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BEHAVIOR OF TRIATOMINES (HEMIPTERA: REDUVIIDAE) VECTORS OF CHAGAS' DISEASE. III. INFLUENCE OF THE NUMBER OF MATINGS ON THE FECUNDITY AND FERTILITY OF *PANSTRONGYLUS MEGISTUS* (BURM., 1835) IN THE LABORATORY

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A study of the effect of mating in the fecundity and fertility of females of P. megistus *fed on pigeon blood every 14 days, was carried out in the laboratory. Two groups were constituted: I – females which mated only once; II – females which stayed always with the males. Only 56.7% of group I females laid fertile eggs, while as much as 90% of group II females laid fertile eggs. The duration of the fertile oviposition was greater in the females which stayed always with the males. Some females of this group were able to mate up to seven times throughout their life-span. This fact render useless sterile males in the control of these insects.*

It is suggested that the components of pigeon's blood used for feeding the triatomines could have an influence upon the fecundity and fertility of the females of the two groups.

Key words: Chagas' disease – reproduction – triatomines – *Panstrongylus megistus*

There is a number of studies indicating that *Panstrongylus megistus* female bugs mate only once and keep laying fertile eggs throughout their life-span. This phenomenon was first mentioned by Neiva (1910), Pinto (1930) and later by Pessoa & Martins (1982). Lima (1940) showed that female triatomines copulated only once although a second mating of short duration could occasionally occur. According to Borba (1972) "*P. megistus* female bugs after a first copulation do not respond to the male attempts".

Another issue regarding the reproductive behavior of *P. megistus* is that there is very little quantitative information about the influence of the number of matings on the fecundity and fertility.

The possibility of only one copulation in female bugs could be important in the epidemilogy of Chagas' disease. This fact that females remain fertile after a single copulation is of great value for the preservation of the species (Ryckman, 1962). The introduction of sterile males in the population might be effective in reducing its reproductive success. This method is know as "authocidal control" (Moya Borja & Borkovek, 1981), and has been applied successfully to control the fly *Dermatobia hominis*, which is know to perform only one copulation during its adult life.

The objective of this work was to determine the influence of mating on the fecundity and fertility of *P. megistus*. This assay is part of a sequence of works that are being published, about the reproduction of *P. megistus* (Lima et al., 1986 a, b).

MATERIAL AND METHODS

Adult insects emerged from 5th instar nymphs, were provided by the Department of Entomology of the Oswaldo Cruz Institute, after sex-screening according to the technique of Espinola (1966). After imaginal ecdyses, adults were marked individually as proposed by Mac Cord et al. (1983) and the bugs were fed on pigeon blood every 14 days. After being kept for ten days, the number of eggs were registered on standardized protocol sheets.

Each female which copulated only once (n = 30), as well as each couple (n = 60), were placed in 7.5 x 5.5cm jars. The number of matings of each couple was evaluated by the number of spermatophores found in the flasks. Each dead male was immediately replaced by another. Experiments finished with the death of the female bugs or with the arrival of infertile eggs. Mean temperature and air humidity varied up to 28.8 ± 2.7°C and 77.7 ± 5.0%, respectively.

RESULTS

Table I shows the results obtained for the oviposition of female *P. megistus* which copulated only once. The table shows minimum and maximum values, as well as means and standard deviations obtained from the mating data. The last oviposition occurred between 1 and 79 ($\bar{X}$ = 9.8 ± 16.2) days before the

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TABLE I

Oviposition and fertility of eggs of *P. megistus* (n = 30) which copulated only once, fed every 14 days

Identification of the couples	Period of preoviposition (Days)	Total number of eggs/laid	Fertile eggs		Days of observation
			Number	(%)	
113	19	46	34	73.9	44
23	17	161	6	3.7	198
55	14	29	6	20.7	49
211	18	159	97	61.0	218
35	13	91	–	–	85
112	12	274	88	32.1	218
125	19	284	26	9.2	225
32	12	98	–	–	186
111	–	–	–	–	190
124	16	116	–	–	101
115	15	234	–	–	218
131	12	67	–	–	36
122	19	161	–	–	88
31	17	12	–	–	38
42	12	99	–	–	99
242	14	61	19	31.1	98
424	14	118	109	92.4	98
423	17	43	15	34.8	34
333	16	6	2	33.3	23
14	12	18	–	–	33
233	15	17	4	23.5	26
243	14	2	1	50.0	18
3	19	86	52	39.5	170
25	11	124	58	46.8	170
41	14	5	–	–	14
52	15	45	24	53.3	26
112	17	18	12	66.7	30
133	15	4	–	–	24
143	15	183	150	81.9	170
45	16	32	–	–	25
Total	–	2561	703	27.5	–
$\bar{X}$ (s)*	14.6 ± 3.6	86.4 ± 80.0	–	–	98.4 ± 74.7

*Mean and standard deviation.

death of the females or the end of the assays, while the last fertile oviposition occurred between 1 and 98 ($\bar{X}$ = 29.4 ± 43.9) days before the death of the females or the end of the assays.

Eggs of unusual aspect and/or color were observed during the present investigation. They were infertile eggs, laid between the 17th and the 27th day ($\bar{X}$ = 20.7 ± 4.5) before the death of the females or the end of the assays. The maximum daily oviposition rate varied from 0 to 20 ($\bar{X}$ = 7.5 ± 4.7) eggs/female/day. After copulation female bugs were observed between 6 and 218 days, the oviposition having occurred between 4 and 205 days and the fertility of eggs between 14 and 135 days.

Some females laid a large amount of eggs with a high fertility rate (females number 143, 211 and 424) but thirteen of the thirty females tested (43.4%) laid infertile eggs, although copulation had occurred, due to extrusion of the spermatophore.

Table II shows the result of oviposition and fertility of eggs of female bugs that remained permanently mated. The minimum and maximum numbers were: from 1 to 7 spermatophores ($\bar{X}$ = 2.6 ± 1.6). The number of eggs per female varied from 1 to 18 eggs per female per day. The last fertile oviposition, before the death of the females or the end of the test, occurred between 1 and 101 days. Twelve of the thirty females laid infertile eggs, which appeared between 9 and 58 days before the death of the females or the end of the experiments. The thirty couples were observed between 11 and 148 days.

Four female bugs presented discrepancies in the pattern of oviposition as compared with the other 26, whose behavior was normal:

Female no. 5 – Presented the longest period of preovipositon (44 days). Released the greatest number of spermatophores (seven), six of these released in the preoviposition period. In 121 days of observation, laid 71 eggs, with a low fertility rate (46.5%).

Female no. 21 – Was observed for 121 days,

TABLE II

Oviposition and fertility of eggs from females of *P. megistus* (n =30) permanently mated, fed every 14 days

Identification of the couples	Period of preoviposition (days)	Total Number of eggs laid	Fertile eggs		Days of observation
			Number	(%)	
422	13	192	162	84.4	138
413	14	181	86	47.5	138
414	16	4	–	–	20
415	27	53	21	39.6	59
421	17	288	240	83.3	138
155	17	210	203	96.7	145
24	12	143	113	79.0	127
14	25	76	60	78.9	148
231	14	83	50	60.2	127
23	16	24	6	25.0	148
142	25	19	13	72.6	88
143	17	45	12	26.7	105
241	12	293	280	95.6	148
222	14	12	10	83.3	33
243	–	–	–	–	109
223	15	16	15	93.8	11
122	14	12	10	83.3	12
245	19	50	4	8.0	148
151	11	81	50	61.7	105
2	15	64	40	62.5	82
3	15	21	7	33.3	62
4	14	38	11	28.9	119
5	44	71	33	46.5	121
11	17	215	176	80.8	118
12	16	95	85	89.5	82
13	16	12	7	58.3	96
14	18	78	28	35.8	148
15	18	121	97	80.2	113
21	27	2	–	–	112
22	23	20	2	10.0	148
Total	–	2529	1821	72.3	–
$\bar{X}$ (s)*	17.4 ±7.2	83.8 ±84.2	–	–	104.9 ±42.2

* Mean and standard deviation.

releasing in this period three spermatophores and only two eggs, both infertile.

Female no. 22 – Was observed for 148 days, releasing three spermatophores and 20 eggs, of which only 2 were fertile.

Female no. 24 – In 109 days of observation, it released two spermatophores and did not lay eggs.

When the couples were placed in a large jar, at the time of feeding, the mere joining of a great number of males and females together stimulated them to copulate, even with 14 days of food deprivation. This way it was possible to see that some females were able to copulate throughout its whole life-span, showing that age does not seem to influence this aspect. In several couples, the spermatophore apeared in the flask after 100 days of observation. It was also observed that, when a mating occurred, in the feeding jar, some male bugs approached and made strong attempts to copulate with the couple or even with other males.

Comparing Table I with Table II, it can be seen that females that remained with the males had a significant chance of fertilization, since 90% were able to lay fertile eggs, while only 56.7% of those which copulated only once layed fertile eggs. The length of fertile oviposition was also greater in the females which remained with the males. In relation to the other items of the two tables, there was a similarity in the two results, except for the number of spermatophores released per female, since those which copulated only once released only one spermatophore.

DISCUSSION AND CONCLUSION

The present results are in contrast with those of Pinto, 1930; Borba, 1972; Pessoa & Martins, 1982, who found that *P. megistus* copulates only once, and after the first mating, it does not accept the male attempts to copulate (Borba, 1972). Galliard (1936) concluded that one single mating was enough to keep *Rhodnius prolixus* eggs fertile throughout the whole female's life-span.

Observations carried out in this experiment,

have found between 1 and 7 spermatophores per female ($\bar{\bar{X}}$ = 2.6 ± 1.6 copulation/couple) indicating that each female can copulate at least up to seven times. This number is much lower than the value found for *Triatoma brasiliensis*, whose pairs copulate an average of $\bar{X}$ = 23.5 ± 5.48 times throughout its life-span. (Brasileiro, 1982).

The females which copulated only once laid the same amount of eggs as those who performed several copulations, although the fertility of the eggs was lower, some of them even infertile. This may be due to the long period of endogamy to which the insects were submitted. Perlowagora-Szumlewicz (1976), working with eight species of triatomines, among which *P. megistus, T. brasiliensis* and *Rhodnius neglectus,* with the first generation in the laboratory, found the fertility of eggs from females which mated once or twice to be the same as those females permanently mated. According to that author, there is a greather chance of the female being fertilized after several rather than after one single mating. In *R. neglectus* and in *T. brasiliensis*, females which mated only once and were regularly fed also had a fertility rate lower than the females which mated several times (Costa et al., 1967; Brasileiro, 1982, respectively). In *R. neglectus*, the authors found that females which mated only once laid fertile eggs until eight or nine months after copulation, while those with multiple matings became fertile throughout the whole life-span (Costa et al., 1967). In *T. brasiliensis*, only 53.2% of the females laid fertile eggs and the hatchings occurred almost always in the first third of the female's life-span (Brasileiro, 1982).

The present results also show that some females lay fertile eggs for many months, after one single copulation. These data are important for the epidemiology of Chagas' disease because they indicate that the female, once mated, can keep the eggs fertile for a long period, leaving new descendents and increasing the chance of forming new colonies of triatomines and thus, the spread of the insect.

The couples fed every 14 days and kept together had a preoviposition period of $\bar{X}$ = 17.4 ± 7.2 days. The values have similar averages as found by Perlowagora-Szumlewicz (1976), for *P. megistus* which was of $\bar{X}$ = 16.8 ± 22.2 days of preoviposition, but the amount of eggs was lower. This may also be due to the long period of endogamy to which the triatomines of the present study were submitted. According to Heitzmann-Fontenelle (1980/1981), forced endogamy triggers a delay in the development of *P. megistus* with a lower duration of the nymphal stages, as well as a decrease in the female adult phase, in the number of ovipositions, in the number of eggs per oviposition and in the percentage of viable eggs. The present results also suggest that the 14-day interval for feeding may have influenced the ovipositon and fertility of eggs. This interval was based on the one used by Zeledón et al. (1970), who fed *Triatoma dimidiata* every two weeks, for the same kind of observation. Shorter intervals may increase ovipositon, thus resulting in a higher fertility rate.

In the results of this work, the mortality was slightly higher than expected. As discussed above, prolonged endogamy may have been responsible for these alterations. However, as some of the dead insects, specially males presented some changes such as strong abdominal swelling, even several days after feeding and with the genitalia completely extruded, the question was raised (Garcia, personal communication) that this phenomenon could be related to the poultry blood which served as a meal for the triatomines. It is possible that a shortage of hemoglobin (Sherlock, 1979), vitamins or proteins in the pigeon diet could influence the insect's spermatogenesis, since several females released spermatophores and did not lay fertile eggs although some of them became fertile later. According to Meickle & Farlane (1964), a vitamin E deficient diet in the cricket *Achaeta domesticus* impairs the function of spermatozoids in males, and the females which mated under those conditions had a delayed oviposition. Garcia & Azambuja (1985) found that protein deficiency in *R. prolixus* may inhibit oogenesis, which depends on the relationship between the ingestion of proteins and the function of the *corpus allatum*. Yet they say oogenesis is not controled by rising the hormone levels in response to abdominal swelling and that, in nature the insects obtain proteins from the blood of vertebrates.

From the results obtained, one may conclude: 1) permanent couples, periodically fed, may copulate several times and the number of copulations seems to be important to keep the female fertile. Females which mated only once had a low fertility rate; 2) one single mating may not be enough to keep the *P. megistus* female fertile. The female of *P. megistus* may, after the first mating, accept other males once or several times throughout its life; 3) the utilization of sterile males in the biological control of *P. megistus* is not recommended. This method would only be useful if the female did not accept other males after the first mating.

RESUMO

Estudou-se, em laboratório, a influência do acasalamento na fecundidade e fertilidade de

fêmeas de *P. megistus*, alimentadas a cada 14 dias. Foram formados dois grupos: I – fêmeas que copularam uma única vez; II – fêmeas que permaneceram sempre com os machos. Das fêmeas do grupo I, apenas 56,7% puseram ovos férteis, enquanto que 90% das fêmeas do grupo II tiveram ovos férteis. A duração da ovipostura fértil foi também maior nas fêmeas do grupo II. Destas últimas, algumas foram capazes de copular até sete vezes ao longo de sua vida, o que inviabiliza o uso de machos estéreis no controle desses insetos.

Os autores discutem também a influência na fecundidade e fertilidade das fêmeas dos dois grupos e na longevidade dos insetos, de componentes no sangue dos pombos que serviram de repasto aos triatomíneos.

Palavras-chave: doença de Chagas – reprodução – triatomíneos – *Panstrongylus megistus*

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