

EFFECTS OF MATING ON FEMALE MATING INHIBITION AND EGG PRODUCTION
IN THE BLACK CARPET BEETLE, ATTAGENUS MEGATOMA F.

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ABSTRACT: Black carpet beetle, *Attagenus megatoma*, females became nonreceptive to mating attempts by males immediately after mating. This nonreceptivity usually diminished with time, although about 40% of the females mated only once before they died. Mating stimulated the corpora allata of the female to produce juvenile hormone which was responsible for final maturation of the eggs. The signal that the female had mated was passed from the abdomen to the head via the ventral nerve cord within 20 min after mating. The mating nonreceptivity exhibited after mating by females was not caused by juvenile hormone.

INTRODUCTION: Insects use cues to trigger changes in physiology and behavior to synchronize their actions with the appropriate set of environmental conditions. Mating in many insects serves as such a cue although mating itself may be triggered by other cues [1]. This paper describes the effects of mating on the production of eggs, and the start of a mating refractory period in black carpet beetle (*Attagenus megatoma* F.) females.

EXPERIMENTAL: Our unpublished preliminary experiments showed that the behavior of mated females was very different from that of unmated females. The formerly receptive female began actively avoiding male mating attempts immediately after mating. Although the female is attractive to males after mating, she rests with the tip of her abdomen pressed against the bottom of the container and does not evert her genitalia, a procedure necessary for mating. In a preliminary test, 3 groups of 20 females each were exposed for 10 min to males 24, 48, or 96 hr after previous mating. No females remated after 24 hr, 3 remated after 48 hr, and 11 remated after 96 hr.

Effect of Mating on Female Willingness to Remate - Our preliminary observations suggested that there was a mating inhibition brought about by the first mating. This response is similar to one we have observed previously in *Trogoderma inclusum* LeConte [2]. The following experiment describes the method used to determine the percentage of females that were inhibited from mating a second time. Because our observations had shown that *A. megatoma* females expel their spermatophore within ca. 24 hr after mating, we could not determine the number of matings by counting the number of spermatophores in the bursa of the female. We therefore

used the sterile male method previously used for *T. inclusum*[2]. Thus, in brief, second matings were determined by observing whether females previously mated to sterile males produced viable offspring when they were subsequently placed with normal males. Production of offspring was taken as proof of remating. (Preliminary experiments indicated that 3- to 4-day-old adult *A. megatoma* males were sterilized with 20,000 R of gamma irradiation).

In the present test, 6- to 8-day-old virgin *A. megatoma* females were paired with either sterile or normal 6- to 8-day-old virgin males in 15 X 45-mm vials and observed until they mated. Mated females were then placed singly with a 6- to 9-day-old virgin male (either sterile or normal depending on the test) in 22 X 45-mm oviposition vials containing 10 ml of rearing media. The pair remained in the vial until both died. The vial was examined for the presence of larvae ca. 6 weeks later.

About 40% of the females mated only once (Table I). Of those females that did remate, the number of progeny per female was reduced by about one-half. This reduction in offspring suggests that remating occurred after most of the eggs had been laid.

TABLE I. Effect of mating on remating in black carpet beetles.

1st male	2nd male	No. of females	% females producing larvae	% females not remating*	Average no. larvae/females with larvae
Normal	Normal	80	90%		77.4
Sterile	Normal	180	50%	40%	38.2

*Treatment subtracted from control.

Effect of Mating on Egg Production - Probably the most obvious effect of mating on the female was the effect on egg production: In this species, only mated females mature and lay eggs. In virgin females, the terminal oocyte of each ovariole grows to ca. 375 μ in length when they enter what appears to be a resting stage. If mating does not occur, these oocytes rarely grow to full size (>500 μ). To determine the effect of mating on egg development, we made the following experiment. Six to 7-day-old females were dissected during 3 time intervals after mating. The length of the terminal oocyte of the largest ovariole from each female was recorded. Data from 10 females were averaged for each of the 3 groups. The oocytes resumed growth within 7 1/2 hr after the females mated (Table II). The oocytes grew to mature size in about 25 hr. Oviposition followed mating after 26-48 hr.

Mechanisms Involved in Change to Mated Physiological State - Because juvenile hormone has been associated with egg production in several insects [3,4,5], the effect on egg maturation of juvenile hormone was studied. Five to 6-day-old virgin females were treated topically with 10 μ g *Cecropia* juvenile hormone (Hoffmann-LaRoche #R06-9550, ENT #33972A) in 1 μ l of acetone (Table III).

TABLE II. Effect of mating on oocyte maturation.

Hr. after mating	Terminal* oocyte length (μ) \pm S.E.
0 - 1	311 \pm 9.5
7 1/2 - 8 1/2	356 \pm 7.7
16 1/2 - 17 1/2	342 \pm 20.0

*Most advanced.

TABLE III. Effect of mating and JH on oocyte development of 5-6-day-old black carpet beetles.

Female treatment	Terminal oocyte size (μ) \pm S.E.	% females with mature eggs
Mated	477 \pm 30	0
Virgin + acetone	325 \pm 9	0
Virgin + JH in acetone	473 \pm 29	30

The females were dissected 21-23 hr after the treatment, and the terminal oocyte from the most advanced ovariole of each female was measured. Data were averaged from 10 females in each group, and the test was replicated 3 times. The results show that juvenile hormone brought about the same effect in virgin females as did mating except that some of the juvenile hormone-treated females had produced mature eggs by 21-23 hr posttreatment whereas the mated females had not. Mature eggs were formed somewhat faster after juvenile hormone treatment than after mating, probably because the corpora allata required a few hours to release threshold levels of juvenile hormone into the haemolymph of mated females. Virgin females were treated with juvenile hormone and checked for mating receptivity after 24 hr. No difference was found between their willingness to mate and the willingness of untreated virgins to mate. Apparently juvenile hormone was not the agent that brought about the mating refractory period seen in mated females.

To determine how the mating stimulus is transferred from the abdomen to the corpora allata, we made an experiment to determine the effect of removal of part of the female reproductive system at different time intervals after mating. The ovipositor, bursa copulatrix, spermatophore, spermatheca, and both accessory glands opening into the spermatheca were removed at 0-5, 5-10, 10-15, 15-20, 30-35, and 60-65 min after mating. Twenty-four to 25 hr after mating the females were checked for mature eggs by dissection. Females matured eggs in the absence of the excised reproductive organs listed if the removal was done 5 min or longer after mating. From other unpublished data we know that sperm do not enter the spermatheca until 5 to 9 hr after mating; therefore, the presence of sperm in the spermatheca can be eliminated as the primary mating stimulus.

TABLE IV. Effect of ventral nerve cord (VNC) severance between the 4th and 5th abdominal segments on the ability of mated black carpet beetle females to produce mature eggs. Females were mated and/or operated at 6-7-, dissected at 8-9 days of age (after 45-50 hr).

Treatment	Percent surviving ^a females with chorionated eggs	Number of surviving females	95% binomial confidence interval
Mated, anesthetized ^b and VNC severed:			
0-5 min postmating	0	21	0-16.0
5-10 min postmating	23.8	21	8.3-46.8
10-15 min postmating	40.0	20	19.1-64.0
15-20 min postmating	65.2	23	44.4-83.4
30-40 min postmating	76.2	21	53.2-91.7
60-70 min postmating	70.8	24	50.9-87.2
Mated, anesthetized and sham operated ^c			
	68.7	147	60.9-76.3
Mated, anesthetized 0-5 min postmating			
	68.0	25	46.5-85.0
Mated, untreated			
	88.0	25	68.8-97.4
Virgin, untreated			
	0	20	0-16.9
Virgin, anesthetized and VNC severed			
	0	38	0-9.1

a Overall mortality in operated groups = 1.5%

b Anesthetized in ice water.

c As there was no significant difference in egg production between insects sham operated at 0-5, 5-10, 10-15, 15-20, 30-40, and 60-70 min postmating, these groups were combined.

Another experiment was done to determine the role of the ventral nerve cord (VNC) in relaying the mating stimulus from the abdomen to the corpora allata. The VNC of 6 to 7-day-old females was severed between the 4th and 5th abdominal segments during the following intervals after the completion of copulation: 0-5, 5-10, 10-15, 15-20, 30-40, and 60-70 min. Females were dissected 45-50 hr postmating, and the presence or absence of mature eggs was recorded. Results for the treatment groups as well as appropriate controls are given in Table IV. An intact VNC was required for up to ca. 20 min after mating if egg maturation was to be stimulated by copulatory act.

DISCUSSION: Mating is an important cue for triggering the physiological and behavioral changes exhibited by the mated *A. megatoma* female. The virgin female produces sex pheromone to lure males for mating. She is receptive to the male and performs those behaviors necessary for mating to occur. She matures oocytes to only 25% of their maximum volume. The latter is important if this relatively short-lived species, which may or may not feed as an adult, is to conserve her energy resources and yet be ready to produce mature eggs soon after mating. The mated female, on the other hand, shows behavioral patterns that can be termed "matedness", which are very different from virgin behavior. Mated females are nonreceptive to males, and eggs are matured and laid. Both of these events are manifested very shortly after mating.

Some tentative conclusions can be drawn about the mechanisms that result in the change from virgin to mated status based on the work reported here. Some event happening within 20 min of mating triggers a chain of physiological events that culminate in the various behaviors associated with a mated female. The VNC is somehow stimulated and probably conducts the "matedness" signal to the brain. This culminates, through an unknown number of intermediate steps, in the stimulation of the corpora allata to produce juvenile hormone which ultimately results in final egg maturation. The corpora allata are "downstream", however, of the point where the effects of mating cause the female mating refractory period. It is indeed possible that the 2 parameters studied--egg production and female mating refractory period--have entirely different chains of events that are stimulated at mating and that culminate in the observed effects of mating. Research is currently underway to further elucidate the mechanisms of these 2 phenomena.

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