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SPERM COMPETITION IN THE NAMIB DESERT BEETLE, *ONYMACRIS UNGUICULARIS*

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Abstract—Sperm competition occurs when a female remates before her initial sperm supply is exhausted. Sperm remained viable in a female *Onymacris unguicularis* (Haag) (Tenebrionidae: Coleoptera) for 7 weeks, but laboratory observations showed a mean number of 5 days between matings. An anatomical basis for sperm displacement was indicated whereby the previous spermatophore was ejected from the female during a subsequent mating. Using the sterile-male technique, there was shown to be a large measure of sperm precedence from the last male to mate with the female.

Key Word Index: Sperm displacement; sperm precedence; sperm viability; mating frequency; irradiation sterilisation; tenebrionid

INTRODUCTION

Sperm competition refers to the competition, within a single female, between sperm from different males for fertilisation of the ova (Parker, 1970a). It is important to note that sperm competition can only occur if the female remates before her initial sperm supply is exhausted (Pruzan-Hotchkiss *et al.*, 1981). Sperm precedence (i.e. non-random sperm use) and sperm displacement (i.e. the replacement at a subsequent mating of the stored sperm of a previous mating) are two components of sperm competition (Gromko and Pyle, 1978). Some degree of sperm competition apparently occurs in most cases of multiple mating (Parker, 1970b).

In the majority of sperm competition studies sperm precedence is shown. It is common that the last male to mate fertilises most of the eggs (e.g. Clarke and Sheppard, 1962; Lefevre and Johnson, 1962; Smith, 1979; McVey and Smittle, 1984) although some cases are known in which sperm of earlier matings is used preferentially to fertilise eggs (e.g. Holmes, 1974; Retnakaren, 1974; Bullini *et al.*, 1976). Sperm precedence can also be incomplete (e.g. Schlager, 1960; Parker, 1970b; Vick *et al.*, 1971; Huettel *et al.*, 1976). Where sperm displacement does not occur and sperm from multiple matings are mixed as in *Gryllus bimaculatus*, "sperm are utilised in proportion to their numerical representation in the spermatheca" (Simmons, 1987).

Anatomical features of the reproductive tract may play an important role in sperm competition. Schlager (1960) related the results of his experiments on sperm precedence in *Tribolium castaneum* to the reproductive structure of the female, where the most recently deposited sperm probably remain in the anterior end of the vagina and are used first in the fertilisation of eggs. Subsequently deposited sperm would displace and thus take precedence over all previously deposited sperm. This advantage deteriorated with time as sperm became mixed in storage. Brower (1975) cited an anatomical basis for positional advantage of sperm whereby, in the Indian meal moth, spermatophore placement next to the opening of the seminal duct prevented migration of sperm from previously deposited spermatophores. Walker's (1980) review demonstrated that the extent of last male sperm precedence was dependent on the shape of the spermathecae.

Studies providing evidence of sperm displacement are not common. Etman and Hooper (1979) provided strong circumstantial evidence that mating induces the female to expel prior sperm from the spermatheca in *Spondoplera litura* while Waage (1979) described the scooping of prior sperm from the bursa copulatrix and spermathecae by the male *Calopteryx maculata*. Further studies using the anatomical approach to work on sperm displacement include comparisons of stored sperm volumes before, during and after copulation. These have indicated between 40–50% sperm removal in *Lestes vigilax* (Waage, 1982), 88–100% removal in *Calopteryx maculata* (Waage, 1979) and 98% removal in *Calopteryx dimidita* (Waage, 1984).

During studies on the multiple mating common in the Namib tenebrionid beetle, *Onymacris unguicularis* (Haag), an anatomical basis for sperm displacement was observed. As sperm competition only occurs if the female remates before her initial sperm supply is exhausted, the first part of this paper (1) endeavours to show that sperm competition does indeed occur in

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O. unguicularis. The paper then (2) describes anatomical events internally in the female reproductive organs during copulation. The third section (3) relates to experiments on sperm precedence using the sterile male technique.

MATERIALS AND METHODS

Experimental animals

Onymacris unguicularis beetles were collected by hand from sand dunes adjacent to the Kuiseb River in the Namib Desert. Freshly collected beetles were used for each set of experiments. They were kept in containers (12 cm high × 50 cm × 25 cm) filled with dune sand to a depth of 6 cm with a 12:12 light–dark cycle. Overhead lamps heated the sand surface to 30–40°C to simulate the temperatures of their natural environment (Holm and Edney, 1973); at night the sand cooled to room temperature. Lettuce and oats were provided *ad libitum*. Once per week the beetles were removed from their containers, chilled to 4°C and sprayed with water to emulate the fog conditions under which they normally obtain water (Hamilton and Seely, 1976).

(1) Experiments to determine occurrence of sperm competition

Sperm viability. Sperm viability, i.e. the length of time that sperm in the female reproductive tract are capable of fertilising eggs, was determined. Fertility was defined as "the percentage of 1st-instar larvae that emerge from an accurately known number of eggs" (Park *et al.*, 1961). Two sets of 18 females were kept in separate containers with males for 4 weeks. One set of females was then isolated from males and the other set was used as a control group. The sand in each container was sieved once per week to remove the eggs which were kept at 30°C until they hatched. Eggs that had not hatched after 5 weeks were counted as infertile.

Mating frequency. Equal numbers of male and female beetles were numbered for identification and were kept in containers as described above. The beetles were observed from 10.00–16.00 h daily for 5-day periods over 5 weeks. Successful copulation, i.e. observation of copulation and evidence of the spermatophore tail protruding from the female, was recorded for each female.

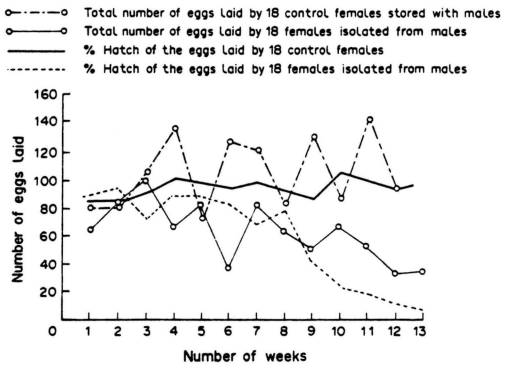

Fig. 1. Number of eggs laid by control female *O. unguicularis* and those isolated from males for 13 weeks. The number of hatchlings is also indicated. $n = 18$.

(2) Dissections of beetles in copula

Mating of beetles was observed and carefully timed. Males and females *in copula* were plunged into liquid nitrogen at 1 min intervals after the start of copulation. Fertilised female beetles were dissected 10, 20, 30, 40, 60 min and 12 and 24 h after the onset of copulation. Dissections ($n = 112$) were performed in insect ringer (Bradbury, 1973).

(3) Sperm precedence using sterile male techniques

Sterile male *O. unguicularis* were produced by irradiation with a ^{60}cobalt source. A range of doses was administered to establish the minimal radiation dosage necessary to achieve complete sterility. Egg fertility from females mated with these males was determined.

Successful breeding of *O. unguicularis* had not been achieved and therefore no source of virgin females was available. Female beetles were kept isolated from males as described above for 20 weeks by which time they were laying few if any eggs and those that were laid did not hatch. These isolated females could therefore be presumed to contain inactive sperm. Fifteen isolated females were placed with sterile males and 10 isolated females were placed with untreated males. Each pair was placed in a separate container. The pairs were maintained under the laboratory conditions described above for 34 days, except that the eggs were removed thrice weekly. The 15 isolated females were then placed with untreated males. The experiment was repeated reversing the periods spent with fertile males and sterile males.

RESULTS

(1) Experiments to determine occurrence of sperm competition

Sperm viability. The control group of 18 females, kept with males, laid 1261 eggs over a 12-week period. These eggs showed 83% fertility. The experimental group, isolated from males, laid 816 eggs in the same time period. The number of eggs laid was consistently lower than the control group. Eggs laid by the experimental females showed 80% fertility until the 7–9th week. Fertility declined and by 12 weeks only 6% of the eggs hatched (Fig. 1).

Mating frequency. Of the females observed over a 5-week period, one died, two never mated and all of the 12 beetles that were mated, mated more than once. If the incidence of multiple mating is defined as the percentage of mated females that mated more than once (Byers, 1978), then there was 100% multiple mating. The mean number of matings per mated female over the 5-week period was 3

(SD ± 2.6; $n = 12$; range 2–11). The mean number of days between mating was 5(SD ± 5.1; $n = 38$; range = 1–19).

(2) Internal events in the female during copulation

Copulation began when the female opened the posterior end of her abdomen and allowed intromission of the aedeagus of the male. A tube then appeared from the male as a temporary elongation of the ejaculatory duct [Fig. 2(1)]. This penile or spermatophoral tube passed through the vagina of the female and into the bursa copulatrix. At this stage the tube contained a coiled core of white opaque secretion surrounded by a thin translucent layer [Fig. 2(2)]. The penile tube passed through the "old" spermatophore in the bursa copulatrix until it reached the top of the bursa. Two simultaneous [Fig. 2(3)] events then occurred.

(i) A bulb appeared in the penile tube and just posterior to it the tube became bent.

(ii) The top of the tube opened and its contents were expelled [Figs 2(4), 3]. The fluid contents of the penile tube appeared to be under pressure. This was observed in unsuccessful copulations where white fluid streamed from the tip of the tube.

The formation of the new spermatophore caused the old spermatophore to be pushed into the vagina and finally to be ejected completely [Fig. 2(5)]. A bleb, consisting of the remains of the bulb and coiled penile tube within the old spermatophore, appeared at the rear end of the female about 12 min after the start of copulation ($x = 12$ min ± SD 4.1; $n = 25$). The bleb fell off after a short time ($x = 37$ min ± SD 25.8; $n = 25$) [Fig. 2(6)]. Copulation time was 7.8 mins (SD ± 2.08; $n = 60$) so the male had withdrawn from the female before the expulsion of the bleb.

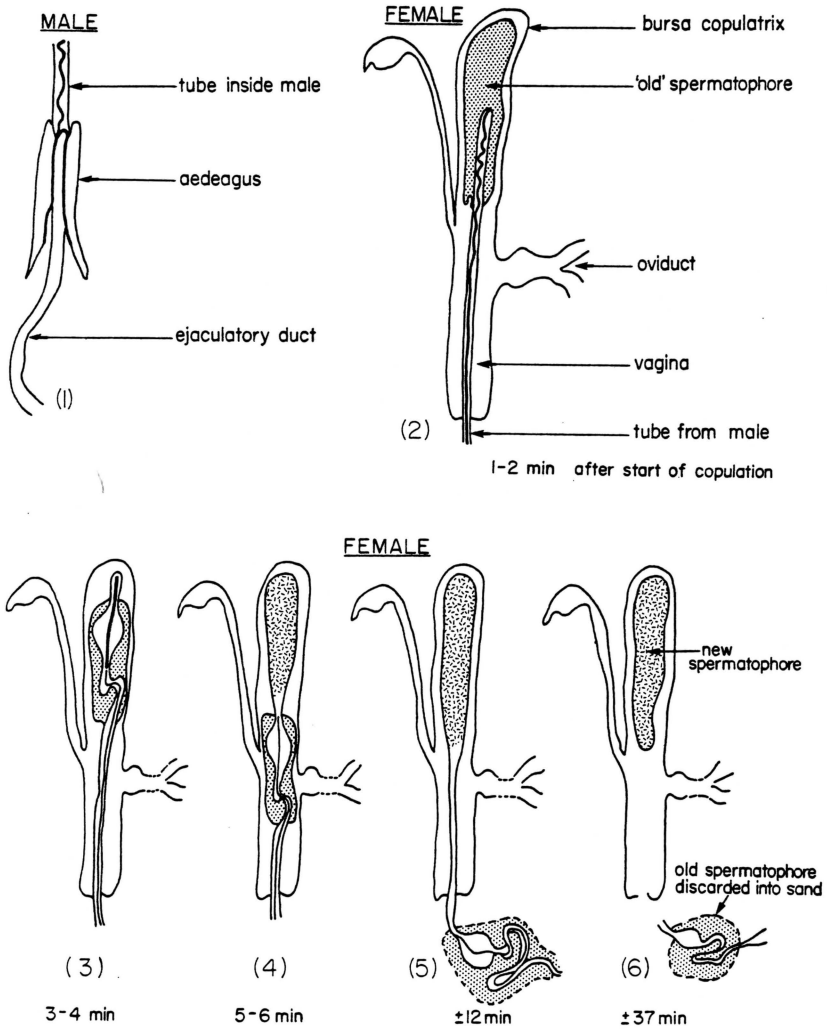

Fig. 2. Internal details of copulation of *O. unguicularis*. (1) Aedeagus of male showing ejaculatory duct with penile tube extending from it. The penile tube is formed inside the female vagina. (2) Male penile tube penetrating old spermatophore in bursa copulatrix of female. (3) Bulb-like structure forms in penile tube near apex of old spermatophore. (4) Penile tube releases new sperm forcing old spermatophore into vagina. (5) Old spermatophore ejected. (6) New spermatophore fills bursa copulatrix.

The new spermatophore took its final shape in the bursa copulatrix of the female. When viewed by means of phase contrast, the spermatophore did not appear to have any definite structural pattern; nor did the sperm appear enclosed in specialised sperm sacs. It seemed rather that the sperm were distributed throughout the seminal fluid from the male and that the spermatophore had a simple, membrane bound, opaque, tubular structure (Fig. 3).

In line with its uncomplicated structure, there was no programmed sequence of swelling and/or rupturing of the spermatophore before liberation of the sperm to the spermatheca as is often found in other insects, e.g. *T. molitor* (Gadzama and Happ, 1974). There was therefore no indication of when the sperm were liberated from the spermatophore or when they migrated into the spermathecal gland, the elongate structure with the swollen tip shown on the left of the bursa (Fig. 2).

During the next 2 weeks the new spermatophore became increasingly translucent in places, losing its white opaque appearance, and the shape of the bursa copulatrix changed. It was swollen, spherical and upright immediately after copulation, but gradually became smaller and increasingly flattened until the upper third was bent over and curled back on itself. The same sequence of events occurred whether it was a "new" male introduced to the female or whether the male and female had been stored together for a long period. The sequence was also independent of the time that had elapsed since the last mating.

(3) Production of sterile males

The results obtained are summarised in Table 1. Complete sterility was caused by exposure to 6000 rads, but beetles exposed to 5000 rads showed greatly reduced fertility without lethal effects and this was therefore the treatment of choice.

(4) Experiments on sperm precedence, using sterile-male technique

(a) The 10 isolated females that were placed with non-treated males to act as a control group, laid 132 eggs during the 34-day period. These eggs hatched 101 larvae, showing 77% fertility.

The 15 isolated females that were placed with sterile males for 34 days laid 189 eggs during this period from which 14 larvae hatched, which is a 7% fertility rate. These same 15 females, when placed with fertile males for a further 7 days, laid 73 eggs which produced 52 larvae—a fertility rate of 71%.

(b) Thirteen isolated *O. unguicularis* females were kept for 4 weeks with untreated males. During this time, 11 females laid 227 eggs and 168 larvae were obtained from these eggs (74% fertility). Sterile males were then put into the containers to replace the fertile males. During the following 4 weeks, the 11 females laid 198 eggs from which 40 larvae hatched (20% fertility).

In both 4(a) and (b), there was no significant difference in the number of eggs laid by females with treated males or with untreated males ($P = 0.2$).

The extent of sperm competition was calculated using the method suggested by Boorman and Parker (1976). For the purposes of the calculation results from normal matings were pooled. A total of 1620 eggs were laid of which 1316 hatched giving 81.2% fertility. Proportion of eggs fertilised by the second male, P_2 values, for normal followed by irradiated male matings and irradiated followed by normal male matings were found to be 0.777 and 0.869 respectively. The overall estimate was 0.823.

Table 1. Production of sterile males using ^{60}Co radiation

Dosage (rads)	Number of eggs laid	Percentage fertile eggs
0	61	82
1000	70	68
2000	91	33
3000	43	20
4000	105	12
5000	68	4
6000	34	0
7000 males	16	0
8000 died	29	0

DISCUSSION

Females kept with males laid more eggs than those isolated from males and the number of eggs laid decreased with increasing time of separation. This implied that the females received some additional material in the form of ovipositional stimulant or ejaculate nutrient. Further experiments (De Villiers, 1984) indicated that material from the ejaculate was absorbed by the female but the exact nature of the contribution is still unknown.

Fertility of eggs in *O. unguicularis* only declined 7–9 weeks after female beetles had been separated from males. However, the average number of matings for females is 3 times in 35 days. This indicates that sperm competition does occur as the female remates before her initial sperm supply is exhausted.

In insect evolution, the production of a spermatophore is considered to represent the primitive condition. There is, however, an evolutionary trend from spermatophores formed in the male before copulation and then placed in the vulva of the female, through to the situation where the intromittant organ of the male penetrates into the supermathecal duct of the female and the spermatophore is formed in the female (Parker, 1970b). An example of the former is the situation in *Tenebrio molitor* where a fully formed spermatophore can be found protruding from the male gonopore, if the male and the female beetles are separated from each other about 30s after the onset of copulation (Gadzama and Happ, 1974). *O. unguicularis* is an example of the latter more advanced condition where the spermatophore is formed within the bursa copulatrix.

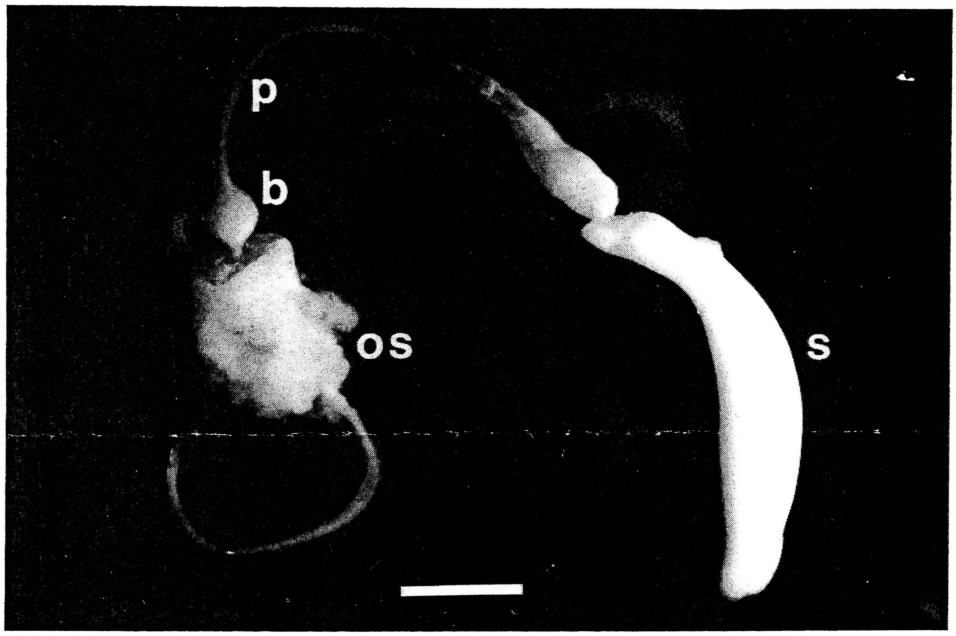

Fig. 3. Partly formed spermatophore dissected from recently mated female *O. unguicularis*. s = new spermatophore, os = old spermatophore, p = penile tube, b = bulb. The old spermatophore has been eased back to reveal the bulb: × 160.

Evidence for the ejection of the previous spermatophore from the female *O unguicularis* during copulation included the following:

(i) The fact that two spermatophores were never found in the bursa copulatrix of a female ($n = 215$ dissections).

(ii) Twice during dissections in saline, the spontaneous formation of the kink below the bulb in the penile tube was observed—on both occasions the "old" spermatophore was visible around the bulb, but as the kink formed, this spermatophore was pulled back to surround the kink.

(iii) All dissections showed the penile tube passing through the "old" spermatophore which then appeared as an entity behind the "new" one.

(iv) The marked difference in colour and composition between the "old" and the "new" spermatophore. Dissections showed a white, opaque and free-flowing fluid forming a mass around the opening of the tube from the male and apparently pushing back the "old" spermatophore, which was basically gelatinous and translucent.

(v) The appearance of an expulsion mass from the rear end of the female 10–15 min after copulation has occurred.

In *O. unguicularis*, there was always a spermatophore present in the bursa copulatrix of gravid females. The females had sperm in the spermatophore and the spermathecal gland. It is not known how quickly, following copulation, that sperm was transferred to the spermathecal gland. The situation in *O. unguicularis* is similar to that in the dragon fly, *Erythemis simplicicollis*, which stores only 5% of the total sperm in the spermathecae and the remainder in the bursa (Waage, in McVey and Smittle, 1984). Other dragon flies have a higher proportion of sperm stored in the spermathecae e.g. *Orthetrum chrysostigma* with 74% (Miller, 1984). The exact proportions were not measured in this study. The dissections gave no evidence of sperm displacement from the spermathecal gland. This could be the area where sperm mixing occurs.

Although there was anatomical evidence for sperm displacement, the question of sperm precedence still remained, as sperm precedence could not be inferred from anatomical studies (McVey and Smittle, 1984). The "old" spermatophores changed in appearance becoming smaller, more gelatinous and translucent with time indicating that sperm may have left the structure. When the spermatophore was removed not all sperm were displaced.

Results of the experiments using sterile males indicated that there was a large measure of sperm precedence from the last male to mate with the female but the calculations of P_2 values indicate that sperm precedence was incomplete when compared to the study by McVey and Smittle (1984) where male *E. simplicicollis* were found to fertilise on average 99.5% of eggs laid immediately after copulation. In experiment 4(a), the immediate batch of eggs laid by females after introduction of normal males showed 71% fertility as opposed to 7% fertility of eggs during a 34-day period with irradiated males. This gave a definite indication of sperm precedence.

The higher fertility of eggs from experimental *O. unguicularis* females first stored with non-treated males prior to the introduction of irradiated males (4b), was an indication that the spermathecal gland might be involved in long-term storage and maintenance of sperm.

Many sperm competition studies do not clearly differentiate between sperm displacement and sperm precedence. It is noteworthy that in this study of *Onymacris unguicularis* it was shown that there is an anatomical basis for sperm displacement that largely explains sperm precedence. There is also evidence that not all sperm are removed when the spermatophore is ejected and that although a high degree of precedence of the newest sperm occurs, sperm competition is incomplete.

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REFERENCES

Boorman E. and Parker G. A. (1976) Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol. Ent.* **1,** 145–155.

Bradbury S. (1973) *Peacock's Elementary Microtechniques* (4th edn.). Arnold, London.

Brower J. H. (1975) Sperm precedence in the Indian meal moth, *Plodia interpunctella*. *Ann. Ent. Soc. Am.* **68,** 78–80.

Bullini L., Coluzzi M. and Bianchi Bullini A. P. (1976) Biochemical variants in the study of multiple insemination in *Culex pipiens* L. (Diptera, Culicidae). *Bull. Ent. Res.* 65, 683–685.

Byers J. R. (1978) Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae) X. Incidence and level of multiple mating in natural and laboratory populations. *Can. Ent.* **110,** 193–200.

Clark C. A. and Sheppard P. M. (1962) Offspring from double matings in swallowtail butterflies. *Entomologist (Lond.)* **95,** 199–203.

De Villiers P. S. (1984) Aspects of the reproductive biology of the Namib tenebrionid beetle, *Onymacris unguicularis* (Haag). Ph. D. thesis, University of the Witwatersrand, Johannesburg, S. Africa.

Etman A. A. M. and Hooper G. H. S. (1979) Sperm precedence of the last mating in *Spodoptera litura*. *Ann. ent. Soc. Am.* **72,** 119–120.

Gadzama N. M. and Happ G. M. (1974) The structure and evacuation of the spermatophore of *Tenebrio molitor* L. (Coleoptera: tenebrionidae). *Tiss. Cell* **6,** 95–108.

Gromko M. H. and Pyle D. W. (1978) Sperm competition, male fitness, and repeated mating by female *Drosophila melanogaster*. *Evolution* **32,** 588–593.

Hamilton W. J. III and Seely M. K. (1976) Fog basking by the Namib desert beetle, *Onymacris unguicularis*. *Nature* **262,** 284–285.

Holm E. and Edney E. B. (1973) Daily activity of Namib desert Arthropods in relation to climate. *Ecology* **54,** 45–56.

Holmes H. B. (1974) Patterns of sperm competition in *Nasonia vitripennis*. *Can. J. Gen. Cytol.* **16,** 789–795.

Huettel M. D., Calkins C. O. and Hill A. J. (1976) Allozyme markers in the study of sperm precedence in the plum curculio, *Contrachelus nenuphar*. *Ann. ent. Soc. Am.* **69,** 465–468.

Lefevre G. and Johnsson U. B. (1962) Sperm transfer, storage, displacement and utilization in *Drosophila melanogaster*. *Genetics* **47,** 1719–1736.

McVey M. E. and Smittle B. J. (1984) Sperm precedence in the dragonfly *Erythemis simplicicollis*. *J. Insect Physiol.* **30,** 619–628.

Miller P. L. (1984) The structure of the genitalia and the volumes of sperm stored in male and female *Nexiothemis farinosa* (Foerster) and *Orthetrum chrysostigma* (Burmeister) (Odonata Libellulidae). *Odonatologica* **13,** 415–428.

Park T., Mertz D. B. and Petrusewicz K. (1961) Genetic strains of *Tribolium*: their primary characteristics. *Physiol. Zool.* **34,** 62–80.

Parker G. A. (1970a) Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *J. Insect Physiol.* **16,** 1301–1328.

Parker G. A. (1970b) The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae) VII. The origin and evolution of the passive phase. *Evolution* **24,** 774–788.

Pruzan-Hotchkiss A., DeJianne D. and Faro S. H. (1981) Sperm utilization in once- and twice mated *Drosophila pseudoobscura* females. *Am. Nat.* **118,** 37–45.

Retnakaran A. (1974) The mechanism of sperm precedence in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **106,** 1189–1194.

Schlager G. (1960) Sperm precedence in the fertilization of eggs in *Tribolium castaneum*. *Ann. ent. Soc. Am.* **53,** 557–560.

Simmons L. W. (1987) Sperm competition as a mechanism of female choice in the field cricket *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.* **21,** 197–202.

Smith R. L. (1979) Repeated copulation and sperm precedence: paternity assurance for a male brooding water bug. *Science* **205,** 1029–1031.

Vick K. W., Burkholder W. W. and Smittle, B. F. (1971) Duration of mating, refractory period and frequency of second matings in female *Trogoderma inclusum* (Coleoptera: Dermestidae). *Ann. ent. Soc. Am.* **65,** 790–793.

Waage J. K. (1979) Dual function of the damselfly penis: sperm removal and transfer. *Science* **203,** 916–918.

Waage J. K. (1982) Sperm displacement by males *Lestes vigilax* Hagen (Zygoptera: Lestidae). *Odonatologica* **11,** 201–209.

Waage J. K. (1984) Sperm competition and the evolution of odonate mating systems. In *Sperm Competition and the Evolution of Animal Mating Systems* (Edited by Smith R. L.). Academic Press, New York.

Walker W. F. (1980) Sperm utilization strategies in nonsocial insects. *Am. Nat.* **115,** 780–799.