Behavioral Ecology Vol. 14 No. 1: 89–96

Prolonged mate guarding and sperm competition in the weevil *Diaprepes abbreviatus* (L.)

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The hypothesis that prolonged copulatory mate guarding coexists with last male sperm precedence was tested for the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). Male *D. abbreviatus* showed a long copulatory guarding. Both males and females were less likely to remate when prolonged guarding occurred compared with terminating copulation early. Guarding was generally terminated by the struggling behavior of the female. Mating experiments using normal and sterile (X-ray irradiated) males revealed a similar value of last-male sperm precedence for both irradiated and normal males. The P_2 values of normal and sterile males were similar when all oviposited eggs were counted over 30 days. These data made it possible to calculate the expected gain to a male from prolonged guarding compared with leaving a female early and seeking out an additional mate. We show that guarding has the higher fitness. Eggs were deposited in clutches in which normal fertilized eggs were grouped together and were attached to a group of sterile eggs. This, together with identifying the form of the cul-de-sac type spermatheca, allowed us to suggest a unique repositioning process, which has not been described elsewhere, as the likely mechanism by which last-male sperm precedence was achieved. *Key words:* copulation guarding, cul-de-sac spermathecae, sperm precedence. *[Behav Ecol 14:89–96 (2003)]*

Prolonged copulatory guarding is a well-described phenomenon in insects and is usually explained as a male adaptation to avoid sperm competition (Simmons, 2000; Thornhill and Alcock, 1983). Mate-guarding involves tradeoffs for males because it consumes time and energy that could be used for finding and mating additional females. It evolves when a male that remains with a single female has greater fitness than a male that seeks additional mating opportunities. Under this explanation, copulatory guarding is expected only when last-male sperm precedence occurs (McLain, 1989; Parker, 1979; Telford and Dangerfield, 1990): if the male stops guarding and the female remates, then most of the eggs she lays will be fertilized by the final male to mate with her.

An alternative hypothesis exists: females may benefit from prolonged male mate-guarding. This can occur through one of two mechanisms. (1) If female fitness is increased by mating with larger males, if mate guarding and male-male competition results in larger males guarding longer than smaller males do, and if the last male to mate fertilizes most of the female's eggs, then mate guarding can increase female reproductive success through direct or indirect benefits (Harari et al., 1999; Simmons et al., 1996). (2) If prolonged copulatory guarding reduces predation, improves female survivorship (Gwynne, 1989; Sivinski, 1983), enhances foraging efficiency (Wilcox, 1984), or saves females time and energy by preventing harassment by searching males (Rowe, 1992; Waage, 1979a), then female fitness is increased when males guard. These explanations suggest that females may play an important role in determining the occurrence and duration of copulatory guarding behavior and that mate guarding cannot be thought of simply as a male adaptation for sperm competition (Jablonski and Vepsäläinen, 1995; Simmons, 1987).

Experimental studies of sperm precedence in insects have revealed that some degree of sperm competition usually occurs after successive inseminations. In most cases, the ejaculate of the last male to mate achieves more than 50% of the fertilizations (Parker, 1984; Simmons, 2000). Several mechanisms for last-male sperm precedence in insects have been suggested: (1) replacement involves removing of rival sperm before insemination with special structures of the penis (Gage, 1992; Siva-Jothy, 1987; Waage, 1979b) or flushing-out sperm, which occurs when new sperm fills up the storage organ and the previous sperm is pushed out (Otronen, 1990); (2) dilution is quantitative competition in which the second male to mate deposits more sperm than was stored from previous matings (Gage, 1991; Newport and Gromko, 1984; Simmons, 1987); (3) destruction occurs when the effective number of previously stored sperm is reduced by the second male through chemical or physical means (Gack and Peschke, 1994; Harshman and Prout, 1994); and (4) repositioning is spatial competition in which the second male to mate moves the sperm within the female's storage organ so that his sperm are more likely to fertilize the eggs (Eady, 1994a,b; Siva-Jothy, 1987). These are male-centered explanations. Females also play an important role in determining the use of sperm (Birkhead and Parker, 1997; Eberhard, 1996).

Female behavior, morphology, and physiology can also influence the process of transferring or storing sperm, the competitive ability of the ejaculates from different males, and, of course, the probability of remating (Birkhead et al., 1993; Eberhard, 1996).

Preliminary observations revealed that males of the West Indian sugarcane rootstalk borer, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) exhibit intense copulatory

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Received 29 January 2002; revised 8 May 2002; accepted 1 June 2002.

guarding: they maintain genital contact long after insemination has occurred. Rival males are attracted to mated pairs (Harari and Landolt, 1997; Harari et al., 2000), and takeovers are common (Harari et al., 1999). Such behavior results in the prediction that last-male sperm precedence should occur in this species. Last-male sperm precedence has been demonstrated in other curculionids such as the plum curculio, *Conotrachelus nenuphar* (Huettel et al., 1976), and the boll weevil, *Anthonomus grandis* (Bartlett et al., 1968), but not in *D. abbreviatus*. The goals of this study are (1) to evaluate the prediction that prolonged copulatory mate-guarding behavior of *D. abbreviatus* is associated with sperm competition and last-male sperm precedence; (2) to examine the hypothesis that male guarding conveys higher reproductive success for males than does searching for additional females with which to mate; (3) to examine whether mate guarding is likely to increase female reproductive success; and (4) to describe the likely mechanism by which sperm precedence is achieved. (5) In addition, because of the unusual way in which female *D. abbreviatus* lay their eggs, we also describe the pattern of differential sperm use by the female after mating with two males.

METHODS

Adult male and female *D. abbreviatus*

Adult *D. abbreviatus* were collected from ornamental trees near Apopka, Orange County, Florida, on nine different occasions during the spring and summer of 1995–1996. Virgin laboratory-reared females and males were acquired from USDA–ARS, Orlando, Florida, USA. All weevils were sexed in the laboratory (Harari and Landolt, 1997). Up to 50 weevils of each sex were maintained in separate Plexiglas frame cages ($30 \times 30 \times 30$ cm) with five sides of 1-mm mesh screening and a Plexiglas bottom. They were fed green beans and kept on the local day length for that time of year (May–October). A double parafilm sheet (3 cm wide $\times$ 10 cm long) was attached with adhesive tape to the inside wall of the females' cages to provide oviposition substrate. These strips were replaced daily. The Plexiglas cages were kept in different field cages ($3 \times 3 \times 3$ m) located 50 m apart, outside of USDA-ARS in Gainesville, Florida, USA, and exposed to outdoor conditions. Field-collected weevils were held for at least 10 days before the experiments to ensure female sexual receptivity. During experiments, adult male and female weevils were placed together in Plexiglas cages and kept under 12 : 12 h light : dark schedule (dawn at 0700 h).

Copulatory guarding

In *D. abbreviatus* the male remains on the female's back in copulatory position with the aedegus continually inserted for more than 16 h. Preliminary experiments demonstrated that females who mate for only 60 min oviposit as many eggs as do females that mate and were guarded all day. The outcome of male-male competition depends, in large part, on the relative size of the two males, with larger males displacing smaller guarding males more easily (Harari et al., 1999, 2000).

Guarding is generally terminated by the struggling behavior of the female. However, when males terminate guarding, they easily climb off the female's back. A female terminates guarding by shaking her abdomen vigorously from side to side; by dropping on her back to the bottom of the cage, which has the effect of dislodging the male; and by running away. Often the female "struggles," shaking her abdomen repeatedly, for some time before the male is dislodged. When the termination of copulation is preceded by shaking behavior, the female is said to have terminated the guarding. The length of time to dislodge a male begins with the first shake and ends with the male dropping off, with repeated shaking interspersed with periods of other activities. Guarding generally terminates around sunset, and most females oviposit during the night after copulation.

Mate-guarding experiments were conducted in outdoor enclosures beginning at 0700 h on 12–15 October 1996 by using field-collected weevils. Ten females and 10 males were placed in a cage, and pairs were allowed to copulate and guard for the following time periods: (1) 2 h, (2) 3 h, (3) 8 h, and (4) until copulatory guarding was terminated naturally without interference (12–16 h; $n = 3$ cages of 10 pairs for each guarding period). Mating couples were marked with dots of Testors gloss enamel paint with the same color combination for each member of a pair, so we could tell if changes in mating partners had occurred. Couples were interrupted at the designated time by gently separating the mounting males by hand from the females. Dislodged males (treatments 1, 2, and 3) were then placed in a separate cage, males of each treatment in a different cage, with 20 females. Dislodged females (treatments 1, 2, and 3) were placed in a separate cage for each treatment with 20 males. Couples that ceased mating naturally (treatment 4) were placed in different cages for males and for females (10 individuals in a cage, with 20 beetles of the opposite sex, $n = 3$ cages). The beetles in all treatments were allowed to remate and then remain guarding until the pair broke up naturally. If either males or females failed to copulate within 1 h, they were taken from the cage, together with the surplus males and females, to avoid male–male competition and takeovers.

This remating experiment was necessarily confounded by time of day because beetles that were separated after 2 h were set to remate in the morning (0900 h), those separated after 4 h were set to remate in late morning (1100 h), and those separated after 8 h were set to remate in mid-afternoon (1500 h), whereas beetles that were allowed to separate naturally were tested for remating in late afternoon and early evening. As a control for a possible time of day effect, we allowed a second set of beetles to mate for the first time at the same time of day as the remating experiments were conducted. Ten males and 10 females were placed together in a cage at 0900, 1100, and 1500 h and were allowed to mate ($n = 3$ cages at each designated time). As a control for treatment 4, in which males and females ceased mating naturally, unmated males and unmated females were introduced into a control cage (10 couples in a cage; $n = 3$ cages) at the same time as the first couple in treatment 4 was ending copulation. As in the remating experiment, males and females that failed to copulate within 1 h were removed.

General linear model (GLM) with the time of remating, sex, and cage as factors (SYSTAT, 1990) was used to compare the number of remating females and males, and Tukey's comparison was used to identify the source of the significant differences in remating numbers. GLM was also used to compare each time regime with its parallel time in the control, for each sex. GLM analyses were also used to compare the number of males and females that terminated copulatory guarding in each treatment group with time and cage as factors, and Tukey's comparison was used to identify the source of the significant differences in the number of beetles that terminated mating for each gender.

Sperm precedence

To determine the outcome of sperm competition between two males, we used the technique of reciprocal normal/sterile male double-mating described by Boorman and Parker (1976). Two males were allowed to mate with each female. One of the males had been irradiated with X-ray radiation.

Irradiated sperm are capable of fertilizing eggs, but the embryo fails to develop (Boorman and Parker, 1976). Using this method means that viable eggs were sired by the normal male, whereas eggs that failed to develop were sired by the irradiated male. By using a dose of 10 krad (1200 R/min), all eggs fertilized by irradiated sperm failed to develop (see Results). We compared the proportion of eggs that developed from the four treatment groups. Females of group NI ($n = 9$) were first allowed to mate with normal males and were then mated with irradiated males. Females of group IN ($n = 10$) were presented with irradiated males first and then with normal males as second mates. Females of group II ($n = 10$) were presented with irradiated males first and second; NN ($n = 10$), with normal males first and second. IN and NI matings were used to assess the relative competitive ability of normal and irradiated sperm, whereas II and NN matings were performed to correct for variation in natural fertility of males in the population and to assess variation in the effectiveness of the irradiation protocol (Boorman and Parker, 1976)

Only virgin, laboratory-reared weevils were used in these experiments. Ten females were placed in a cage with 30 males. A male-biased sex ratio was used because some irradiated males did not mate. Only large males were used, to control for a possible effect of ejaculate volume and female preference for large males (Harari et al., 1999). When the pairs formed, noncopulating males were removed from the cage. Mated males were allowed to guard the females for 4 h, after which the pair was separated by hand and the female was presented with 30 new virgin males. After the pairs were formed, noncopulating males were removed from the cage. Second mating males were also allowed to guard the females for 4 h. Each round of copulation and guarding was limited to 4 h because two successive males had to mate in the same day while females were still receptive and before they oviposited at night.

Females were allowed to oviposit until only unfertilized eggs were being laid, that is, until their sperm supply was depleted or lost viability. Parafilm sheets with eggs were taken from the cages daily and kept in a greenhouse. After five days of development, the condition of the eggs was categorized by visual inspection: (1) fertilized eggs showed brown head capsules of the developing embryos, (2) fertilized eggs that failed to develop showed no brown head capsules, and (3) unfertilized eggs were clear with no signs of embryonic development. For each treatment group, we measured the number of eggs in each category on each successive day. ANOVA (SYSTAT, 1990) was used to compare the number of eggs oviposited by females after mating in IN, NI, NN, or II treatment groups on the first day of oviposition, with the cumulative number of eggs oviposited during 30 days. The t test was used to compare the number of eggs sired by the second males in IN and NI treatments after the first night after mating and after 30 days of oviposition. The degree of second-male sperm precedence (P_2) was calculated as described by Boorman and Parker (1976).

Pattern of differential sperm use by the female

Females deposited their eggs in clutches with the eggs arranged in rows, which meant that the order of fertilization by the different sires could be distinguished. As described above, eggs sired by a normal male developed a brown head capsule within five days of oviposition, whereas eggs sired by a sterile male had no brown head capsules but could be distinguished from unfertilized clear eggs. The pattern of sperm utilization by females was examined in egg clutches oviposited on the first night after mating with either NI or IN males. The first egg clutches oviposited on parafilm sheets by seven females of each group were taken to the greenhouse.

Table 1
General linear analysis of the effect of guarding time and sex on the rate of remating in the beetle *Diaprepes abbreviatus*

Source	Sum-of-squares	df	Mean-square	F ratio	p
Time	549.229	3	183.076	744.718	.000
Sex	0.188	1	0.188	0.763	.388
Cage	1.583	2	0.792	2.111	.184
Time × sex	1.229	3	0.410	1.667	.190
Time × cage	1.417	6	0.236	0.630	.705
Error	3.000	8	0.375		

$n = 24$, multiple $R = 0.995$, squared multiple $R = 0.989$.

After five days of development, attached eggs, which were deposited sequentially and sired by the same male, were counted separately from attached eggs that were sired by the other male, until all eggs in the clutch were counted. The distribution of the eggs on the sheet was analyzed using a runs test for randomness (Zar, 1988).

The spermathecal type

The type of spermatheca may have a crucial effect on the results of sperm competition (Eberhard, 1996). Dissections of freshly killed, virgin female specimens of *D. abbreviatus* made it possible to observe the muscle attachments, as well as the sclerotized parts of the female genitalia. After cutting the pleural membrane between the sterna and terga, it was possible to remove the entire genital apparatus with a fine-tipped forceps. This dissection included the spermatheca, which is attached by the spermathecal duct to the bursa copulatrix of the female genital system. Fat bodies, connective tissue, and musculature were not cleared with KOH or lactic acid, as is usual with such dissections. This made it easier to study the muscles and their attachments and to interpret the function of these muscles in the transfer of sperm from the spermatheca in the process of fertilization during oviposition.

RESULTS

Copulatory guarding

Males and females did not differ significantly in their tendency to remate after guarding, with a significant effect of guarding duration on remating for both males and females (Table 1). Both males and females were less prone to remate late in the day (Table 2). Almost all males and females (93–96%) remated when copulations were interrupted after 2, 4, and 8 h, but only 16.7 ± 5.8% of males and 20.0% of females remated when guarding ceased naturally after 12–16 h (Table 2). Similar results were obtained for the control groups: almost all control females and males mated when first introduced at the time when beetles of treatments 1, 2 and 3 were remated (0900, 1100, and 1500 h, respectively), but only 23.3% of females and 16.7% of males mated when they were introduced first late in the day (≥1900 h; Table 2).

The vast majority of females (93.3%) that had been guarded for 12–16 h struggled at the time of remating with a new male, and only a few mated a second time (20.0%). When they did, guarding lasted only 35.5 ± 18.4 min (mean ± SE; range, 17–63 min). Control females for treatment 4, which were mating for the first time late in the day, also struggled and were guarded only for a short period of time (23.3 ± 5.80% mating; guarding time, 37 ± 25.4 min; range, 7–72 min).

Males and females differed significantly in their behavior during the termination of guarding (Tables 3 and 4). Mate

Table 2
Percentage of remating after different copulatory guarding durations in *Diaprepes abbreviatus*

Guarding time	Remating (% ± SE)		Control (% ± SE of mating beetles)	
	Females	Males	Females	Males
2 h	96.7 ± 5.8[a]	96.7 ± 5.8[a]	100[a]	100[a]
4 h	100[a]	96.7 ± 5.8[a]	100[a]	96.7 ± 5.8[a]
8 h	93.3 ± 5.8[a]	96.7 ± 5.8[a]	93.3 ± 5.8[a]	96.7 ± 5.8[a]
All day	20.0 ± 10.0[b]	16.7 ± 5.8[b]	23.3 ± 5.8[b]	16.7 ± 5.8[b]

Guarding times 2, 4, and 8 h were ended experimentally, whereas "all day" refers to naturally concluded matings. Controls were allowed to mate for the first time at the same time of day as the remating experimental individuals to control for the effect of time of day.

[a,b] Different letters indicate significant differences (Tukey HSD; comparing numbers of females and males in different timing [columns] and between groups at a similar time [rows]; SYSTAT 1990).

guarding appeared to be terminated by the females in more than 90% of pairs lasting for 12–16 h (treatment 4); termination was shown rarely by females after 8 h of guarding (treatment 3) and was never shown by mated females in treatments 1 and 2. Only 10.0% of the males terminated mating after 12–16 h of guarding, (treatment 4), and as few as 3.3% terminated mating after 8 h of guarding (treatment 3). No guarding male was observed to terminate a mating after only 2 and 4 h of guarding (treatments 1 and 2). The duration of struggling to dislodge a male was highly variable, from 2 to 66 min (30.19 ± 15.31 min, $n = 27$), with long periods of inactivity interspersed with periods of struggling. Males appeared to have little control over these attempts to end mate guarding. Only twice did a dislodged male regain a female, and then only for a few minutes.

Sperm precedence

The number of fertilized eggs laid by females was high on the first night after copulating, dropped sharply on the second day, increased gradually for about a week, and then decreased again (Figure 1A,B). The number of unfertilized eggs oviposited by females increased gradually over time, owing to either sperm depletion and/or diminished sperm viability (Figure 1C,D). Nearly all (99.6 ± 0.4%) eggs hatched from clutches oviposited during the night after mating to normal males (NN), whereas only 74 ± 11.2% of eggs hatched when oviposited during the 30 days after mating with NN males. This means that females oviposited few unfertilized eggs after mating with normal males, and this number gradually increased over time (Figure 1D). There was no significant difference between the number of eggs laid on the first night of oviposition by females mated with two normal males (NN), two sterile males (II), or one normal male and the other sterile (NI and IN) (mean ± SD; 168.0 ± 50.8, 154.4 ± 30.7, 183.0 ± 28.2, 155.7 ± 49.4, respectively; ANOVA $F_{3,35} = 0.995$, $p = .407$). Similar results were obtained over the 30-day period of oviposition (mean ± SD; 2721.5 ± 736.8, 2492.4 ± 41303.7, 2582.1 ± 494.0, 2769.8 ± 510.9, respectively; ANOVA $F_{3,35} = 0.481$, $p = .698$).

The second male to mate, either normal (IN) or irradiated (NI), sired most of the eggs oviposited during the first night after mating. No significant difference was detected between the percentages of eggs sired by the normal males when second, or by the irradiated males when second (mean ± SE; 72.5 ± 7.4%, 74.3 ± 15.9%, respectively; t test, $t = 0.429$, $p = .673$, df = 17). Second male sperm precedence, P_2, with IN males was 0.75 (range, 0.50–0.86) and 0.72 with NI males (range, 0.41–0.98). Last-male sperm precedence continued over 30 days of egg laying, in about the same proportion, and there was no significant difference between the percentage of eggs sired by the second males in IN and NI treatment groups (mean ± SE; 57.6 ± 13.1%, 50.4 ± 10.0%, respectively; t test, $t = 0.464$, $p = .649$, df = 17). P_2 after mating with IN was 0.76 (range, 0.56–0.97), whereas after mating with NI, P_2 was 0.70 (range, 0.47–0.87).

Table 3
General linear analysis of the effect of copulatory guarding duration on the number of males and females that terminated copulation in *Diaprepes abbreviatus* pairs

Source	Sum-of-squares	df	Mean-square	F ratio	p
Females[a]					
Time	178.000	3	59.333	142.400	.000
Cage	0.167	2	0.083	0.200	.824
Error	2.500	6	0.417		
Males[b]					
Time	2.000	3	0.667	1.600	.285
Cage	0.167	2	0.083		.824
Error	2.500	6	0.417		

[a] $n = 12$, multiple $R = .993$, squared multiple $R = .986$.
[b] $n = 12$, multiple $R = .681$, squared multiple $R = .464$.

Pattern of differential sperm use by the female

Eggs were deposited in clutches, and the eggs of different sires (NI or IN) were clumped in small groups (Figure 2). This meant that females mated to a normal male second (IN) oviposited fertilized eggs in bunches separated by smaller bunches of undeveloped eggs sired by the sterile male (mean ± SE; 21.3 ± 3.9 normal eggs, range, 15–32; and 7.2 ± 1.9 undeveloped eggs, range, 4–11; $n = 7$ egg clutches). The same pattern was detected when a female was mated to the sterile male second (NI), with the difference that bunches of undeveloped eggs were separated by smaller bunches of normal developing embryos (mean ± SE; 19.7 ± 4.4 sterile eggs, range, 14–32; and 8.1 ± 2.3 developed eggs, range, 5–13; $n = 7$ egg clutches). In both mating patterns, single unfertilized eggs were scattered individually over the clutch. The pattern of egg deposition according to the different sires was found to be highly nonrandomized, such that eggs sired by one male were more likely to be oviposited next to eggs sired by the same male than next to an egg fertilized by a different male (runs test for randomness; $10.01 > z > 7.61$, $p < .001$, for a total of 14 egg clutches; Zar, 1988).

Table 4
Natural termination of mating after different copulatory guarding durations in *Diaprepes abbreviatus*

Time	Mating termination (% ± SE)	
	Females	Males
2 h	0	0
4 h	0	0
8 h	3.3 ± 5.7[a]	3.3 ± 5.7[a]
All day	90.0 ± 10.0[b]	10.0 ± 10.0[a]

[a,b] Different letters indicate significant differences (Tukey HSD; comparing numbers of females and males that terminated mating in different timing [columns]; SYSTAT 1990).

The spermatheca

The spermatheca (Figure 3) is C-shaped, with a dorsal lobe (the ramus) opening to the spermathecal gland. The sclerotized spermatheca is flexible and has muscle attachments between the base of the body of the spermatheca and its apex. The flexing of these muscles may act to pump sperm out of the spermatheca along with secretions from the spermathecal gland, which pass through the ramus and mix with the sperm. Together the sperm and the secretions are pumped out of the spermatheca through the spermathecal duct to the bursa copulatrix and on to the oviduct, where they meet the ova, which are fertilized by the sperm. Mature eggs pass from the oviduct along the ovipositor to be placed between leaves, along with an adhesive that attaches the eggs to the leaves and keeps the leaves together.

DISCUSSION

Copulatory guarding

D. abbreviatus weevils remain in copula for more than 16 h, with the male riding on the female's back with his aedegus inserted. When guarding ends naturally at dusk, *D. abbreviatus* females are unlikely to remate, but when guarding is interrupted early, most females remate. This pattern is coupled with strong last-male sperm precedence (P_2 = 0.76), as predicted by the hypothesis that mate guarding evolves as a paternity assurance mechanism (Dickinson, 1995; Gwynne, 1984). Weak second-male fertilization success (P_2 = 0.52–0.59) has been found in two other curculionids (Bartlett et al., 1968; Huettel et al., 1976), although apparently no copulatory guarding occurs in these species (Tumlinson JH, personal communication). This suggests that the intensity of copulatory guarding in curculionids is correlated with the degree of sperm precedence, as it is in other species of insects (Gwynne, 1984; McLain, 1989; Rowe, 1992; Smith, 1979; Waage, 1979b).

Our data allow us to estimate the reproductive success associated with guarding. When guarding, the male incurs an opportunity cost (mating with a reduced number of females), but he fertilizes about 75% of the eggs that the female lays and reduces her chances of remating. However, if a male leaves his first female, she is likely to remate before oviposition, and because of last-male sperm precedence, most of her eggs will be fertilized by a second male. If the male leaves, however, he can mate again if he can locate another female and fertilize most of the eggs laid by his second female. To summarize this argument:

Gain from staying, or number of eggs fertilized by male that stays and guards = (number of eggs laid first night ×

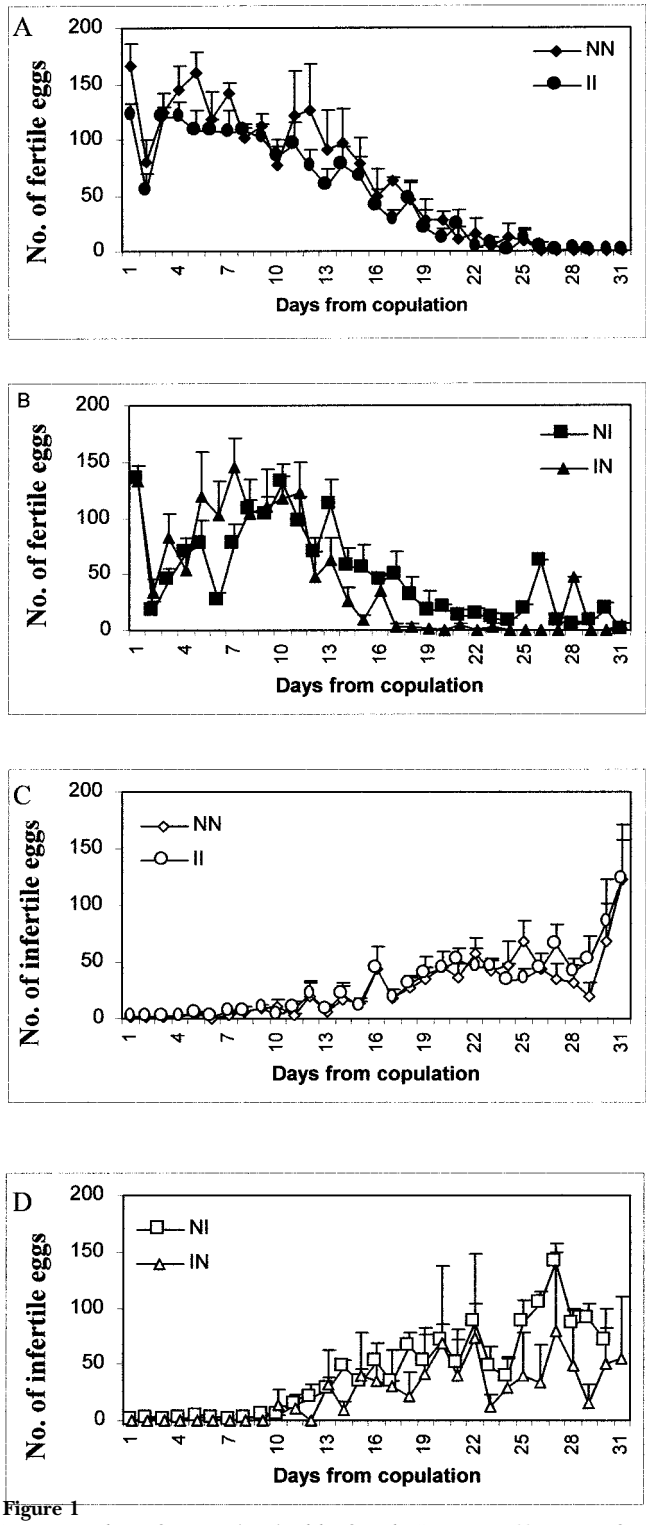

Figure 1
Mean number of eggs oviposited by female *Diaprepes abbreviatus* after double-mating over the 30 days after oviposition. (A) Fertile eggs oviposited after sequential mating with two normal males (NN; n = 10 females) and with two sterile males (II; n = 10 females). (B) Fertile eggs oviposited after sequential mating with a normal male first and an irradiated male second (NI; n = 9 females), and with an irradiated male and a normal male second (IN; n = 10 females). (C) Infertile eggs oviposited after sequential mating with two normal males (NN; n = 10 females) and with two sterile males (II; n = 10 females). (D) Infertile eggs oviposited after sequential mating with a normal male first and an irradiated male second (NI; n = 9 females) and with an irradiated male and a normal male second (IN; n = 10 females).

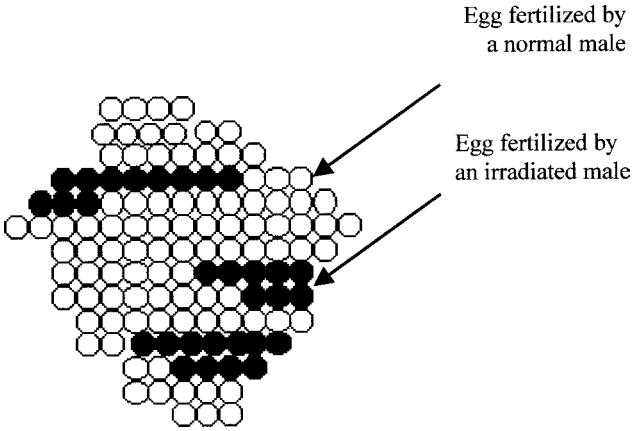

Figure 2
An example of an egg clutch oviposited by a female after a sequential mating with a normal male first and an irradiated male second. Eggs sired by normal males could be distinguished from those sired by irradiated males by their brown head capsules.

paternity × proportion hatching) = (168 × 0.75 × 0.99) = 124.7 eggs.

Gain from leaving, or number of eggs fertilized by male that leaves female 1 and mates with female 2: *female 1* = [number of eggs laid × paternity × probability female will remate (mean of remating females in Table 2) × hatchability] for first night + gain from small portion of females that do not remate = [(168 × 0.25 × 0.97 × 0.99) + (168 × 1 × 0.05 × 0.99)] = 49.0; *female 2* = 124.7 eggs × *P*, where *P* is the probability of finding and mating with a second female; *female 1* + *female 2* = 49.0 + 124.7*P*.

If the probability of finding a female, *P*, is less than 0.60, then males should leave more surviving offspring by guarding than by not guarding. All receptive females are generally guarded, so a male that leaves off guarding would have to take over a female from another male. The probability of a large male taking over a small male is high, whereas the reverse is practically zero. In a laboratory study, the mean probability of a male taking over a male that was guarding a female was 0.37 (Harari et al., 1999). Incorporating this figure into the equation, the probability of winning in male-male competition for the guarded female ($P = 0.37$) reveals that the gain from leaving the female (95.1 fertilized eggs) is less than the expected gain from guarding (124.7 eggs).

Females may also gain from mate guarding. Prolonged copulatory guarding may assist females in flights between foraging patches (Thornhill, 1984), protect females from various dangers (Gwynne, 1989; Sivinski, 1983), or minimize the loss of time and energy required to resist male mating attempts (Gwynne, 1984; Parker, 1984; Waage, 1979a). During the prolonged guarding behavior of *D. abbreviatus*, mating pairs remain on leaves and females continue to feed while in tandem; females do not waste energy in lengthy premating struggles, and they deposit their eggs after guarding has terminated. Large unmated males are attracted to mating couples, and evidence suggests that females benefit from mating with larger males (Harari and Landolt, 1997; Harari et al., 1999). This means that mate guarding in *D. abbreviatus* may be of mutual benefit, because males gain from guarding by reducing sperm competition with other males, whereas females may benefit from being guarded if male-male competition results in larger, better quality males guarding longer and siring more of her progeny.

Gwynne (1984) argues that strong last-male sperm precedence is often associated with high male investment in the female or her offspring. Males may provide protein nourishment to the female during copulation (Freidel and Gillott, 1977) or introduce secretions during mating (Monsma et al., 1990) that greatly increase sperm precedence. We have evidence that male *D. abbreviatus* transfer some materials into the female's genital track along with the sperm; these can be detected in the female hemolymph a few minutes after mating (Harari et al., 1999), but we do not know whether these materials influence sperm precedence patterns.

Sperm precedence

Studies of sperm competition assume no differences in the competitive ability of the two ejaculates (Eady, 1991; Gwynne, 1984). In our study, sterilized males that were mated with females on the day of sterilization died after 3–10 days, whereas normal males did not die after more than 60 days. Mortality after irradiation treatment is known to occur in another curculionid, the boll weevil, *A. grandis,* owing to the destruction of the midgut regenerative cells, which replace secretory cells that are continually sloughed (Reimann and Flint, 1967). Despite differences in survivorship, our data do not reveal differences among males in their ability to fertilize eggs: P_2 values were similar between the normal (0.75 after IN) and irradiated males (0.72 after NI).

Like most other beetles that have been studied (P_2 = 0.52–0.83; Bartlett et al., 1968; Lewis and Austad, 1990), *D. abbreviatus* shows a clear pattern of partial second-male sperm precedence, but the mechanism by which precedence is achieved is unclear. (1) Replacement of previous sperm is associated with very high sperm precedence values (McVey and Smittle, 1984) and implies special penile or other structures (Gage 1992; Waage, 1984) that are capable of removing previously stored sperm, but these are not found in *D. abbreviatus*. Flushing and other mechanisms often result in the appearance of old ejaculate discharged from the female's genitalia (Eady, 1994a; De Villiers and Hanrahan, 1991), but this does not occur in *D. abbreviatus*. (2) The dilution mechanism suggested by Newport and Gromko (1984) implies that sperm are stored as a mixture and released randomly, and that last-male sperm precedence is achieved owing to a high rate of sperm loss right after insemination by both first and second males. In this case last-male sperm precedence will increase over time, as more sperm of previous ejaculates are lost.

This prediction does not agree with our results. In *D. abbreviatus* no significant difference in values for last-male precedence occur over the 30 days of oviposition, that is, to the time when sperm are becoming inviable or used up by the female. These results would be consistent, however, with another possible mechanism for a second-male advantage based on sperm mixing: the second male to mate adds more sperm than the first (Gage, 1991; Parker et al., 1990). However, both sperm-mixing mechanisms assume random release of sperm from the two ejaculates, which is in contrast with our results. Eggs from clutches oviposited by NI or IN females were not randomly distributed, but were aggregated such that eggs fertilized by the irradiated male were more likely to be found together than with eggs fertilized by the normal male (and vice versa). This unique oviposition pattern within a clutch suggests nonrandom fertilization. (3) In the destruction mechanism, later ejaculates cause a reduction in the effective number of previously stored sperm (Eberhard, 1985; Harshman and Prout, 1994; Parker, 1970). This mechanism could not be confirmed or denied for *D. abbreviatus*, but it seems unlikely as it is not obvious how this mechanism could explain the nonrandom pattern of egg laying. (4) Alternatively, the observed second-male advantage in reciprocal IN and NI matings could be the result of sperm

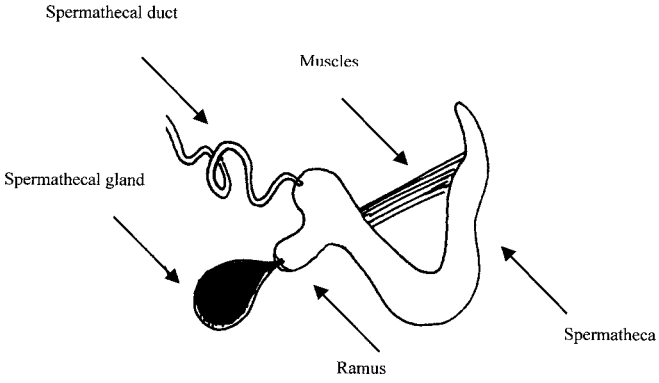

Figure 3
The spermatheca of *Diaprepes abbreviatus* (spermathecal muscles, ramus, and spermathecal gland).

repositioning (Siva-Jothy 1987), which refers to the displacement of previous sperm within the female's storage organ such that the new sperm will be placed closer to the site of fertilization (Smith, 1979; Waage, 1984). Eady (1994a) and Siva-Jothy and Tsubaki (1994) show that sperm repositioning (sperm stratification) results in a reduction of P_2 with time as the second male's sperm is progressively used, improving the location of sperm ejaculated by the previous male. However, P_2 in *D. abbreviatus* remains constant as fertile sperm are gradually reduced. This may be explained by the rate of sperm mortality exceeding sperm utilization, that is, if a decrease in both the first male's sperm and the second male's sperm viability occurred before there was a significant decline in the numbers of the second male's sperm, then females would not utilize all the second male's sperm and would not have the opportunity to make use of all the first male's sperm before it became inviable. The sperm repositioning mechanism is thought to operate best in spermathecae of the cul-de-sac type, in which a single duct is used to place sperm into the spermatheca and lead sperm out of it for fertilization (Eady, 1994a; Siva-Jothy and Tsubaki, 1994). In such spermathecae, the second male to mate pushes the first male's sperm away from the opening and positions his own sperm closer to the exit point, increasing the chance that his sperm will be used to fertilize the eggs. The spermatheca of *D. abbreviatus* is of the cul-de-sac type, and therefore, repositioning of the sperm is the probable mechanism to achieve the second male's sperm precedence.

Cryptic female choice (Eberhard, 1994, 1996) might influence the degree of sperm precedence under any of the suggested mechanisms (Eady, 1994b; Knowlton and Greenwell 1984). Villavaso (1975) has shown that female boll weevils use a spermathecal muscle to limit the amount of sperm displaced by the second male. Similar muscles connecting the apex of the C-shaped spermatheca to its base were observed in *D. abbreviatus*. In addition, a ramus diverges from the spermatheca close to the exit duct. The ramus, which is attached at its apical end to the spermathecal gland, together with the muscles that connect the two parts of the spermatheca, may play a significant role in the pattern of sperm release and in its preferential use by the female to fertilize the eggs. Our assumption is that most of the first male's sperm is repositioned by the second male's sperm, pushed into the apex of the spermatheca, where the first male's sperm is not available for fertilization of the ova. At the same time, a portion of the first male's sperm is forced, by the sperm of the second male, into the ramus. When fertilizing the eggs, females first use sperm of the second male to mate, which is placed closer to the fertilization duct, but frequently insert some sperm of the first male, confined in the ramus, into the fertilization duct. This sperm insertion can be done while contracting the muscles and giving the sperm locked in the ramus a way out. The female may also spatter materials from the gland while pushing the sperm out from the ramus.

Cryptic choice was not tested in this study because the first and the second males were previously chosen to be of the same size. In his evolutionarily stable strategy model for the evolution of guarding, Yamamura (1986) argued that when sperm competition occurs, the optimal strategy is either to guard until oviposition or not to guard at all. The guarding strategy is advantageous (1) when the population is male biased, (2) when high searching efficiency exists, (3) under conditions of high population density, and (4) when the preoviposition period is short. In wild populations of *D. abbreviatus*, as well as in our experimental cages, the operational sex ratio was 1:1; wild population densities varied from low (five couples on a tree) to dense (hundreds of couples on a single tree) (personal observations); and oviposition took place during the night after insemination. Our study suggests several additional factors that should be added to Yamamura's list. Copulatory guarding will be advantageous when (1) unguarded females are receptive to new males after insemination, (2) takeover attempts and intrasexual competition are common, (3) the probability of finding an unmated female is low, and (4) males donate some kind of resource to the female during copulation. These attributes will contribute to the cost of remating and thus increase the success associated with remaining with a mated female. Under these conditions, guarding will result in higher long-term reproductive success than will searching for additional females. When females also benefit from male guarding, directly or indirectly, then no sexual conflict is expected and copulatory guarding should evolve.

We thank W. J. Schroeder and H. Smith, USDA-ARS, Orlando, Florida, for supplying laboratory-reared weevils and B.J. Smittle, FDACS, Division of Plant Industry, Gainesville, Florida, for irradiating the weevils. We also thank Dr. V. Chew, USDA-ARS, South Atlantic Area, for advice on statistical analysis and Dr. J. Sivinski, USDA-ARS, Gainesville, Florida, for helpful comments on an earlier draft. This research was supported by Postdoctoral Fellowship Award No. IF-0183-94 from the United States-Israel Binational Agricultural Research and Development Fund (BARD) and the University of Florida Foundation. C.W.O. was supported in part by grant FLAX 97007 from CSREES (Cooperative State Research, Education, and Extension Service, USDA).

REFERENCES

Bartlett AC, Mattix EB, Wilson NM, 1968. Multiple matings and use of sperm in the boll weevil, *Anthonomus grandis*. Ann Entomol Soc Amer 61:1148–1155.

Birkhead TR, Moller AP, Sutherland WJ, 1993. Why do females make it so difficult for males to fertilize their eggs? J Theor Biol 161:51–60.

Birkhead TR, Parker GA, 1997. Sperm competition and mating systems. In: Behavioural ecology: an evolutionary approach (Krebs JR, Davies NB, eds). Oxford: Blackwell Science; 121–148.

Boorman E, Parker GA, 1976. Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. Ecol Entomol 1:1451–1455.

De Villiers PS, Hanrahan SA, 1991. Sperm competition in the Namib Desert beetle, *Onymacris unguicularis*. Insect Physiol 37:1–8.

Dickinson JL, 1995. Trade-offs between postcopulatory riding and mate location in the blue milkweed beetle. Behav Ecol 6:280–286.

Eady PE, 1991. Sperm competition in *Callosobruchus maculatus*

(Coleoptera: Bruchidae): a comparison of two methods used to estimate paternity. Ecol Entomol 16:45–53.
Eady P, 1994a. Intraspecific variation in sperm precedence in the bruchid beetle *Callosobruchus maculatus*. Ecol Entomol 19:11–16.
Eady P, 1994b. Sperm transfer and storage in relation to sperm competition in *Callosobruchus maculatus*. Behav Ecol Sociobiol 35:23–129.
Eberhard WG, 1985. Sexual selection and animal genitalia. Cambridge: Harvard University Press.
Eberhard WG, 1994. Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. Evolution 48:711–733.
Eberhard WG, 1996. Female control: sexual selection by cryptic female choice. Princeton: Princeton University Press.
Freidel T, Gillott C, 1977. Contribution of male-produced proteins to vitellogenesis in Melanoplus. J Insect Physiol 23:145–151.
Gack C, Peschke K, 1994. Spermathecal morphology, sperm transfer and a novel mechanism of sperm displacement in the rove beetle, *Aleochara curtula* (coleoptera, Staphylinidae). Zoomorphology 114:227–237.
Gage MJG, 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. Anim Behav 42:1036–1037.
Gage MJG, 1992. Removal of rival sperm during copulation in a beetle, *Tenbrio molitor*. Anim Behav 44:487–589.
Gwynne DT, 1984. Male mating effort, confidence of paternity, and insect sperm competition. In: Sperm competition and the evolution of animal mating systems (Smith RL, ed). New York: Academic Press; 117–149.
Gwynne DT, 1989. Does copulation increase the risk of predation? Trends Ecol Evol 4:54–56.
Harari AR, Handler AM, Landolt PJ. 1999. Size assortative mating, male choice and female choice in the curculionid beetle *Diaprepes abbreviatus*. Anim Behav 58:1191–1200
Harari AR, Landolt PJ, 1997. Orientation of sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), to weevil, frass, food odors. J Chem Ecol 23:857–868.
Harari AR, Landolt PJ, Brockmann HJ, 2000. Intrasexual mounting in the beetle *Diaprepes abbreviatus* (L.) Proc R Soc Lond B 267:2071–2079.
Harshman LG, Prout T, 1994. Sperm displacement without sperm transfer in *Drosophila melanogaster*. Evolution 48:758–766.
Huettel MD, Calkins CO, Hill AJ, 1976. Allozyme markers in the study of sperm precedence in the plum curculio, *Conotrachelus nenuphar*. Ann Entomol Soc Amer 69:465–468.
Jablonski P, Vepsäläinen K, 1995. Conflict between the sexes in the water strider, *Gerris lacustris*: a test of two hypotheses for male guarding behavior. Behav Ecol 6:388–396.
Knowlton N, Greenwell SR, 1984. Male sperm competition avoidance mechanisms: the influence of female interests. In: Sperm competition and the evolution of animal mating systems (Smith RL, ed). New York: Academic Press; 62–85.
Lewis SM, Austad SN, 1990. Sources of intraspecific variation in sperm precedence in red flour beetles. Am Nat 135:351–359.
McLain DK, 1989. Prolonged copulation as a post-insemination guarding tactic in a natural population of the ragwort seed bug. Anim Behav 38:659–664.
McVey ME, Smittle BJ, 1984. Sperm precedence in the dragonfly *Erythemis simplicicollis*. J Insect Physiol 30:619–628.
Monsma SA, Harada HI, Wolfner MF, 1990. Synthesis of two *Drosophila* male accessory gland proteins and their fate after transfer to the female during mating. Develop Biol 142:465–475.
Newport MEA, Gromko MH, 1984. The effect of experimental design on female receptivity to remating and its impact on reproductive success in *Drosophila melanogaster*. Evolution 38: 1261–1272.
Otronen M, 1990. Mating behavior and sperm competition in the fly, *Dryomyza anilis*. Behav Ecol Sociobiol 26;349–356.
Parker GA, 1970. Sperm competition and its evolutionary consequences in the insects. Biol Rev 45:525–568.
Parker GA, 1979. Sexual selection and sexual conflict. In: Sexual selection and reproductive competition in insects (Blum MS, Blum NA, eds). New York: Academic Press; 123–166
Parker GA, 1984. Sperm competition and the evolution of animal mating strategies. In: Sperm competition and the evolution of animal mating systems (Smith RL, ed). New York: Academic Press; 2–61.
Parker GA, Simmons LW, Kirk H, 1990. Analyzing sperm competition data: simple models for predicting mechanisms. Behav Ecol Sociobiol 27:55l–65.
Reimann JG, Flint HM, 1967. Irradiation effects on the midguts and testes of the adult boll weevil, *Anthonomus grandis*, determined by histological and shielding studies. Ann Entomol Soc Amer 60: 298–308.
Rowe L, 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Anim Behav 44:189–202.
Simmons LW, 1987. Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus*. Behav Ecol Sociobiol 21:197–202.
Simmons LW, 2000. Sperm competition and its evolutionary consequences in the insects. Princeton: Princeton University Press.
Simmons LW, Stockley P, Jackson RL, Parker GA, 1996. Sperm competition or sperm selection: no evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria*. Behav Ecol Sociobiol 38:199–206.
Siva-Jothy MT, 1987. Variation in copulation duration and the resultant degree of sperm removal in *Orthetrum cancellatum* (L.) (Libellulidae: Odonata). Behav Ecol Sociobiol 20:147–151.
Siva-Jothy MT, Tsubaki Y, 1994. Sperm competition and sperm precedence in the dragonfly *Nanophya pygmaea*. Physiol Entomol 19:363–366.
Sivinski J, 1983. Predation and sperm competition in the evolution of coupling durations, particularly in the stick insect *Diapheromera veliei*. In Orthopteran mating systems: sexual competition in a diverse group of insects (Gwynne DT, Morris GK, eds). Boulder, Colorado: Westview Press; 147–162.
Smith RL, 1979. Repeated copulation and sperm precedence: paternity assurance for a male brooding water bug. Science 205:1029–1031.
Telford SR, Dangerfield JM, 1990. Manipulation of the sex ratio and duration of copulation in the tropical millipede *Alloporus uncinatus*: a test of the copulatory guarding hypothesis. Anim Behav 40: 984–986.
Thornhill R, 1984. Alternative hypotheses for traits believed to have evolved by sperm competition. In: Sperm competition and the evolution of animal mating systems (Smith RL, ed). New York: Academic Press; 151–179
Thornhill R, Alcock J, 1983. The evolution of insect mating systems. Cambridge: Harvard University Press.
Villavaso EJ, 1975. Functions of the spermathecal muscle of the boll weevil, *Anthonomus grandis*. J Insect Physiol 21:1275–1278.
Waage JK, 1979a. Adaptive significance of postcopulatory guarding of mates and nonmates by male *Calopteryx maculata* (Odonata). Behav Ecol Sociobiol 6:47–154.
Waage JK, 1979b. Dual function of the damselfly penis: sperm removal and transfer. Science 203:916–918.
Waage JK, 1984. Sperm competition and the evolution of Odonate mating systems. In: Sperm competition and the evolution of animal mating systems (Smith R, ed). New York: Academic Press; 251–290.
Wilcox RS, 1984. Male copulatory guarding enhances female foraging in a water strider. Behav Ecol Sociobiol 15:171–174.
Yamamura N, 1986. An evolutionarily stable strategy (ESS) model of postcopulatory guarding in insects. Theoret Pop Biol 29: 438–455.
Zar JH, 1998. Biostatistical analysis. New York: Prentice Hall.