Fitness Consequences of Multiple Mating on Female *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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ABSTRACT The effects of multiple mating on fitness in the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), were studied. In the first of a series of experiments, the impact of multiple mating on female fitness was determined by evaluating the effects of a single mating period with one male, continuous exposure to one male, and continuous exposure to five males. Continuous exposure to one male increased lifetime fecundity by extending the period of time progeny were produced compared with a single mating period with one male, although average progeny size was reduced. Exposure to five males significantly reduced female survival and the number and size of progeny produced compared with the other treatments. In the second experiment, the number of progeny and the length of time progeny could be produced from a single copulation were determined. Females became sperm depleted within 7 ± 1 wk after laying 259 ± 22 progeny, but a second mating period 9 wk after the first copulation extended progeny production. In the final experiment, the mechanism for the decline in progeny production at high male densities was determined to be reduced oviposition, caused at least in part by a high proportion of time spent in copula. The number of copulations has costs and benefits for females that may affect population dynamics.

KEY WORDS oviposition, multiple mating, rice weevil, *Sitophilus*, Curculionidae

FEMALE MULTIPLE MATING WITH the same male (repeated matings) or multiple males (polyandry) is a widespread behavior, but the causes and fitness consequences of this behavior are often complex and poorly understood (Ridley 1990, Keller and Reeve 1995). Polyandry has been proposed to evolve because the benefits of multiple matings to females exceed the costs or because males manipulate interactions against the female's best interests. Benefits to females of mating multiple times can be divided into two classes: (1) material benefits such as extra sperm or male-derived substances (Thornhill and Alcock 1983, Newcomer et al. 1999) and (2) genetic benefits (Jennions and Petrie 1997). Genetic benefits include improving on previous copulations by mating with higher-quality males (Thornhill and Alcock 1983, Simmons 1987, Olsson et al. 1996), reducing the risk of genetic incompatibility (Zeh and Zeh 1996, 1997), inbreeding avoidance (Madsen et al. 1992, Tregenza and Wedell 2002), manipulating offspring paternity (Edvardsson and Arnqvist 2000), increasing genetic diversity (Baer and Schmid-Hempel 1999), and genetic bet-hedging (Watson 1991).

Selection on males to increase their fitness may result in female polyandry (Arnqvist and Nilsson 2000, Crudgington and Siva-Jothy 2000, Stutt and Siva-Jothy 2001). Male fitness depends largely on the number of females he inseminates, but females often benefit from limiting the number of mates. This conflict can result in an evolutionary arms race over the number of copulations and at a given point in time either sex may have an advantage (Arnqvist and Rowe 2002). If males can force copulations then female response needs to balance the costs of resistance versus accepting additional copulations (Clutton-Brock and Parker 1995).

The relationship between the number of copulations and female fecundity can be positive (Pardo et al. 1995, Sakurai 1996, Wilson et al. 1999, Jimenez-Perez et al. 2003), neutral (Kraan and Straten 1988, Svard and Wiklund 1988, Kawagoe et al. 2001), or negative (Cook 1999, Orsetti and Rutowski 2003). A variety of other fitness costs can be also be associated with multiple mating: increased risk of predation (Rowe 1994), physical damage (Helversen and Helversen 1991), transmission of infection (Hurst et al. 1995, Rolff and Siva-Jothy 2002), reduction of female life span (Keller and Reeve 1995, Hou and Sheng 1999, Kawagoe et al. 2001), and time lost for feeding and oviposition (Keller and Reeve 1995). The costs and benefits of multiple mating for each sex can have important influences on many aspects of an organism's behavior and ecology, and as a consequence, on the

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effective monitoring and management of pest populations (Sadek 2001).

Three species of *Sitophilus* (*S. granarius* (L.), *S. oryzae* (L.), and *S. zeamais* Motschulsky) (Coleoptera: Curculionidae) are seed parasites and are important worldwide economic pests of cereal grains such as wheat, corn, rice, and sorghum (Longstaff 1981b). These species share the same general life history. Development from egg to adult occurs inside an individual seed. Adults, after a variable period of time after eclosion, emerge from the seed. Adults are long lived, with estimates ranging up to 36 wk, depending on environmental conditions (Longstaff 1981b). Females lay eggs singly in holes excavated in the seed and lay multiple eggs per day and a large number of eggs over their long lifespan (Lathrop 1914, Richards 1947).

Although there are reports that female *Sitophilus* spp. can mate multiple times (Richards 1947, Stubbs 1982, Walgenbach and Burkholder 1987), there is little published information on the reproductive strategies of *Sitophilus* spp., and the fitness consequences of multiple mating are not well understood. The mating behavior of *S. zeamais* was described by Walgenbach and Burkholder (1987) and that of *S. granarius* by Wojcik (1969). Males of *S. granarius* and *S. orzyae* produce an aggregation pheromone that may help facilitate encounters between males and females (Phillips and Burkholder 1981, Phillips et al. 1985). Walgenbach and Burkholder (1987) reported that female *S. zeamais* did not copulate before 3 d after emergence, and for some individuals, this refractory period lasted for as long as 10 wk. Copulation lasted an average of 4.8 h, with a range from 3 to 5.9 h. There was no effect of time of day, presence of light, female age, or mating status of either sex on copulation duration or time until copulation occurred after adding beetles to chamber. Females remated readily out to 10 wk of age, the limit of the study. In *S. granarius*, females had a refractory period of up to 15 d after emergence and took 3–4 d after copulation before viable progeny were produced (Stubbs 1982).

There is some evidence for potential benefits of multiple mating for *Sitophilus* species. Surtees (1964) found that, at 25°C, both isolated female *S. granarius* and females paired with males survived and laid eggs for a similar period of time. However, at 20°C, the period of time over which eggs were laid by isolated females was reduced compared with paired females. Longstaff (1981a) showed that, at low population and high population densities, fecundity was reduced in *S. oryzae.* Stubbs (1982) found that the greater the length of time a male *S. granarius* was held with a female, the greater the progeny production.

The overall objective of this study was to evaluate the costs and benefits of multiple mating for female rice weevils, *S. oryzae.* In the first of a series of experiments, the impact of multiple mating on female fitness was determined by evaluating the influence of a single mating period with one male, continuous exposure to one male, and continuous exposure to five males on female fitness. In the second experiment, the number of progeny that can be produced from a single mating and the role of sperm depletion in limiting progeny production were assessed. Correlated information about the length of the refractory period before mating and copulation duration on reproduction for this species was also determined. In the final experiment, the mechanism for the decline in progeny production at high male densities was assessed.

Materials and Methods

Impact of Exposure to Males on Female Fitness. The effects of different levels of exposure to males on female survival, lifetime progeny production, and progeny size were assessed. For this and subsequent experiments, a laboratory colony of *S. oryzae* maintained on wheat (*Triticum aestivum* L.) was used. The environmental conditions for colony production and all experimental tests were 27°C, 70% RH, and 24-h dark. Newly emerged *S. oryzae* were sieved from wheat kernels <12 h after emergence. This time period was selected based on previous published research for *S. zeamais* (Walgenbach and Burkholder 1987). Beetles were sexed based on rostrum characters (Halstead 1963), and males and females were held separately in 30-ml plastic cups with lids with ≈4 g of wheat. A small dot of colored nail polish was added to the dorsal surface of the thorax of females to facilitate identification. Beetles were held singly for an additional 3 d to attain reproductive maturity before initiating the experiment.

The following treatments were prepared: virgin females, females with a single mating period with one male, females with continuous exposure to one male, and females with continuous exposure to five males. Virgin females were held in cups with wheat for 48 h before the start of the experiment and then transferred to a new cup of wheat (30-ml plastic cups with ≈4 g of wheat). Single mating period females were confined with one male for 24 h before the start of the experiment and then the male was removed, and the female was transferred to a new cup with wheat. In the continuous exposure to one male treatment, females were held with one male for 24 h before start of the experiment, and then female and male were transferred to new cup with wheat. The continuous exposure to five males treatment was similar to the previous treatment, except females were transferred to experimental cups containing five males. Ten replicates of each treatment were set up.

Experimental cups were held until female death. At 2-d intervals for 20 transfers, and thereafter at 7-d intervals, survival was assessed, and all live weevils were transferred to new experimental cups. If a male was dead at the time of transfer, it was removed and replaced with a new male from the colony that was at least 1 mo old. If a female was dead at time of the transfer, that replication was terminated. After removal of the weevils, the experimental cups were checked daily, and number of adult progeny that had emerged was counted beginning at 4 wk and continuing until 8 wk after transfer. All progeny produced at the first (2 d), fourth (8 d), and eighth (16 d) transfers

were also sexed, and elytra length, elytra width, and body weight were measured (Campbell 2002).

Lifetime Fecundity After One Copulation and Recovery from Sperm Depletion. To determine the impact of a single copulation on lifetime fecundity, newly emerged virgin females were exposed to a single male daily until copulation occurred, and then females were transferred to oviposition cups where female survival and progeny production were determined. To evaluate if decline in progeny production was the result of sperm depletion, approximately one-half of the females were exposed to a second male at 9 wk after copulation: a period of time at which, based on prior experiment, females with a single copulation period were no longer laying eggs.

Females ($n = 72$) were collected within 12 h after emergence from infested wheat kernels held individually in 96-well tissue culture plates (Becton Dickinson, Lincoln Park, NJ). Based on findings from previous experiments, individuals were kept isolated from time of emergence to avoid any copulations from occurring. Females were placed in plastic cups with ≈50 wheat kernels and exposed to individual males daily for 3-h periods until copulation (Walgenbach and Burkholder 1987) was observed. Males were collected from colony jars and were at least 1 mo after emergence when used in experiments. Two sexes were marked with different colors of nail polish as described above to facilitate identification. A male and female pair was added daily to a new cup with 50 kernels of wheat for a period of 3 h and observed at 15-min intervals. The female was recorded as mated if weevils were observed to be in copula for at least four observation intervals (i.e., at least 1 h). If copulation did not occur that day, females were returned to their initial plastic cup and held individually until the next day when they were tested again. The number of days until copulation occurred and duration of copulation were recorded for each individual female.

After mating, females were transferred individually to cups with ≈150 kernels of wheat. At weekly intervals, females were transferred to new cups, and the grain from the old cups was held for at least 8 wk. Cups were checked as described above, and the number of adult progeny produced per week was determined. After 9 wk, when females were predicted to be sperm depleted based on previous experiments, 33 of the 72 females were randomly selected and exposed to a second male for a 24-h period. After removal of the male, the females continued to be held and transferred as described above. Females were held until their death or the termination of the experiment after 18 wk. The following parameters were determined from these experiments: total number of eggs laid, period of time over which eggs were laid, number of eggs laid before and after second copulation, and female life span.

Mechanism for Reduced Progeny Production with Continuous Exposure to Multiple Males. Two experiments were performed to evaluate the mechanism that generates the decline in number of progeny produced by females confined with multiple males. In the first experiment, the hypothesis that the decline in progeny production was caused by a decrease in oviposition and that this decrease is a result of presence of males not just of crowding was assessed. Wheat (158 ± 2 kernels) was added to 30-ml plastic cups, beetles were collected, and females were marked and mated using techniques described in previous experiments. One of the following treatments was added to each cup: one virgin female, one mated male, one mated female, one mated female and one virgin female, one mated female and five virgin females, one mated female and one male, or one mated female and five males. Cups were held for 48 h. Five replicates were performed of each treatment. After weevils were removed, the wheat kernels were stained using the acid fuchsin technique developed by Frankenfeld (1948) and modified by Pedersen (1979) so that egg plugs (oviposition hole in which an egg and egg plug have been deposited, i.e., egg cavities (Kanaujia and Levinson 1981) could be easily identified. The wheat kernels were individually inspected, and the number of egg plugs was determined.

To determine the mechanism by which males reduce female fitness, an experiment was conducted in which the time spent by females in copula and on wheat kernels was assessed under different male densities. Females and males were collected from individually isolated infested kernels as described above. Males were marked on the thorax with a small dot of nail polish to facilitate identification. Females and males were paired and allowed to copulate over a 48-h period before the start of the observations. Males and females were randomly assigned to the following treatments: one mated female with one mated male and one mated female with five mated males. Combinations of males and females were added to 30-ml clear plastic cups containing ≈30 kernels of wheat. The cups were randomly assigned to a position on one of three shelves in an incubator, where they could be seen through an observation window in the door (Percival Scientific, Perry, IA). When observations were not being made, the observation window was covered with an opaque door to maintain dark conditions.

Cups were observed twice a day, under red light conditions, at ≈0800 and 1700 hours for 4 wk. At each time-point, survival, location of the female, and whether or not she was in copula were recorded. If any of these conditions could not be determined by looking through the window, at the end of all the observations, the door to the incubator was opened, and the cups in question were observed more closely. The weevils were transferred weekly to new cups with wheat. Male survival was assessed at each transfer, and when necessary, dead males were replaced with males at least 1 mo old from the colony.

Analysis. Analysis of variance (ANOVA) or general linear model (GLM) procedures with post hoc Tukey's multiple range tests, correlation analysis, and *t*-tests were performed using Systat version eight for Windows (SPSS, Chicago, IL). Survival analysis using Kaplan-Meier Estimation and log-rank tests were performed using SAS version 9 (SAS Institute, Cary, NC).

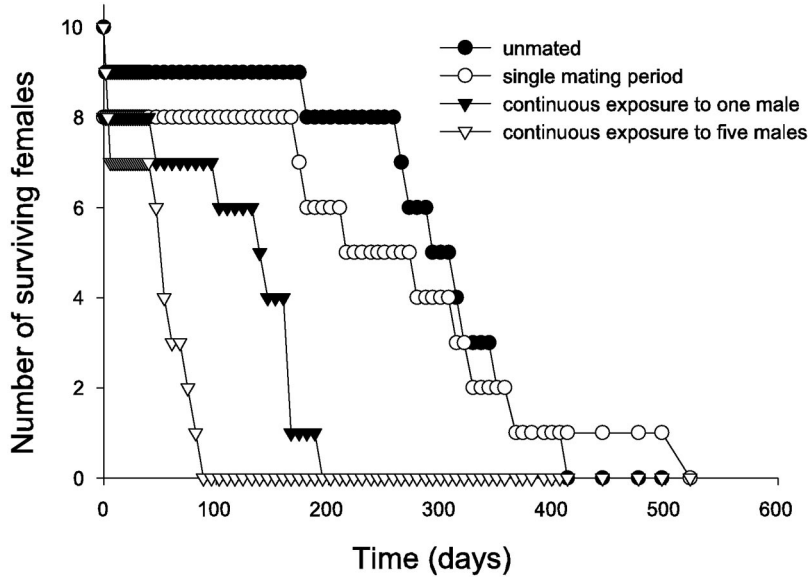

Fig. 1. Survival of female *S. oryzae* exposed to the following treatments: no mating, single mating period before start of the experiment, continuous exposure to one male, and continuous exposure to five males.

Data are presented as mean ± SE. Contingency tables and log-likelihood ratio test analysis were performed based on approaches in Zar (1999).

Results

Impact of Exposure to Males on Female Fitness. The presence of males had a significant influence on the female rice weevil survival (Fig. 1). Survival functions using Kaplan-Meier estimation were significantly different among the treatments (log-rank test: $\chi^2 = 51.49$, df = 3, $P < 0.0001$). Female survival was longest in the unmated (307 ± 22 d, $N = 9$) and single mating period (295 ± 38 d, $N = 8$) treatments. In contrast, continuous exposure of females to a single male reduced survival to 140 ± 16 d ($N = 8$). Females held with five males ($N = 8$) had all died by 89 d, with a mean survival of 52 ± 10 d. Based on pair-wise comparisons using log-rank tests, survival functions for unmated females and females with a single mating period were not significantly different, and females with continuous exposure to one or five males were not significantly different, but both groups were significantly different from each other.

Female lifetime fecundity (i.e., adult progeny produced) was greatly reduced in the five male treatment (14 ± 5 progeny, $N = 8$) compared with continuous exposure to a single male (461 ± 76 progeny, $N = 8$) and a single mating period (210 ± 63 progeny, $N = 8$) treatments (Fig. 2). Total progeny production was significantly different among all treatments based on GLM procedure and Tukey's multiple range test ($F = 18.547$; df = 3,29; $P < 0.0001$). In the single mating period treatment, two females did not produce any progeny, and in the continuous exposure to a single male, one female did not produce any progeny. One individual in the no mating period did produce progeny (160 progeny total), indicating that copulation had occurred before sorting of the weevils from the colonies. Subsequent experiments have shown that some females do copulate within the first 24 h. There was no difference in the period of time over which progeny were produced among the treatments with exposure to males, except for females held with five males compared with females held with one male (GLM and Tukey test: $F = 5.715$; df = 2,16; $P = 0.025$).

In the single mating period treatment, it appeared that females became sperm depleted. The bulk of the progeny production after one 24-h mating interval lasted for 38 ± 7 d, but the average lifespan of females was 295 ± 38 d (Fig. 2). In several cases, progeny production continued at a very low level—typically less than one individual per day. However, in one case, after a period of 44 d with no progeny produced, there was a small spike of progeny production over a 3-wk period that produced 52 additional progeny. Females in the single mating period that produced progeny continued to survive for an average of 231 ± 19 d after ceasing progeny production. In contrast, females in the continuous exposure to one male and five male treatments survived after stopping progeny production for an average of 36 ± 15 and 22.8 ± 5.6 d, respectively. This postreproductive survival period was significantly longer in the single mating period treatment compared with the other two treatments that did not differ from each other (GLM and Tukey test: $F = 57.428$; df = 2,17; $P < 0.0001$). During the first 38 d of the study, for females that laid eggs, the total number of eggs laid was not significantly different between the single mating period (244 ± 51 eggs) and the continuous exposure to one male (212 ± 28 eggs) treatments (*t*-test: $t = 0.605$, $P = 0.556$).

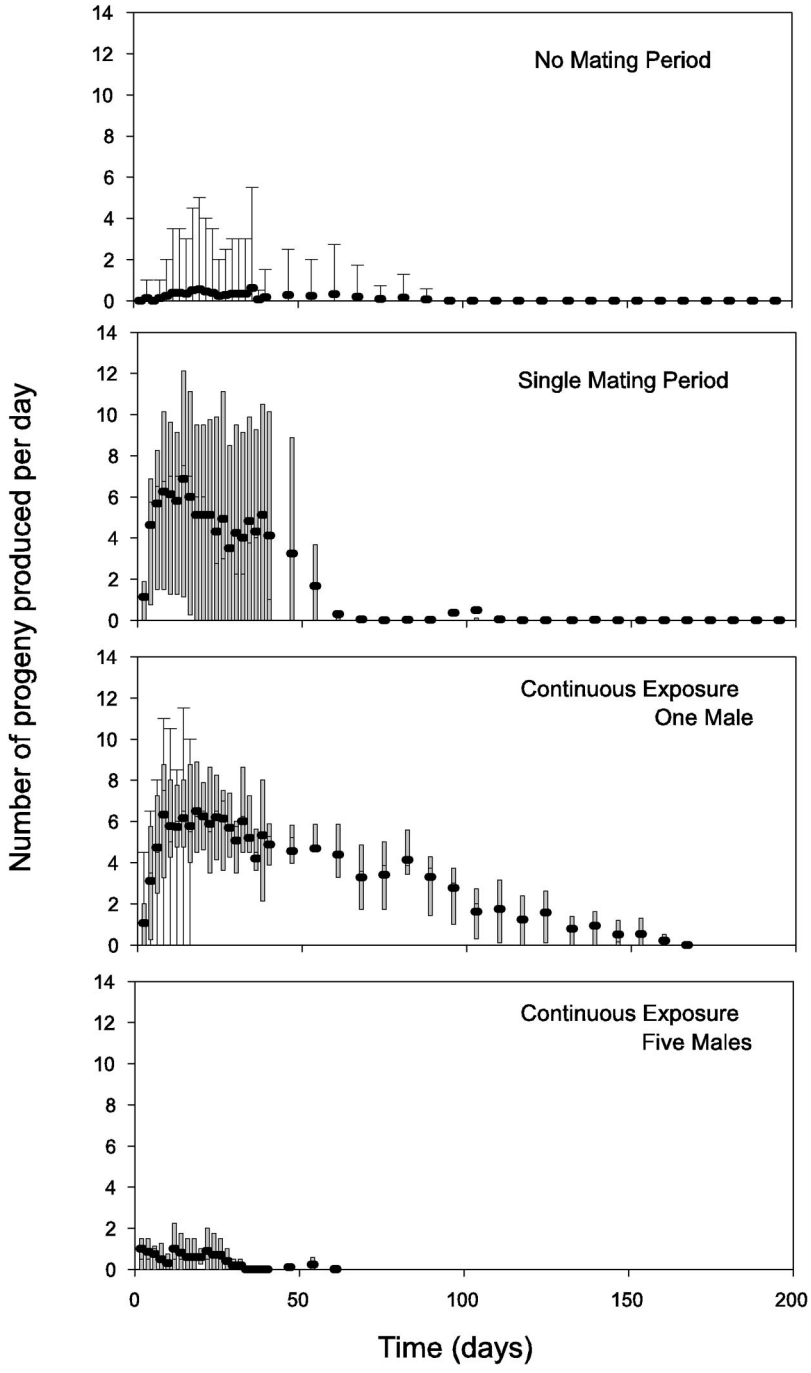

Fig. 2. Female *S. oryzae* progeny production over time when exposed to the following treatments: no mating, single mating period before start of the experiment, continuous exposure to one male, and continuous exposure to five males. Data are presented as box plots, with the box representing 50% of data and bars indicating 10th and 90th percentiles of the data. The thick black line indicates the mean of the data.

Different levels of exposure to males impacted progeny size. All three measures—elytra length, elytra width, and weight—showed the same pattern, but only elytra length data and analysis are presented. Progeny elytra length was significantly longer in the single mating period treatment (1.561 ± 0.004 mm; $N = 214$) compared with continuous exposure to single male treatment (1.528 ± 0.005 mm; $N = 235$), and both were longer than those exposed to five males treatment (1.505 ± 0.009 mm; $N = 57$; GLM and Tukey

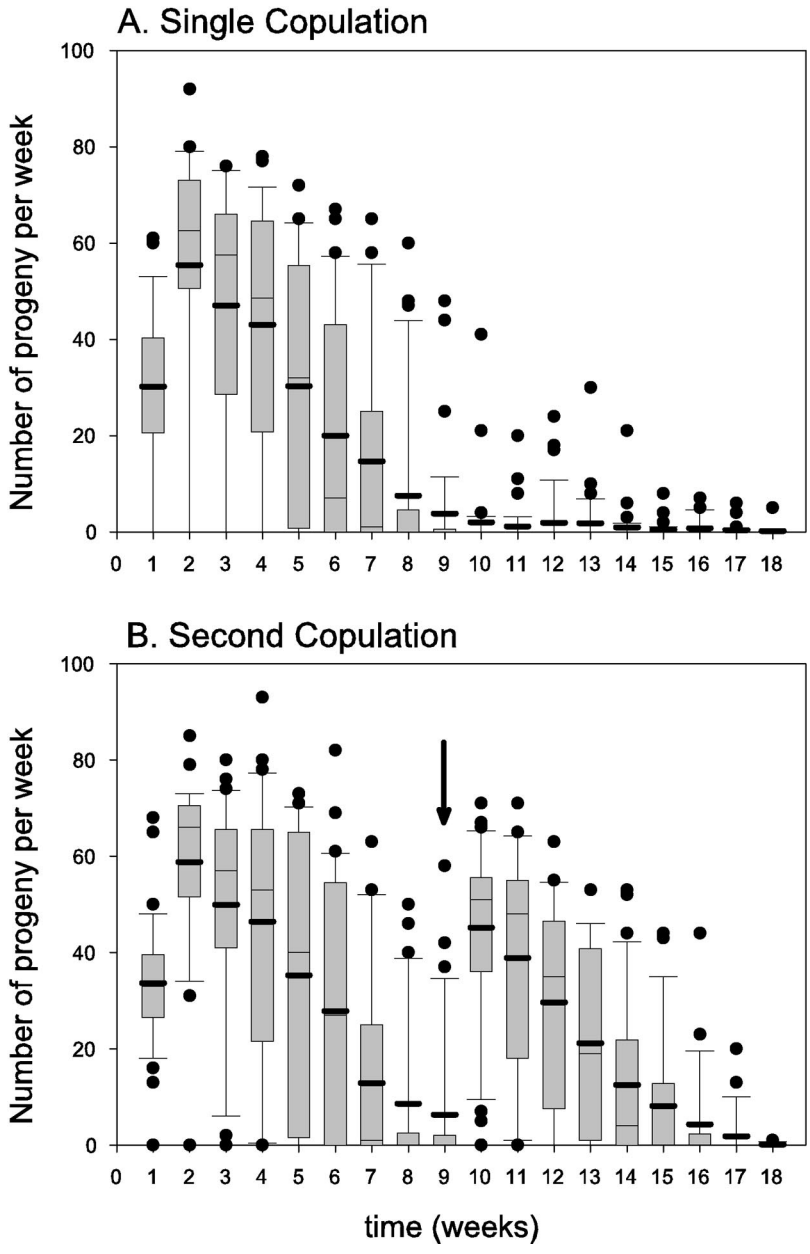

Fig. 3. Female *S. oryzae* progeny production over time when exposed to the following treatments: (A) single copulation before start of the experiment and (B) a second 24-h mating period at 9 wk after initial copulation. Data are presented as box plots, with the box representing 50% of the data, bars indicating 10th and 90th percentiles of the data, and black circles indicating data points outside this range. The thick black line indicates the data mean and the thin line the median.

analysis: $F = 23.244$; df = 2,503; $P < 0.0001$). Transfer time (first, fourth, or eighth transfer) was not a significant factor for any of the progeny size measurements (GLM: $P > 0.05$). Sex ratio (0.51 for single mating period; 0.53 for continuous exposure to one male; and 0.56 for continuous exposure to five males) was also not significantly different among the levels of exposure to males (contingency table and log-likelihood ratio test: $G = 0.643$; $G_{2,\ 0.05} = 5.991$).

Lifetime Fecundity After One Copulation and Recovery from Sperm Depletion. The time after emergence until females were observed in copula was 1.6 ± 0.1 d ($N = 71$; range, 1–4 d), and the average duration of copulation was 7.6 ± 0.3 observation intervals, or on average, at least 120 min in duration. Females were able to produce 259 ± 22 progeny ($N = 38$) over a period of 7 ± 1 wk with a single copulation (Fig. 3A). After a single mating, 10.5% (4 of 38 females) of the

females did not produce any progeny. Most females (34 of 38 females, 89%) survived to 18 wk when the experiment was terminated. On average, progeny production peaked at 2 wk and declined until 8 wk, when it leveled off. Most females produced a similar number of progeny per week throughout the reproductive period, with a sharp drop to a couple progeny per week before dropping to zero. However, the timing of this drop varied among individuals and some females had a long tail of declining progeny production, and in some cases, female progeny production dropping to zero for >1 wk followed by another short period of progeny production.

There were poor linear correlations between refractory period before copulation or duration of copulation and most of the fitness parameters measured (i.e., egg number, lifespan, progeny production period length). However, copulation duration and total number of eggs produced had a Pearson correlation coefficient of $r = 0.525$ ($P = 0.0007$). There was a good correlation between the length of time eggs were laid and the total number of eggs laid ($r = 0.621$; $P < 0.0001$), but a nonsignificant correlation between lifespan and total number of eggs laid ($r = 0.213$; $P = 0.2001$).

When females were allowed to copulate with another male over a 24-h period after 9 wk, there was an increase in number of progeny produced (GLM: $F = 25.323$; df = 1,69; $P < 0.0001$) and the period of time over which they were produced (GLM: $F = 23.514$; df = 1,69; $P < 0.0001$) compared with the single copulation treatment (Fig. 3B). Females were able to produce 437 ± 28 progeny ($N = 33$) and produce progeny over a period of 11.2 ± 0.6 wk with a second period of copulation. Longevity of the females was not different between single copulation and the second copulation period females (GLM: $F = 0.159$; df = 1,69; $P = 0.691$).

Progeny production in the 9 wk before the second copulation period was not different from the total progeny production in the single copulation treatment (*t*-test: $t = 0.915$; df = 32; $P = 0.367$), but females produced fewer progeny after the second copulation period. The mean number of progeny produced in the first 9 wk was greater (279 ± 23 adults) than the number of progeny produced in the second 9 wk after the second copulation period (158 ± 17 adults; paired *t*-test: $t = 4.316$; df = 32; $P < 0.0001$). There were poor linear correlations between the refractory period between emergence and copulation or duration of copulation and all of the fitness parameters measured (i.e., egg number, lifespan, progeny production period length). There was a strong correlation between the length of time eggs were laid and the total number of eggs laid ($r = 0.768$; $P < 0.001$), but a nonsignificant correlation between lifespan and total number of eggs laid ($r = -0.074$; $P = 0.6817$).

Mechanism for Reduced Progeny Production with Continuous Exposure to Males. There was an effect of exposure to males on the number of egg plugs deposited onto wheat kernels that corresponds with the patterns seen in progeny production (Fig. 4). Number of egg plugs was significantly different across the treatments (GLM and Tukey test: $F = 24.06$; df = 6,28; $P < 0.001$). However, the number of egg plugs deposited by mated females was significantly lower only in the five male treatment. In treatments where the mated female was held with one or five virgin females, there was no impact on the number of egg plugs. In both the single male and single virgin female treatments, there were no egg plugs found in the wheat.

The mechanism by which females deposit fewer egg plugs and produce fewer progeny seems to result, at least in part, from females copulating more frequently (Fig. 5A). There was no apparent pattern over time in the proportion of females in copula or off the wheat (Fig. 5B). This indicates that females remain receptive to males for at least 4 wk and are not, at least within the constraints of this bioassay, driven from the wheat. Across all the time-points, the mean proportion of females in copula was less in the one male treatment (0.23 ± 0.02) compared with the five male treatment (0.68 ± 0.02; ANOVA on arcsine [square root] transformed data: $F = 139.05$; df = 1,90; $P < 0.0001$). Across all time-points, the mean proportion of females on the wheat kernels was the same in the one male (0.88 ± 0.02) and five male (0.88 ± 0.02) treatments (ANOVA on arcsine [square root] transformed data: $F = 0.000$; df = 1,90; $P < 0.992$).

Discussion

Sitophilus oryzae females, after an initial refractory period, are able to copulate throughout their lifespan. This refractory period is shorter than that reported by Walgenbach and Burkhoder (1987) for *S. zeamais* reared under similar temperature and relative humidity conditions. Although not specifically addressed here, males were also observed to mate multiple times throughout their life. The male refractory period between emergence and mating is 2.3 ± 0.1 d (range, 1–5 d; $n = 68$), similar to that of females (unpublished data). In *S. oryzae*, additional copulations with a single male enabled females to extend the period of time that they produce progeny compared with females with only one copulation period. This suggests that a single mating may not provide adequate sperm to fertilize all of the eggs a female can lay in her life or that gonadotropic factors may become depleted (Ramaswamy et al. 1997).

Continuous exposure to one male reduced female overall longevity compared with females with one copulation period. Before becoming apparently sperm depleted, females in both of these treatments produced the same number of progeny per day. Thus, the benefits of multiple mating, in terms of increased progeny production, occurred later in life. Although survival among virgin females averaged 307 ± 22 d, it is unknown how long individual beetles survive under natural conditions where mortality risks are presumably higher. Increases in lifetime fecundity with multiple mating and corresponding detrimental effects on female longevity have been reported for other insect species (Ridley 1988, Arnqvist and Nilsson 2000). A

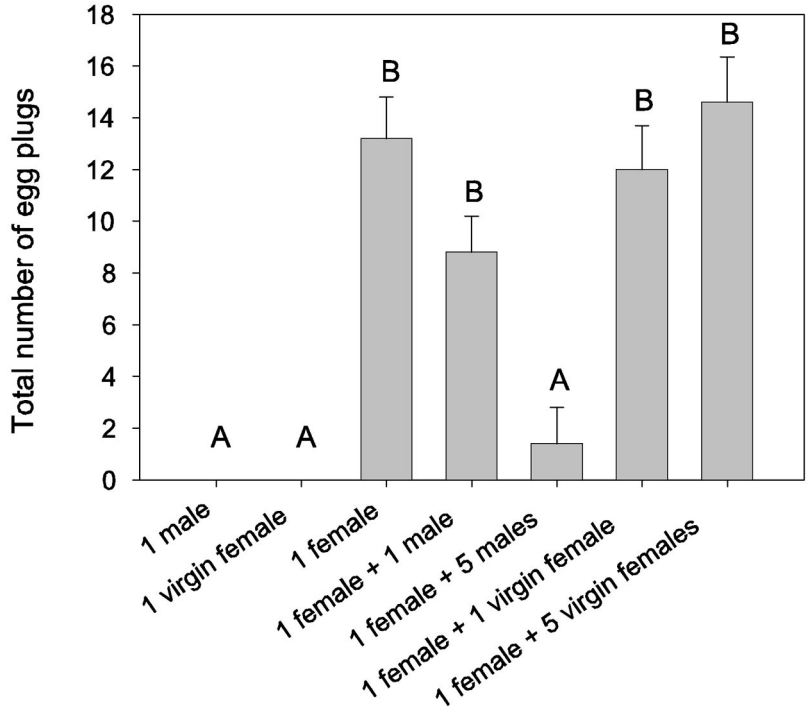

Fig. 4. The mean ± SE number of egg plugs produced during a 48-h period by one mated male, one virgin female, one mated female, one mated female and one male, one mated female and five males, one mated female and one virgin female, and one mated female and five virgin females.

further benefit to multiple mating is the avoidance of unsuccessful copulations, perhaps caused by males failing to provide sperm or seminal factors. In this study, 10.5% of females with a single copulation with a virgin male failed to produce any progeny. Research addressing the age distribution and reproductive status of females and males collected in the field is needed to clarify this issue. The availability of age grading procedures using near infrared (NIR) analysis (Perez-Mendoza et al. 2004) could facilitate this research. In addition, studies addressing the influence of multiple copulations on males are needed.

An additional cost to females from the continuous exposure to a single male and the corresponding multiple copulations versus a single copulation is the decrease in progeny size. This is likely to negatively effect the fitness of the progeny, because larger adult size is correlated with increased fitness in many species of insects (Andersson 1994). The fitness consequences of body size for *S. oryzae*, however, are not well documented. Richards (1947) reported that heavier females had more eggs in their ovaries as virgins and had a greater oviposition rate after mating than lighter females, although interpretation of these data is complicated because number of eggs and body weight are likely correlated. When larger rice weevil males compete with smaller males for copulations with females, larger males spend more time in copula than smaller males and 73% of the time are the first to copulate (unpublished data). The mechanism for the observed decline in progeny size is unknown. Increased stress placed on females from multiple copulations, increased male harassment, or seminal factors may have impacted progeny size. Eady et al. (2000) found that polyandry decreased egg-to-adult survival in *Callosobruchus maculatus* (F.) and proposed that male ejaculate-derived ovipositional stimulants may have been involved. Alternatively, previous research has indicated that progeny size is strongly influenced by the size and quality of the seed in which the eggs were laid (Ungsunantwiwat and Mills 1985, Campbell 2002), and increased adult density may lead to more feeding damage in seeds and a reduction in the quality of the environment for progeny development.

The progeny production benefits of continuous exposure to males versus a single mating period were density dependent. At the higher male density, progeny production was greatly reduced compared with the other two mated female treatments. In addition, the negative effects on female survival and progeny size were even greater at the higher male density. Observations of oviposition and frequency of copulation suggest that, with increasing male density, females spend more time in copula and lay fewer eggs. The precise mechanism for the decline in number of eggs laid is unknown but likely results at least in part from time lost because of frequent copulations. Injury and interference from male–male competition for copulations may also be a factor, because males interrupt ongoing copulations and can expel a copulat-

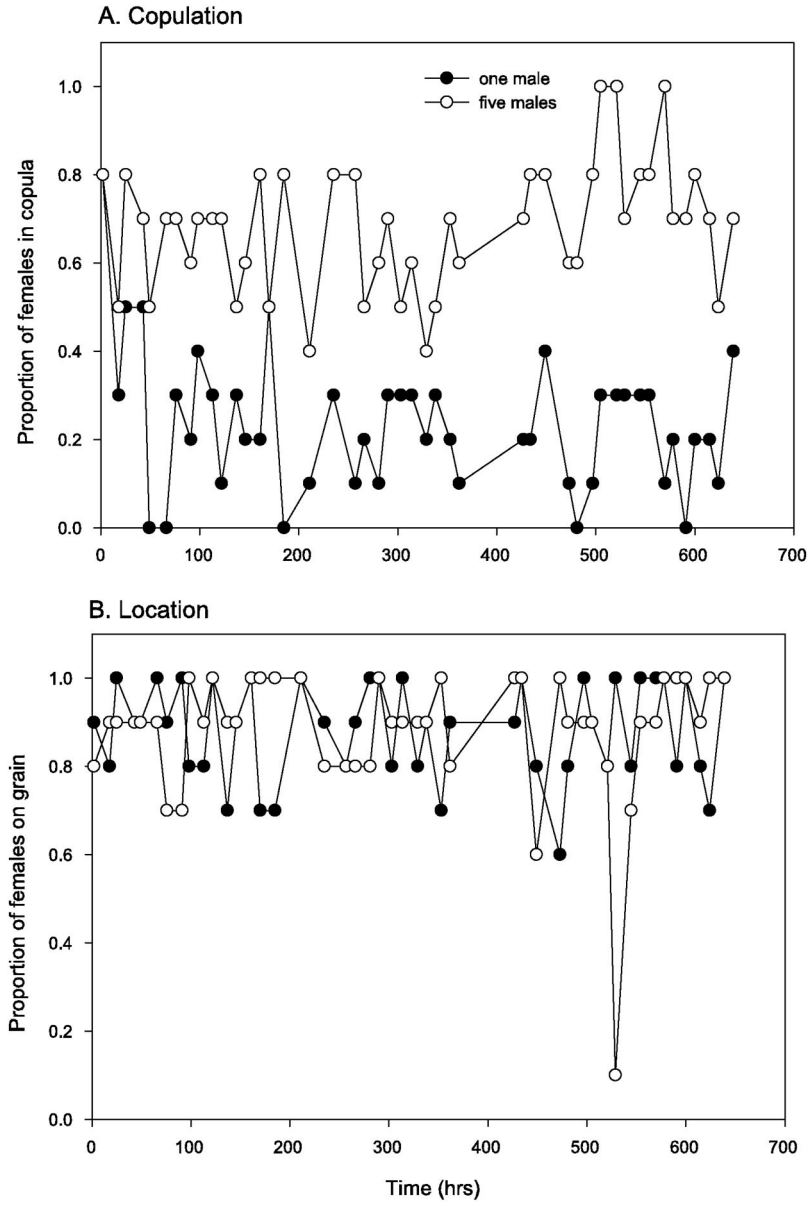

Fig. 5. The proportion of replicates with the female observed to be in copula (A) or off the wheat kernels (B) at observations made over a 4-wk period for treatments with mated females held with one male or with five males.

ing male (unpublished data). Male accessory gland secretions have also been reported to have negative effects on female fitness in other insects. In *Drosophila melanogaster*, accessory gland products cause higher mortality and reduce egg production (Fowler and Partridge 1989, Chapman et al. 1993, 1995).

Interactions between males and females under more natural field conditions are not well understood. *Sitophilus* species exploit a wide range of resources and a wide range of patch sizes, so making generalizations about probability of encounters between males and females is difficult. In large bulk grain storages, the densities of *Sitophilus* spp. are often low (Reed et al. 2003), although this information is seldom collected at a spatial scale appropriate for evaluating individual interactions between beetles because localized high-density spots in bulk grain can occur. In grain spillage situations, and presumably in other small patch situations, *Sitophilus* densities can be much higher (Arthur et al. 2005). Longstaff (1981a) showed an Allee effect on *S. oryzae* fecundity with increasing density. Negative effects on reproduction resulting from not enough and too much multiple mating could contribute to this phenomenon. Rice weevils have a one to one sex ratio; therefore, critical factors in evaluating the effect of density will be the time between

copulations for males versus females and the probability of encounter between the sexes. Further research in this area is needed, because failure by females to regulate the number of copulations optimally can result from a range of factors, including that male–female encounters are typically rare under more natural situations. Also, in larger spatial contexts, females may be better able to control encountering males, and differences in factors such as female mobility after copulation may be important in reducing male encounters after sufficient copulations.

Female multiple mating is a widespread behavior that is often costly for females. In *S. oryzae*, females clearly gain benefits from multiple mating in terms of extending the period of time oviposition can occur, but potentially incur high costs as well especially as the number of copulations increase. Because females do not seem to regulate the number of copulations to maximize their own fitness, the frequent copulations, even when they clearly negatively impact female fitness, probably result from females attempting to curtail male harassment (Thornhill and Alcock 1983, Clutton-Brock, Parker 1995). Whether this is because of an evolutionary arms race over the rate of mating or because of a lack of selection pressure under natural conditions because of low male–female encounters remains to be determined. It is also unclear if the benefits in terms of increased length of the reproductive period actually can occur in the field because lifespan under these conditions is likely to be less than in the laboratory. Regardless of the frequency of multiple mating under more natural conditions, this study provides important baseline data on the reproduction in this major stored-product pest that has implications for understanding inter- and intrasex interactions.

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