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Fecundity of the blood-feeding insect *Rhodnius prolixus* increases in successive periods of egg production

R. G. Chiang and J. A. Chiang

Natural Science and Mathematics Division, Redeemer College, Ancaster, Ontario L9K 1J4 (Canada)
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Abstract. The fecundity of the blood-feeding insect, *Rhodnius prolixus*, was observed to increase in successive periods of egg production, each period being triggered by a single large blood meal. As previously published, the fecundity of mated animals was significantly higher than that of unmated animals for the first period of egg production. For a second period of egg production, fecundity increased significantly in both mated and unmated animals. By the fourth period, fecundity had returned to first-feed values for mated animals, but remained high for unmated animals, and the fecundity of mated and unmated animals was not significantly different. Thus, during successive periods of egg production, the processes which maintain fecundity of unmated animals below that of mated animals are overcome.
Key words. Insect; fecundity; egg production; E value; *Rhodnius prolixus*.

The fecundity of *Rhodnius prolixus* has been well documented with respect to the nutritional status[1,2] and the mated state of the female[3]. Earlier work has shown that egg production is almost absent in unfed females[4,5], whereas females which receive a blood meal produce a 'full complement' of eggs. In addition to the requirement for food, a distinct difference is observed between the fecundity of an unmated female and a mated female if both animals are of comparable size and have imbibed similar quantities of blood. This information has been reported in literature as the 'E value', which expresses fecundity as the number of eggs made in relation to the initial weight of the animal and the size of its blood meal[6,7]. Fed unmated females have been shown to have E values ranging from 0.60 to 1.10, whereas mated females had E values ranging from 1.58 to 2.20[7]. Fecundity has been explored extensively for animals which have been fed once. However, the fecundity of animals fed at regular intervals over a longer period of time has not been examined to the same extent[8,9]. The intent of the present study was to gain insight into the mechanisms underlying the production of eggs by documenting the E values of mated and unmated females during successive periods of egg production. Interestingly, by the fourth period of egg production, the fecundity of unmated animals had increased to the level found in mated animals.

Materials and methods

Insects used in this study were reared as part of a colony which was maintained in a dark, humid incubator at a temperature of 28 °C. Day 8–10, post-emergent adult females were randomly selected for mating and placed in individual containers with a mature, recently fed, male. Mating was considered to have occurred if, within one to two days, spermatophore casings were present in the container. Mating was later confirmed by the development of fertile eggs.

On day 11 post-emergence the dorsal abdominal surface of mated and unmated females was marked for identification purposes with a felt-tip pen. These animals were individually weighed, and placed in a communal jar for feeding. All animals were fed on rabbit blood. Once fed, the females were re-weighed and their new weights recorded. To permit the monitoring of egg-laying, each female was then placed in an individual container containing a piece of filter paper on which the eggs were laid. The number of eggs laid were recorded on a daily basis. On day 23 postfeeding the animals were weighed, fed to repletion, re-weighed and isolated once more to permit us to monitor egg-laying for a further 23 days. Animals were maintained for a total of 3 or 4 feeds, spanning a time period of 4.5 months. This procedure was repeated 6 times (i.e. 6 separate runs) and for each run 10–15 mated and 10–15 unmated animals were monitored. Some mated animals were killed after the third period of egg production, so there was a smaller number of mated animals in the fourth period of egg production. In total, the oviposition of approximately 100 animals was recorded over four successive periods of egg production.

We observed previously that by day 23 postfeeding, mated animals have laid all their eggs, whereas unmated animals usually have 1 to 4 eggs remaining in their ovaries. In these earlier studies, animals were killed to estimate the number of eggs produced, 21 days after a single blood meal. Since the animals could not be killed

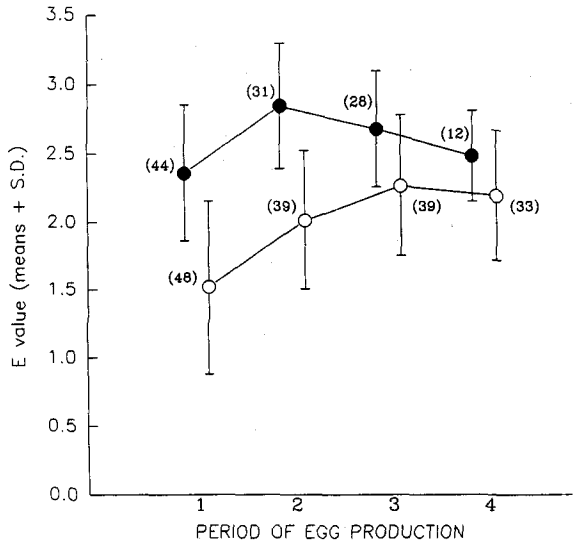

Figure 1. Changes in the fecundity (E value) of mated (closed circles) and unmated (open circles) *Rhodnius prolixus* during successive periods of egg production. E values were calculated by dividing the number of eggs produced by day 26 postfeeding by the product of the initial weight (in mg) and the weight of the blood meal (in mg) and multiplying by 1000. Each period of egg production was initiated by a single large blood meal, and extended over approximately 3 weeks. E values are expressed as means ± SD, and sample sizes are in parenthesis.

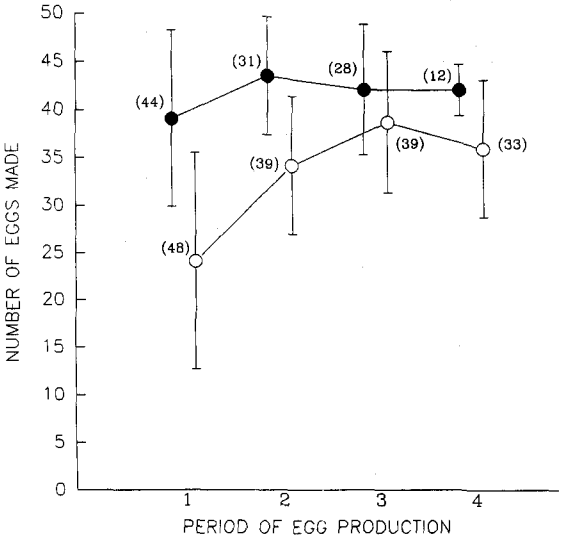

Figure 2. Changes in the number of eggs produced by mated (closed circles) and unmated (open circles) *Rhodnius prolixus* during successive periods of egg production. Number of eggs is expressed as means ± SD, and sample sizes are in parenthesis.

in the present study until three weeks after the fourth feed, an alternative approach was employed to determine the total number of eggs produced after a blood meal. It has been noted that feeding is a stimulus for egg-laying, such that chorionated eggs present in the ovary will be laid within 1 to 2 days after ingestion of a blood meal[10]. Furthermore, there exists a lag time between the ingestion of the blood meal and the appearance of the first chorionated egg. This lag time is approximately 3 days[11]. To ensure that all eggs produced during a given period of egg production were counted, we also included any eggs that were laid within 3 days following the feed that initiated the next period of egg production.

For the first 3 feeds, E values were calculated by dividing the total number of eggs laid by day 26 postfeeding by the product of the initial weight and the weight of the blood meal and multiplying by 1000 (refs 6, 7). On the fourth and final feed, animals were killed on day 23 postfeeding, and the number of eggs noted. Use of the E value parameter provides the basis for comparing fecundity between the runs and also for comparison with previously published work. Statistical analysis was carried out using Student's *t*-test.

Results and discussion

Three weeks after their first blood meal, 44 mated animals laid an average of 39.05 ± 9.20 eggs, whereas 48 unmated animals laid an average of 24.01 ± 11.41 eggs. This greater fecundity of mated animals is typical for *Rhodnius*. In earlier studies depicting this variability, animals were killed after the period of egg production to ensure that any mature eggs remaining in the ovary were counted. As noted above, we accounted for the possibility of mature eggs remaining in the ovary on day 23 postfeeding by including eggs laid within three days following the blood meal initiating the next period of egg production. Of the 71 mated animals monitored after their second, third and fourth feeds only 6 (8%) laid eggs within 3 days of feeding. Of the 107 virgin animals monitored similarly, 28 (26%) laid eggs within 3 days of feeding. This result is in keeping with our previous observation that, by day 21 postfeeding, a greater number of unmated animals than mated animals have eggs in their ovaries.

To allow for a more direct comparison with previously published results, the E values were calculated. Following a single blood meal, mated animal E values were 2.36 ± 0.50 and unmated animal E values were 1.49 ± 0.65, reflecting the significantly lower number of eggs produced by unmated animals (Student's *t*-test, $p < 1\%$). These mean E values are notably higher than those reported previously[7], and may reflect a difference in the environment of the insects, or in the rabbits used to supply blood. All previously published E values were calculated using animals raised and fed on rabbits at York University; the E values of the present study were based on insects raised and fed on rabbits at Redeemer College. Preliminary studies using the same strain of insect suggest that blood from different rabbits can lead to the production of different maximum numbers of eggs after a blood meal. Other studies have found that

variations in the ingested food can affect fecundity in a number of insects (houseflies[12], mosquitoes[13], *Rhodnius*[1,14,15]).

Following the second feed, the fecundity of unmated and mated animals increased, as reflected in the increase in both the mean number of eggs laid, and the mean E values of both groups. This increase was greater for the unmated animals compared to the mated animals (see figs 1 and 2). Upon being fed to repletion a third time, the unmated animals showed a further increase in their fecundity, whereas the fecundity of mated animals returned to a level close to that following the first feed. Following the third period of egg production there was no significant difference between the number of eggs produced by mated and unmated animals (Student's *t*-test). A fourth feed did not elicit any further increase in fecundity in either group of animals, and for this period of egg production, there was no significant difference between the E values of mated and unmated animals. There is therefore an increase in the fecundity of unmated animals as a result of multiple feeds, so that the ability of the unmated to convert ingested food into eggs is no longer statistically different from that of the mated animal.

The increase in the fecundity of the unmated animal with successive periods of egg production provides an interesting insight into the control of egg production in these animals. It has been previously shown[11] that unmated and mated animals, following a single blood meal, will produce eggs at the same rate, but the unmated animal will stop egg production after approximately 7–8 days, whereas the mated animal will continue to produce eggs for at least 14 days. As a result, the mated animal produces more eggs than the unmated animal. It has been suggested that the lower egg production of unmated animals might result from the inhibition of the release of juvenile hormone (JH) from the corpora allata (CA) of these animals[6], coupled with the release of an antigonadotropin from the abdominal neurosecretory organs[3]. This antigonadotropin would prevent any JH that might be present in the hemolymph from causing the eggs to mature. Conversely, the continued production of eggs in the mated animals may result from a steady supply of JH from the CA, achieved by the removal of a factor from the brain that inhibits the CA[16], combined with the absence of an antigonadotropin. The inhibitory factor from the brain could be rendered inoperative by a spermathecal factor released by the spermatheca when sperm are present within it[8].

Our finding that feeding alone can increase the fecundity of unmated animals suggests some modifications of the preceeding working hypothesis. If the spermathecal factor is responsible for the increased ability to produce eggs, then multiple feeding reduces the need for the spermathecal factor, and/or takes the place of such a factor and is able to remove the inhibition of the CA by the brain. No sperm are present in unmated animals. The ability to increase fecundity without the presence of sperm in the spermatheca follows a logical path, if one assumes that in unmated animals the conservation of nutrients would be advantageous until mating and fertilization have taken place. In the presence of adequate nutrients, there would be little need to conserve nutrients, and hence, limit egg production.

A hypothesis to explain the present findings can be constructed by separating the increase in fecundity as a result of feeding (i.e. fed versus unfed animals) from the increase in fecundity as a result of mating (i.e. unmated versus mated animals). We will assume that either act (feeding or mating) causes activity of the CA leading to the release of JH. It is possible, therefore, that a mated animal, in its first feed, has experienced two bouts of CA activity; the first at mating, and the second at feeding. On the other hand, the unmated animal after its first feed has experienced only one bout of CA activation. At its second feed, the unmated animal experiences its second bout of CA activity, and as a result, coupled with the animal's previous exposure to JH, more eggs are produced. This second bout of CA activity might result in the greater production of JH and/or an increase in the sensitivity of JH for those tissues involved in egg production. Interestingly, Davey and Singleton[5] did observe that a greater number of eggs was produced by mated unfed animals compared to unmated unfed animals, which is in keeping with the notion that mating alone could result in activation of the CA. In addition, the levels of JH have been seen to increase in the females of various insect species after copulation[17–19]. Nevertheless, the mating stimulus and the feeding stimulus for egg production are inherently different, since at least three feeds are needed for unmated animals, as a population, to reach a level of fecundity similar to that of mated animals.

Although the present findings cannot ascertain the exact mechanisms by which egg production is controlled in *Rhodnius*, they do provide some additional information that can be used to evaluate our current understanding of this matter. Clearly, the difference between unmated and mated animals is reduced by successive periods of egg production. This increased fecundity may result from a reduction in the inhibition of the CA by the brain, and/or increased activation of the CA by another mechanism, and/or the activation of tissues involved in the egg production process. Whatever is the precise means by which this occurs, the present findings illustrate the need to consider that unmated and mated animals fed a second time do not behave like those given a single blood meal. Indeed, if the inhibition of the CA in unmated animals during the first feed were maintained, it might be expected that during successive periods of egg production unmated animals would show

a marked reduction in fecundity compared to mated animals. Since this does not occur, studies should now be undertaken to determine whether active inhibition of the CA, or the absence of a factor that stimulates the CA, is the mechanism responsible for reduced fecundity in unmated animals during their first period of egg production.

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