LIST OF PUBLICATIONS

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Abstracts

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Primary stimulus for oviposition and egg production in the house cricket *Gryllodes sigillatus* (Walker)

By N B Itgi, V K Biradar and S B Mathad

**Abstract**

One-day-old adult crickets of *Gryllodes sigillatus* were maintained in egg incubators at 30 ± 1 °C, 12 L:12 D photoperiod and at 80 ± 5 % RH conditions to investigate the primary stimulus on oviposition and egg production. Mated females produced 5-6 times (2448 eggs/female) more number of eggs than virgins (513). The mere physical insemination of spermatophore, male pheromonal activity and male accessory gland secretions did not stimulate oviposition and egg production. Multiple matings by multiple males did not increase or decrease the egg laying, egg production and fertility among the mated females. And it seemed to be that the egg production was not correlated with the amount of sperms present in the spermathecae. The stimulus was achieved only when the sperms were present in the spermathecae and after releasing them in the female reproductive tract. Further, the consecutive matings were essential for the additional stimulus. The spermathecal gland plays a significant role in the reproduction of the cricket *G. sigillatus*.

1 Introduction

Investigations were made on mating effects and its influence role on oocyte maturation, oviposition and egg production in different species of insects (Engelmann 1970). It provided a stimulus for increased oviposition in *Rhodnius prolixus* (Coles 1965, Davey 1967), *Tenebrio molitor* (Mordue 1965), *Aedes aegypti* (Leahy and Craig 1965) and *Pleurotettix guttiventris* (Bentur and Mathad 1974). According to Yamoaka and Hirao (1976) in *Bombyx mori* a certain ovipositoinal stimulating substance was existed in male reproductive tract and effective only after transferring to female. Whereas in *A. aegypti, Drosophila melanogaster* and *Culex pipiens* (Leahy 1967), *Melanoplus sanguinipes* (Pickford et al., 1969) and *Schistocerca gregaria* (Leahy 1973) observed that the accessory gland fluid enhanced the rate of oviposition. But in *Cimex lectularius* egg maturation was stimulated only after spermatozoa reaching the corpora semiinals (Davis 1965). Therefore, the present study has been planned to investigate the primary stimulus for oviposition and egg production in the cricket *G. sigillatus*.

2 Materials and methods

2.1 General

The house cricket of *G. sigillatus* was reared in our laboratory and rearing techniques were same after Mathad and Dakhshavani (1972) for the cricket *P. guttiventris* with some modifications. For these experiments male and female crickets were selected from the stock culture and were unmated.
prior to experiments. Experimental insect jars provided concentrated poultry feed, folded filter paper for space and shelter and coarse wet sand in 5 ml cups as water source and oviposition site were maintained in the Bell electric egg incubator at 30 ± 1°C, 12 L:12 D photoperiod and 80 ± 5 % RH conditions. The eggs laid by a female every day were recorded and the females were dissected at the termination of the experiment to note the number of eggs retained. The egg production refers to the number of eggs oviposited and those retained in the oviduct. Fecundity was expressed as the number of eggs oviposited and those retained in the oviduct. Fecundity was expressed as the number of eggs oviposited and those retained in the oviduct. Comparison of means was calculated by 't' test.

2.2 Effect of different stimuli

46 female crickets were selected on the day of emergence in to adult and constituted into A, Virgin females, B, Females, mere insemination of spermatoocyte, C, Females, only the presence of male, D, Females, mated with testecotomised males and E, Females, male accessory gland secretion recipients. In the treatment B, same aged male crickets were allowed to mate in a normal condition at every day and after mating spermatoocyte were removed immediately from the bursa copulatrix. In C, male and female crickets were separated by making a partition in the experimental jars by steelmesh which permit the auditory, olfactory, tactile and pheromonal communication and prevented mating. In the treatment D, males were testecotomised on the day of their imaginal moulting into adult and were allowed to mate from 6th day onwards. In E, accessory glands were removed from 20 male crickets of G. sigillatus after 15 days of their emergence and homogenized for 15 minutes by tissue homogenizer in 5 ml of insect ringer's solution. The homogenized fluid (0.02 ml) was injected by microsyringe to virgin female crickets in haemocoel of the abdominal region. The experiment was conducted for 60 days.

2.3 An adequate supply of sperms by multiple males

18 females and 35 males crickets were selected from the stock culture which were emerged on the same day and grouped into A, one female with one male, B, one female with two males and C, one female with three males. Spermatoocytes were collected every day from the experimental jars and mating rates were assessed by per week. Experiment was terminated when the insects mortality reached more than 50 %.

2.4 Role of spermathecae and sperm

One-day-old male and female crickets were selected from the stock culture and constituted into A, normal female and male (controls), B, spermathecae deprived females, spermathecae were removed on the day of their emergence and allowed males to mate, C, spermathecae deprived females after shedding of spermatoocyte, spermathecae were removed, D, one-week-old normal males, females. Spermatoocytes were allowed to oviposited for two days and spermathecae were removed on 8th day. Females and males allowed in the experimental jar, and E, normal females and males were selected and allowed to oviposit for 15 days and on 16th day spermathecae were removed. In all these treatments females and males were kept together in the experimental jar till the termination of experiment.

3 Results

3.1 First experiment

Results are presented in table 1. The rate of fecundity, oviposition and egg production in B, C, D and E were not significantly different (p>0.05) among the treatments when compared to that of virgins (controls).

3.2 Second experiment

Results are presented in table 2. Statistical comparison of these results of an average fecundity, fertility and egg production showed no significant difference in the treatments A, B and C.
Primary stimulus for oviposition and egg production in *G. sigillatus*

Table 1: Fecundity, oviposition and egg production under different stimuli in the house cricket *Gryllodes sigillatus* (Walker)

<table>
<thead>
<tr>
<th>Group</th>
<th>No of insects</th>
<th>Treatments</th>
<th>Fecundity (eggs laid/female/day)</th>
<th>Oviposition (%)</th>
<th>Egg production/female (Av)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>Virgin females</td>
<td>60 ± 20 a</td>
<td>34 ± 2</td>
<td>513</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>Mere insemination of spermatophore or only mating stimuli</td>
<td>55 ± 10 b</td>
<td>33 ± 4</td>
<td>552</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>Only presence of male or pheromonal stimuli</td>
<td>50 ± 15 c</td>
<td>32 ± 0</td>
<td>507</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>Testec tomised males mated with normal females</td>
<td>54 ± 15 d</td>
<td>32 ± 5</td>
<td>511</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>Accessory gland fluid injected to virgins</td>
<td>60 ± 15 e</td>
<td>35 ± 7</td>
<td>515</td>
</tr>
</tbody>
</table>

There is no statistical significant difference in fecundity, oviposition and egg production among these treatments.

Table 2: Effect of multiple matings on fecundity, fertility and egg production of the cricket *Gryllodes sigillatus* (Walker) among the mated insects

<table>
<thead>
<tr>
<th>Group</th>
<th>No of insect pairs</th>
<th>Treatments female</th>
<th>male</th>
<th>Fecundity (eggs laid/female/day)</th>
<th>Fertility (%)</th>
<th>Egg production (Av)</th>
<th>Mating rate/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>1 1</td>
<td></td>
<td>370 ± 30</td>
<td>92 ± 1.67</td>
<td>2152</td>
<td>1.42 ± 0.36</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>1 2</td>
<td></td>
<td>380 ± 42</td>
<td>94 ± 2.35</td>
<td>2135</td>
<td>2.0 ± 0.24</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>1 3</td>
<td></td>
<td>393 ± 61</td>
<td>93 ± 1.5</td>
<td>2198</td>
<td>4.15 ± 1.44</td>
</tr>
</tbody>
</table>

Comparison of means: Fecundity P > 0.05 NS, fertility P > 0.05 NS, egg production P > 0.05 NS and mating rate P > 0.001 S

Although the adequate supply of sperms by multiple matings of multiple males (B and C) the mating rates were significantly differed when compared with that of A (p<0.001)

3.3 Third experiment

Results are presented in Table 3 and the figure.

Normal mated female crickets laid their eggs because the presence of spermatozoa in the spermathecae in the treatments A, D and E. In order to test, the mated and oviposited females were deprived of their spermathecae in D, and E. Statistical comparison of these results showed a significant difference in fecundity, egg production and oviposition (d-d' = p<0.001 and e-e' = p<0.001). In the treatments B, C, D, and E, the rate of fecundity, oviposition and egg production were decreased and significantly not differed when compared with that of virgins (Exp 1 A).
Table 3 Effects on fecundity, egg production and oviposition in spermathecae deprived female crickets of *Gryllodes sigillatus* at different treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>No of Treatments</th>
<th>Treatments</th>
<th>Fecundity (egg laid/female/day)</th>
<th>Egg production (mean)</th>
<th>Oviposition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>Control</td>
<td>372 ± 51 a</td>
<td>2437</td>
<td>92 ± 21</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>Spermathecae deprived females on the day of emergence</td>
<td>563 ± 121 b</td>
<td>465</td>
<td>170 ± 15</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>Spermathecae deprived females after shedding of spermatophore</td>
<td>50 ± 10 c</td>
<td>503</td>
<td>167 ± 21</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>Normal females mated and oviposition for a period of two days</td>
<td>970 ± 15 d</td>
<td>1933</td>
<td></td>
</tr>
<tr>
<td>D₁</td>
<td>7</td>
<td>Spermathecae deprived females after two days of oviposition (group D)</td>
<td>51 ± 12 d'</td>
<td>4317</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>Normal females mated and oviposition for a period of 15 days</td>
<td>4247 ± 792 e</td>
<td>632</td>
<td></td>
</tr>
<tr>
<td>E₁</td>
<td>10</td>
<td>Spermathecae deprived females after 15 days of oviposition (group E)</td>
<td>393 ± 6 e'</td>
<td>402</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of means: a-b = P < 0.001 S, a-c = P < 0.001 S, a-d = P < 0.001 S, a-d' = P < 0.001 S, and a-e' = P > 0.001 S, a-e = P > 0.5 N S
4 Discussion

It is the fact that copulation accompanied by transfer of a spermatophore, sperm and accessory secretions changes the subsequent reproductive behaviour of orthopteran females. Literature varies on the relative importance of male accessory gland secretion and sperm on oviposition in different species of insects. Pickford et al. (1969) reviewed the effect of mating on fecundity and demonstrated that the accessory gland secretion that provide stimulus for oviposition in the grasshopper Melanoplus sanguinipes (F). Similar opinion have been made in the insects A. aegypti, D. melanogaster and Culex pipiens (Leahy 1967) and Schistocerca gregaria (Leahy 1973). Whereas, the maturation of egg was stimulated only after spermatozoa reaching the corpora seminaria in Culex lectularius (Davis 1965), females with eupyrene sperm in their spermathecae were less attractive to males and layed more eggs in tobacco budworm (Raulston et al. 1975). There are some differences of opinion concerning the nature of the primary stimulus. Truman and Riddiford (1974) concluded that the presence of sperm in the bursa copulatrix cause the release of a hormonal factor leading to the production of an oviposition stimulating hormone by the corpora cardiaca in the saturniid moth. Our results pertaining to first experiment emphasized that the only physical act of mating stimuli/physical insemination of spermatophore or male pheromonal activity or testectomised male mating (presuming that accessory gland secretions were passed to females) and injecting the male accessory gland secretions did not stimulate the oviposition and egg production in the G. sigillatus.

The investigations in the S. gregaria and Locusta migratoria (Engelmann 1970), R. prolixus (Coles 1965, Davey 1967), T. molitor (Mordue 1965), A. aegypti (Leahy and Craig 1965) and P. guttentos (Bentur and Mathad 1974) were indicated that mated females laid more number of eggs. Our findings supported the above investigations. Further, adequate supply of sperm by multiple matings of multiple males did not decrease or increase the rate of oviposition and egg production among the mated females, although there was a significant increase in mating rates. Therefore, the increase in mating rate/mates beyond the optimal have no influence on reproduction in this cricket.

The most difficult task is to determine whether the presence of sperm or accessory gland secretion that in the spermathecae which stimulate oviposition. Experiments with castrated males (Truman and Riddiford 1974) showed that although a spermatophore was transferred such mating did not stimulate the female to oviposit. Finding on pink bollworm females with a spermatophore but only apyrene sperm in the spermathecae oviposited few eggs and also would seem to indicate the need for eupyrene sperm but did not rule out the role of accessory gland secretions (LaChance et al. 1975). Further, the investigations on the same species, females that mated with irradiated males and normal amount sperm in the spermathecae laid significantly fewer eggs than females that mated with control males and concluded that the radiation affects sperm or secretions in the duplex region of the ejaculatory duct or secretions present in the accessory gland (LaChance et al. 1977, 1978). Kumta and Tappel (1961) demonstrated that a dose of 20 krad would affect the integrity of the accessory gland secretions. When an aqueous
extract of male reproductive tracts was injected into the haemocoel of female silkworm, *Bombyx mori* egg laying was achieved and suggested that the ovipositional stimulating factor is neither a lipid nor a large protein (Yamaoka and Hirao 1976). Leopold (1976) came to the same conclusion concerning the ovipositional stimulating factor in the house fly.

Present results with the *G. sigillatus* supported the idea that sperms must be present in the spermathecae to elicit the ovipositional response. Because, the matured females laid their eggs within 24 h after mating. If the spermathecae were removed these females behave like virgins. Further, the testiconomised males produced the spermatophore and their accessory gland secretions that have passed through mating to females did not stimulate the oviposition and egg production. Whereas accessory gland removed males did not form the spermatophore and failed to transfer to females (by oral discussion with V K Biradar). The stimuli was only achieved when there were sperms in the spermathecae (Exp 3). The ovipositional response (1) the spermatophore with accessory gland secretions without spermatozoa, (2) spermatophore with sperms in it and spermathecae. In these 2nd has highest effect on oviposition and egg production. It is, therefore considered to be the most potent releaser of substance and sperms must be present in the spermathecae to stimulate investigations of LaChance et al. (1973), Karpenko and North (1973), MacFarlane and Tsao (1974) and Yamaoka and Hirao (1976) were supported our views. Investigations would be needed on nervous integration, hormonal stimulation, components of male accessory gland secretions etc., and our informations on this aspect extends in most of these studies are in progress.

Acknowledgements

We express our sincere thanks to Prof. (Smt.) S S Mathad, Head of the Zoology department of University College for the encouragement and thanks are also due to Dr. V B Nadkarni Professor and Head, Zoology Department, Karnataka University, Dharwad for the laboratory facilities.

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