MULTIPLE MATING BY LASIODERMA SERRICORNE (F.) - EFFECTS ON FERTILITY AND FECUNDITY

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ABSTRACT: A series of single-pair mating experiments with adult *Lasioderma serricorne* (F.) was conducted to determine the significance of multiple matings by both males and females in terms of the effects upon male fertility and female fecundity. The effect of multiple mating by females was determined by recording the numbers of viable progeny produced by females that had mated 1, 2 or 3 times. Patterns of sperm utilization were established by the use of an autosomal genetic marker. Male fertility rates were determined by observing the numbers of viable offspring produced by females after mating with males that had mated one or more times. The relationship of these results to insect age at first mating, mating duration and oviposition will be discussed.

The cigarette beetle, *Lasioderma serricorne* (F.), is an important cosmopolitan pest of a wide variety of stored products, and as such has been the subject of more than 200 research papers during the past 60 years. Despite this volume of literature relatively little information regarding reproductive behavior is available. It has often been reported [1, 2, 3, 4, 5] that adults frequently remate. However, the frequency with which multiple mating occurs and its role in the reproductive process are unknown.

The purpose of this paper is to report results of experiments that were carried out to determine the number of times that both male and female beetles will mate, and to find out what effect, if any, multiple mating has upon male fertility and female fecundity in *L. serricorne*.

METHODS AND MATERIALS: Beetles used in this study were obtained from a colony that has been maintained in the Gainesville laboratory for several years. Insects were reared and handled as pupae and young adults as previously described [6].

Multiple mating by females - The numbers of times that individual females would mate were established by recording the occurrence of successive copulations over a 12-day period. Initially, sexually mature females (4-6 days old) were mated with 6-8 day old virgin males. Matings took place in 15.5 X 50-mm glass vials during the 4th-8th hr of the photophase. A 1-hr period was allotted for the commencement of copulation. After mating, males were discarded and females were placed in 27 X 55 mm oviposition

vials containing ca. 3 g of the rearing medium and allowed to oviposit for 3 days. After this time, females were returned to the mating vials and exposed to new males for 1 hr. The females (remated or not) were then placed on fresh medium for 3 more days. Third and 4th matings were set up after an additional 3 and 6 days, respectively. Unmated females served as controls. To establish that insemination and not merely copulation was occurring at each mating, several females were selected immediately after the first and second matings and were dissected to determine if sperm were present in the bursa copulatrix. Approximately 1 hr is required for the complete transfer of sperm from the bursa to the spermatheca; therefore, the presence of sperm in the bursa is evidence of a recent (<1 hr) insemination. The numbers of offspring produced by females that had mated 1, 2, or 3 times were recorded by larval counts ca. 3 weeks thereafter. The experiment was replicated 3 times with each replication comprised initially of 20 females.

Multiple mating by males - Male mating frequency was determined by exposing individual sexually mature males, 6-8 days old at the outset, to single virgin females daily for 10 consecutive days. Single-pair matings were carried out following the method described above. The mating history of each male was recorded, and immediately following the 2nd-9th matings all females were dissected and checked for the presence of sperm. Transfer of sperm by test males was taken as evidence of fertility. Females from the 1st and 10th matings were allowed to oviposit, and the resulting progeny were counted ca. 3 weeks later.

Sperm utilization - The pattern of sperm utilization by twice-mated females was established by using single-pair matings and an autosomal semi-dominant genetic marker (black body) [7]. In this strain, the homozygous mutant is black and the heterozygote is dark brown. Wild-type beetles are reddish brown. Initially, wild type females were mated to wild type males and black females were mated to black males. Males were discarded after mating and the females were kept on oviposition medium for the next 3 days. After this time, they were returned to the mating vials and wild type females were mated to black males and black females were mated to wild type males. Remated females were then put on fresh oviposition medium and not further disturbed. Upon eclosion, the numbers and phenotypes of all 2nd mating progeny were noted. Progeny counts only were made of the offspring derived from the 1st matings. The occurrence of dark brown (heterozygous) individuals among the progeny produced following the 2nd mating was evidence that sperm from that mating had been utilized. The experiment was replicated 4 times using an initial 20 females of either phenotype/replication.

RESULTS: Multiple mating by females - Nearly all of the test females mated at least 2 times, approximately 10% mated 3 times and no 4th matings were observed (Table 1). By the date of the 4th mating, the females were 13-16 days old and most completed oviposition or had died before this time. The average numbers of

TABLE I. Incidence of multiple mating by *L. serricorne* females.

Number of matings	N	% females mated
1	60	96.7
2	58	94.8
3	56	12.7
4	7	0.0

offspring produced by females that had mated 1, 2, or 3 times were not significantly different (Table II). Unmated females produced no offspring. Dissection of newly mated females indicated that sperm were transferred at each mating.

TABLE II. Average numbers of offspring produced by *L. serricorne* females following 1, 2, or 3 matings.

Number of matings	N	X number of offspring (±se)
1	51	68.0(±2.4)
2	48	72.1(±1.4)
3	7	75.3(±6.3)

Multiple mating by males - More than 90% of the males used in this test mated at least 6 times during the 10-day period. The mean number of matings/male was 7.2. Sperm were transferred in more than 95% of the cases. Approximately 1/4 (23.3%) mated each day of the test. Mortality during the test was less than 5%. There was no significant difference between the fertility of males that had mated 1 or 10 times. Females that were mated to previously unmated males (N = 18) produced 77.9±2.7 (se) offspring while females that mated with males that had mated 9 times previously (N = 14) produced 71.0±5.0 (se) progeny.

Sperm utilization - The pattern of sperm utilization by twice-mated females is summarized in Table III. Approximately 85% of the offspring produced following a second mating, in either series of crosses, were heterozygous and thus were derived from

TABLE III. Distribution of homozygous and heterozygous offspring among twice-mated wild-type and mutant *L. serricorne* females.

		% progeny of 2nd mating:		$\bar{X}$ number of offspring/
Female	N	Homozygous	Heterozygous	female (±se)[a]
Wild-type	44	16.6(±1.9)	83.4(±1.9)	74.2(±4.5)
Mutant	51	15.7(5.1)	84.3(5.1)	67.3(±4.3)

[a]Both matings.

sperm obtained at the 2nd mating. There was no significant difference between the total numbers of offspring produced by black and wild-type females. Considerable individual variation was noted in both series of crosses. Among the twice mated wild type females, 15.9% produced no heterozygous offspring, 18.2% produced only heterozygous progeny and the remaining 65.9% produced both phenotypes. For black females, only 7.8% produced all black offspring, 31.4% had only dark brown progeny and the remaining 60.8% produced both homo- and heterozygous individuals. As not all of the test females remated, it was possible to compare the average numbers of offspring produced by both once and twice mated females. Twice mated females (black and wild type) produced a mean 70.5±3.9 (se) while once mated (both phenotypes) females averaged 73.4±4.1 progeny.

DISCUSSION: The results of these experiments indicate that while nearly all *L. serricorne* females were receptive to a 2nd mating and that some will even mate a 3rd time, multiple mating in this species had no significant effect upon overall female fecundity. It is likely that overall fecundity is more a function of female weight [8] (i.e., larval nutrition) and the age at which the adult female begins to oviposit [9]. The occurrence of heterozygous offspring among the progeny of twice-mated females does, however, indicate that sperm received in a second mating were utilized. It is possible that multiple mating in *L. serricorne* serves as a mechanism to increase genetic recombination.

Lasioderma serricorne males showed no loss of fertility at advanced ages (up to 16 days) and after many matings. These results suggest that individual males may be capable of impregnating a considerable number of females and that, for example, a control program for this species based upon selective removal of the male (e.g. pheromone trapping) would have to be extremely efficient before significant reduction in mating would result.

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