BEHAVIOR

Influence of Oviposition Substrate on Female Receptivity to Multiple Mating in *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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ABSTRACT The influence of oviposition substrate on female receptivity to remating is examined in *Callosobruchus maculatus* (F.) by comparing responses of nonvirgin females, maintained with or without access to oviposition substrate, to subsequent courting males. We demonstrate that females maintained without oviposition substrate, and thus prevented from ovipositing, readily reject additional male attempts to copulate. Females allowed unrestricted access to oviposition substrate, on the other hand, remate readily with subsequent courting males. These treatment differences are not the result of differences in male behavior; males responded similarly to females in each treatment. We then discuss four hypotheses which may explain these observed treatment differences.

KEY WORDS male harassment, mating behavior, sexual selection

IN MOST SPECIES of insects, both males and females mate multiple times (Ridley 1988). The advantages of multiple mating by males generally are well understood (Thornhill & Alcock 1983). However, for females, the advantages of multiple mating are less clear. The evolution of variation in female mating frequency among species thus has been the subject of extensive investigation (reviewed in Walker 1980; Fox 1993a).

In addition to among-species and among-population variation, mating frequency generally varies among females, within populations. Although some of this variation may be genetic (Pyle & Gromko 1981, Gromko & Newport 1988), much also may be caused by environmental sources (Harshman et al. 1988). In this study we investigate one environmental source of variation in female mating frequency of *Callosobruchus maculatus* (F.). We examine the influence of oviposition substrate on female receptivity to remating by comparing responses of nonvirgin females, maintained with or without access to oviposition substrate, to subsequent courting males. We demonstrate that females prevented from egg laying can, and do, readily reject additional male attempts to copulate. Females allowed unrestricted access to oviposition substrate, on the other hand, remate readily with subsequent courting males.

Materials and Methods

C. maculatus is a cosmopolitan pest of stored legumes (Fabaceae). Females of *C. maculatus* generally will mate multiple times in their lifetime, although only one, or occasionally two, matings are required to fertilize all their eggs (C. Fox, unpublished data). Following mating, females commence egg laying (often within minutes, if seeds are available), cementing their eggs to the surface of the host seeds (Messina 1991). Collection and rearing details for the population used here are discussed in Fox (1993b; Bay Area population).

When a male encounters a female, he generally raises his antennae and waves them as he approaches her (Rup 1986). Upon contacting the female, he antennates her all over, and then mounts from the rear. While clasping the female with his pro- and mesothoracic legs, the male antennates and palpates the sides of the female. Simultaneously, he extends his aedeagus under the female's abdomen and strikes her with his aedeagus, attempting to contact her genitalia. During this sequence there are at least two ways in which a female can reject a male and thus control mating. First, she can prevent mounting by fleeing or kicking as a male approaches. Second, she can refuse to expose her genitalia after a male has mounted. In these experiments, females were never observed rejecting a male once he was mounted, although rejections by fleeing or kicking were common.

Virgin female beetles were collected from host seeds and presented with an individual virgin

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male within ≈12 h of adult emergence. The female was allowed to copulate once. Following mating, half of these females were transferred to 60-mm petri dishes containing an oviposition substrate (≈5 g azuki seeds, *Vigna angularis*). The remaining females were transferred to empty 60-mm petri dishes.

At 12-h intervals following their initial mating, each female was transferred to an empty 35-mm petri dish and presented with a virgin male. Each female remained confined with a male until either copulation occurred or the female rejected the male. Two types of rejections were defined. In rejection type 1 (R1), the male pursued the female and attempted to mount her two times within 15 min but was unsuccessful at initiating copulation because either the female kicked him with her rear legs or the female successfully fled from the male (a male pursued a female for two continuous circuits around the edge of the petri dish without pausing or losing contact with each other). In rejection type 2 (R2), the male pursued the female within the petri dish (not necessarily continuously), attempting to mount her once, but was unsuccessful at initiating copulation within 15 min because the female kicked or avoided the male (as described).

Rejections of type R1 are thus unambiguous rejections—the females clearly refused to mate. Rejections of type R2 may be complicated by male responses to a female. These males attempted to mate once, but it is unclear if they subsequently gave up attempting to locate the female again or, instead, were unsuccessful at locating the female again. If a male demonstrated no interest in the female within 5 min of introduction to the petri dish (failed to pursue the female and attempt mounting), females were offered a fresh virgin male, and the trial was repeated. If the second male also demonstrated no interest in the female, the male was labeled as *nonresponsive* to the female. All males either copulated with a female or were classified into one of these three categories (R1, R2, or nonresponsive). In all cases, a male that mounted a female without protest from the female was successful at initiating copulation.

This experiment was replicated twice, using a different laboratory generation of the beetles in each replicate. The time at which females first accepted another male was recorded in 12-h intervals.

Voucher specimens from this study have been deposited in the National Museum of Natural History, Washington, DC.

Results and Discussion

We found that female *C. maculatus* prevented from egg laying (maintained without oviposition substrate) readily rejected male advances; females without oviposition substrate delayed remating longer than females that were allowed to oviposit (Fig. 1; Mann–Whitney U-tests; replicate 1, $U = 10.0$, $P < 0.001$; replicate 2, $U = 0.0$, $P < 0.001$). We also found that few females, whether egg laying or not, remated with the first male presented to them (Fig. 1).

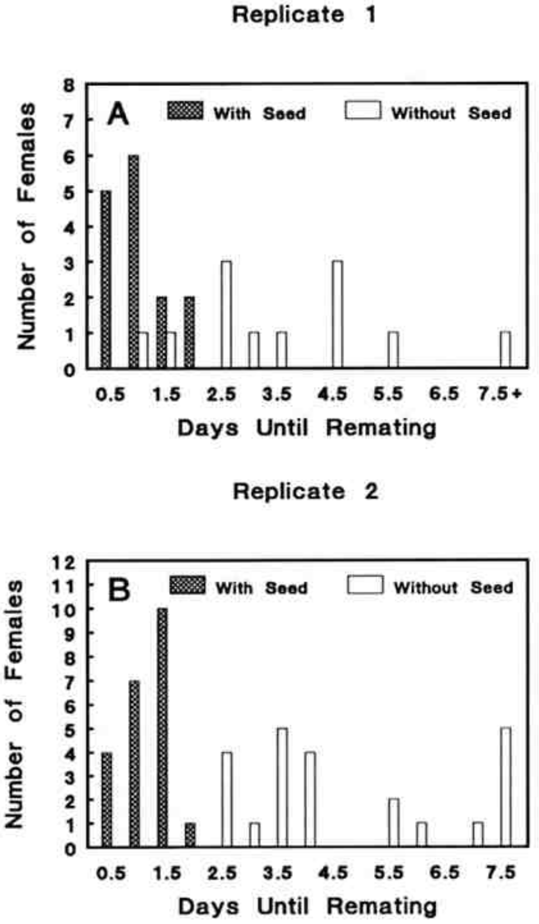

Fig. 1. Time at which females first accept a second mate when presented with males at 12-h intervals. Replicates 1 and 2 used different laboratory generations.

The observed differences in time to remating cannot be explained by differences in male response to females in the two treatments (Table 1). More than half the males in each treatment unambiguously attempted to copulate with the females and were either rejected (R1) or mated successfully. There was no difference among treatments in frequency of attempted copulations (matings + R1 rejections), R2 rejections, or nonresponsive males (Table 1).

Our results indicate that females of *C. maculatus* can, and will, readily reject male attempts at copulation. In addition, females that do not have access to oviposition substrate and thus are prevented from laying eggs, reject males much

Table 1. Effect of oviposition treatment on male courtship behavior in *Callosobruchus maculatus*

Day	Male behavior	No. pairs Without oviposition substrate	 With oviposition substrate
0.5	Attempted copulation (mated + R1)	13	12
	R2 rejections	10	9
	Nonresponsive	0	2
		$X^2 = 2.09$ NS	
1.0	Attempted copulation (mated + R1)	16	12
	R2 rejections	7	6
	Nonresponsive	0	1
		$X^2 = 1.28$ NS	
1.5	Attempted copulation (mated + R1)	18	11
	R2 rejections	5	1
	Nonresponsive	0	0
		$X^2 = 0.98$ NS[a]	

For X^2 analyses, matings and R1 rejections by females are lumped together as attempted copulations by the male to test the hypothesis that male behaviors are heterogeneous across treatments: matings and R1 rejections represent the same male behavior (attempted copulations) but different female responses. Data are for replicate 2 only; these data were not recorded for replicate 1. Chi-square analyses compare the effect of treatment on the distribution of male responses, within each day. Separate X^2 analyses were performed for each day (df = 2 for day 0.5 and 1; df = 1 for day 1.5).

NS, not significant.

[a] Test performed as two-way contingency after deleting nonresponsive category.

more frequently than egg-laying females. This rejection of copulation attempts is particularly interesting because males contribute ≈5% of their body weight to the female in their ejaculate (Fox 1993c), and this contribution can be used by the female for somatic maintenance (Fox 1993a), as well as for egg production (Fox 1993a and c). Females in either a storage environment or natural environment rarely feed as adults, and the resulting nutrient stress has serious effects on mortality as well as potential lifetime egg production (Fox 1993a,c), presuming the female eventually finds an oviposition substrate. Nutrients obtained during mating offset some of these negative effects (Fox 1993a,c).

At this time, it is unclear why females prevented from egg laying reject males more readily than egg-laying females. However, at least four hypotheses may explain the observed treatment differences. First, although the extent to which a single spermatophore fills a female's spermatheca is unknown, it is possible that a female is unable to receive a second spermatophore until this first spermatophore has been absorbed partially. If egg-laying females absorb the spermatophore faster than females not laying eggs, either into their somatic tissues or directly into their eggs, we would expect egg-laying females to become capable of remating sooner than females not laying eggs.

Second, the cost of remating may be sufficiently high that females remate only to replenish diminishing sperm supplies and avoid remating when sperm are not limited. However, Fox (unpublished data) has demonstrated that most females of the population used in this study (Bay Area population) need only mate one time to fertilize all their eggs; thus females of neither treatment should need to remate to replenish sperm supplies.

Third, if females remate to increase genetic diversity among offspring (Caldwell & Rankin 1974), females that have not used sperm from the first male would receive very little benefit from remating because of very high second-male sperm precedent (Eady 1991). Thus, females may refuse remating until at least some eggs have been laid. This hypothesis also may explain why approximately half of the females that had access to oviposition substrate rejected at least one male before eventually remating.

Finally, female tolerance of male harassment may be flexible, so that egg-laying females are less tolerant of harassment than females that are prevented from egg laying. The *convenience-polyandry* hypothesis suggests that females mate multiply because accepting multiple copulations is less costly to the female than resisting harassment by persistent males (Parker 1970). If the costs of multiple mating are sensitive to variation in environmental conditions, such as access to oviposition substrate, then a female's tolerance of harassment may be flexible. To our knowledge, this convenience-polyandry hypothesis has only been tested once; Rowe (1992) concluded that male harassment can explain the evolution of multiple mating in the water strider, *Gerris buenoi* Kirkaldy. In our experiments with *C. maculatus*, although both experimental groups of females would suffer some of the same costs of male harassment, such as physical damage or depletion of energy reserves, the two groups of females will differ in other costs of remating. For example, time may be a more valuable resource for females that are laying eggs and thus not expendable for male avoidance; copulation almost always is completed in under 5 min, whereas males may pursue a female for more than 10 min if the female resists copulation. Similarly, females that have had recent success in locating host seeds may be chased away from this high-quality patch if they flee from a male, whereas recently unsuccessful females have not encountered a patch that they can lose.

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