D. melanogaster* matings. They postulated that sperm from a second mating physically displaces the previously stored sperm. The mechanism of displacement may be flooding the female's reproductive tract with more sperm than can be stored. In *D. melanogaster* the number of sperm stored in the female is 500 - 700, yet the male transfers up to 5 times that number. The excess sperm that are not stored may be used to flush the female's reproductive tract and displace previously stored sperm (Gromko et al., In Press; Parker, 1982). Experiments have verified second male precedence, indicating that sperm from the first male may not be present within the spermatheca (Boorman and Parker, 1976; Lefevre and Jonsson, 1962; Prout and Bundgaard, 1977).

Economopoulos and Gordon (1972) noted that a second mating results in the loss of some or all of the sperm stored in the female milkweed bug, *Oncopeltus fasciatus*. Castrated males are unable to displace stored sperm, therefore, the mechanism of displacement can not just be a simple "washing out" of the spermatheca by male seminal fluid.

Sperm in the Indian meal moth, *Plodia interpunctella*, migrate from the spermatophore, through the seminal duct and common oviduct, into the spermathecal duct and into an elongate spermatheca. Sperm from a second mating pushes sperm stored in the spermatheca back so that sperm closest to the spermathecal duct is used first (Brower, 1975).

Pair et al. (1977), working with sterile backcrosses of the tobacco budworm, *Heliothis virescens*, found two types of sperm,
nucleated eupyrene sperm and anucleate apyrene sperm. Females dissected following mating with a normal *Heliothis virescens* male show eupyrene sperm in the spermatheca. Females mated first with a normal male, and then with a sterile backcross of a *H. virescens* male show anucleate apyrene sperm stored in the spermatheca. The normal eupyrene sperm has been displaced and replaced by the apyrene sperm.

All Lepidoptera produce 2 types of sperm, nucleated eupyrene sperm which fertilize eggs, and the anucleate apyrene sperm. Apyrene sperm are produced in large numbers, are immediately activated during ejaculation by a secretion from the male reproductive tract, show more vigorous motility than eupyrene sperm, and migrate to the spermatheca from the bursa copulatrix. These factors have lead to the hypothesis that the function of the apyrene sperm is to displace sperm that are present in the spermatheca from previous matings (Silberglied *et al.*, 1984).

Dissections of doubly-mated female armyworms, *Spodoptera litura*, showed that sperm displacement occurs with the spermatheca being emptied and filled again (Etman and Hooper, 1979). Immediately following a second mating, 55% of the females examined show sperm in the spermatheca. This percentage drops until at 30 min following a double mating no females show sperm in the spermatheca. At 1 hr after the second mating sperm are again present in 50% of females examined. The mechanism of displacement and replacement of the sperm is not known.

As has been reviewed, the male damselfly, *C. maculata*, removes stored sperm with its penis prior to ejaculation. Fincke (1984) showed that the male damselfly, *Enallagma hageni*, displaces 87% of the previously stored sperm from within a female. The penal morphology of
most damselflies and dargonflies suggests that sperm displacement is very common in members of the order Odonata (Waage, In Press).

Sivinski (1980) suggested that certain unusual sperm forms may have evolved in order to resist displacement due to sperm competition. For example, barbs found on grasshopper sperm may have evolved in order to maintain a position within the spermatheca and to resist displacement by another male's sperm.

MATING BEHAVIOUR IN FIELD CRICKETS.

There are good reasons to suspect that sperm competition occurs in field crickets. Sperm competition occurs in other Orthoptera, including the cockroach, B. germanica (Cochran, 1979), 3 species of grasshoppers, S. gregaria (Hunter-Jones, 1960), P. texanus (Nabours, 1927), and L. migratoria (Parker and Smith, 1975), and the decorated cricket, Gryllodes supplicans (Sakaluk, Unpub MS). Female crickets show the necessary preadaptations for sperm competition to occur, multiple mating by the female prior to oviposition, storage of sperm within the female, long sperm life, and efficient sperm use so that ejaculates can overlap (Parker, 1970a). As well, males exhibit post-copulatory guarding behaviour that may have evolved in the context of sperm competition. This section presents a composite review of cricket mating behaviour, from several species, with specific reference to aspects of cricket biology essential to an understanding of sperm competition and the experiments performed here.

a) Acoustical Behaviour

A calling song is produced by the male rubbing a scraping edge on the inner margin of the lower tegmen (forewing) against a row
of file teeth on the underside of the upper tegmen. Each song is species specific and the specificity is produced by varying the pulse rate (wingstroke rate) and the length and spacing of pulse groups. A group of pulses is regarded as a chirp or trill (Alexander, 1967).

Calling song transmission in crickets has a genetic basis. Hybrids derived by crossing *Teleogryllus commodus* and *T. oceanicus* produced offspring with intermediate calling song patterns. Rearing the crickets under different conditions of temperature, diet, light cycle, time of year, and population density did not affect adult song production. Genotype was the only factor affecting the calling song pattern. Backcrosses of hybrid males to females with the maternal genotype revealed that calling song was polygenic in origin. Three traits were examined more closely and it was found that gene(s) controlling the intertrill interval were carried on the sex chromosome while the number of pulses per trill and trills per phrase were autosomal traits. The genetic basis for song patterns is expressed as genetic control of the neurons responsible for operation of the wing muscles (Bentley, 1971).

Song reception also has a genetic basis and may result from the same genetic template as song production. In phonotaxis experiments, where females were placed in a Y maze and presented with the choice of walking towards or away from a song type, females consistently preferred conspecific song to songs produced by other species of crickets. Hybrid females from a *T. oceanicus* female and *T. commodus* male cross preferred hybrid calls over either parental call (Hoy and Paul, 1973).

*G. integer* males occur in closely spaced groups where a male
can either be a caller or a non-calling, satellite male though intermediate forms of behaviour occur. Calling males call for longer durations than non-calling males. Calling males are also parasitized by the larvae of the fly, *Euphasiopteryx ochracea* (Diptera: Tachinidae), more than non-callers (Cade, 1975; 1979a). Cade (1981a) selected high and low calling lines and demonstrated that the duration in calling has a genetic basis with a realized heritability of 0.50 for the high line, and 0.53 for the low line. Thus calling duration also is under genetic control, and selection pressures exerted by parasitizing flies and male-male competition for females would affect the gene frequency in a population.

Time of calling is also dependent on the sexual readiness of the male. *G. campestris* males will call only when there is stimulation of spermatophore pouch by the presence of a spermatophore (Huber, 1962). Spermatophore formation appears to show diel periodicity under an alternating light cycle of 12 hours dark:12 hours light in *Acheta domesticus*, so that spermatophores are formed during the light phase, and removed in the dark phase (McFarlane, 1968). Cade and Wyatt (1984) found that the age of first spermatophore production, expressed in days from adult moult, was 4.2 days in *G. pennsylvanicus*, 4.8 days in *G. veletis*, 3.7 days in *T. africanus*, and 4.4 days in *G. integer*. There were no significant differences among species for the age of first spermatophore production. Age of first spermatophore production corresponded to the age of first calling song production in the species surveyed.

b) Pre-Courtship Phase

Cricket courtship can be divided into 4 periods: mate attraction
and pre-courtship, courtship, copulation, and post-copulatory phases. During the mate attraction or pre-courtship phase male field crickets call from shallow burrows or depressions in the ground. Calling, in the pre-courtship phase, functions to attract female crickets to the male's position in the field. Both males and females will respond to male calling song and will move towards the source (Boake, 1983), though males do not respond to a calling song as strongly or as consistently as females do (Cade, 1981b; Pollack, 1982). The female's phonotactic response to the calling song allows her to find a male in order to mate.

c) Courtship

Courtship is initiated when the male and female contact each other; species specific pheromones are important in close range communication. Male antennal contact of female body parts elicited courtship behaviour in *A. domesticus* and *T. oceanicus* (Hardy and Shaw, 1983) and *T. commodus* (Loher and Rence, 1978). Contact chemoreception may help individuals to identify conspecifics. Airborne pheromones may also play a role in identifying correct sex and species in *A. domesticus* and *G. integer* (Otte and Cade, 1976). Pheromones may also play a role in initiating male calling song. Paul (1976) tested male response to 4 possible test stimuli, untreated control paper towel, paper towel conditioned with live adult females, paper towel conditioned with live adult males, and live adult females, in the ground crickets *Allonemobius fasciatus*, *A. allardi*, *A. tinnulus* and *P. ambitiosus*. For each species, greater than 75% of males produced a calling song when exposed to either paper conditioned with female scent, or live females. Few males responded to paper conditioned with
male scent or control paper. Thus, for these species of ground crickets, pheromones may play a role in the production of calling song.

Following the initial contact with a female cricket, the male turns his body away from the female and begins to rock in a courtship dance. The male will also antennate the female and begin to produce the courtship song (Alexader and Otte, 1967). The courtship song consists of soft rustling pulses interspersed with louder ticks (Alexader, 1961). The role of the courtship song in the mating sequence is unclear. Khalifa (1950) reported that A. domesticus females will not mount male courting crickets that have had their tegmina removed. Crankshaw (1979) reported that 74% of all courtships performed by A. domesticus males resulted in successful matings. When males had their tegmina removed so that courtship calls could not be produced, none of the males mated. When recordings of courtship song were played to a female placed with a wing-removed male, 62% of the mating attempts were successful. In T. oceanicus, 13% of the courtship attempts accompanied by courtship calls, resulted in successful matings, while only 1% of attempted courtships without courtship call resulted in a female mounting the male and copulation did not occur. Dominant males were more likely to perform the courtship call than subordinate males and females may be using the courtship call to determine dominance rather than species, especially since courtship songs are not species specific (Burk, 1983). Boake and Capranica (1982) silenced Amphiascuta maya males, by covering the file teeth on the wings with wax so that courtship song could not be produced. Silenced males were able to maintain dominance if it had
been established prior to treatment, but were unable to achieve dominance if introduced to a new group of males. Silenced dominant males also attempted copulations less frequently than untreated dominant males. However, silenced males showed no higher failure rate when attempting courtship than non-silenced control males. Boake (1984) concluded that chirps or courtship song is not an essential signal from a male to a female during mating but functions to determine dominance.

Other studies have indicated that the courtship song may not be necessary for mating to occur. Loher and Rence (1978) deafened female crickets by removing their forelegs, however, these crickets could respond to the male and would mate providing their antenna were intact. However, crickets may be able to detect song through spiracles and substrate vibrations, thus removing their forelegs may not completely prevent reception of the song stimulus.

While producing the courtship song the male antennates the female and backs towards her. The courtship phase is concluded when the female begins to antennate the male and touch his cerci. When this happens, the male stops producing the courtship call and lowers his wings. The female mounts the male and copulation occurs (Alexander, 1961; Alexander and Otte, 1967).

d) Copulation

During copulation the male transfers sperm to the female using a spermatophore. When the female has mounted the male the male inserts the tip of his median epiphallus between the base of the female's ovipositor and the subgenital plate. The male extrudes a spermatophore and inserts it with the use of a guiding rod into the
female. The spermatophore tube is placed inside the spermathecal duct while the bulk of the spermatophore remains outside the female. Studies of copulation in *Gryllus assimilis* (Spann, 1934) and an unnamed *Gryllus* sp. (Alexander and Otte, 1967) reported hook like projections on the spermatophore tube. These hooks are fastened to the muscles around the ovipositor with the tube of the spermatophore inserted into the bursa. Copula duration in crickets is short, generally lasting approximately 30 sec (Spann, 1934). Following copulation the female dismounts (Alexander and Otte, 1967).

e) Post-Copulatory Phase

The spermatophore empties by an osmotic process and the sperm move from the tip of the spermatophore tube to the spermatheca by their own movement. In *A. domesticus* the full amount of sperm and most of the seminal fluid are transferred to the female's spermatheca in an hour (Khalifa, 1949). *A. domesticus* females observed in an arena had a mean spermatophore attachment time of 29.3 min, while females that were isolated from males immediately after mating showed a mean attachment time of 38.3 min. There was no significant difference between these attachment times, thus the presence or absence of males does not seem to affect spermatophore attachment time in *A. domesticus* (Sakaluk and Cade, 1980). Offspring production, measured as the number of nymphs hatched, was significantly correlated with spermatophore attachment time in *A. domesticus*, and this may be the result of increased sperm transfer due to longer spermatophore attachment (Sakaluk and Cade, 1983). In *T. commodus*, complete sperm transfer occurs in approximately 68 min (Loher and Rence, 1978).

*G. supplicans* males (Orthoptera: Gryllidae) transfer a
spermatophlax, a large gelatinous mass attached to the spermatophore ampulla, to the female at mating. The female removes the spermatophlax and feeds on it. Females finish eating the spermatophlax on the average 39.8 min after mating, and remove the sperm ampulla 12.2 min later. There was a significant linear relationship between the duration of time a female spends feeding on a spermatophlax and the duration of spermatophore ampulla attachment. There was also a significant linear relationship between the duration of ampulla attachment and the number of sperm transferred to the female's spermatheca, with maximum sperm transfer occurring after 55 min of spermatophore attachment (Sakaluk, 1984).

Post-copulatory behaviour in field crickets consists of mate guarding, where the male stands beside or at 90° to the female and antennates her. In *A. domesticus*, if the female moves away the male may rapidly move backwards and forwards (Khalifa, 1950). Alexander (1961) reported rapid exploratory locomotion by the male if the guarded female moved away, followed by rapid antennation of the female if she was located by the male. *T. commodus* males will respond to movement by the female with antennae vibration, rapid body rocking, or chasing the female (Loher and Renee, 1978). Studies with *T. commodus* in the laboratory show that the female will remain with the male (n = 92) or leave the burrow to oviposit (n = 68) following copulation (Evans, 1983).

Males are unable to copulate again until they have formed another spermatophore (Alexander and Otte, 1967). Female crickets will mate repeatedly without ovipositing (Alexander, 1961; Evans, 1983). *A. domesticus* females mated a mean of 2.8 times when observed for 2 hrs
each night for 15 nights. In this period of observation only 4 out of 67 females mated only once (Salaluk and Cade, 1980). In laboratory experiments all T. commodus females mated more than once prior to ovipositing, and most mated more than 4 times (Evans, 1983). Females eventually refused to copulate unless they oviposited and became receptive again only after oviposition occurred (Alexander, 1961).

Control of oviposition in females crickets is complex and may be influenced by hormones, diurnal thermoperiods, the frequency of mating, and the availability of suitable oviposition sites. Loher (1979a) found that injections of prostaglandin increased oviposition in virgin T. commodus females while saline injections did not increase oviposition. Later work with T. commodus females (Loher et al., 1981) showed that precursors to prostaglandin are present in virgin females, and transfer of the prostaglandin synthetase in mating leads to the production of prostaglandin 2 hrs after mating. Egg laying also begins 2 - 24 hrs after mating, thus indicating that prostaglandin is necessary for oviposition in T. commodus females. Destephano and Brady (1977) found prostaglandin synthetase present in testes, seminal vesicles, vas deferens and spermatophores produced in A. domesticus males. Prostaglandin synthetase activity is also found in the bursa copulatrix, spermatheca and oviducts in mated female crickets. Prostaglandin is present in mated, female crickets but not virgins indicating that they are synthesized after mating. Multiple mating by female crickets may have evolved in the context of female requirements for prostaglandin synthetase transfer from males (Salaluk and Cade, 1983).

Other hormones that influence oviposition are ecdysone and
20-hydroxyecdysone. Injections of these hormones increase the fecundity of mated *G. bimaculatus* females 2.5 times when compared to sham treated controls. Ecdosteriod concentrations are high in the testes of *G. bimaculatus* males but are low in the spermatophores. It is unknown if they are transferred from male to female during mating (Behrens and Hoffmann, 1983).

Changes in diurnal thermoperiod also affect oviposition in *G. bimaculatus* females. Females housed in an environment that changed from 26°C to 14°C on either a 2:2 hr, 7:7 hr or 8:8 hr cycle produced more eggs than females held at any constant temperature (Behrens et al., 1983). This increase in egg laying may be related to hormone production in the female cricket. Female crickets also show a circadian rhythm of oviposition. Mated *T. commodus* females exposed to 12 hr light:12 hr dark photoperiod showed increased oviposition in the light phase of the cycle (Loher, 1979b).

Loher and Edson (1973) showed that sexual receptivity in *T. commodus* females is present throughout life and is not temporarily abolished by mating or oviposition. First oviposition did not occur until 5-8 days after the post-adult moult, no matter when the female mated. The number of eggs a female laid was not influenced by the frequency of mating, however more than 1 copulation may be necessary to ensure fertilization. Mated females not only laid more eggs, but they produce more eggs than virgin females.

Sakaluk and Cade (1980) showed that the number of matings may be important to progeny production in crickets. *A. domesticus* females that have been mated twice to a male produce significantly more offspring than females who were mated only once to a male. Early
experiments with *G. integer* did not show a significant difference in the production of offspring between females who had mated once and females who had mated twice. Subsequent experiments (Sakaluk and Cade, 1983) with a larger sample size, showed that singly-mated females, including females that mated but left no nymphs produced a mean of 368.1 nymphs per female, while doubly-mated females produced a mean of 815.6 nymphs per female. These means for progeny production for singly and doubly-mated females are significantly different from each other.

Female oviposition may also be affected by the availability of a suitable oviposition site. Alexander (1961) reported that female crickets will oviposit only in damp substrate. If a damp surface is not available for oviposition the female will probe at the dry substrate without depositing eggs. Alexander postulated that the ovipositor has a sensory apparatus that must detect dampness for oviposition to occur.

*T. commodus* females deposit more eggs in moist conditions than in dry conditions (Evans, 1983). Laboratory experiments with artificial habitats showed that males tend to burrow in moist areas as well. Also, females tended to oviposit in the male's burrow or calling site (61% of observed ovipositions in a 48 hr period). Females that did not oviposit at a calling site tended to oviposit in areas where other females had oviposited.

Mating Behaviour in *G. integer*

*G. integer*, the insect used in this study, is a field cricket ranging from western Texas to western Florida, north into Missouri and
south into Mexico. It is characterized by its trilling song, which differentiates it from the field crickets that shares its range. It occurs in grassy habitats and has a 2 generational life cycle with no diapause (Alexander and Cade, Unpub MS).

Mating behaviour in *G. integer* follows the same sequence as mating in the cricket species mentioned in the previous section. Male *G. integer* use a calling song to attract females to their position in fields and grassy habitats. Males exhibit variation in sexual behaviour. A male can be a caller, a non-caller or satellite male, or can switch from one behaviour to another. Calling males call for longer periods and at greater intensities than non-calling males. Satellite or non-calling males call infrequently, or will begin to call when a nearby calling male stops calling. Calling males and satellite males also differ in their reactions to broadcasts of *G. integer* cricket song. In response to taped song some males stopped calling and became stationary satellite males. Other males respond by attacking either the speaker or a male cricket tethered in front of the speaker. Calling males will also differ in respect to changes in intensity of their song after exposure to taped playbacks of calling song. Aggressive males showed an increase in song intensity following exposure to taped calling song, while those who respond nonaggressively to taped cricket song decreased the intensity of their song (Cade, 1979a). As has been mentioned previously, the duration of calling by a individual male appears to have a genetic basis (Cade, 1981a).

While calling serves to attract females to the male's location, it also attracts a parasitic fly, *Euphasiopteryx ochracea*, which lays
its larvae on the calling male. *G. integer* males that are parasitized by *E. ochracea* larvae show reduced calling time and a shorter life span than non-parasitized males (Cade, 1975; 1979a; 1984).

Mating proceeds through courtship and copulation as in other field crickets, production of the courting song and antennation of the female by the male is followed by the female mounting the male and copulation occurs (Graham, 1982; Salaluk and Cade, 1980; 1983 Sandford, 1971).

Female *G. integer* maintained as isolated pairs with a male showed an average spermatophore retention time of 61.5 min. Female *G. integer* mated in an arena situation and allowed to remain in the arena after mating showed spermatophore retention times of 30.5 min (Sakaluk and Cade, 1980).

*G. integer* males are able remate rapidly, within 15 min of their last mating (Alexander, 1962; Sandford, 1971). Female *G. integer* will also remate rapidly, females mated an average of 4.4 times over 2 nights of continuous observation in an arena setting, and an average of 3.3 times in 6 hrs of continuous observation when maintained as isolated pairs with a male (Sakaluk and Cade, 1980).

*G. integer* shows a diel periodicity for calling song production with the peak of calling song production occurring 8 - 11 hrs past sunset, or immediately before or during sunrise (Cade, 1979a). In the field the number of matings peaks at dawn (Cade, pers. comm.). However, courting song production and male and female mating rates do not vary with changes in the light and dark cycle in a laboratory environment (Graham, 1982). The laboratory, however, does not have a distinct dawn phase, rather there is a sudden transition from the dark
phase to the light phase.

Oviposition in G. integer appears, as in other crickets, to require a moist substrate. Nymphs hatch 12 - 49 days after first observed oviposition. Observations of G. integer nymphs hatching show that the nymph works its way to the surface of the substrate by undulating movements while still enclosed in a transparent membrane. When the nymph reaches the surface of the substrate it frees itself from the membrane head first (Sandford, 1971).

Specific Behaviour Indicating that Sperm Competition May Occur.

As reviewed previously there are 4 necessary preadaptations for the evolution of sperm competition. These are, multiple mating by the female prior to oviposition, sperm storage within the female, long sperm life, and efficient sperm usage so that ejaculates can overlap (Parker, 1970a). Field crickets show these pre-adaptations. Female crickets can mate several times before ovipositing (Alexander, 1961; Evans, 1983; Sakaluk and Cade, 1980). Sperm are stored in female crickets inside the spermatheca (Spann, 1934). Finally, experiments show that singly mated female A. domesticus and G. integer do not show the sperm depletion patterns that would be expected if the animal had an inefficient sperm utilization pattern. Thus it is likely that ejaculates can overlap in these species (Sakaluk and Cade, 1983).

There are other indications that sperm competition may operate in field crickets, and these also may indicate the order of precedence that may be found in the cricket. The first of these is the shape of the spermatheca. Spann (1934) reported that the shape of the
spermatheca in female G. assimilis is oval, and is approximately 3 mm long and 1.5 mm wide. This indicates that sperm precedence values may tend toward last male priority.

A second indication that sperm competition may have evolved in field crickets is the evolution of possible adaptations to sperm competition. One such counter-adaptation is post-copulatory mate guarding of the female by the male. Khalifa (1950) interpreted mate guarding as having evolved in order to prevent the female from destroying the spermatophore. While there is a relationship between spermatophore attachment time and the average number of offspring produced for A. domesticus in unguarded females (Sakaluk and Cade, 1983), mate guarding does not seem to increase spermatophore attachment time when compared to attachment times for unguarded females (Khalifa, 1950; Sakaluk and Cade, 1980). Loher and Renee (1978) reported that males will prevent attempts by the female to remove the spermatophore and hypothesized that the length of time occupied by mate guarding should correlate with the length of time to empty the spermatophore. Mate guarding in T. commodus lasts on the average 83 min, while it takes 68 min for the spermatophore to empty (Loher and Renee, 1978).

Mate guarding may also function in preventing females from remating with another male (Alexander, 1961; Khalifa, 1950; Loher and Renee, 1978). This can be adaptive because doubly-mated females lay more eggs and have a lower percentage of mating failure than singly-mated females (Salaluk and Cade, 1980; 1983). Males who mate more then once with a female will have more offspring in the next generation.
By preventing females from mating with other males, male mate guarding can function to prevent sperm competition from other males. Mate guarding episodes last approximately 80 min in *T. commodus* (Loher and Renee, 1978). The first stage of oviposition, probing with the ovipositor, also begins approximately 60 min post copulation (Loher, 1979b). Thus mate guarding overlaps with the time necessary to induce oviposition. If second male precedence occurs in field crickets sperm from the last male to mate with the female will fertilize most of her eggs. By guarding the female until she oviposits the male assures that his sperm will be used to fertilize at least some of the eggs laid. Also, the duration of mate guarding in *T. commodus* lasts approximately the length of time that it takes for the male to regain sexual readiness, approximately 2 hrs (Loher, 1981), and may help to keep the female close by until the male is able to mate again.

Another possible counter-adaptation to sperm competition is the induction of a female refractory period by the male. Female crickets do not show a refractory period and are capable of mating immediately following copulation (Loher and Renee, 1978). However, mating blocks the phonotactic response of the female to the male calling song in *T. commodus* (Loher, 1981). The mechanism by which this blocking occurs is not known. Females deprived of contact with males become increasingly phonotactic following the third day of isolation. When males were added to an aquarium containing male-deprived female *G. veletis*, the level of female phonotaxis decreased to that of females who had been constantly exposed to males. It is not known if mating occurred between those females and the introduced males (Cade, 1979b). While this is not a refractory period in the strict sense of
the term (females are able to remate) it may be adaptive for the male. If females are able to response to the courtship song by mating, but do not respond to male calling song, a male may be able to monopolize the female and have her close by in order to remate when he has produced a new spermatophore. *Gryllus* sp. courtship song has a very low intensity and therefore is not audible over long distances (Alexander, 1961; Boake, 1983). Since satellite males occur in some species of this genus (Cade, 1979a), including *G. integer*, the lack of phonotaxis in just mated females may serve to monopolize the female and prevent satellite males from copulating with the females and decrease the probability of sperm competition occurring.

The evidence presented in this section makes it seem likely that sperm competition occurs in the field cricket, and that mate guarding and the depression of female receptivity following mating have evolved in response to sperm competition.
MATERIALS AND METHODS

In this study 4 experiments were performed. The radiation and sterilization experiment was designed to determine the amount of radiation necessary to sterilize *G. integer* males. The diapause experiment was designed to determine if *G. integer* eggs diapause under laboratory conditions. Competition experiment 1 was designed to determine if sperm competition occurs in *G. integer*, and to determine the order of sperm precedence if it does. Competition experiment 2 also was intended to determine if sperm competition occurs and involved different *G. integer* cultures and modifications of the mating procedure.

In this section I describe the culturing of insects, the method of irradiating males, the mating procedure, the oviposition routine, and the research protocol for the 4 experiments.

a) Culturing of Insects.

Crickets used in all experiments were obtained from laboratory stock cultures derived from field captured *G. integer* adults. In all cases, except where noted, cultures had been reared in the lab for more than 8 generations, and crickets were used without considering generation and stock differences. Cultures were maintained in 45 l garbage cans with egg carton shelters. Both the room lights, and lights suspended in the garbage cans were on a 12 hr:12 hr Light:Dark cycle. Food, Purina Cat Chow® and lettuce, was provided ad lib and water was available from test tubes plugged with absorbant cotton and dishes of moist vermiculite. The temperature at the surface of the vermiculite was approximately 30°C with the lights on.
Adults were removed from culture within 24 hrs of the adult moult and were housed individually in 500 ml waxed paper containers (American Can Co.) with clear plastic lids. Each container was labelled with the date of removal from culture so that the age of the individual would be known. As male *G. integer* become sexually mature at 3 - 5 days post adult moult, all crickets removed within 24 hr of the adult moult would be virgins. Individuals were provided with Purina Cat Chow® as food, water in a small vial plugged with absorbent cotton, and a piece of egg carton as shelter. Containers were placed in the laboratory and maintained at approximately 20°C.

b) Irradiation Procedure

Male crickets were irradiated in a 500 ml waxed paper container, similar to the ones described as housing the crickets, that had been divided into 4 approximately equal sized compartments with cardboard barriers. This was done in order to allow up to 4 males to be irradiated at once. As the crickets could move between compartments the males were also individually marked with Liquid Paper® Correction Fluid with a pattern of small dots on the male's pronotum. Control crickets, males that were not irradiated, were placed in a similar container as the irradiated males and were marked in a similar manner.

Irradiation was accomplished by exposing the males to gamma radiation from a Gammacell 220 (Atomic Energy of Canada Limited). Unless otherwise noted the container with the control males was placed on a shelf in the room containing the Gammacell 220. The drawer of the Gammacell 220 was lowered into the irradiation position and the time of exposure was monitored with a stopwatch. Following exposure to the radiation the crickets were returned to the laboratory.
Determination of the amount of time necessary to reach an exposure level involves obtaining the current amount of radiation emitted per min by the Gammacell, and is based on time since installation and the half life of Cobalt-60. Details on this calculation, a sample calculation, and the times used in the following experiments are given in Appendix I.

Crickets were returned to their individual containers, numbered and marked as to the experimental group the individual was assigned to and whether it was an N or R male. Any variation in the irradiation procedure will be noted for each experiment.

c) Mating Procedure

Mating patterns will be discussed separately for each experiment. However, the following procedure was followed for all matings. Matings took place in the lab during the light phase of the L:D cycle, generally starting between 08:00 - 09:00 hr. Mating chambers consisted of a 500 ml waxed paper container each containing a piece of egg carton shelter. Two female G. integer (one marked with a drop of Liquid Paper®correction fluid) were placed into a mating chamber. A male was added and the crickets were observed continuously. When a successful mating occurred, defined as the transfer of a spermatophore from the male to a female, the mated female was removed from the mating chamber and isolated for 60 min in a small test tube. Isolation insured that the female was unable to remove the spermatophore while sperm were transferred. If the female did remove the spermatophore prior to the end of the isolation period, the duration of spermatophore attachment was recorded and the female was retained in the test tube for the full 60 min. Occasionally, a
spermatophore was removed while transferring the female to the test tube. Due to the shortness of the spermatophore attachment time in these cases, this mating was classed as a failure and the female was immediately returned to the mating chamber. Following mating, males were returned to their home container for the isolation period. Unmated females were sacrificed or returned to their home container for use in future experiments.

Following the 60 min isolation period, the mated female would, depending on the mating pattern used, be returned to the mating chamber with the next male in the mating sequence, or if the mating pattern had been completed, the female would be placed in an oviposition dish.

d) Oviposition Procedure

Oviposition was allowed to occur in one of two types of dishes. In the radiation and sterilization experiment, and in the diapause experiment, a large oviposition dish was used, consisting of a 500 ml waxed paper container filled with approximately 250 ml of moist vermiculite. Food was provided in shallow dishes and was changed daily. Females were able to obtain water from the moist vermiculite. Water tubes were not provided as females will oviposit in them (pers. obs.). Oviposition dishes were maintained on a laboratory bench under a 12:12 L:D cycle for the oviposition period.

In competition experiment 1 and 2, a small oviposition dish was used consisting of a 500 ml waxed paper container containing the top or bottom of a 60 X 15 mm petri dish filled with approximately 25 ml of moist vermiculite. Food was provided in shallow dishes and was changed daily.
In all cases, the moisture level of the vermiculite was checked daily, and dishes were sprayed with water if they felt dry to the touch. Females were allowed to oviposit for 10 - 11 days after all matings were complete, unless otherwise noted.

e) Determination of Sperm Motility

Following oviposition, females were removed from the oviposition dishes and were sacrificed. A squash mount preparation of the spermatheca was examined for the presence of motile sperm. To prepare a squash mount the spermatheca was dissected out of the female and placed on a clean microscope slide. The spermatheca was covered with a drop of 0.9% saline solution and then cut in half. A coverslip was lowered onto the microscope slide and the cut spermatheca was gently squashed. The squash mount was examined with a Leitz Laborlux 11® binocular compound microscope on 100X, and then 400X, magnification. Movement of the spermatozoa was readily visible without staining. Sperm were scored as either motile or non-motile.

f) Incubation Procedure

With the exception of the diapause experiment, eggs were incubated for 8 weeks from the day the female was sacrificed or until 2 weeks after the last nymph emerged, whichever period was longer. Oviposition dishes in the diapause experiment were incubated for 350+ days. In all cases dishes were checked daily for the presence of nymphs and were moistened with water if needed. Emerging nymphs were mouth aspirated daily and the number of nymphs emerging from each dish was recorded. Except where noted, dishes were incubated in the lab on a bench under 12:12 L:D cycle and at normal room temperatures.
Following the incubation period dishes were examined for unhatched eggs. The soil in the dish was allowed to dry at room temperature. Small amounts of soil were placed in a petri dish and examined under a Wild® dissecting microscope at 100X magnification until all the soil in the dish was examined. All unhatched eggs were counted; any nymphs that had hatched but not emerged from the soil found in the oviposition dish, were also counted and added to the total number of nymphs hatched.

Radiation and Sterilization Experiment

This experiment was designed to determine the dosage of radiation necessary to sterilize male G. integer. Adult male crickets were removed from culture and housed individually. When sexually mature, a male was randomly assigned to either the R (irradiated male) group, or the N (control) group. Males in the R group were exposed to 7,000 rad of gamma irradiation. (For the time necessary to give exposure to 7,000 rad see Appendix I.) Control males in this case were treated as described above, with the exception of being placed in the radiation drawer of the Gammacell 220, without the drawer being lowered into the irradiation position, for the same length of time that the R males were irradiated.

Females were mated twice to the same male, either an R or N male, and were then allowed to oviposit. Following 10 - 11 days of oviposition the females were sacrificed.

In this experiment, oviposition dishes were incubated in a garbage can identical to the ones used for stock cultures. The plastic lid on the oviposition dish was replaced by a cheesecloth
covering to increase air circulation in the dish and to decrease mold formation on the soil in the dishes. Dishes were placed in the garbage can and kept warm with a 100 watt light bulb suspended in the can. The light bulb was set on a 12:12 L:D cycle. During the incubation period, dishes were monitored every day according to the protocol discussed above.

Motility of sperm following exposure to irradiation was determined using males and females from a related species, *Gryllus rubens*. (For a discussion concerning the relationship between *G. rubens* and *G. integer* see Appendix III.) Sexually mature male *G. rubens* were exposed to 7,000 rad of gamma irradiation and then placed, with an equal number of females, in a large jar with food, water, and egg carton shelters. Jars were maintained in the lab on a 12:12 LD cycle, at room temperature (approximately 23°C). Following 48 hrs, females were removed from the jars and the spermatheca was dissected out and examined for the presence of motile sperm using the procedure described above.

Diapause Experiment

This experiment was designed in order to determine if *G. integer* eggs diapaused under laboratory conditions. Whether or not *G. integer* diapaused in the laboratory was important so that the incubation period set for the competition experiments would be long enough to insure that all viable eggs hatched. Females were mated twice to the same male, in all cases a normal untreated male, and were then allowed to oviposit 10 days. Following the oviposition period, the females were sacrificed.
Oviposition dishes were incubated as described in the radiation experiment. Dishes were incubated for 350+ days, and were monitored daily for emerging nymphs that were mouth aspirated, counted, and recorded. The vermiculite was moistened if dry. Following the end of incubation, the dishes were not examined for unhatched eggs and were discarded.

Competition Experiment 1

Competition experiment 1 was designed to test whether sperm competition was occurring in *G. integer*, and to determine the order of sperm precedence. The irradiated male technique was used to test for precedence. Three experimental groups were designated: an RN group, where a female would be mated twice to an R male and then mated twice to an N male; an NR group, where a female would be mated twice to an N male and then mated twice to an R male; and an NN group, where a female would be mated twice to an N male and then mated twice to a second N male. The rationale for using 2 matings with each male is presented in Appendix IX.

In this experiment, all matings for one experimental group were completed before the next experimental group was started. Pairs of adult males were matched for age. One male would be designated the R male and would be exposed to 7,000 rad of gamma radiation, the dose necessary to induce sterility. The control male would not be exposed to gamma radiation, but was placed in a chamber similar to the irradiation container and set on a shelf in the room housing the Gammacell 220 while the R cricket was being irradiated. The control chamber was covered with aluminum foil to match the darkness of the
container inside the Gammacell 220. Males assigned to the NN experimental group were treated identically to the N males in the other experimental groups.

Matings followed the pattern outlined in Figure 1. Two females were placed inside the mating chamber with the first male of the mating pattern. The first female to mate with the male would become the experimental female, and the non-mated female would be returned to her home container. The female would be mated twice to the first male of the mating pattern, and then mated twice with the second male in the mating pattern. A 60 min isolation period followed each mating, to insure that each female did not remove the attached spermatophore prior to maximum sperm transfer. All matings took place on the same day. No males were used in more than one experiment.

Following 10 days of oviposition, females were sacrificed and dissected, as has been discussed previously. Oviposition dishes were kept at approximately 25°C, on 12:12 L:D cycle for 8 eight weeks, or 2 week following last nymph emergence, whichever period was longer.

Competition Experiment 2

Competition experiment 2 was designed as a replicate of competition experiment 1 with some important modifications. Results obtained in competition experiment 1 indicated that preferential usage of sperm from irradiated males may have been occurring. As well, the results obtained in competition experiment 1 were at variance with those obtained for other Orthoptera, therefore a replicate experiment was designed. All crickets used in competition experiment 2 were obtained from stock derived from the same parents. This was the
Figure 1  A Flowchart Showing the Mating Pattern for Females in Competition Experiment 1.
Figure 1. A Flowchart Showing the Mating Pattern Followed for Females in Competition Experiment 1.
second lab reared generation for this stock line. The same line was used in order to control for effects due to fecundity differences that may exist among lines. Adult males were treated in a manner similar to those in competition experiment 1. The same experimental groups, NR, RN, and NN were used, however, the assignment to experimental groups was random and matings involving all experimental groups were performed at the same time. Some males were used in more than one mating experiment, however no male pair was used more than once.

Matings involved a similar pattern to those done in competition experiment 1, this procedure is summarized in Figure 2. Two females were placed with the first male of the experimental pair. The female that mated with the male was retained for the duration of the experiment and the non-mated female was returned to its home container. Females were mated twice to the first male of the sequence, and then mated twice to the second male in the sequence with a 60 min isolation period following each mating, as described in the general procedures section. Females were given the opportunity to remate following each isolation period. Females that did not remate on the same day were given access to the next male in the sequence for 2 days, or until the mating sequence was complete.

The oviposition routine also varied from that used in competition experiment 1. Females were allowed access to an oviposition dish if they had mated. Females who had completed the mating sequence were placed into a fresh oviposition chamber for an 8 day oviposition period. Following the 8 day oviposition period, the female was transferred into a new oviposition chamber. Females were transferred twice yielding 3 oviposition dishes per female. This was done to
Figure 2. A Flowchart Showing the Mating Pattern for Females in Competition Experiment 2.
Figure 2:
A Flowchart Showing the Mating Pattern for Females in Competition Experiment 2

1. Mate with First Male
   - In Progress Dish
   - Incubate IP Dish
   - Examine IP Dish for Unhatched Eggs

2. Remate with First Male
   - In Progress Dish
   - Incubate IP Dish
   - Examine IP Dish for Unhatched Eggs

3. Mate with Second Male
   - In Progress Dish
   - Incubate IP Dish
   - Examine IP Dish for Unhatched Eggs

4. Remate with Second Male
   - Oviposition Dish 1
   - Incubate Dish 1
   - Examine Dish 1 for Eggs

5. Oviposition Dish 2
   - Incubate Dish 2
   - Examine Dish 2 for Eggs

6. Oviposition Dish 3
   - Incubate Dish 3
   - Examine Dish 3 for Eggs
determine if there was any change in precedence values as a function of time since mating.

Each oviposition dish was incubated for 8 weeks following the removal of the female, or for 2 weeks from the day of last nymph emergence, whichever was longer. Dishes were maintained as described above.
RESULTS

In this section I present the results of the 4 experiments I performed. Life history data for the individuals used in the 2 competition experiments, including age of males at mating, age of males at death, and age of females at mating, are presented in Appendices IV and VI. All ages represent the number of days post adult moult.

Radiation and Sterilization Experiment

The mean age of the R group males on the day of mating was 8.5 days, ranging from 5 - 11 days (S.D = 1.6, N = 15). The mean age of the N group males on the day of mating was 8.0 days, ranging from 6 - 10 days (S.D. = 1.6, N = 5). There was no significant difference between the two groups for the age of the male at mating (Mann-Whitney U test, U = 31.5, p > 0.05).

The mean age of death for males in the R group was 22.8 days, ranging from 16 - 28 days (S.D. = 3.0, N = 15). The mean age of death for males in the N group was 59.2 days, ranging from 27 - 77 days (S.D. = 19.0, N= 5). There was a significant difference between the two groups of males for age of death (U = 1, p < 0.05, two-tailed).

Examination of the spermatheca of female G. rubens that had been mated with irradiated male G. rubens revealed motile sperm in the spermatheca in 8 out of 12 females examined. Of the remaining 4 females, I was unable to find the spermatheca for 3 females and the spermatheca was empty in 1 female.

Productivity is defined as total number of eggs laid by a female
in an oviposition period, and is the sum of the nymphs emerged and the unhatched eggs counted for a female. The mean productivity of females in the R and N groups is presented in Table 2. There was no significant difference in mean productivity between females mated in the R group and females mated in the N group.

The mean proportion of eggs hatched in the R group and the N group is presented in Table 3. There was a significant difference in the proportion of eggs hatched between the two groups, with a lower proportion hatched occurring following mating with an R male.

Diapause Experiment

Mean time from termination of the female to first nymph emergence was 22.2 days, with a range of 18 - 31 days (S.D = 4.9, N = 6). Mean number of days of nymph emergence was 28.5 days, with a range of 9 - 51 days (S.D. = 19.1, N = 6). Figure 3 shows the mean number of nymphs emerging per day from the first day of hatch. No nymphs emerged from day 51 following the first day of nymph emergence until cultures were terminated following a minimum of 300 days additional oviposition for all dishes. Two oviposition dishes, not included in the data above, did not hatch a nymph in incubation periods of 368 and 381 days.

Competition Experiment 1

Life history data for each treatment group, including age of males at mating, age of female at mating, and age of males at death are presented in Appendix IV. Within and between group comparisons for these parameters are also presented in Appendix IV. There was
TABLE 2  Productivity (nymphs + unhatched eggs) of females mated with R males or mated with N males in the radiation and sterilization experiment.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>X PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>337.5</td>
<td>201.4</td>
<td>65 - 733</td>
<td>15</td>
</tr>
<tr>
<td>N</td>
<td>291.2</td>
<td>128.3</td>
<td>153 - 500</td>
<td>5</td>
</tr>
</tbody>
</table>

U = 35.5,  p > 0.05.
TABLE 3 Proportion of eggs hatched following mating with an R male, or following mating with an N male in the radiation and sterilization experiment.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>X PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>0.01</td>
<td>0.02</td>
<td>0 - 0.04</td>
<td>15</td>
</tr>
<tr>
<td>N</td>
<td>0.67</td>
<td>0.27</td>
<td>0.19 - 0.86</td>
<td>5</td>
</tr>
</tbody>
</table>

U = 0, p < 0.05, one-tailed.
Figure 3. Mean Nymph Emergence per Day, Following First Nymph Emergence, in the Diapause Experiment. ($\bar{x} \pm 1$ S.D.)
FIGURE 3.

N=6

MEAN NUMBER OF NYMPHS (± 1 S.D.)

DAY FOLLOWING FIRST NYMPH EMERGENCE
one significant difference among these life history parameters. There was a significant difference between the age of death of R males belonging to both the NR and RN groups and N males belonging to the NN, the NR, and the RN groups. These data are presented in Table IV - 8.

Examination of the females' spermatheca in order to determine sperm motility revealed that 3 out of 5 females in the NN group, and all females in the NR and RN groups (N = 16 in each group) showed motile sperm present in the spermatheca following 10 days of oviposition. I was unable to find the spermatheca in 2 out of 5 females in the NN group.

Mean productivity (nymphs plus unhatched eggs) for each of the treatment groups is presented in Table 4. A Kruskal-Wallis test revealed no significant difference in mean productivity between the 3 treatment groups.

Proportion of eggs hatched in the treatment groups is presented in Table 5. A Kruskal-Wallis test revealed a significant difference in the proportion of eggs hatched between the 3 treatment groups. Pairwise combinations of Mann-Whitney U tests revealed that each group was significantly different than the other groups for the proportion of eggs hatched.

The proportion of eggs fertilized by the second male to mate in the NR treatment group was 0.98. The proportion of eggs fertilized by the second male to mate in the RN treatment group was 0.15. The mean of these two values, $P = 0.57$. The calculation of $P$ for competition experiment 1 can be found in Appendix V.
TABLE 4  Between group productivity (nymphs + unhatched eggs) in competition experiment 1.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>$\bar{x}$ PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>262.2</td>
<td>134.2</td>
<td>0 - 492</td>
<td>13</td>
</tr>
<tr>
<td>NR</td>
<td>319.6</td>
<td>283.1</td>
<td>0 - 1001</td>
<td>16</td>
</tr>
<tr>
<td>RN</td>
<td>340.4</td>
<td>136.1</td>
<td>113 - 576</td>
<td>16</td>
</tr>
</tbody>
</table>

$H = 1.70$, $p > 0.05$. 
TABLE 5  Between group differences in proportion of eggs hatched in competition experiment 1.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>0.68</td>
<td>0.26</td>
<td>0 - 0.90</td>
<td>13</td>
</tr>
<tr>
<td>NR</td>
<td>0.02</td>
<td>0.05</td>
<td>0 - 0.18</td>
<td>16</td>
</tr>
<tr>
<td>RN</td>
<td>0.11</td>
<td>0.09</td>
<td>0 - 0.26</td>
<td>16</td>
</tr>
</tbody>
</table>

* H adj. 26.53, p < 0.05.

NN vs NR  U = 12.9, p < 0.05, two-tailed.

NN vs RN  U = 15.5, p < 0.05, two-tailed.

NR vs RN  U = 41, p < 0.05, two-tailed.

* H adj. used rather than H as there were a high number of tied scores.
Competition Experiment 2

Life history data for Competition Experiment 2, including within and between group differences in age of males at mating, age of males at death, age of females at mating, and within group differences for spermatophore attachment time and oviposition period are in Appendix VI. There was a significant difference in the age at mating of the first male between males assigned to the NR group and males assigned to the NN and the RN groups. These data are presented in Table VI - 3. As well, there was a significant difference in the age of death of R males assigned to the NR group when compared to R males assigned to the RN group. These data are presented in Table VI - 6. In general, R males assigned to either the NR or the RN group died significantly sooner than N males assigned to any group. These data are presented in Table VI - 9. There were no other significant differences in life history in competition experiment 2.

In Progress (IP) dishes were those that females were allowed access to if they had not completed the mating pattern in 1 day. Within group comparisons for mean productivity (nymphs plus unhatched eggs) and the proportion of eggs hatched between the In Progress (IP) dish and other oviposition dishes are presented in Appendix VII. As well, between group comparisons for productivity (nymphs plus unhatched eggs) and proportion of eggs hatched in the In Progress (IP) dishes are presented in Appendix VII. The IP dish showed significantly different mean productivity than oviposition dish 2 in both the NN and RN groups. These data are presented in Table VII - 1. There were no other significant within group productivity differences between the IP and other oviposition dishes. The mean proportion of
eggs hatched in the IP dish of the RN group was significantly lower than the mean proportion of eggs hatched in any other oviposition dish in the RN group. These data are presented in Table VII - 2. There were no other significant within group differences for the mean proportion of eggs hatched between the IP dish and any other dish.

The mean proportion of eggs hatched in the IP dish in the RN group was significantly lower than the mean proportion of eggs hatched in the IP dish for any other treatment group. These data are presented in Table VII - 3.

Examination of the spermatheca for the presence of motile sperm, following the oviposition period, revealed that motile sperm were present in 9 out of 9 females examined in the NN group, and 7 out of 7 females examined in the NR group. As well, motile sperm were present in 8 out of 9 females examined in the RN group. I was unable to find the spermatheca in 1 female in the RN group.

Within group comparisons of the mean productivity for the 3 oviposition dishes are in Table 6. Within the RN group the mean productivity of dish 3 was significantly different than the mean productivity of both dish 1 and dish 2. There were no other significant productivity differences within the groups.

The mean proportion of eggs hatched from the 3 oviposition dishes can be found in Table 7. There were no within group differences for the mean proportion of eggs hatched in oviposition dishes 1, 2, or 3.

The mean productivity (nymphs plus unhatched eggs) for each oviposition dish, compared between the 3 groups is shown in Table 8. There were no significant differences between the 3 groups for mean productivity in any oviposition dish.
TABLE 6  Within group differences in productivity for oviposition dishes 1, 2, and 3 in competition experiment 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DISH</th>
<th>( \overline{x} ) PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A</td>
<td>NN</td>
<td>1 155.6</td>
<td>146.0</td>
<td>24 - 488</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 200.7</td>
<td>119.7</td>
<td>93 - 479</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 128.8</td>
<td>101.6</td>
<td>10 - 325</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( H = 3.90, P &gt; 0.05. )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>NR</td>
<td>1 165.0</td>
<td>120.5</td>
<td>8 - 356</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 156.9</td>
<td>112.4</td>
<td>5 - 317</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 98.8</td>
<td>71.0</td>
<td>0 - 214</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( H = 2.19, P &gt; 0.05. )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6C</td>
<td>RN</td>
<td>1 181.3</td>
<td>83.9</td>
<td>12 - 315</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 193.2</td>
<td>76.6</td>
<td>32 - 302</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 124.2</td>
<td>58.8</td>
<td>18 - 261</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( H = 6.64, P &lt; 0.05. )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6C 1 vs 2  \( U = 70, p > 0.05. \)

2 vs 3  \( U = 30, p < 0.05, \) two-tailed.

1 vs 3  \( U = 37.5, p < 0.05, \) two-tailed.
TABLE 7  Within group differences in proportion of eggs hatched in oviposition dishes 1, 2, and 3 in competition experiment 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DISH</th>
<th>PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>7A</td>
<td>NN</td>
<td>1</td>
<td>0.26</td>
<td>0.23</td>
<td>0.03 - 0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.25</td>
<td>0.19</td>
<td>0.03 - 0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.26</td>
<td>0.23</td>
<td>0.01 - 0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H adj = 0.06, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7B</td>
<td>NR</td>
<td>1</td>
<td>0.15</td>
<td>0.23</td>
<td>0 - 0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.12</td>
<td>0.15</td>
<td>0 - 0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.07</td>
<td>0.07</td>
<td>0 - 0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H adj = 0.79, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7C</td>
<td>RN</td>
<td>1</td>
<td>0.19</td>
<td>0.20</td>
<td>0 - 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.18</td>
<td>0.18</td>
<td>0.2 - 0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.22</td>
<td>0.20</td>
<td>0.3 - 0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H adj = 0.37, p &gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 8  Between group differences in productivity for dishes 1, 2, and 3 in competition experiment 2.

<table>
<thead>
<tr>
<th>DISH</th>
<th>GROUP</th>
<th>X PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NN</td>
<td>155.6</td>
<td>146.0</td>
<td>24 - 488</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>165.0</td>
<td>120.5</td>
<td>8 - 356</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>RN</td>
<td>181.3</td>
<td>83.9</td>
<td>12 - 315</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H = .76, p &gt; 0.05.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>NN</td>
<td>200.7</td>
<td>119.7</td>
<td>93 - 479</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>156.9</td>
<td>112.4</td>
<td>5 - 317</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>RN</td>
<td>193.2</td>
<td>71.6</td>
<td>32 - 302</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H = .6, p &gt; 0.05.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NN</td>
<td>128.8</td>
<td>101.6</td>
<td>10 - 325</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>98.8</td>
<td>71.0</td>
<td>0 - 214</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>RN</td>
<td>124.2</td>
<td>58.8</td>
<td>18 - 261</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H = .87, p &gt; 0.05.</td>
<td></td>
</tr>
</tbody>
</table>
Total productivity is defined as the sum of the number of nymphs plus unhatched eggs across all 3 oviposition dishes. The mean total productivity is shown in Table 9. There were no significant differences between groups for total productivity.

The mean proportion of eggs hatched for oviposition dishes 1, 2, and 3 is shown in Table 10. There were no significant differences in the mean proportion of eggs hatched between the 3 treatment groups for either dish 1 or 2. For dish 3 the mean proportion of eggs hatched in the NR group was 0.07, and this value was significantly different than the proportion of eggs hatched in the NN group but not the RN group.

Aggregate proportion hatched (the mean proportion hatched over all oviposition dishes) is shown in Table 11. The mean aggregate proportion hatched for the NR group was significantly different than the mean aggregate proportion hatched for the NN group but not the RN group.

The proportion of eggs fertilized by the second male to mate in an NR pattern was 0.52, while the proportion of eggs fertilized by the second male to mate in an RN pattern was 0.72. This gives a mean $\bar{P}$ of 0.62. The calculation of $\bar{P}$ for competition experiment 2 can be found in Appendix VIII.
TABLE 9  Between group total productivity in competition experiment 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>455.2</td>
<td>320.4</td>
<td>24 - 1103</td>
<td>11</td>
</tr>
<tr>
<td>NR</td>
<td>402.7</td>
<td>270.2</td>
<td>13 - 706</td>
<td>11</td>
</tr>
<tr>
<td>RN</td>
<td>498.7</td>
<td>186.5</td>
<td>62 - 768</td>
<td>12</td>
</tr>
</tbody>
</table>

H = 0.75, p > 0.05.
TABLE 10  Between group differences in proportion hatched for 3 treatment groups for dishes 1, 2, and 3 in competition experiment 2.

<table>
<thead>
<tr>
<th>DISH</th>
<th>GROUP</th>
<th>\overline{x} PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10A</td>
<td>NN</td>
<td>0.26</td>
<td>0.23</td>
<td>0.03 - 0.74</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>0.15</td>
<td>0.23</td>
<td>0 - 0.78</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>RN</td>
<td>0.19</td>
<td>0.20</td>
<td>0 - 0.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{H adj} = 2.68, \textit{p &gt; 0.05}.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10B</td>
<td>NN</td>
<td>0.25</td>
<td>0.19</td>
<td>0.3 - 0.53</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>0.12</td>
<td>0.15</td>
<td>0 - 0.49</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>RN</td>
<td>0.18</td>
<td>0.18</td>
<td>0.02 - 0.56</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{H adj} = 3.3, \textit{p &gt; 0.05}.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10C</td>
<td>NN</td>
<td>0.28</td>
<td>0.23</td>
<td>0.01 - 0.79</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>0.07</td>
<td>0.07</td>
<td>0 - 0.19</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>RN</td>
<td>0.22</td>
<td>0.19</td>
<td>0.03 - 0.65</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{H adj} = 6.96, \textit{p &lt; 0.05}.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10C NN vs NR  \textit{U} = 14, \textit{p < 0.05}, two-tailed.

NN vs RN  \textit{U} = 50.5, \textit{p > 0.05}.

NR vs RN  \textit{U} = 30, \textit{p > 0.05}.
TABLE 11 Between group aggregate proportion hatched in competition experiment 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>$\bar{x}$ PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>0.26</td>
<td>0.17</td>
<td>0.1 - 0.65</td>
<td>11</td>
</tr>
<tr>
<td>NR</td>
<td>0.13</td>
<td>0.16</td>
<td>0 - 0.55</td>
<td>11</td>
</tr>
<tr>
<td>RN</td>
<td>0.19</td>
<td>0.11</td>
<td>0.02 - 0.41</td>
<td>12</td>
</tr>
</tbody>
</table>

$H_{adj} = 7.77, p < 0.05.$

NN vs NR $U = 21.5, p < 0.05$, two-tailed.

NN vs RN $U = 49, p > 0.05.$

NR vs RN $U = 33.5, p > 0.05.$
DISCUSSION

In this section I will discuss the data presented in the previous section concerning the effects of radiation on adult male *G. integer*, the diapause or non-diapause of *G. integer* in the lab, and the results of the 2 competition experiments. Conclusions will be drawn, based on the results obtained in the competition experiments, as to the type of sperm utilization pattern shown by *G. integer*. Also, the sperm utilization pattern shown by *G. integer* will be used to discuss the evolution of male and female mating behaviour in field crickets.

Sperm Utilization Patterns in *Gryllus integer*.

In the radiation and sterilization experiment the proportion of eggs hatched from females mated with normal males was much greater than that for females mated with males exposed to 7,000 rad of gamma radiation. The proportion of eggs hatched by females in the R group was so low, that 7,000 rad was apparently sufficient to sterilize adult male *G. integer*. This dose was therefore used in subsequent experiments.

These results are comparable to those reported elsewhere on other species of insects. Sterilization of male insects is reported to occur at doses ranging from 3,000 rad in *Rhagoletis pomonella* (Myers et al., 1976) up to 20,000 rad in the decorated cricket, *Gryllodes supplicans* (Sakaluk, Unpub MS). The dose used in this study was in the midrange of those presented in Appendix I.

The low proportion of eggs hatched from females mated to R males may be due to 3 reasons. The first is that the sperm from males exposed to 7,000 rad of gamma radiation is unable to fertilize eggs,
that is, sperm inactivation has occurred. The second is that sperm from males exposed to radiation have had dominant lethal mutations induced, such that the sperm can fertilize eggs, but these eggs are inviable and cannot hatch. Thirdly, the sperm transfer or insemination mechanism was damaged by the radiation, in such a way that sperm were not being transferred to the female.

In this connection, Lim et al., (1972) revealed significant chromosomal abnormalities in 6 species of crickets following exposure to up to 2,500 rad of gamma radiation, and I observed motile sperm present in the spermatheca of female \textit{G. rubens} following mating with irradiated conspecific males. Thus it seems likely that sterilization of male \textit{G. integer} following irradiation with 7,000 rad of gamma radiation is due to a dominant lethality being induced in the chromosomal complement of sperm, such that the zygote is formed but killed during development. Also, the unhatched eggs belonging to the R group appeared to contain embryos, while the unhatched eggs from the N group did not (pers. obs.). Therefore, it seems that the observed results were not due to sperm inactivation.

It was also unlikely that the low proportion of eggs hatched following mating with R males was due to a defect in sperm transfer, or the insemination process. Female \textit{P. guttiventris}, \textit{G. bimaculatus}, and \textit{T. commodus} show a rapid increase in oviposition rates following mating when compared to unmated females (Bentur et al., 1977; Loher and Edson, 1973). However, virgin female \textit{T. commodus} and female \textit{T. commodus} mated with testectomized males do not show any significant difference in the number of eggs laid following mating, indicating that a factor produced in the testis of the male is responsible for
the increase in oviposition following mating. Later experiments showed that this mating factor is prostaglandin synthetase (Destephano and Brady, 1977; Loher, 1979a). If the insemination process was not operating correctly in the pairs of crickets used in the radiation and sterilization experiment presented here, this factor could not be transferred to the female and the females in the R group would show significantly lower productivity (nymphs plus unhatched eggs) than females in the N group. The lack of significant difference in mean productivity between female G. integer mated with N males, and those mated with R males, indicated that radiation did not affect the male's ability to transfer materials to the female during insemination.

A second result of exposure to 7,000 rad of gamma radiation was that the life span of R group males was significantly shortened compared to the lifespan of males assigned to the N group. These results were consistent throughout both competition experiments as well, in each case males assigned to the N group lived significantly longer than males assigned to the R group. The results reported here concerning the lifespan of male crickets exposed to gamma radiation confirm those presented in 1971 by Hunter and Krithayakirn. They found decreased longevity in both male and female adult A. domesticus exposed to 4,000 - 10,000 rad of gamma radiation. Therefore 7,000 rad of gamma radiation was an appropriate dose of radiation for use with the sterile male technique to determine the sperm utilization pattern in female G. integer. Reduced longevity in G. integer and other species is thought to result from deleterious mutations.

In the diapause experiment, no eggs hatched after 51 days of
nymph emergence, indicating that there was no diapause under laboratory conditions for G. integer. The pattern of nymph emergence shown in this experiment was very similar to the pattern of progeny production found by Sakaluk and Cade (1983) for both singly and doubly-mated A. domesticus and singly and doubly-mated G. integer. In these studies nymph emergence peaked within the first 10 days of emergence then decreases until no more nymphs are produced. G. integer females are therefore different from G. firmus females, a species in which field collected females captured in fall lay both diapausing and non-diapausing eggs within the same batch (Ibrahim and Walker, 1980; T.J. Walker, 1980). The absence of diapausing eggs indicated that 8 weeks of incubation is sufficient for all viable eggs to hatch. In order to insure that all viable eggs had hatched in competition experiments 1 and 2, I set the incubation period as either 8 weeks following removal of the females, or 2 weeks past the last recorded date of nymph emergence, which ever was longer.

There are 3 possible results of competition experiments: first male precedence, last male precedence, or sperm mixing. With first male precedence the proportion of eggs hatched from the NR group is not significantly different from the NN group since the first male to mate, the N male, fertilizes most of the eggs laid by the female. The proportion of eggs hatched in the RN group is significantly lower than that in the NN and NR groups, and should approach zero. With second male precedence the proportion of eggs hatched in the RN group is not significantly different than the proportion of eggs hatched in the NN group, and both are significantly greater than the proportion of eggs hatched in the NR group. With sperm mixing the proportion of
eggs hatched in the NR and RN groups is not significantly different, and the proportion of eggs hatched in the 2 treatment groups will be lower than the proportion of eggs hatched in the NN group.

Sperm utilization patterns are not discrete but rather are continuous. For example, the pattern shown by a species may lie somewhere between sperm mixing and last male precedence, or sperm mixing and first male precedence. In this case the results obtained by the sterile male technique may fit none of the patterns described, but will tend toward one pattern over the others. The previous 3 predictions will be used, along with $P$ values, to discuss what sperm utilization pattern occurs in *C. integer*.

In competition experiment 1 there was no difference in mean productivity (nymphs plus unhatched eggs) for females mated in any treatment group or the control group, therefore there were probably no differences between males in any group in terms of ejaculate composition or mechanism of sperm transfer for reasons discussed previously.

The significant difference in the proportion of eggs hatched between the 2 treatment groups and the control group indicated that there are differential sperm utilization patterns among the 3 treatment groups. Closer examination of the mean proportion hatched values showed that they best approximate a pattern of sperm mixing: although the mean proportion hatched for the NR and the RN group (0.02 and 0.11 respectively) are statistically different, they are both closer to each other than they are to the mean proportion hatched of the NN group (0.68). The $P$ value of 0.57 indicated that the sperm utilization pattern shown by *C. integer* is sperm mixing, tending
toward a slight advantage to the second male to mate. Therefore it seems that following an NR or RN mating the sperm contained within the spermatheca mix and approximately equal proportions of eggs are fertilized by sperm from each male.

These data however are confounded by the proportion of eggs fertilized by the R male for the 2 treatment groups. The proportion of eggs fertilized by the R male in the NR group was 0.98, while the proportion of eggs fertilized by the R male in the RN group was 0.85. Therefore sperm from the R males were fertilizing more eggs than sperm from the N males, regardless of the order of mating. It is unclear why the sperm from R males should be fertilizing more eggs than sperm from N males, even under conditions of sperm mixing. One possible explanation is that R sperm out-compete N sperm through increased motility. The opposite effect can also occur, such that, irradiation damages the male’s sperm making affected cells less competitive than sperm from normal males. Ignatowicz et al., (1983) exposed male mold mites, *Tyrophagus putrescentiae*, to either 60, 80, or 100 krad of gamma radiation. Females mated with R males in either an NR or RN pattern laid many eggs and the proportion of eggs hatched was between 0.89 and 0.99, regardless of mating order or the radiation dosage used. Females mated with R males alone had very low fecundity, and no eggs hatched. It was in order to control for differences in the ability of sperm from the R males and the N males to fertilize eggs that reciprocal crosses are used, that is, both NR and RN mating patterns.

In order to test to see if the apparent preferential usage of sperm from R males was a real phenomenon, and to replicate the
experiment, since sperm mixing is not the case in other Orthoptera, experiment 2 was performed.

In competition experiment 2 there were 2 significant differences in life history parameters between males assigned to the NR group and males assigned to the other groups. The first was that the first male to mate in the NR group (the N male) was significantly younger at the day of mating than the first male in the RN group (the R male) or the first male in the NN group (the N1 male). While this difference was statistically significant, the first males to mate in the NR group were sexually mature and able to mate successfully. Therefore it is unlikely that the difference in the age of the first male to mate between the NR group and males belonging to the RN group and the NN group could affect the ability of the sperm from these males to fertilize eggs.

The other significant difference in life history parameters in competition experiment 2 was that R males assigned to the NR group died at a significantly younger age than R males assigned to the RN group. This difference is perhaps anomalous and was not related to a difference in the age of irradiation between males assigned to the 2 groups as there was no significant difference between the 2 groups for age at irradiation. While the R males assigned to the NR group did die at a significantly younger age than RN group R males, their death occurred after they had completed their matings, and their ability to copulate with the female was no different than that of other males (pers. obs.).

For both treatment groups and the control group, the productivity (nymphs plus unhatched eggs) for oviposition dish 3 (oviposition
period 17 - 24 days post-mating) was lower than the productivity of
dish 2 (oviposition period 9 - 16 days post-mating), although this was
only statistically significant for the RN group. Behrens et al.,
(1983) showed that the number of eggs laid by a female per day peaks
rapidly at approximately 10 - 20 days post-adult moult and then
decreases gradually in G. bimaculatus with time, even though the
females were allowed constant access to males. Sakaluk (Unpub MS)
found a decrease in the numbers of eggs fertilized by males following
16 days of oviposition in an experiment where females were not allowed
access to males following 2 matings. In competition experiment 2,
females were not allowed access to males following the completion of 4
matings. If the decrease in productivity is related to presence of an
egg laying hormone, or sperm supplies, then a decrease in the numbers
of eggs laid may be expected as time since mating increases. In this
connection Destephano and Brady (1977) and Loher (1979a) showed that
prostaglandins are responsible for the increase in oviposition rates
in mated female crickets and that the male cricket transfers the
prostaglandin synthase enzyme to the female at mating. Therefore the
significant decrease in productivity in oviposition dish 3 of the RN
group may be related to decreased level of prostaglandins in the
females.

Another possible explanation for the decreased mean productivity
for oviposition dish 3, is that following oviposition periods of
greater 16 days, females may show depleted sperm supplies, and are
unable to fertilize eggs. This is unlikely to be the case however
because 8 of 9 females examined at termination in the RN group, and
all females examined in the NR and NN groups had motile sperm
remaining in their spermatheca following oviposition. In any case, such differences in productivity should not affect the outcome of sperm competition experiments.

There were no significant within group differences between the oviposition dishes for the proportion of eggs hatched for either of the treatment or control groups in oviposition dish 1, 2, or 3. This indicates that the sperm utilization pattern following double matings with 2 males is constant and fixed over a female's lifetime. The lack of significant differences in productivity (nymphs plus unhatched eggs) between groups for any dish or for dishes 1, 2, and 3 combined (total productivity) indicated that equal numbers of eggs were being laid in the control and 2 treatment groups.

The mean aggregate proportion hatched (the sum of the hatched eggs/sum of the productivity for the 3 oviposition dishes) in competition experiment 2, revealed that the NR group showed a significantly lower proportion hatched than the NN group, however, this was not significantly different than the RN group. The RN group was not significantly different than the NN group for mean aggregate proportion of eggs hatched. This is consistent with an explanation of sperm mixing with a slight advantage to the last male to mate.

When the data are examined comparing individual oviposition dishes, it is found that there were no significant differences between any of the 3 groups in terms of the proportion of eggs hatched for either oviposition dish 1 or 2. It is only in oviposition dish 3 that the proportion of eggs hatched in the NR group is found to be significantly lower than the proportion of eggs hatched in either the NN or the RN group.
The lack of significant difference between the RN and the NN group in terms of the proportion of eggs hatched may be due to the low proportion of eggs hatched in the NN group in competition experiment 2. The low proportion hatched in this group is especially noticeable when the proportion hatched in the NN group of competition experiment 2 is compared to the proportion of eggs hatched in the NN group of competition experiment 1, or the N group of the radiation and sterilization experiment. This may be the result of 3 factors.

Mating for competition experiment 2 occurred from January 1984 until March 1984. Thus mating and nymph emergence was occurring during the winter months. Bate (1972) found that female A. domesticus collected as penultimate instar nymphs, then reared under constant temperature conditions in the laboratory, showed no effect of temperature but a specific effect of season on presence or absence of egg laying following mating and numbers of egg batches laid. This effect was such that significantly more females mating in the winter than mating in the spring failed to lay eggs. It is not known if this effect would occur in laboratory reared cultures of crickets.

Another explanation for the low proportion hatched in the NN group of competition experiment 2 is that the temperature of the lab was too low for successful incubation. Behrens et al., (1983) reported that at a temperature of 20°C hatching success of G. bimaculatus is 18% and that hatching success increases with increasing temperature. The temperature in the laboratory during competition experiment 2 was approximately 20°C, and was higher (25°C) during competition experiment 1.

A third explanation for the low proportion of eggs hatched in the
NN group of competition experiment 2 may be that the crickets obtained were from stock that showed a lower natural fertility level, when compared to females used in either the radiation and sterilization or competition experiment 1. In general, some females will always show low hatching success when compared to other females under identical conditions. Sakaluk and Cade (1980; 1983) found between 2 - 13% of doubly-mated *G. integer* females did not produce progeny.

As all females used in competition experiment 2 were obtained from the same stock, treated in the same manner, and all groups were being mated and allowed to oviposit at the same time of year, then the low proportion of eggs hatched following mating with N males should have affected all groups equally. In any case $P$ values (Boorman and Parker, 1976) take into account the natural infertility of the line used in the experiment and thus acts to remove the confounding variable of low fertility in the NN females. The $P$ value of 0.62, obtained in competition experiment 2, supports the conclusion of sperm mixing with slight second male advantage, found in competition experiment 1. The range of $P$ values of 0.52 following an NR mating and 0.72 following an RN mating, show that the apparent preferential use of R sperm found in competition experiment 1 did not occur in competition experiment 2.

The results obtained in both competition experiment 1 and 2 indicate that sperm mixing occurs in *G. integer*, with a slight advantage to the second male to mate. Sperm utilization patterns vary among the Orthoptera that have been studied. For example, the grasshoppers *Schistocera gregaria* and *Paratettix texanus*, both show second male precedence as a sperm utilization pattern (Hunter-Jones,
1960; Nabors, 1927). The grasshopper *Locusta migratoria* shows second male precedence when the female is allowed to oviposit between matings. This, however, changes so that there is a slight first male advantage if the second mating follows immediately after the first mating (Parker and Smith, 1975). First male precedence is also found in the German cockroach, *Blattella germanica*, if the female is not allowed to oviposit in between matings. If remating is delayed by oviposition, the sperm from the second male gradually replaced that of the first male, indicating that the sperm utilization pattern is one where the sperm belonging to the second male gradually replaced the first male's sperm (Cochran, 1979).

A sperm utilization pattern has been determined for only one other member of the family Gryllidae; that is the decorated cricket, *Gryllodes supplicans*. Sakaluk (Unpub MS) also found sperm mixing occurs, but with a slight advantage accruing to the first male to mate. A slight advantage to the first male to mate in *G. supplicans* may be due to differences in spermathecal shape between *G. supplicans* and *G. integer*. W.F. Walker (1980) found that females in monogamous species, or species that tend toward first male precedence have spheriod spermatheca, while females in species with last male precedence tend to have elongate or tubular spermatheca. The spermatheca of female *G. integer* tends to be intermediate or oval, and is slightly elongate. A photograph of a *G. integer* spermatheca is presented in Figure 4. This would be consistent with a sperm utilization pattern of sperm mixing, with a slight advantage to the second male to mate. In *G. supplicans* the spermathecal shape changes with repeated matings, becoming more oval with an increased number of
Figure 4. Dissected Female *Gryllus integer*, showing Spermatheca.
(Spermatheca is large white oval area)
Figure 4.
matings (Sakaluk, Unpub MS). Thus spermathecal shape may influence the difference between sperm utilization patterns in G. integer and G. supplicans.

Sperm Competition as a Selection Pressure

Sperm competition has been invoked as a selection pressure driving the evolution of a range of behaviour in crickets including male post-copulatory mate guarding, female non-receptivity to male calling song, nuptial feeding by males, and multiple mating by females. Post-copulatory mate guarding is generally thought to have evolved in the context of second male precedence, and is thought to function to prevent sperm displacement by subsequent males to mate (Parker, 1970; In Press). Post-copulatory mate guarding should not evolve under a sperm utilization pattern of first male precedence, as sperm from the first male to mate fertilizes the majority of the eggs laid by the female. For example, the spider Frontinella pyramitela, exhibits first male precedence and pre-copulatory guarding of the immature female by the male (Austad, 1982; In Press)

Under a sperm utilization pattern of sperm mixing, post-copulatory mate guarding is likely to have evolved in the context of 2 functions. One is to prevent the female from removing the spermatophore prior to its being emptied. The other is to monopolize the female for future matings. Both functions act to increase the number of sperm belonging to a male that are stored in the spermatheca. If the number of fertilizations a male can obtain is related to the proportion of stored sperm that belong to him, increasing the number of sperm stored in the spermatheca will increase
the number of eggs he can fertilize.

Sakaluk (1984; Unpub MS) has demonstrated that the duration of spermatophore ampulla attachment is positively related to the percentage of eggs fertilized in the cricket, *G. supplicans*. In both competition experiment 1 and 2, spermatophore attachment time was not related to either the proportion of eggs hatched or productivity. These results are not conclusive however because of the double mating with each male, that is, in all cases females had at least 1 mating with each male that had a spermatophore attachment time greater then 30 minutes. Therefore the effect of relative proportion of the number of sperm stored in the spermatheca on the sperm utilization pattern cannot be determined for *G. integer*.

The blocking of female response to male calling song (Boake, 1983; Cade, 1979b; Loher, 1981), but not to courtship song, after mating may also function to monopolize females for future matings, and allows a male to increase the relative number of sperm he has stored within a female's spermatheca. In order for this behaviour to have evolved as a response to a selection pressure of sperm competition the female non-response to calling song would have to be male induced. There is no evidence that the female non-responsiveness to calling song in crickets is induced by a substance passed from the male to the female at mating.

Theoretically, when males show significant amounts of paternal investment in offspring, there should be strong selection pressures on the males to have high levels of paternity assurance and therefore, strong precedence patterns in one direction or the other (Gwynne, In Press). Parker (In Press) concluded that what determines the
The relationship between the amount of male parental investment and the male's expenditure on paternity assurance mechanisms is the availability of both males and females to find mates. When mates are easily available there will not be as strong of a selection pressure on males to assure paternity in the presence of male parental investment. It seems that mates are not a limiting resource in crickets, and thus 2 members of the family Gryllidae, such as \textit{G. integer} and \textit{G. supplicans}, can evolve sperm mixing as a sperm utilization pattern, as well as evolving different amounts of male paternal investment.

The sperm utilization pattern shown by an insect may also have an effect on the evolution of multiple mating by females of that species, especially if an increase in genetic diversity is postulated as a selection pressure towards the evolution of multiple mating. Lloyd (1979) hypothesized that females may mate with the first male available and will remate if a "better quality" male is found. If last male precedence is operating as a sperm utilization pattern this is a reasonable supposition, the "better quality" male will fertilize the female's offspring and her inclusive fitness will be increased. However, under a sperm utilization pattern of sperm mixing, remating with a "better quality" male will only benefit half a female's offspring, assuming only 2 matings with complete sperm mixing, and it unknown whether this would be a strong enough selection pressure to drive the evolution of multiple mating. Also, although there is some evidence that \textit{G. integer} females may be discriminating

1. This is the true \textit{G. integer}, and is a different species from the one used in these experiments.
among males based on trill duration (Hedrick, pers. comm.), there is no evidence that females are preferentially mating with favoured males (Graham, 1982), or that the female discrimination is based on a genetic advantage.

Multiple mating is also thought to have evolved in order to increase genetic diversity among offspring from a single batch of eggs laid by a female (Richmond and Erhman, 1972). Strong precedence of either first or last male to mate would negate any advantage of increased genetic diversity following multiple mating, however sperm mixing as a utilization pattern would allow females to benefit from multiple paternity.

While increased genetic diversity among a females's offspring may increase that female's reproductive fitness, especially under changing climatic conditions, there is no evidence that multiple mating will increase the genetic diversity among a batch of eggs any more than a single mating will (Williams, 1975). Therefore, even under conditions of sperm mixing, the evolution of multiple mating in order to increase the genetic diversity of a female's offspring is unlikely.

For crickets, it seems that the greatest benefit to females who mate multiply is to maintain high levels of nymph production (Sakaluk and Cade, 1980; 1983). Doubly-mated female *A. domesticus* and *G. integer* produce significantly more offspring than singly-mated females, and this increase in production may be related to increased levels of prostaglandins (Loher, 1979), or increased numbers of sperm available for fertilization (Sakaluk and Cade, 1983).

In all cases, the importance of sperm competition as a selection pressure in the evolution of behaviour depends on the temporal
relationship between mating and oviposition. If females are mating, and ovipositing following each mating, there may be fewer sperm to compete in subsequent matings. Any tendency toward last male precedence in these individuals may be more strongly expressed. If females are mating more than once prior to ovipositing there will be large numbers of sperm present within the spermatheca, and therefore more sperm in competition for fertilizations. Thus sperm mixing as a sperm utilization pattern will be favoured and tendencies to last male precedence will be reduced.

The $P$ values for G. integer that have been determined in this study, 0.57 and 0.62, indicate approximate equality between different males mated with the same female in terms of offspring production. In crickets, where males show variation in mating behaviour (Cade, 1979a), it allows a researcher to measure more directly the fitness of the different behavioural patterns in terms of the number of copulations achieved, since ejaculates have a roughly equal probability of fertilizing eggs.
SUMMARY AND CONCLUSIONS

Sperm competition is the competition between ejaculates for the fertilization of eggs laid by a female following a double mating. Four sperm utilization or precedence patterns are possible: first male precedence, where sperm from the first male is used to fertilize eggs laid following a double mating; second male precedence, where sperm from the second male to mate is used to fertilize eggs laid following a double mating; "all-or-none" pattern, where sperm from either male fertilizes all eggs laid by a female following a double mating but which male's sperm used is random; and sperm mixing, where sperm from both males to mate is used equally in fertilizing eggs laid by a female following multiple mating.

There are 4 techniques that can be used to study sperm competition. Hybrid crosses, genetic markers, electrophoretic techniques, or sterile males can be used to determine paternity of offspring. By determining the paternity of the offspring following a double mating the sperm utilization pattern can be determined.

Sperm competition occurs in a wide variety of animals, but has been most commonly studied in insects. This is because insects fulfill the pre-adaptations that are necessary for sperm competition to evolve. Female insects mate multiply, often with different males, prior to oviposition. Female insects store sperm in a sperm storage organ, the spermatheca, and the sperm remain viable during storage. Finally, sperm are used in such a manner that large amounts remain in the spermatheca following oviposition, and thus ejaculates can overlap. Other animals that show these preadaptations include,
spiders, isopods, birds, rodents, ground squirrels, and possibly snakes.

The sperm precedence pattern shown by a species may be the result of several factors. One of these is a genotypic difference between the two males leading to differential fertilizing ability of the males' sperm. Another is spermathecal shape, such that, females with elongate spermathecae tend to show second male precedence, while females with ovoid spermathecae tend to show first male precedence. Other factors affecting the precedence pattern shown by an animal include, male sexual experience, weight of the male, and the length of time the male and female interact.

Sperm competition is thought to be a selective pressure leading to the evolution of male behaviour that functions to displace previously stored sperm from the female, or to prevent displacement of sperm. These counter-adaptations to sperm competition include, physical removal of sperm from the sperm storage organ, insertion of a mate plug in the female genital opening so that future copulations are prevented, male induced refractory periods, such that, females do not respond to male courtship, and mate guarding following copulation such that males are unable to gain access to the mated female.

In this study 4 experiments were designed to determine if sperm competition occurs in the field cricket, *Gryllus integer*. The first of these, the radiation and sterilization experiment, determined that 7,000 rad of gamma radiation sterilized adult *G. integer* males. This sterilization was likely to be due to a dominant lethal mutation of the sperm chromosomes, such that, the sperm were able to fertilize eggs, but the eggs laid were inviable. Longevity of the males was
also affected, and males exposed to gamma radiation showed a significantly shorter lifespan than untreated males.

The diapause experiment determined that there was no laboratory diapause for G. integer eggs. These results were used to determine the incubation time for the oviposition dishes in the competition experiments.

In competition experiment 1 the proportion of eggs hatched in the 3 groups (females mated with a normal male then with a normal male; females mated with a normal male then with a sterile male, females mated with a sterile male then with a normal male) were significantly different from each other. The results most closely resembled a pattern of hatching expected under sperm mixing. Calculation of the proportion of eggs fertilized by the second male to mate following a double mating (Boorman and Parker, 1976) in this experiment yielded a value of 0.57, or 57% of the eggs laid following a double mating were fertilized by the second male to mate.

This experiment was replicated in competition experiment 2. In competition experiment 2 the aggregate proportion of eggs hatched for females mated with a normal male then with a sterile male, was significantly different than the proportion hatched in the other 2 groups. Again, this resembles the pattern expected if sperm mixing was occurring in G. integer. The proportion of eggs fertilized by the second male to mate following a double mating (Boorman and Parker, 1976) was 0.62, that is, 62% of the fertilizations following a double mating could be attributed to the second male.

A sperm utilization pattern of sperm mixing in G. integer was used to interpret the evolution of male behaviour in field crickets,
including post-copulatory mate guarding by the male, the lack of female phonotaxis to male calling song following mating, nuptial feeding by males, and the evolution of multiple mating by females. As well, the determination of a sperm utilization pattern of sperm mixing in G. integer is an important addition to the body of field research using this species, because it allows the fitness of males using alternative mating strategies to be quantified in terms of the number of matings obtained by each male.
LITERATURE CITED


Alexander, R.D. and D. Otte 1967 The Evolution of Genitalia and Mating Behaviour in Crickets (Gryllidae) and other Orthoptera. University of Michigan, Miscellaneous Publications No. 133.


Bate, J. 1972 Variation in Fecundity of *Acheta domesticus* (Insecta, Orthoptera, Gryllidae) in Relation to Season and Temperture. Pedobiologia, Bd. 12: 1 - 5.


Cade, W.H. 1979b Effect of Male Deprivation on Female Phonotaxis in Field Crickets (Orthoptera: Gryllidae; Gryllus) Can Ent. 111: 741 - 744.


Cochran, D.G. 1979 A Genetic Determination of Insemination Frequency and Sperm Precedence in the German Cockroach. Ent exp. & appl. 26: 259 - 266.


Crankshaw, O.S. 1979 Female Choice in Relation to Calling and Courtship Songs in Acheta domesticus. Ani Behav. 27: 1274 - 1275.


Gwynne, D.T. 1982 Mate Selection by Female Katydids (Orthoptera: Tettigoniidae, Conocephalus nigropleurum). Animal Behav. 30: 734 - 738.


Hardy, T.N. and K.C. Shaw 1983 The role of chemoreception in sex recognition by male crickets: Acheta domesticus and Teleogryllus oceanicus. Physiol Entomol. 8: 151 - 166.


Hatch, S.A. 1983 Mechanism and Ecological Significance of Sperm Storage in the Northern Fulmar with Reference to its Occurrence in Other Birds. The Auk 100: 593 - 600.


Huber, F. 1962 Central Nervous Control of Sound Production in Crickets and Some Speculation on its Evolution. Evolution 16: 429 - 442.


Khalifa, A. 1950 Sexual Behaviour in *Gryllus domesticus* L. Behaviour 2: 264 - 274.


Taber, S. 1955 Sperm Distribution in the Spermatheca of Multiple Mated Queen Honey Bees. J. Econ. Entomol. 48: 522 - 525.


## APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix I</td>
<td>Radiation Biology</td>
<td>160</td>
</tr>
<tr>
<td>Appendix II</td>
<td>Formula for $\bar{P}$, and a Sample Calculation</td>
<td>166</td>
</tr>
<tr>
<td>Appendix III</td>
<td>Relationship Between the Species, <em>Gryllus rubens</em> and <em>G. integer</em></td>
<td>170</td>
</tr>
<tr>
<td>Appendix IV</td>
<td>Life History Data, Competition Experiment 1</td>
<td>173</td>
</tr>
<tr>
<td>Appendix V</td>
<td>Calculation of $\bar{P}$ for Data Obtained in Competition Experiment 1</td>
<td>183</td>
</tr>
<tr>
<td>Appendix VI</td>
<td>Life History Data, Competition Experiment 2</td>
<td>184</td>
</tr>
<tr>
<td>Appendix VII</td>
<td>Productivity and Proportion of Eggs Hatched for the In Progress (IP) Dishes, Competition Experiment 2</td>
<td>196</td>
</tr>
<tr>
<td>Appendix VIII</td>
<td>Calculation of $\bar{P}$ for Data Obtained in Competition Experiment 2</td>
<td>201</td>
</tr>
<tr>
<td>Appendix IX</td>
<td>The Use of 4 Matings in the Competition Experiments</td>
<td>202</td>
</tr>
</tbody>
</table>
APPENDIX I Radiation Biology

Radiation, such as gamma radiation, damages biological systems by ionizing the proteins, nucleic acids, lipids, and carbohydrates that make up cell components. Gamma rays are electromagnetic radiations with wavelengths in the range of $10^{-8} - 10^{-11}$ cm. Gamma radiation is produced when a nucleus releases energy. The amount of gamma radiation an object is exposed to is expressed in roentgen (R), a unit of exposure based on the ionization that the radiation source produces in the air. Units of absorption, that is the amount of radiation that an object absorbs, are known as rads. In soft tissue the absorbed dose per roentgen is between 0.93 - 0.98 rad, therefore the number of rads an object absorbs is approximately equal to the number of roentgens that it is exposed to (Casarett, 1968).

This property of radiation has been utilized in order to sterilize insects. When sterilized male insects are released in the environment and mate with wild females, infertile eggs are laid. By continuing to release males into the population for several generations the total number of individuals of the species decreases (Casarett, 1968).

Sterilization of male insects with irradiation occurs in 3 ways. One is sperm inactivation. Sperm inactivation occurs when the gamete is killed and is no longer able to form a zygote. The other way sterilization can occur is if a dominant lethality is induced in the sperm such that the zygote is formed but is killed sometime during development. Dominant lethality is induced by lower doses of radiation than sperm inactivation (von Borstal, 1963). Thirdly, sterilization can occur if the sperm transfer mechanism is damaged by
radiation.

When insects are being sterilized for use in sperm competition experiments the sperm should be able to compete with the sperm from normal untreated males for fertilizations. Doses of radiation used to sterilize male insects in these experiments should be the minimum necessary to induce sterility, so that sperm inactivation does not occur.

For examples of insects that have been sterilized with gamma radiation, and the doses used to induce the sterility, see Table I - 1.

Few studies have examined the effect of exposure to ionizing radiation in field crickets, and most of those have concentrated on survival following radiation of nymphs. Menhinick and Crossley (1968) exposed 4 ages of *Acheta domesticus* (Orthoptera: Gryllidae), small nymphs, medium nymphs, large nymphs, and young adults, to doses of gamma radiation ranging from 0 - 512 kiloroentgens. They found decreasing sensitivity to radiation (as measured by time in days for 50% of individuals to die) as the nymphs became older. Adults were less affected then nymphs.

These results were replicated by Jobin et al. (1970) who exposed *A. domesticus* eggs and nymphs to gamma radiation ranging from 50 - 2000 roentgens. Again the measure of radiation sensitivity was lethality. Embryos showed the most sensitivity to radiation. Radiosensitivity gradually decreased as development proceeded with last instar nymphs showing the greatest resistance to the effects of radiation.

Later work by Hunter and Krithayakiern (1971) used adult *A.*
TABLE I - 1 Examples of Insects Sterilized with Gamma Radiation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>% Infertility</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIPTERA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delia antiqua</td>
<td>4 krad</td>
<td>95.0</td>
<td>McEwen et al., 1984</td>
</tr>
<tr>
<td>Anopheles stephensae</td>
<td>7 krad</td>
<td>96.5</td>
<td>Sharma et al., 1978</td>
</tr>
<tr>
<td>Anopheles stephensae</td>
<td>8 krad</td>
<td>97.2</td>
<td>Sharma et al., 1978</td>
</tr>
<tr>
<td>A. quadrivittatus</td>
<td>8 - 13 krad</td>
<td>n/a</td>
<td>Stone, 1963</td>
</tr>
<tr>
<td>Aedes aegypti</td>
<td>11 - 18 krad</td>
<td>n/a</td>
<td>Stone, 1963</td>
</tr>
<tr>
<td>Culex tarsalis</td>
<td>15 krad</td>
<td>n/a</td>
<td>Stone, 1963</td>
</tr>
<tr>
<td>Dacus oleae</td>
<td>15 - 18 krad</td>
<td>100.0</td>
<td>Thomou, 1963</td>
</tr>
<tr>
<td>Dacus dorsalis</td>
<td>10 krad</td>
<td>n/a</td>
<td>Stone, 1963</td>
</tr>
<tr>
<td>Dacus cucurbitae</td>
<td>10 krad</td>
<td>n/a</td>
<td>Stone, 1963</td>
</tr>
<tr>
<td>Ceratitis capitata</td>
<td>10 krad</td>
<td>n/a</td>
<td>Stone, 1963</td>
</tr>
<tr>
<td>Scatophaga stercoraria</td>
<td>10 krad</td>
<td>n/a</td>
<td>Parker, 1970b</td>
</tr>
<tr>
<td>Rhagoletis pomonella</td>
<td>3 krad</td>
<td>n/a</td>
<td>Myers et al., 1976</td>
</tr>
<tr>
<td><strong>LEPIDOPTERA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichoplusia ni</td>
<td>30 krad</td>
<td>96.5</td>
<td>North and Holt, 1968</td>
</tr>
<tr>
<td><strong>COLEOPTERA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callosobruchus maculatus</td>
<td>15 krad</td>
<td>n/a</td>
<td>Ahmend et al., 1977</td>
</tr>
<tr>
<td><strong>ORTHOPTERA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gryllodes suppgicans</td>
<td>20 krad</td>
<td>n/a</td>
<td>Sakaluk, Unpub MS</td>
</tr>
</tbody>
</table>
domesticus exclusively. It was found that, for both females and males, there was a sharp decrease in life expectancy in crickets exposed to between 4,000 - 10,000 roentegens of gamma radiation, but within that range of doses, there was no difference in mean life expectancy.

Lim et al. (1972) exposed 6 species of Gryllidae; G. assimilis, G. bimaculatus, G. pennsylvanicus, A. domesticus, S. marginatus, and Allonemobius allardi, to gamma radiation. Both nymphs and adults were exposed to the gamma radiation and the dosage varied from 1 krad to 2.5 krad. Cytological studies of irradiated individuals revealed significant chromosomal fragmentation, and other abnormalities including chromatid gaps, and unequal chromosome segregation. The type or amount of cytological damage did not correlate with either species or dose. Breeding experiments with irradiated females mated with normal males, and irradiated males mated with normal females showed that eggs would be laid but would not hatch. However, no data were presented to support this, and the radiation doses used in these experiments were not reported.

In order to determine the length of time to expose an animal to a given dose of radiation one uses the half life of Co of 5.3 years, and the time elapsed since installation of the Gammacell 220, to determine the current output of the Gammacell 220. The following example illustrates this process by showing how I determined the output of the Gammacell 220 (expressed in rads/min) at the start of my experiments in August 1982. The date of installation of the Gammacell was December 1960 and its output at that time was 2.17 + 0.8 x 10 rads/hour. The elapsed time to August 1982 was 21.6 years, a time greater than 4 half life periods. The emission rate following 4 half
life periods was 225 rads per min. August 1982 exceeded the 4 half life period by approximately 4 months. Using Table I - 2, I determined that the decay rate for 4 months was 0.9571. Multiplying the dose rate of 225 rads/min by the decay rate yielded an output of 215.3 rads/min. The length of time necessary to expose crickets to 7,000 rads would be 32.5 min.

I calculated the Gammacell 220 output 4 times during the course of this research. The dates, the experiments performed, output rates, and the time necessary to achieve exposure to 7,000 rads were as follows: August 1982, Radiation and Sterilization, 215 rads/min, 32.5 min; June 1983, Competition Experiment 1, 193 rads/min, 36 min; December 1983, Competition Experiment 2, 175 rads/min, 40 min; September 1984, Motility of R Sperm, 140 rads/min, 50 min. The last output rate (September 1984) was obtained from Dr. A. Croy, Brock University, and was calculated using a different method from the one described above.
Table I - 2 Decay Factor of $^{60}$Co, used in calculating the output of the Gammacell 220.

<table>
<thead>
<tr>
<th>MONTHS</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.9931</td>
<td>0.9783</td>
<td>0.9676</td>
<td>0.9571</td>
<td>0.9477</td>
<td>0.9383</td>
<td>0.9292</td>
<td>0.9194</td>
<td>0.9089</td>
<td>0.8981</td>
</tr>
<tr>
<td>10</td>
<td>0.9851</td>
<td>0.9703</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.9603</td>
</tr>
<tr>
<td>20</td>
<td>0.9632</td>
<td>0.9544</td>
<td>0.9559</td>
<td>0.9572</td>
<td>0.9587</td>
<td>0.9600</td>
<td>0.9611</td>
<td>0.9621</td>
<td>0.9631</td>
<td>0.9642</td>
</tr>
<tr>
<td>30</td>
<td>0.9430</td>
<td>0.9317</td>
<td>0.9348</td>
<td>0.9380</td>
<td>0.9412</td>
<td>0.9444</td>
<td>0.9476</td>
<td>0.9508</td>
<td>0.9540</td>
<td>0.9572</td>
</tr>
<tr>
<td>40</td>
<td>0.9242</td>
<td>0.9161</td>
<td>0.9189</td>
<td>0.9221</td>
<td>0.9253</td>
<td>0.9286</td>
<td>0.9319</td>
<td>0.9352</td>
<td>0.9385</td>
<td>0.9419</td>
</tr>
<tr>
<td>50</td>
<td>0.9063</td>
<td>0.8984</td>
<td>0.8930</td>
<td>0.8927</td>
<td>0.8980</td>
<td>0.9034</td>
<td>0.9088</td>
<td>0.9142</td>
<td>0.9196</td>
<td>0.9251</td>
</tr>
<tr>
<td>60</td>
<td>0.8891</td>
<td>0.8804</td>
<td>0.8789</td>
<td>0.8846</td>
<td>0.8905</td>
<td>0.8964</td>
<td>0.9024</td>
<td>0.9084</td>
<td>0.9144</td>
<td>0.9206</td>
</tr>
<tr>
<td>70</td>
<td>0.8720</td>
<td>0.8639</td>
<td>0.8634</td>
<td>0.8693</td>
<td>0.8752</td>
<td>0.8811</td>
<td>0.8870</td>
<td>0.8930</td>
<td>0.8990</td>
<td>0.9050</td>
</tr>
<tr>
<td>80</td>
<td>0.8550</td>
<td>0.8469</td>
<td>0.8464</td>
<td>0.8524</td>
<td>0.8583</td>
<td>0.8642</td>
<td>0.8701</td>
<td>0.8760</td>
<td>0.8820</td>
<td>0.8880</td>
</tr>
<tr>
<td>90</td>
<td>0.8380</td>
<td>0.8299</td>
<td>0.8294</td>
<td>0.8354</td>
<td>0.8413</td>
<td>0.8473</td>
<td>0.8533</td>
<td>0.8593</td>
<td>0.8653</td>
<td>0.8713</td>
</tr>
<tr>
<td>100</td>
<td>0.8210</td>
<td>0.8129</td>
<td>0.8124</td>
<td>0.8184</td>
<td>0.8243</td>
<td>0.8303</td>
<td>0.8363</td>
<td>0.8423</td>
<td>0.8483</td>
<td>0.8543</td>
</tr>
<tr>
<td>110</td>
<td>0.8040</td>
<td>0.7959</td>
<td>0.7954</td>
<td>0.8014</td>
<td>0.8073</td>
<td>0.8133</td>
<td>0.8193</td>
<td>0.8253</td>
<td>0.8313</td>
<td>0.8373</td>
</tr>
<tr>
<td>120</td>
<td>0.7870</td>
<td>0.7789</td>
<td>0.7784</td>
<td>0.7844</td>
<td>0.7903</td>
<td>0.7963</td>
<td>0.8023</td>
<td>0.8083</td>
<td>0.8143</td>
<td>0.8203</td>
</tr>
<tr>
<td>130</td>
<td>0.7700</td>
<td>0.7619</td>
<td>0.7614</td>
<td>0.7674</td>
<td>0.7733</td>
<td>0.7793</td>
<td>0.7853</td>
<td>0.7913</td>
<td>0.7973</td>
<td>0.8033</td>
</tr>
<tr>
<td>140</td>
<td>0.7530</td>
<td>0.7449</td>
<td>0.7444</td>
<td>0.7504</td>
<td>0.7563</td>
<td>0.7623</td>
<td>0.7683</td>
<td>0.7743</td>
<td>0.7803</td>
<td>0.7863</td>
</tr>
</tbody>
</table>

T $\frac{1}{2}$ = 5.27 YEARS

Published by the Atomic Energy Agency of Canada Limited.
APPENDIX II  Formula for $P_R$, and a Sample Calculation.

Ideally, a female insect mated with an N male should show 100% fertility, or a proportion of eggs hatched of 1.0, and a female mated with an R male should show 0% fertility, or a proportion of eggs hatched of 0.0. However, all female insects show a low level of "natural infertility" that is, there is a small proportion of eggs that do not hatch following an N mating. As well, unless very high doses of radiation are used, there will be a low proportion of eggs that will hatch following mating with an R male. It was in order to account for these values that Boorman and Parker (1976) developed a formula for $P_R$, or the proportion of eggs fertilized by an R male following either an RN or an NR mating.

\[
P_R = (1 - x) + \left( \frac{z}{p} \right) \left( \frac{1 - x}{p} \right)
\]

where: $x$ is the proportion of eggs hatched following an NR mating or RN mating, $p$ is the proportion of eggs hatched following an N mating and $z$ is the proportion of eggs hatched following an R mating. In this formula, the expression $1 - \frac{x}{p}$ is the depression in fertility caused by fertilizations obtained by the R sperm, while the second part of the formula, $\frac{z}{p} \cdot \frac{1 - x}{p}$, is the expected proportion of eggs that hatch due to fertilizations obtained by the R sperm.

This formula assumes that $p$, the proportion of eggs hatched following an N mating is independant of the number of matings that a female insect has. While multiple mating increases productivity in the crickets *A. domesticus* and *G. integer* (Sakaluk and Cade, 1980; 1983), it is not known if this is a result of increased numbers of
eggs produced, or an increase in the proportion of eggs hatched. In other insects, for example *D. melanogaster*, the proportion of eggs hatched is independant of the number of matings a female engages in (Lefevre and Jonsson, 1962).

The values of P obtained using Boorman and Parker's R formula can be used to determine P or the proportion of eggs fertilized by the second male to mate following a double mating. In an NR mating the second male to mate is the R male therefore \( P = \frac{2}{P} \). In an RN mating the second male to mate is the N male, therefore \( P = R \). The proportion of eggs fertilized by the second male is \( 1 - P \). When reciprocal matings are performed \( \bar{P} \) can be determined. This is the proportion of offspring following a double mating that are due to fertilizations by the second male in both mating patterns. If \( \bar{P} = 1.0 \) than complete second male precedence is occurring, that is, all eggs laid are fertilized by the second male. Complete first male precedence results in a \( \bar{P} \) value of 0.0, while sperm mixing results in a \( \bar{P} \) value of 0.5.

A sample calculation of \( \bar{P} \) for *D. melanogaster* follows. These data were obtained from the data published by Boorman and Parker (1976).

In this experiment:

\[ \begin{align*}
  p &= \text{the proportion of eggs hatched following an N mating} = 0.97, \\
  z &= \text{the proportion of eggs hatched following an R mating} = 0.12, \\
  x_{NR} &= \text{the proportion of eggs hatched following an NR mating} = 0.15, \\
  x_{RN} &= \text{the proportion of eggs hatched following an RN mating} = 0.91. 
\end{align*} \]
If \( P = \frac{1 - x}{p} + \frac{z}{p} \cdot \frac{1 - \frac{x}{p}}{1 - \frac{z}{p}} \)

then \( P = 1 - \frac{.15}{.97} + \frac{.12}{.97} \cdot \frac{1 - .97}{1 - .12} \cdot \frac{.97}{.97} \)

\[= 1 - .15 + .12 \cdot \frac{1 - .97}{1 - .12} \cdot \frac{.97}{.97} \]

\[= .85 + .12 \cdot \frac{.85}{.88} \]

\[= .85 + .12 \cdot .97 \]

\[= .85 + .12 \]

\[= .97 \]

Therefore the proportion of eggs fertilized by the R male following the NR mating was .97.

If \( P \) is calculated using the same formula, \( R(NR) \)

then \( P = 1 - \frac{.91}{.97} + \frac{.12}{.97} \cdot \frac{1 - .97}{1 - .12} \cdot \frac{.12}{.97} \)

\[= 1 - .94 + .12 \cdot \frac{1 - .94}{1 - .12} \cdot \frac{.12}{.97} \]

\[= .06 + .12 \cdot \frac{.06}{.88} \]

\[= .06 + .12 \cdot .07 \]

\[= .06 + .01 \]

\[= .07 \]

Therefore, the proportion of eggs fertilized by the R male following
an RN mating is 0.07.

The proportion of eggs fertilized by the second male following the NR mating is \( P_{2(NR)} = P_{R(NR)} = 0.97 \), while the proportion of eggs fertilized by the second male to mate after an RN mating is 

\[
P_{2(RN)} = 1 - P_{R(RN)} = 1 - 0.07 = 0.93.
\]

Mean \( P \) or \( \bar{P} = \frac{0.97 + 0.93}{2} = \frac{2}{2} \) is 0.95, that is, the proportion of eggs fertilized by the second male to mate following a double mating is 0.95.

Calculation of \( \bar{P} \) uses unweighted means in order to obtain the values for the variables \( x, p, \) and \( z \). This is done because weighted means give too much emphasis to some females and less to others. As well, each egg is not an individual sampling unit, rather each female is the sampling unit, and the variables \( x, p, \) and \( z \) should be based on values obtained from each female.
Appendix III  Relationship Between the Species, *Gryllus rubens* and *G. integer*.

The *G. rubens* used in the radiation and sterilization experiment, to determine the motility of sperm from males exposed to 7,000 rad of gamma irradiation, were obtained from cultures derived from field collected individuals from Eastern Oklahoma. *G. rubens* and *G. integer* are very similar in life cycle, habitat, range, and are morphologically indistinguishable (Alexander and Cade, Unpub MS). The 2 species can, however, be distinguished on the basis of calling song. *G. integer* has a pulse rate of 77 - 88 pulses per sec at 25°C, while *G. rubens* has a mean pulse rate of 46 - 64 pulses per sec at 25°C. In order to ascertain that the species used was *G. rubens* recording were made of male calling song and were then examined in order to determine the pulse rate of the song. Recordings were made at 25°C using a Sony model TC - 105 tape recorder. Sonagrams were made of the recorded calling song on a Kay sonagraph (Model 7029 Kay Elemetrics Corp. Pine Brook New Jersey). A sample sonagraph is shown in Fig III - 1a. The pulse rate of this male, determined on the basis of this sonagraph, was 58 pulses per second, and is within the published range available for *G. rubens*. As well, this sonagraph resembles the published sonagraphs for *G. rubens* (Walker, 1964).

Fig III - 1b shows a representative sonagraph of the calling song from a male *G. integer*. (Sonagrams of *G. integer* obtained from E. Baslayga, Brock University.) The pulse rate for this male was 85 pulses per second. For more information on speciation in crickets, and the relationship between different cricket species see Alexander, 1968.

Although the male and female *G. rubens* are a different species
Figure III - 1 Sonagraphs of Calling Song from male *Gryllus rubens* and *G. integer*.

1A A sonagraph of calling song from a male *G. rubens*.

1B A sonagraph of a calling song from a male *G. integer*. 
from *G. integer*, the morphological similarity between the two species makes it unlikely that results obtained with *G. rubens*, concerning the motility of sperm following exposure of males to radiation, would be different than those of male *G. integer*. 
APPENDIX IV  Life History Data, Competition Experiment 1.

Within Group Differences

The mean age of mating for the first and second male to mate within a treatment group is presented in Table IV - 1. A Mann-Whitney U test revealed no significant difference between the first and the second male to mate within a treatment group for age at mating.

Occasionally a female would be able to remove a spermatophore during the 60 min isolation period. Within group tests between females with all spermatophore attachment times $\geq$ 30 min and females with at least 1 spermatophore attachment time $< 30$ min revealed no significant differences between these two type of females for proportion of eggs hatched or productivity. The proportion of eggs hatched for females in each group with spermatophore attachment times $\geq$ 30 min and $< 30$ min on 1 occasion are presented in Table IV - 2. The productivity for females in each group with spermatophore attachment times of $\geq 30$ min and $< 30$ min on 1 occasion are presented in Table IV - 3.

Between Group Differences

Table IV - 4 shows the mean age of mating for the first male to mate. There was no significant difference between any treatment group for the age of the first male to mate on the day of mating.

Table IV - 5 shows the mean age of mating for the second male to mate. Again there was no significant difference between any treatment group for the age of the second male to mate with a females on the day of mating.

Table IV - 6 shows the mean age of mating for females. There was
### TABLE IV - 1 Mean age at mating of the first and second male to mate within the 3 treatment groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>x AGE AT MATING (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N1</td>
<td>9.2</td>
<td>2.5</td>
<td>7 - 14</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>8.9</td>
<td>1.7</td>
<td>7 - 12</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>U = 83.5, p &gt; 0.05.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td>N</td>
<td>8.5</td>
<td>0.7</td>
<td>8 - 10</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>8.6</td>
<td>0.8</td>
<td>8 - 10</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>U = 125, p &gt; 0.05.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RN</td>
<td>R</td>
<td>8.5</td>
<td>.6</td>
<td>7 - 9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>8.4</td>
<td>.5</td>
<td>8 - 9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>U = 116.5, p &gt; 0.05.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE IV - 2 Within group differences in proportion of eggs hatched between females with spermatophore attachment times ≥ 30 min for all matings, and females with spermatophore attachment times < 30 min for at least 1 mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>FEMALES</th>
<th>PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 30 min</td>
<td>0.67</td>
<td>0.29</td>
<td>0 - 0.90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>&lt; 30 min</td>
<td>0.72</td>
<td>0.15</td>
<td>0.57 - 0.87</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>U = 13, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 30 min</td>
<td>0.02</td>
<td>0.05</td>
<td>0 - 0.18</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>&lt; 30 min</td>
<td>0.00</td>
<td>0.00</td>
<td>0 - 0.01</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U = 11.5, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 30 min</td>
<td>0.10</td>
<td>0.10</td>
<td>0 - 0.26</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>&lt; 30 min</td>
<td>0.12</td>
<td>0.08</td>
<td>0 - 0.22</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U = 23, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE IV - 3 Within group differences in productivity (nymphs + unhatched eggs) between females with spermatophore attachment times ≥ 30 min for all matings, and females with spermatophore attachment times < 30 min for at least 1 mating

<table>
<thead>
<tr>
<th>GROUP</th>
<th>FEMALES</th>
<th>% PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 30 min</td>
<td>257.9</td>
<td>141.6</td>
<td>0 - 492</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>&lt; 30 min</td>
<td>276.3</td>
<td>131.8</td>
<td>190 - 428</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U = 14, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 30 min</td>
<td>364.0</td>
<td>294.7</td>
<td>0 - 1001</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>&lt; 30 min</td>
<td>127.3</td>
<td>109.4</td>
<td>1 - 191</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U = 9, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 30 min</td>
<td>373.0</td>
<td>137.1</td>
<td>113 - 576</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>&lt; 30 min</td>
<td>268.8</td>
<td>114.3</td>
<td>117 - 422</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U = 17, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE IV - 4 Between group differences in the age of the first male in the mating pattern, on the day of mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>( \bar{\text{AGE}} ) (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N1</td>
<td>9.2</td>
<td>2.5</td>
<td>7 - 14</td>
<td>13</td>
</tr>
<tr>
<td>NR</td>
<td>N</td>
<td>8.5</td>
<td>0.7</td>
<td>8 - 10</td>
<td>16</td>
</tr>
<tr>
<td>RN</td>
<td>R</td>
<td>8.5</td>
<td>0.6</td>
<td>7 - 9</td>
<td>16</td>
</tr>
</tbody>
</table>

H adj = .11, p > 0.05.
TABLE IV - 5 Between group differences in the age of the second male in the mating pattern, on the day of mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>$\bar{x}$ AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N2</td>
<td>8.9</td>
<td>1.7</td>
<td>7 - 12</td>
<td>13</td>
</tr>
<tr>
<td>NR</td>
<td>R</td>
<td>8.6</td>
<td>0.8</td>
<td>8 - 10</td>
<td>16</td>
</tr>
<tr>
<td>RN</td>
<td>N</td>
<td>8.4</td>
<td>0.5</td>
<td>8 - 9</td>
<td>16</td>
</tr>
</tbody>
</table>

$H \ adj = 0.24$, $p > 0.05$. 
TABLE IV - 6 Between group differences in the age of the female at the day of mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>10.0</td>
<td>4.0</td>
<td>4 - 18</td>
<td>13</td>
</tr>
<tr>
<td>NR</td>
<td>8.9</td>
<td>1.3</td>
<td>6 - 11</td>
<td>16</td>
</tr>
<tr>
<td>RN</td>
<td>9.6</td>
<td>1.2</td>
<td>7 - 11</td>
<td>16</td>
</tr>
</tbody>
</table>

H adj = 2.1, p > 0.05.
no significant difference between any group for the age of the female at mating.

Table IV - 7 shows the mean age of death of the males, for all groups. There was no significant difference in the age of death of the R males in the NR and RN groups. There was also no significant difference in the age of death of the N males from the NN group, the NR group, and the RN group. When the R males were tested against the N males for age of death a significant difference in the age of death of males belonging to the two groups was found. These data are presented in Table IV - 8.
TABLE IV - 7 Between group differences in the age of male death for N males and R males.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N1</td>
<td>43.4</td>
<td>15.6</td>
<td>15 - 75</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>49.9</td>
<td>21.5</td>
<td>28 - 74</td>
<td>13</td>
</tr>
<tr>
<td>NR</td>
<td>N</td>
<td>56.1</td>
<td>20.3</td>
<td>22 - 96</td>
<td>14</td>
</tr>
<tr>
<td>RN</td>
<td>N</td>
<td>49.3</td>
<td>17.7</td>
<td>23 - 79</td>
<td>15</td>
</tr>
</tbody>
</table>

H adj = 2.21, p > 0.05.

|       | R    | 21.9     | 3.6  | 18 - 34  | 16 |
|       | R    | 21.6     | 2.7  | 16 - 25  | 16 |

U = 111.5, p > 0.05.
TABLE IV - 8 Difference in age of male death between R males and N males, 3 treatment groups lumped for analysis.

<table>
<thead>
<tr>
<th>MALES</th>
<th>$\bar{x}$ AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>21.8</td>
<td>3.1</td>
<td>16 - 34</td>
<td>32</td>
</tr>
<tr>
<td>N</td>
<td>50.0</td>
<td>19.0</td>
<td>15 - 96</td>
<td>53</td>
</tr>
</tbody>
</table>

$z = -7.08$, $p < 0.05$, one-tailed.
APPENDIX V Calculation of $P_2$ for data obtained in competition experiment 1.

In this experiment:

$p =$ the proportion of eggs hatched following an NN mating $= 0.68,$

$z =$ the proportion of eggs hatched following an R mating $= 0.01,$

$x =$ the proportion of eggs hatched following an NR mating $= 0.02,$

$x =$ the proportion of eggs hatched following an RN mating $= 0.11.$

\[
\begin{align*}
P_{R(NR)} &= 1 - .02 + .01 \cdot \frac{.02}{.68} \\
       &= 1 - .03 + .01 \cdot \frac{.03}{.99} \\
       &= .97 + .01 \cdot .97  \\
       &= .97 + .01  \\
       &= .98 \\

P_{R(RN)} &= 1 - .11 + .01 \cdot \frac{.11}{.68} \\
       &= 1 - .16 + .01 \cdot \frac{.16}{.99} \\
       &= .84 + .01 \cdot .84 \\
       &= .84 + .01 \\
       &= .85 \\

\text{thus } P_{R(NR)} &= P_{2(NR)} = .98, \text{ while } P_{R(RN)} = 1 - P_{2(RN)} = .15 \\
\text{and mean } P &= \frac{.98 + .15}{2} = .57.
\end{align*}
\]
APPENDIX VI  Life History Data, Competition Experiment 2

Within Group Differences

The age of the first and second male to mate is presented in Table VI - 1. There was no significant difference between the first and second male to mate in terms of age on the day of mating when tested with a Mann-Whitney U test.

Only 1 female in each of the NN and RN groups, and no females in the NR group, showed at least one spermatophore attachment time < 30 min. Therefore I was not able to test this for any effect on proportion of eggs hatched or productivity. Also, since there were no significant differences between females with all spermatophore attachment times ≥ 30 min., and females with at least one spermatophore attachment time ≤ 30 min, in terms of productivity and proportion of eggs hatched in competition experiment 1, I felt that I could combine these groups of females for subsequent analysis.

Table VI - 2 shows productivity (nymphs and unhatched eggs) for females who had a total oviposition time of < 24 days, 24 days, or > 24 days. Within each group there was no significant difference in productivity for females with different oviposition periods.

Between Group Differences

Table VI - 3 shows the age of the first male to mating in a mating pattern at the day of mating for the three treatment groups. A Kruskal-Wallis test showed that there was a significant difference between the 3 treatment groups for the age of the first male at mating. Pairwise combinations of Mann-Whitney U tests revealed that the age of mating for males assigned to the the NR group was significantly different than the age of mating for males assigned to
TABLE VI - 1 Within group analysis for difference in age of first and second males to mate, at day of mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>$\bar{X}$ AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N1</td>
<td>16.5</td>
<td>4.2</td>
<td>9 - 22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>14.3</td>
<td>3.6</td>
<td>8 - 22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>U = 39.5, p &gt; 0.05.</td>
</tr>
<tr>
<td>NR</td>
<td>N</td>
<td>12.2</td>
<td>3.0</td>
<td>8 - 17</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>13.6</td>
<td>3.6</td>
<td>9 - 20</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>U = 47.5, p &gt; 0.05.</td>
</tr>
<tr>
<td>RN</td>
<td>R</td>
<td>15.7</td>
<td>3.4</td>
<td>10 - 22</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>14.4</td>
<td>3.4</td>
<td>9 - 21</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>U = 53.5, p &gt; 0.05.</td>
</tr>
</tbody>
</table>
TABLE VI - 2 Within group productivity differences in females with oviposition periods of < 24 days, 24 days, > 24 days, for dishes 1, 2, and 3.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PERIOD</th>
<th>( \bar{X} ) PRODUCTIVITY</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>&lt; 24</td>
<td>221.3</td>
<td>227.4</td>
<td>24 - 470</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>485.8</td>
<td>359.9</td>
<td>236 - 1103</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&gt; 24</td>
<td>638.0</td>
<td>260.9</td>
<td>347 - 851</td>
<td>3</td>
</tr>
</tbody>
</table>

\( H = 3.44, p > 0.05. \)

| NR    | < 24   | 359.5                         | 398.1| 78 - 641 | 2 |
|       | 24     | 320.5                         | 269.2| 13 - 625 | 6 |
|       | > 24   | 596.0                         | 162.8| 409 - 706 | 3 |

\( H = 2.73, p > 0.05. \)

| RN    | < 24   | NONE                          |      |        |   |
|       | 24     | 496.1                         | 216.2| 62 - 768 | 9 |
|       | > 24   | 506.3                         | 64.7 | 432 - 550 | 3 |

\( U = 13, p > 0.05. \)
TABLE VI - 3 Between group differences in the age of the first male to mate on day of mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>X AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N1</td>
<td>16.5</td>
<td>4.2</td>
<td>9 - 22</td>
<td>11</td>
</tr>
<tr>
<td>NR</td>
<td>N</td>
<td>12.2</td>
<td>3.0</td>
<td>8 - 17</td>
<td>11</td>
</tr>
<tr>
<td>RN</td>
<td>R</td>
<td>15.7</td>
<td>3.4</td>
<td>10 - 22</td>
<td>12</td>
</tr>
</tbody>
</table>

H adj = 7.01, p < 0.05.

NN vs NR  U = 25.5, p < 0.05, two-tailed.

NN vs RN  U = 55, p > 0.05.

NR vs RN  U = 31, p < 0.05, two-tailed.
either the NN or RN group.

Table VI - 4 shows the age of the second male to mate at the day of mating. A Kruskal-Wallis test determined no significant difference in the age of mating between the three treatment groups.

The age of the female at the day of mating is shown in Table VI - 5. There were no significant differences between treatment groups for the age of the female at mating.

There was a significant difference in the age of death of R males assigned to the NR group, compared to R males assigned to the RN group, when tested with a Mann-Whitney U test. These data are presented in Table VI - 6. This difference in the age of death is not related to age of irradiation of the males, as there were no significant differences between the 2 treatment groups for the age of irradiation. The mean age of irradiation of males assigned to the NR group was 11.9 days post adult moult while the mean age of irradiation of males assigned to the RN group was 13.5 days post adult moult. These data are presented in Table VI - 7. Age of death of N males is presented in Table VI - 8. There was no significant difference between the N males for age of death for either group of males in the NN group or the N males in the NR or RN treatment groups.

Since there was no difference in the age of death of N males belonging to the different groups, I lumped all N males into one group in order to compare the age of death of N males to the age of death of R males. These data are presented in Table VI - 9. The lumped group of N males was tested separately against R males from the NR group and R males from the RN group using Mann-Whitney U tests. These tests revealed that R males died significantly sooner the N
TABLE VI - 4 Between group differences in age of the second male to mate at the day of mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N2</td>
<td>14.3</td>
<td>3.6</td>
<td>8 - 22</td>
<td>11</td>
</tr>
<tr>
<td>NR</td>
<td>R</td>
<td>13.6</td>
<td>3.6</td>
<td>9 - 20</td>
<td>11</td>
</tr>
<tr>
<td>RN</td>
<td>N</td>
<td>14.4</td>
<td>3.4</td>
<td>9 - 21</td>
<td>12</td>
</tr>
</tbody>
</table>

H adj = .764, p > 0.05.
TABLE VI - 5 Between group differences in age of female at mating.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>( \bar{x} ) AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>18.4</td>
<td>6.0</td>
<td>11 - 28</td>
<td>11</td>
</tr>
<tr>
<td>NR</td>
<td>19.9</td>
<td>4.2</td>
<td>14 - 27</td>
<td>10</td>
</tr>
<tr>
<td>RN</td>
<td>21.7</td>
<td>6.1</td>
<td>14 - 31</td>
<td>12</td>
</tr>
</tbody>
</table>

\[ H_{adj} = 2.63, p > 0.05. \]
TABLE VI - 6  Age of death of R males assigned to NR group, compared to the age of death of R males assigned to the RN group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>X Age (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>24.8</td>
<td>4.2</td>
<td>20 - 32</td>
<td>10</td>
</tr>
<tr>
<td>RN</td>
<td>30.2</td>
<td>4.0</td>
<td>23 - 37</td>
<td>12</td>
</tr>
</tbody>
</table>

U = 31, p < 0.05, two-tailed.
TABLE VI - 7 Age of irradiation of R males assigned to NR group, Compared to the age of irradiation of R males assigned to the RN group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>X AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>11.9</td>
<td>3.2</td>
<td>8 - 19</td>
<td>10</td>
</tr>
<tr>
<td>RN</td>
<td>13.5</td>
<td>2.7</td>
<td>9 - 18</td>
<td>12</td>
</tr>
</tbody>
</table>

U = 38, p > 0.05.
TABLE VI - 8 Between group differences in age of death of N males assigned to the 3 treatment groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>[\bar{x}] AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>N1</td>
<td>69.2</td>
<td>23.0</td>
<td>33 - 93</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>61.0</td>
<td>27.7</td>
<td>37 - 113</td>
<td>8</td>
</tr>
<tr>
<td>NR</td>
<td>N</td>
<td>56.3</td>
<td>33.1</td>
<td>20 - 113</td>
<td>8</td>
</tr>
<tr>
<td>RN</td>
<td>N</td>
<td>63.8</td>
<td>26.0</td>
<td>24 - 99</td>
<td>9</td>
</tr>
</tbody>
</table>

H adj = .912, p > 0.05.
TABLE VI - 9 Age of death of N males (all groups combined) compared to R males from the NR group and the RN group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALES</th>
<th>( \bar{X} ) AGE (days)</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>R</td>
<td>24.8</td>
<td>4.2</td>
<td>20 - 32</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>N all</td>
<td>62.9</td>
<td>25.6</td>
<td>30 - 113</td>
<td>20</td>
</tr>
</tbody>
</table>

\( U = 1, p < 0.05, \) one-tailed.

| RN    | R     | 29.3                   | 3.9  | 23 - 34 | 11 |
|       | N all | 62.9                   | 25.6 | 30 - 113 | 20 |

\( U = 7.5, p < 0.05, \) one-tailed.
males for either group.
Appendix VII  Productivity and Proportion of Eggs Hatched for In Progress (IP) dishes, in Competition Experiment 2.

In Progress, or IP dishes, were the oviposition dishes that females were allowed access to if they had not completed the mating pattern in one day. Not every female required a second day to complete the mating sequence, thus data for IP dishes is not available for every female. There were 2 females, 1 in the NN group, and 1 in the RN group who required 3 days to complete the mating pattern and thus have 2 IP dishes. These dishes have not been included in any analysis.

The productivity (nymphs plus unhatched eggs) of IP dishes compared to oviposition dishes 1, 2, and 3 for each group is shown in Table VII - 1. A Mann-Whitney U test revealed a significant productivity difference between the IP dish and oviposition dish 2 in both the the NN group and the RN group. There were no other significant within group differences in terms of productivity between the IP dish and other oviposition dishes for any group.

Mean productivity of the IP dishes in the NN group was 48.2 nymphs plus unhatched eggs (S.D. = 43.4, Range 0 - 115, N = 6); in the NR group $\bar{x} = 145.2$ (S.D. = 55.8, Range 60 - 216, N = 5); and in the RN group $\bar{x} = 90.7$ (S.D. = 91.2, range 2 - 219, N = 7). A Kruskal-Wallis test revealed no significant difference in mean productivity between the IP dishes belonging to the 3 groups ($H = 4.9$, $P > 0.05$).

The proportion of eggs hatched in the IP dish, and oviposition dishes 1,2, and 3 for each group are presented in Table VII - 2. A Kruskal-Wallis test revealed no significant difference in terms of the proportion of eggs hatched in the IP dish and oviposition dish 1,2, and 3 in either the NN or the NR groups. Within the RN group the
Table VII - 1 Within Group Productivity (nymphs plus unhatched eggs) Between the In Progress (IP) Dishes, and Oviposition Dish 1, 2, and 3.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DISH</th>
<th>Mean Productivity</th>
<th>S.D.</th>
<th>Range</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NN</td>
<td>IP 48.2</td>
<td>43.4</td>
<td>0 - 115</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1  155.7</td>
<td>146.0</td>
<td>24 - 488</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2  200.7</td>
<td>119.7</td>
<td>93 - 479</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3  128.8</td>
<td>101.6</td>
<td>10 - 325</td>
<td>10</td>
</tr>
</tbody>
</table>

H = 10.31, p < 0.05.

IP vs 1, U = 14, p > 0.05.

IP vs 2, U = 2, p < 0.05, two-tailed.

IP vs 3, U = 15, p > 0.05.

<table>
<thead>
<tr>
<th>B</th>
<th>NR</th>
<th>IP 145.2</th>
<th>55.8</th>
<th>60 - 216</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1  165.0</td>
<td>120.5</td>
<td>8 - 356</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2  157.0</td>
<td>112.4</td>
<td>5 - 317</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3  98.8</td>
<td>71.0</td>
<td>0 - 214</td>
<td>9</td>
</tr>
</tbody>
</table>

H = 2.56, p > 0.05

<table>
<thead>
<tr>
<th>C</th>
<th>RN</th>
<th>IP 90.7</th>
<th>91.1</th>
<th>2 - 219</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1  181.3</td>
<td>83.9</td>
<td>12 - 315</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2  193.2</td>
<td>76.6</td>
<td>32 - 302</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3  124.2</td>
<td>58.7</td>
<td>18 - 261</td>
<td>12</td>
</tr>
</tbody>
</table>

H = 9.58, p < 0.05.

IP vs 1, U = 20.5, p > 0.05.

IP vs 2, U = 16.5, p < 0.05, two-tailed.

IP vs 3, U = 27.0  p > 0.05.
Table VII - 2 Within group differences in the proportion of eggs hatched in the In Progress (IP) dish, and in oviposition dishes 1, 2, and 3, in competition experiment 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DISH</th>
<th>PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NN</td>
<td>IP 0.19 0.17 0.17</td>
<td>0.17</td>
<td>0 - 0.50</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.29 0.17 0.23</td>
<td>0.23</td>
<td>0.03 - 0.74</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.25 0.19 0.19</td>
<td>0.19</td>
<td>0.03 - 0.53</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.29 0.17 0.23</td>
<td>0.23</td>
<td>0.01 - 0.79</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>H = 3.35, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>NR</td>
<td>IP 0.19 0.17 0.17</td>
<td>0.17</td>
<td>0.02 - 0.47</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.15 0.17 0.29</td>
<td>0.29</td>
<td>0 - 0.78</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.12 0.17 0.15</td>
<td>0.15</td>
<td>0 - 0.49</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.07 0.07 0.07</td>
<td>0.07</td>
<td>0 - 0.19</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>H = 3.22, p &gt; 0.05.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>RN</td>
<td>IP 0 0 0</td>
<td>0</td>
<td>0 - 0.60</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.19 0.20 0.20</td>
<td>0.20</td>
<td>0 - 0.60</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.18 0.18 0.18</td>
<td>0.18</td>
<td>0.20 - 0.56</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.22 0.20 0.20</td>
<td>0.20</td>
<td>0.30 - 0.65</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>H = 17.71, p &lt; 0.05.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IP vs 1, U = 7, p < 0.05, one-tailed.
IP vs 2, U = 0, p < 0.05, one-tailed.
IP vs 3, U = 0, p < 0.05, one-tailed.
proportion of eggs hatched in the IP dish was significantly lower than the proportion of eggs hatched in oviposition dish 1, 2, and 3. This is to be expected as the eggs laid by females in the IP dishes in this group would be fertilized by R sperm alone.

The proportion of eggs hatched in the IP dishes alone for the 3 groups is presented in Table VII - 3. A Mann-Whitney U test revealed that the proportion of eggs hatched in the IP dish belonging to the RN group was significantly lower than the proportion of eggs hatched in the IP dish for the NN or the NR group. This too would be expected as the eggs laid in the IP dish for females belonging to the RN group would be fertilized by R sperm, while the eggs laid in the IP dishes of the other 2 groups would be fertilized by N sperm.
Table VII - 3 Differences in the proportion of eggs hatched between the IP dishes for the 3 treatment groups, in competition experiment 2.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>% PROPORTION HATCHED</th>
<th>S.D.</th>
<th>RANGE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>0.19</td>
<td>0.17</td>
<td>0 - 0.50</td>
<td>6</td>
</tr>
<tr>
<td>NR</td>
<td>0.19</td>
<td>0.17</td>
<td>0.20 - 0.47</td>
<td>5</td>
</tr>
<tr>
<td>RN</td>
<td>0</td>
<td>0</td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

H = 11.10, p < 0.05.

NN vs NR, U = 14, p > 0.05.

NN vs RN, U = 3.5 p < 0.05, one-tailed.

NR vs RN, U = 0, p < 0.05, one-tailed.
APPENDIX VIII Calculation of $\bar{P}$ for data obtained in competition experiment 2.

In this experiment:

$p =$ the proportion of eggs fertilized following an NN mating = 0.26,

$z =$ the proportion of eggs fertilized following an R mating = 0.01,

$x =$ the proportion of eggs fertilized following an NR mating = 0.13,

$y =$ the proportion of eggs fertilized following an RN mating = 0.19,

\[
\begin{align*}
P & = 1 - .13 + .01 \cdot \frac{1}{.26} + .26 \\ & = 1 - .5 + .04 \cdot \frac{1}{1 - .04} \\ & = .5 + .04 \cdot .96 \\ & = .5 + .04 \cdot .52 \\ & = .5 + .02 \\ & = .52 \\
\end{align*}
\]

\[
\begin{align*}
P & = 1 - .19 + .01 \cdot \frac{1}{.26} + .26 \\ & = 1 - .73 + .04 \cdot \frac{1}{1 - .04} \\ & = .27 + .04 \cdot .96 \\ & = .27 + .04 \cdot .28 \\ & = .27 + .01 \\ & = .28 \\
\end{align*}
\]

thus, $\bar{P} = P = .52$, and $\bar{P} = 1 - P = .72$,

\[
\begin{align*}
\frac{\bar{P}}{2} & = \frac{.52}{2} \text{ and } \frac{\bar{P}}{2} = \frac{.72}{2} \\
\end{align*}
\]

and mean $\bar{P} = \frac{.52 + .72}{2} = .62$
Appendix IX  The Use of 4 Matings in the Competition Experiments.

When designing the mating protocol for the competition experiments it was necessary to insure that females were inseminated by sperm from both males. Sakaluk and Cade (1980, 1983) reported that between 24.1 - 50% of singly-mated female *Gryllus integer* did not produce offspring, while between 2.2 - 12.5% of doubly-mated *G. integer* did not produce offspring. Therefore, in order to insure that females were successfully inseminated by both males, females were doubly-mated to each male in the mating pattern.

Alexander (1961) reported that female crickets copulate repeatedly. Eventually the female would no longer respond to male courtship and would become receptive again only after ovipositing. Alexander hypothesized that females become non-receptive to male courtship when the spermatheca was full of sperm and would re-mate when sperm had been used. Laboratory experiments with *Teleogryllus commodus* (Evans, 1983), *Acheta domesticus* and *Gryllus integer* (Sakaluk and Cade, 1980) have demonstrated that female crickets will readily mate multiply. Therefore, the assumption was made that females who had completed the mating pattern had not filled the spermatheca and thus sperm from each male would be stored.