EFFECT OF MALE MESADENE SECRETIONS ON FEMALES OF *CANTHON CYANELLUS CYANELLUS* (COLEOPTERA: SCARABAEIDAE)

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ABSTRACT

This study determined the effect of male mesadene secretions on females of *Canthon cyanellus cyanellus* LECONTE, both when they were inseminated normally and when the secretions were transplanted to virgin females. In the first case, mating took place when the ovary was immature, triggering ovarian maturation, egg laying and nest building. In the second case, the transplantation of male mesadene secretions to virgin females initiated ovarian maturation, but neither egg laying nor nest building took place. Virgin females that did not receive the secretions had no ovarian maturation and did not lay eggs or build nests. It is therefore possible that male mesadene secretions induce ovarian maturation. In the present study, this inducement was greater in inseminated females than in those receiving transplanted secretions.

Key Words: Mesadene secretions, Transplant, Ovarian maturation, Scarabaeinae

RESUMEN

En este trabajo se determinó el efecto que tienen las secreciones de las mesadenias del macho sobre las hembras de *Canthon cyanellus cyanellus*, cuando son inseminadas normalmente, o cuando las secreciones son trasplantadas a hembras vírgenes. En el primer caso la cópula tuvo lugar cuando el ovario estaba inmaduro, desencadenando la maduración ovárica, la oviposición y la construcción del nido. En el segundo caso, el trasplante de las secreciones de las mesadenias a hembras vírgenes inició la maduración ovárica, pero no la oviposición ni la construcción del nido. Las hembras vírgenes que no recibieron las secreciones no maduraron el ovario ni hubo oviposición o construcción del nido. Es posible que las secreciones mesadénicas del macho induzcan la maduración ovárica. Esta inducción fue mayor en las hembras inseminadas que en las que recibieron el trasplante de las secreciones.

In various species of Scarabaeinae (Scarabaeidae), mating takes place shortly after the female emerges (Monteith & Storey 1981; Klemperer 1982) or before egg laying (Halffter & López 1977; Halffter et al. 1980; Huerta et al. 1981; Monteith & Storey 1981; Anduaga & Huerta 1983; Sato & Hiramatsu 1993). In the above-mentioned cases, mating is necessary for egg laying and nesting to begin.

In the dung beetles, *Canthon indigaceus chevrolati* HAROLD and *Copris incertus* SAY, the first mating, which occurs during the pre-nesting period, when the ovary is still immature, is indispensable for ovarian maturation, egg laying and nesting to occur (Martínez & Cruz 1990; Martínez et al. 1996).

In *Canthon cyanellus cyanellus* LECONTE, the first mating is at 10 days after female emergence. This occurs half way through the pre-nesting period, which lasts about 20 days. During this period ovarian maturation occurs, only food balls are produced and there is no nesting. Afterwards, during the nesting period, other matings may occur (Martínez 1992).

During mating, the male of *C. c. cyanellus* produces a spermatophore containing abundant seminal fluid which consists principally of the secretions of the accessory glands (mesadenes). Most of this seminal fluid has a high concentration of proteins, although it also contains glycogen and acid mucopolysaccharides (Cruz & Martínez 1992). This is also the case in other Coleoptera (Anderson 1950; Landa 1960; Gerber, et al. 1971; Gundevia & Ramamurty 1977; Huignard et al. 1977; Peferoen & de Loof 1983; Black & Happ 1985).

The objective of this study was to determine the effect of male mesadene secretions in *C. c. cyanellus* upon ovarian maturation and female reproductive behavior.

Materials and Methods

This study was carried out on adult *Canthon cyanellus cyanellus*, of known age and raised in the laboratory. Insects were kept at 27°C, 70% RH, a photoperiod of 14:10 hours and were fed beef.

Females were tested in one of four manners: 1) a female was kept together with a male from the time of emergence (n = 63), 2) virgin females were isolated from the time of emergence (n = 57), 3) virgin females received transplants of mesadene secretions from mature males at 10 days old (n = 31), and 4) virgin females had sterile Ringer-Ephrussi solution injected at 10 days old (n = 25).

The females in categories 1 and 2 were sacrificed at 5, 10, 15, 20, and 25 days of age, with approximately 10 females per age group. At 10 days of age the females in categories 3 and 4 received the secretion transplant or the injection of Ringer solution. It was allowed to take effect for 5, 10 or 15 days. The females in these last two categories were sacrificed at 15, 20 and 25 days of age. The were about 5 females in these age groups.

To carry out mesadene transplantation, the reservoir, a structure in which glandular secretions are stored, was obtained from 20-30 day-old males. Females were anaesthetized with ethyl acetate for 3 minutes, which allowed them to recover without complications. The elytra and wings of anaesthetized females were lifted carefully, and the reservoir was placed in the dorsal region of the abdomen. Using an entomological pin, a dorsal puncture was made through which glandular secretions were introduced into the abdominal cavity. It was not necessary to use sealer, as the wound healed quickly on its own. Females recovered in about 5-10 minutes. After a 24-hours period they were put inside a terrarium (Cruz 1994).

The reproductive systems from all four groups were dissected out in Ringer-Ephrussi solution. Each ovary was measured and drawn to scale with the aid of a camera lucida. The ovary and vaginal frotis together with the spermatheca from each female were obtained and dyed *in toto* using the Feulgen-green light technique. The presence of the spermatophore in the vagina or of spermatozoids in the spermatheca indicated that the female had been inseminated.

The length of the basal oocyte was analyzed in each age group and the different categories were compared. Sample sizes were 5 to 17 females per age group. Since these were small samples, a 95% confidence interval was calculated using the formula $\bar{x} \pm \alpha_{n-1}/\sqrt{n}$ (1.96), where $\bar{x}$ is the sampling median and α_{n-1} is the standard deviation. Analysis of variance (ANOVA) was used to compare means.

Female reproductive behavior was categorized according to the following factors: if they were inseminated, if they laid eggs, and either made food or nest balls.

RESULTS

The female reproductive system in *C. cyanellus cyanellus* is similar to that of other Scarabaeinae: it consists of a single left ovary with one ovariole, an oviduct, vagina and spermatheca with its accessory gland.

Females with Males

In these females, the first basal oocyte measured 0.7 mm 5 days after eclosion. By the 10th day it had more than doubled in size to 1.5 mm. By the 15th day it had again doubled in size. After 20 days, it was almost egg-laying size, which in this species is 3 mm (Fig. 1; Table 1). Females in this group copulated between 10 and 15 days of age. Spermatozoids were observed in the spermatheca, and the spermatophore was in the vagina. By 15 days after eclosion, all of the females dissected had been inseminated but had not yet begun to nest. By 20 days, most of the females had already laid a first egg, and the second basal oocyte was close to egg-laying size. At the age of 25 days, all females were making their first nest with 1 to 7 nest balls, corresponding at 1 to 7 eggs laid.

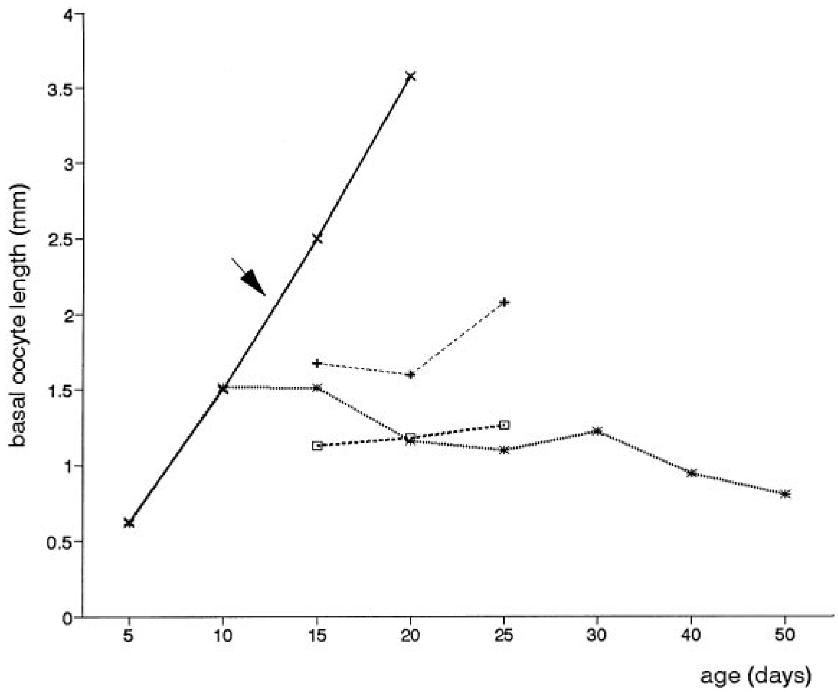

Fig. 1. First basal oocyte development in *Canthon cyanellus cyanellus* females. (X) Females with male since emergence, the arrow shows the age at which first copula occurs; (*)Virgin females; (+) Virgin females with transplant from 10 days of age on; (□) Virgin females injected with Ringer's solution.

TABLE 1. BASAL OOCYTE MATURATION IN FEMALES OF *CANTHON CYANELLUS CYANELLUS* KEPT WITH A MALE SINCE EMERGENCE (F-M), VIRGIN FEMALES (VF), VIRGIN FEMALES WITH TRANSPLANT (VF-T) AND VIRGIN FEMALES INJECTED WITH RINGER'S SOLUTION (VF-R). ($\bar{x}$ ± SE)(N) NUMBER OF FEMALES PER AGE.

	Basal oocyte length (mm)			
Age (days)	F-M	VF	VF-T	VF-R
5	0.72 ± 0.10(10)	0.61 ± 0.17(11)	—	—
10	1.50 ± 0.12(14)	1.52 ± 0.15(12)	—	—
	mating			
15	2.50 ± 0.15(17)	1.51 ± 0.09(12)	1.68 ± 0.15(10)	1.13 ± 0.17(10)
20	3.58 ± 0.10(10)	1.16 ± 0.10(10)	1.60 ± 0.13(10)	1.18 ± 0.08(10)
25	2.65 ± 0.21(12)	1.10 ± 0.10(12)	2.08 ± 0.17(11)	1.26 ± 0.31(5)

Virgin Females

Until the 10th day, the development of the first basal oocyte in these females was similar to that of females of the same age that had been kept with males. On the 15th day, the size of the basal oocyte remained almost identical to that observed at 10 days. From day 20 to 25, however, it gradually diminished in size, and was reabsorbed in females more than 50 days old (Fig. 1: Table 1).

The ovaries of virgin females did not fully mature, and the oocytes entered into reabsorption. Virgin females only made food balls, and did not construct nest balls or nest.

Virgin Females with Male Mesadene Secretion Transplant

The transplant of secretions was performed at 10 days of age due to our observation that the first mating tends to occur around this age. At day 15 the first basal oocyte measured an average approximately 1.6 mm; this size did not change by 20 days of age. However, at 25 days of age, the first basal oocyte became substantially larger (Fig. 1; Table 1). All females in this group initiated first basal oocyte maturation, but oocytes did not reach egg-laying size even at 25 days.

Although they initiated ovary maturation, these females did not lay eggs, make nest balls or nest; their only activity was the production of food balls.

Virgin Females Injected with Ringer's Solution

In these females, first basal oocyte size did not noticeably increase (Fig. 1; Table 1); furthermore, neither egg laying, ovary maturation or nest making took place. Activity was limited to making food balls which they did not turn into nest balls.

DISCUSSION

In *Canthon cyanellus cyanellus* the development of the first basal oocyte in virgins was compared with those of females kept together with the male since the time of emergence. After 15 days, the size of the first basal oocyte was no longer comparable between the two groups ($F_{(1,27)}$: 26.8; $p < 0.01$). In females which were inseminated by a male, the ovary was bigger than in individuals kept alone. This difference was greatest at 20 days of age ($F_{(1,19)}$: 210; $p < 0.01$) even compared to 25-day-old females ($F_{(1,22)}$: 41.8; $p < 0.01$).

These results demonstrate that the first mating triggers the final maturation of the basal oocyte and the ovary, and, in some yet unknown way, induces egg laying and nesting. This has also been demonstrated to be true in *Canthon indigaceus chevrolati* and *Copris incertus*. In these two beetles, virgin females neither finish ovary maturation, lay eggs, nor make nests (Martínez & Cruz 1990; Martínez et al. 1996).

A comparison of the size of the first basal oocyte in females which were inseminated and in those which received male mesadene transplant showed marked differences from the age of 15 days ($F_{(1,25)}$: 14.0; $p < 0.01$) until 20 days ($F_{(1,18)}$:136; $p < 0.01$), but differences were non significant at 25 days ($F_{(1,21)}$: 4.1); the effects of mating and glandular secretions are, therefore, not comparable. The slow increase in basal oocyte size in females that received the transplant suggests that egg laying size could be reached at a more advanced age, although this was never confirmed through observation.

When virgin females were compared with those that received the transplant of male mesadene secretions, it became clear that the secretions do have a positive effect on ovarian maturation. After 20 days of age, the basal oocyte size was larger in the females receiving the transplant than in virgins ($F_{(1,18)}$: 6.4; $p < 0.05$). The greatest difference was observed during the period between 20 and 25 days of age ($F_{(1,21)}$: 24.6; $p < 0.01$): the ovary continued to mature up to an advanced stage, but oviposition did not take place during the period of observation. In females over 30 days old, basal oocyte size diminished in unaltered virgins but not in those that had received the secretion transplant.

An analysis of females with the transplant compared to those with Ringer's solution injections at various ages yielded the following data: the only significant difference observed was between treatments ($F_{(1,4)}$: 14.4; $p < 0.05$) regardless of age ($F_{(4,50)}$: 1.4; non significant). Virgin females which received male mesadene transplants had greater oocyte size than virgin females injected only with Ringer's solution.

When *C. c. cyanellus* virgin females received the transplant of male mesadene secretions, the ovary matured up to an advanced stage, but oviposition never took place. Females can receive the stimulus that induces ovarian maturation either during copulation or through the transplant of secretions. We do not yet know which of the components in these secretions act directly upon the ovary to induce vitellogenesis and ovary maturation, but the abundance of certain proteins may indicate that they are responsible.

In the weevil *Acanthoscelides obtectus* SAY (Huignard 1984) and in the mosquito *Aedes taeniorhyncus* (WIEDEMANN) (Borovsky 1985), vitellogenesis is induced by proteins called paragonial substances found in secretions of the male glands. In *A. obtectus* these substances are distributed through the haemolymph to the female's head, thorax and abdomen soon after mating and induce ovarian maturation (Huignard 1978). In other species of flies, and a grasshopper in which virgin females received secretions or male accessory gland extracts, ovarian maturation and egg laying resulted (Merle 1968; Leahy 1973; Burnet et al. 1973; Ramalingan & Craig 1976). Egg laying in various species of Orthoptera is also controlled by the paragonial substances (Pickford et al. 1969; Leahy 1973; Friedel & Gillott 1976) and in one butterfly (Santhosh-Babu & Prabhu 1987). In various species of Diptera, these substances control not only oviposition but the sexual receptiveness of females after mating (Burnet et al. 1973; Baumann 1974; Ramalingan & Craig 1976; Young & Downe 1987; Ohashi et al. 1991; Spencer et al. 1992).

In various species of insects, after lysis of the spermatophore into the vagina, the spermatozoids and part of the seminal fluid pass into the spermatheca. The remainder of the seminal fluid enters the haemolymph, where it subsequently reaches target sites directly or via a hormone that controls reproductive output (Raabe 1986).

Although it has been confirmed that the first mating is necessary for ovarian maturation and egg laying in *Canthon indigaceus chevrolati*, *Copris incertus* and *Canthon c. cyanellus,* only in *C. c. cyanellus* has it been show that the male mesadene secretions induce ovarian maturation and egg laying. However, in the dung beetle genus *Sisyphus* LATREILLE, virgin females sometimes make a few brood balls that contain infertile eggs or no eggs at all (Paschalidis 1974), suggesting that in this group the male mesadene secretions is not necessary to induce ovarian maturation and egg laying.

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