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COPULATION AND EGG-PRODUCTION IN *RHODNIUS PROLIXUS*: THE ROLE OF THE SPERMATHECAE

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INTRODUCTION

In *Rhodnius*, as in many other insects, virgin females lay eggs very much more slowly than do mated females (Buxton, 1930; Khalifa, 1950). Nutrition is also important; even a mated female will lay no eggs if she is not fed (Buxton, 1930). The present work ignores the effects of nutrition in that all of the females used in the experiments were allowed to feed to repletion at regular intervals. This paper presents results which suggest that the primary stimulus for egg production resulting from mating emanates from the spermathecae.

MATERIALS AND METHODS

The insects used in this experiment were maintained at 28° C. in the laboratory and all of the experimental animals were fed at intervals of about 10 days. In those experiments which involved mating, the experimental females were placed individually with two recently fed males and checked every 20 min. to see whether mating was taking place. After mating had occurred the males were removed and 24 hr. later the container was examined for the spermatophore which is shed by the female if the mating is successful.

The eggs of individual females were counted daily. For each group of females subjected to a particular treatment the average cumulative number of eggs per female was plotted against the time in days. This procedure yielded a graph in which periods of egg-laying alternated with periods of relative inactivity, the latter usually occurring immediately after a meal. During the periods of activity the rate of oviposition was approximately linear; straight lines were fitted by inspection. Typical graphs appear in Fig. 1. The results of the experiments can, therefore, be expressed as the slopes of the lines during the period of activity, giving the average number of eggs laid per female per day. Only the data for the first three cycles of oviposition are considered in this paper.

The surgical manipulations are quite straightforward, and were carried out on unanaesthetized animals fastened down by means of plasticine. The various organs were approached through incisions in the ventral abdomen which were sealed up with a wax of low melting-point. In those experiments involving transplantations, it was necessary to swab the abdomen of the donor thoroughly with 70% alcohol. The transplanted organs were not passed through a Ringer's solution. The degree of mortality varied with the operation; for the experiments under consideration here it was never more than 20%.

EXPERIMENTAL RESULTS

Virgin females. Unmated females fed 21 days after emergence began to lay eggs 2 days later. Oviposition has continued for 140 days at the time of writing and is still in progress. Table 1, treatment 1, gives the slopes of the lines for the first three cycles of oviposition. It will be seen that an unmated female lays an average of about 2·5 eggs per day.

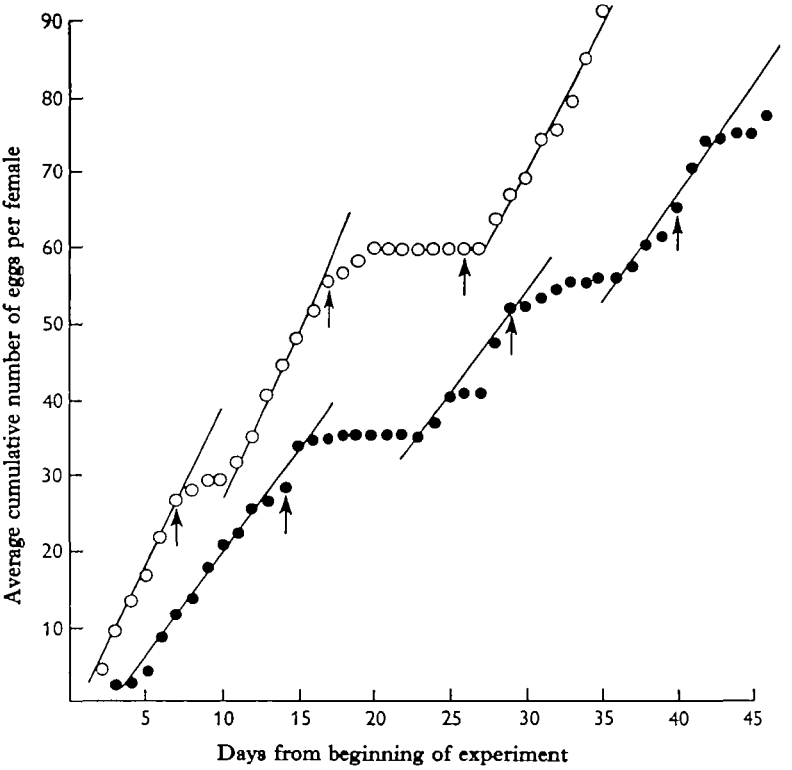

Fig. 1. A graph showing the time-course of the average cumulative number of eggs laid by virgin (solid circles) and mated (open circles) females. The arrows indicate feedings.

Table 1. *The average rate of egg production for each of the first three cycles of oviposition in females subjected to various treatments*

Treatment	No. of females	No. of eggs per female per day		
		Cycle I	Cycle II	Cycle III
1. Unmated	7	2·6	2·4	2·5
2. Normal mating	8	4·4	4·2	4·0
3. Males lacking seminal vesicles	8	1·4	1·6	2·0
4. Males lacking opaque accessory glands	7	2·0	2·6	3·0
5. Virgin females—no spermathecae	4	2·1	2·3	3·8
6. Mated females—no spermathecae	4	3·2	3·3	2·5
7. Unmated females receiving virgin spermathecae	6	2·8	2·4	2·4
8. Unmated females receiving mated spermathecae	5	4·7	3·0	4·4
9. Unmated females receiving seminal vesicles	5	2·7	2·8	2·3
10. Unmated females receiving transparent accessory glands	7	2·6	2·5	2·8

Oviposition in mated females. The females involved in this experiment were mated 4 weeks after emergence and began to oviposit on the second day after mating. They laid eggs very much more rapidly than did the unmated females (Fig. 1 and Table 1, treatment 2), the rate being about 4·3 eggs per female per day. These females began to die comparatively early after mating. One female of the eight involved died 22 days after mating, and the experiment was discontinued 35 days after mating when two others died.

Females mated with males lacking their seminal vesicles. The females involved in this experiment were from the same emergence that provided the females for the previous experiment. They were mated 4 weeks after emergence to males which had been deprived of their seminal vesicles. This procedure results in the production of an otherwise normal spermatophore into which spermatozoa are not incorporated. As a consequence, no spermatozoa reach the spermathecae. This experiment was discontinued after 45 days by which time three of the eight females involved had died. From Table 1, treatment 3, it is apparent that these females laid eggs at the rate of about 1·6 eggs per female per day which is less than that determined for unmated females.

Females mated with males lacking their opaque accessory glands. When males which have had their opaque accessory glands removed mate with normal females, a spermatophore containing spermatozoa is produced, but the spermatozoa are not taken into the spermathecae (Davey, 1958). Eight females were successfully mated to males which had been deprived of their opaque accessory glands. Their oviposition was followed for 45 days, when they were dissected. One female was found to have spermatozoa in one spermatheca. Five of the eggs laid by this female hatched, and the number of eggs laid was consistently higher than for any of the other females in the experiment. The figures presented in Table 1, treatment 4, do not, therefore, include the data for this female. It will be seen that the rate of oviposition for the remaining females was about 2·5 eggs per female per day, which is characteristic of virgin females.

Females lacking their spermathecae. The evidence thus far indicates that a mated female will not lay eggs rapidly unless there are spermatozoa present in the spermathecae. In order to test this hypothesis further the egg production of mated and unmated females which had been deprived of their spermathecae was examined. This operation, impinging as it does on the very ducts through which the eggs pass to the outside, resulted in many anomalies. While mortality was not particularly high, some of the females laid no eggs at all, although the body was distended with fully developed eggs. In a few cases, dissection of such females showed that the ovaries had ruptured, releasing eggs into the body cavity. Other females laid only a few black eggs. As a result of these abnormalities, only four mated and four unmated females are included in the data for this experiment.

From treatments 5 and 6 in Table 1 it is clear that, perhaps because of the small numbers involved, the rate of oviposition is more variable in this experiment. Nevertheless, for females lacking their spermathecae, the rate of oviposition is no greater among mated individuals than among virgins. Furthermore, the rate of oviposition among these females, at a level of about 2·8 eggs per female per day, is near that for intact, unmated females.

The effects of implanting spermathecae. While it would be useful to study the effects

of mating on females with denervated spermathecae, this operation has thus far proved to be too difficult. As an alternative, the possibility of a blood-borne factor being involved was investigated by implanting spermathecae from virgin or recently mated females into recently moulted virgins. Five females receiving mated spermathecae and six receiving virgin spermathecae survived to the end of the experiment, which ran for 120 days. From Table 1, treatments 7 and 8, it is shown that the rate of oviposition of females receiving virgin spermathecae is about 2·5. The rate of oviposition of females receiving mated spermathecae, on the other hand, is rather variable, but considerably higher. At an average level of 4·0 for the first three cycles, it is more nearly characteristic of the rate for mated females. Since these results represent an important finding, the slopes of the first eight cycles were determined in each case. When these additional data are considered, the average rate of oviposition of females receiving mated spermathecae is $4{\cdot}4 \pm 0{\cdot}9$ S.D. For females receiving virgin spermathecae it is $2{\cdot}5 \pm 0{\cdot}3$ S.D.

The effects of implanting seminal vesicles. In order to determine whether the effects observed with implanted spermathecae were simply due to spermatozoa, one seminal vesicle from an unmated male was implanted into each of several virgin females. This represented perhaps a hundred times the amount of spermatozoa contained in the spermathecae of a female. As a control for this experiment one transparent accessory gland from a similar male was implanted into each control female. This was taken to represent an amount of protein roughly equal to that in the seminal vesicles. As treatments 9 and 10 in the table demonstrate, neither implant raised the rate of oviposition above the level characteristic of virgin females.

DISCUSSION

The results presented here suggest that a blood-borne factor from spermathecae containing spermatozoa increases the rate of oviposition in *Rhodnius*. This tentative conclusion is, of course, based on the results of the experiments involving the implanting of mated spermathecae. The fact that implanting spermathecae from virgin females does not increase the rate of oviposition demonstrates that the surgical manipulation alone is not responsible for the effect. The factor apparently does not emanate from the spermatozoa, since implanting massive quantities of spermatozoa has no effect on the rate of egg production. On the other hand, it may be agreed that the spermatozoa in the spermathecae are physiologically different from those in the seminal vesicles, and that once in the spermathecae, the spermatozoa secrete the necessary factor. At any rate, it is clear that the spermathecae and the spermatozoa contained in them co-operate to produce the postulated substance. These results are supported by the experiments involving males which had been operated in two ways, both of which resulted in matings which were normal except for the fact that spermatozoa did not reach the spermathecae. In these cases the increase in egg production which follows normal matings did not occur. Again, females lacking their spermathecae received completely normal spermatophores, yet the expected increase in egg production did not occur. It has not been possible to design an experiment which specifically eliminates a nervous control of oviposition by the spermathecae. All that can be said is that the explanation of the results thus far does not require such a hypothesis. Another possibility which must be mentioned is that mated spermathecae remove

from the blood some factor which inhibits egg production. While this is held to be unlikely on *a priori* grounds, the results of the present experiments do not eliminate the possibility. The inhibiting factor might be a metabolite which is required by the fertilized spermathecae. Certainly the cells which secrete into the lumen of the spermathecae become more active in mated females (Davey & Webster, 1964, unpublished data).

In the house cricket, *Acheta domesticalis*, a dietary deficiency in vitamin E prevents the function of spermatozoa in the males, which are otherwise normal. Preliminary experiments showed that when such males are placed with normal females, oviposition is greatly delayed (Mickle & McFarlane, 1964). While these early experiments are inconclusive, there is a possible parallel with the present work.

Vitellogenesis in *Rhodnius*, as in other insects, is under the control of the corpus allatum (Wigglesworth, 1936). In those insects in which the relationship between various stimuli and egg production has been carefully examined, the stimulus appears to operate through the corpus allatum. Thus, in *Leucophea*, mating involves the abolition of the inhibition of the corpus allatum by the central nervous system (Engelmann, 1960). In *Schistocerca*, the presence of males brings about precocious maturation of females accompanied by enlargement of the corpus allatum (Highnam, 1962). In many insects, the corpus allatum itself appears to be under the control of the neurosecretory system in the brain (references in Highnam, 1964). In *Rhodnius*, it is thought to be unlikely that the spermathecae would exercise any direct control over the ovaries. Presumably the factor from the spermathecae intervenes somewhere in the endocrine chain which controls vitellogenesis. Studies are now under way to determine which links in the chain are involved.

SUMMARY

1. Mated females of *Rhodnius prolixus* lay eggs at approximately twice the rate of unmated females.

2. Males which have had their seminal vesicles or opaque accessory glands removed produce spermatophores at mating, but no spermatozoa appear in the spermathecae of the females. The females involved in such matings do not exhibit an increase in egg-production.

3. When females lacking their spermathecae are mated to normal males, the increase in egg production which follows normal matings does not materialize.

4. Implanting spermathecae from mated females into virgin females increases the egg-production of the host. Implanting spermathecae from virgin females or seminal vesicles or transparent accessory glands from males has no effect on the egg-production of the donor.

5. It is tentatively concluded that a blood-borne factor from the spermathecae containing spermatozoa is the primary stimulus to increased egg production in a mated female.

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