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INFLUENCE OF MATING ON OVARIAN FOLLICLE DEVELOPMENT IN *TRIATOMA INFESTANS* (KLUG, 1834)

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This work examines the influence of mating on ovarian follicle development in Triatoma infestans. *The observations were carried out on both virgin and mated females, which were killed at various times after their emergence. There was no difference in the ovarian development of both experimental groups during the first gonadotrofic cycle. By the 7th day mated females as well as virgin females showed vitellogenic oocytes. The coriogenesis and ovulation process began on the 13th day after imaginal moulting. However we could observe that egg-laying was dependent on mating. Mated females laid eggs whereas virgin females did not lay eggs. However ovarian production was significantly greater in the mated females. It is suggested that in* T. infestans *mating stimulates egg-laying but it does not influence the oogenesis and ovulation process.*

Key words: *Triatoma infestans* – mating – ovarian development

In most insects mating regulates female reproductive activity, by modifying sexual receptivity, permitting egg-laying or stimulating oogenesis (Engelman, 1970; Huignard, 1974; Gerber, 1975).

Some aspects about the influence of mating on the triatomine's reproductive activity have been studied by various authors (Ryckman, 1962; Davey, 1967; Costa et al., 1967; Pratt & Davey, 1972; Mundall, 1978; Lima et al., 1987). In *Triatoma brasiliensis,* females which had mated several times laid more eggs than those which had only mated once (Brasileiro, 1984). Mating also affected longevity, fertility and egg hatching in this species (Brasileiro, 1982).

Although work has been done on oogenesis, fertility and fecundity (Danilov, 1966; Perlowagora, 1969; Asin & Crocco, 1990), there are no references that relate *T. infestans* mating to their ovarian development.

Considering that the reproductive capacity of one species is important in its population dynamics regulation, the aim of this work was to investigate the influence of mating on the ovarian development in *T. infestans.*

MATERIALS AND METHODS

Triatoma infestans adult females free of infection and moulted in the laboratory from the fifth nymphal stage were used. The insects were supplied by Chagas' National Service (Córdoba, República Argentina), and were kept at room temperature (27 ± 1 °C) and in 60-70% relative humidity. They were fed weekly *ad-libitum* on *Columba livia.*

After emergence, insects were put into two experimental groups, with 56 females in each one.

Group 1 – virgin females – Females without males from emergence to being killed.

Group 2 – mated females – Females with males from emergence to being killed.

Mating fulfilment was verified by the presence of spermatophorous in the flask containing the mated insects which was controlled daily.

Seven females from each group were killed at 1, 3, 5, 7, 11, 13, 17, and 21 days after emergence following Asin & Crocco (1990).

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The following variables were quantified:

At the moment of emergence – Fresh weight of insects: variable used to estimate the quantity of blood contained in the insect's crop (Montenegro, 1983).

During the experimental period – (time from the moment of emergence to the being killed) – (a) Blood ingested, this variable was calculated from the difference in insect weight before and after each feeding; (b) Total blood ingested, obtained by the summation of the intakes of all the females during the experimental period; (c) Age of female at the beginning of egg laying; (d) Number of eggs laid by each female.

At the moment of being killed – (a) Insect's crop fresh weight; (b) Ovarian development: estimated as the oocytes number for different phases of oogenesis (Asin & Crocco, 1990); (c) Number of eggs present in the female genital tracks, or ovulated eggs; (d) Blood consumption calculated according to the following equation: fresh consumption = initial blood + total intake – residual blood (Montenegro & Pasina, 1984); (e) Net ovarian production defined as: N. O. P. = ovulated egg weight (mg) + laid egg weight (mg). Laid egg weight was obtained by weighing the just laid egg batch; (f) Consumption food convertion efficiency to an ovarian production (C. F. C. E. O. P.) which was estimated as: C. F. C. E. O. P. = ovarian production x 100/consumed blood (Montenegro & Pasina, 1984).

Dissections were carried out under a stereoscopic miscroscope. Weights were measured on a Mettler balance (0.01 mg precision).

For comparison between means, the Student t-test was performed. Correlation analysis and linear regression were carried out on certain variables (Sokal & Rohlf, 1979).

RESULTS

Ovarian follicle development – The percentage of oocytes present at different phases of oogenesis in virgin and mated female ovaries on various days after emergence is shown in Table I. There was no significant variation between virgin and mated groups ($p > 0.05$). Vitellogenic oocytes could be observed in virgin as well as in mated females seven days after emergence, and corionated oocytes from the 13th day onwards.

The percentage of virgin and mated females that ovulated and/or laid eggs from the 13th day after emergence is shown in Table II. At this moment the beginning of ovulation was registered for 71% of the virgin females and 42% of the mated females.

Also at this time 42% of the mated females began the oviposition while during the whole experimental period there was no oviposition in virgin females.

The mean number of ovulated and laid eggs for both experimental groups is shown in Table

TABLE I

Percentage of oocytes present at different phases of oogenesis in virgin and mated females of *Triatoma infestans*

Days after emergence	Females	% previtellogenic oocytes X ± SD[a]	% vitellogenic oocytes X ± SD	% chorionated oocytes X ± SD
7th day	virgin	81.9 ± 17	30.3 ± 3	–
	mated	81.5 ± 29.9	41.5 ± 25	–
11th day	virgin	79.0 ± 16.3	31.3 ± 2.8	–
	mated	50.0 ± 34.4	44.0 ± 17	–
13th day	virgin	60.3 ± 14.8	22.8 ± 8.5	19.5 ± 4.2
	mated	73.6 ± 19.2	23.4 ± 8.0	14.7 ± 7
17th day	virgin	71.5 ± 6.9	25.0 ± 6.1	7.9 ± 5.2
	mated	54.7 ± 9.5	24.4 ± 9.4	25.3 ± 3.3
21st day	virgin	75.5 ± 13.4	23.6 ± 7.7	4.2 ± 7.8
	mated	52.0 ± 12.5	36.6 ± 11.8	13.1 ± 10

a: SD = Standard deviation.

TABLE II

Percentage of virgin and mated females that ovulated and/or laid eggs

Days after emergence	Females	Virgin		Mated	
		ovulated n (%)	laid n (%)	ovulated n (%)	laid n (%)
13th day	7	5 (71)	0	3 (42)	3 (42)
17th day	7	6 (80)	0	6 (85)	6 (85)
21st day	7	5 (70)	0	7 (100)	3 (45)

TABLE III

Mean number of ovulated and/or laid eggs by virgin and mated females

Days after emergence	Ovulated eggs		Laid eggs	
	virgin female X ± SD[a]	mated female X ± SD	virgin female X ± SD	mated female X ± SD
13th day	4 ± 5	4 ± 2	0	5 ± 3
17th day	6 ± 4	3 ± 1[b]	0	21 ± 10[c]
21st day	10 ± 7[d]	2 ± 1[b]	0	26 ± 16[c]

a: Standard deviation.
b: significant differences between virgin and mated females ($p < 0.001$).
c: significant differences between mated females of the 13th day with mated females of the 17th day and the 21st day ($p < 0.001$).
d: significant differences between virgin females of the 17th day with virgin females of the 21st day ($p < 0.001$).

III. Thirteen days after emergence these variables did not show significant differences between the groups. However, on the 17th and 21st days the mean number of ovulated eggs increased significantly in the virgin females ($p < 0.001$). This variable reached values of 6,4 ± 4,3 and 10,2 ± 7,4 eggs respectively. On these dates, no oviposition was registered for the virgin females while for the mated females the mean number of eggs laid increased from 5,3 ± 2,8 on the 13th day to 26,3 ± 16,5 on the 21st day.

Evolution of net ovarian production in virgin and mated females along time – The values obtained for net ovarian production are shown in Table IV. In virgin females, this variable had no significant changes from the 13th to the 21st day after emergence. On the other hand, in mated females net ovarian production increased significantly 17 days after emergence reaching a mean value of 60,07 ± 35,06 mg.

Finally, during the experimental period (21 days) net ovarian production was significantly greater in mated females, their mean value being 44 ± 41 mg, against 15 ± 17 mg obtained in virgin females.

Relationship between blood-consumption and ovarian production – There was a linear relationship between these variables in both experimental groups with a significant coeficient of correlation (Table V). These results show that an increase in blood consumption is related to an increase in ovarian production.

Efficiency of consumed blood convertion to ovarian production – The mean blood consumption during the first 21 days after emergence in both experimental groups is shown in Table VI.

This variable showed no differences between virgin and mated females. However, mated females were more efficient in the transformation of consumed blood to the ovarian production in comparison with virgin females ($p < 0.001$).

TABLE IV

Net ovarian production (mg ovulated eggs + mg laid eggs), in virgin and mated females of *Triatoma infestans*

Days after emergence	Virgin female X ± SD[a]	Mated female X ± SD	Differences between groups
13	9,1 ± 14,2	11,6 ± 14,6	NS
17	13,2 ± 13,7	60,0 ± 35,0[b]	P < 0.001
21	21,1 ± 22,9	40,1 ± 49,6[b]	NS

a: SD = Standard deviations.
b: significant differences between mated females of 13 days and mated females of 17 and 21 days after emergence (p < 0.001).

TABLE V

Regression and correlation analysis between blood consumption (X) and ovarian production (Y) (mg)

Group	n	r	P <	Linear Regresion equation
Virgin female	21	0,74	0.01	y = 343,7 + 5,71 X
Mated female	21	0,66	0.01	y = 329,8 + 3,50 X

TABLE VI

Fresh blood consumption and efficiency of consumed blood convertion to ovarian production (ECCOP) in virgin and mated females of *Triatoma infestans*

	Virgin female		Mated female		Differences between groups
	n	X ± SD[a]	n	X ± SD	
Blood Consumption	35	324 ± 166	35	350 ± 219	NS
ECCOP	21	2,7 ± 2,9	21	7,2 ± 7,0	p < 0.001

a: SD = Standard deviation.

DISCUSSION

In many insects, mating not only supplies spermatozoids, but it can also modify the female reproductive activity (Huignard, 1974). Present results demonstrate that in *T. infestans,* mating does not interfere in the ovarian follicle development. This is supported by the absence of significant differences in the mean number of oocytes during different phases of oogenesis in virgin as well as in mated females from the 7th to the 21st day after emergence. In both groups we could observe vitellogenic oocytes on the 7th day after emergence, and oocytes in chorion formation on the 13th day after emergence. These data agree with those for the same specie by Asin & Crocco (1990). They found that, females kept with males from their emergence began the vitellogenesis around the 6th day and the chorion formation on about the 11th day. Up to the present time it has not been reported what happens in the virgin female ovary.

A similar pattern of ovarian development, in virgin and mated females during the first cycle of oogenesis, was also discovered in *Rhodnius prolixus* (Pratt & Davey, 1972). These authors support the view that the first cycle of oogenesis is the same for both groups, whereas the second cycle is inhibited in virgin females.

In other insects such as *Diploptera puntacta* (Engelman, 1959) and *Acanthoscelidae obtectus* (Huignard, 1975) mating stimulates the oogenesis. In *T. infestans* the oocyte maturation seems to be independent of this factor.

We found no influence of mating on ovulation, this process started at the 13th day after emergence in both virgin and mated females.

However in *T. infestans* mating stimulated oviposition. The mated females began laying on the 13th day after emergence, while no oviposition was registered in virgin females during the first gonadotrofic cycle.

This allows us to propose that in the virgin female there may be an egg retention in the genital tracks, which is proved by a significantly greater mean number of ovulated eggs in comparison to the mated females. For mated females, ovulation and oviposition were observed while the oocytes were maturing in the ovary.

These results coincide with Regis (1974), who found a stimulating action of mating on egg laying in *T. infestans*. He also observed eggs retained in the virgin female genital tracks. This author pointed out that the ovulation process was not modified by mating, but it was conditioned by the moment at which the first blood feed was offered to the insect.

However, we found that egg retention in the virgin female ovary does not interfere with the continuity of oogenesis, at least during the first 21 days of adult life. Similar results were found by Regis (1979), who observed that egg retention did not modify the oocyte production.

Besides mating, feeding is another factor which affects on the triatomine female reproduction (Costa et al., 1967; Zeledon et al., 1970; Regis, 1979; Brasileiro, 1982, 1984). Our results demonstrate a strong correlation between blood consumption and total ovarian production in virgin as well as in mated females. However the quantity of blood consumed in both experimental groups was similar, which could indicate that mating does not influence the speed at which insects consume blood, at least during the first 21 days after emergence.

The relationship between blood consumption and oogenesis in *T. infestans*, was analyzed by Montenegro (1989). This author concluded that mating increases consumption speed and the consumed blood convertion efficiency to ovaric material. These results indicate that mated females are more efficient in transforming blood consumed to ovaric material. Our differences with Montenegro (1989) with respect to the consumption variable, are probably due to the fact that our observations were carried out during the first cycle of oocyte maturation, while Montenegro (1989) worked with mated females which were subjected to a period of fasting before the experimentation began.

Similar results to ours were found by Coles (1965) in *R. prolixus*, who concluded that in the first cycle of ovarian production, both virgin and mated females digest ingested blood at the same speed and suggested, in terms of the ovary's dry weight, that virgin females retained their eggs while mated ones laid them.

Finally we are able to conclude that during the first cycle of ovarian maturing in *T. infestans*, mating does not interfere with the oogenesis process, or inhibit the beginning of the ovulation, but it exerts a stimulating action on the oviposition.

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