Further Studies on the Reiterative Mating Ability in Males of *Spodoptera litura* F. (Lepidoptera: Noctuidae)

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(Received February 5, 1973)

In this paper, the reiterative mating ability of males of *Spodoptera litura* F. was investigated under semi-field conditions. Many males mated 4–7 times during their life in the laboratory condition, so that it was considered that they were able to mate several times even under field conditions. Such a reiterative mating ability in the male parallels its length of longevity. The male most actively mated at the 3rd night from emergence. When a single male was successively paired with a fresh virgin female for several nights, the fertilization rate of eggs in the female was not greatly changed by the difference in the order of matings in that male. The females which mated successfully laid their eggs in the largest numbers on the 2nd night following the night of mating, but the female which failed to mate (virgin female) did so on the 5th night.

INTRODUCTION

In the last several years, research on sex pheromones has advanced greatly in various aspects. Along these lines, mating behaviour has also been studied extensively in many species of insects. Mating behaviour of *Spodoptera litura* F. was already been studied by Miyashita and Fuwa (1972) and Oyama (1972), but the detailed picture of mating behaviour is not yet clearly understood.

In particular, reiterative mating ability in males is considered to be one of the most important problem in relation to the control of this insect by employment of sex pheromones.

In this paper, we have reported the results of experiments made on the reiterative mating ability in the adult males of *Spodoptera litura* F. under semi-field conditions.

MATERIALS AND METHODS

Insects used were reared on an artificial diet1 in the laboratory. Experiments were conducted under semi-field conditions: i.e., temperature, humidity and light

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1 See Table 3 in the appendix.
conditions were not controlled, but the insects were protected from rain and direct sunshine. Males were kept individually in a big glass bottle (12 cm in diam. and 7 cm in height), and a single fresh virgin female was put into each bottle every day. The females were removed from the bottle the next morning, and placed individually in a small bottle (9 cm in diam. and 7 cm in height) for observation of egg-laying frequency. Eggs produced by the females were removed from the bottles daily and kept separately in a small glass tube in order to maintain a humid environment for the egg. Larvae from hatched eggs and the remnant unhatched eggs were preserved in alcohol and counted under a binocular. In this case, the larvae which emerged normally and those found intact but deceased in the egg shell were counted as "fertilized eggs". Any others were counted as "unfertilized eggs". All females were dissected after death in order to ascertain mating success or failure.

The inner wall of the bottles were covered with paper, and a strip of thick paper was inserted as a foothold for the insect together with small dish containing a 10\% solution of honey which served as food. The experiment was repeated 4 times during the period from June to August. The periods during which the experiments were conducted and the number of males used are shown in Table 1.

Table 1. Periods of Experimental Scheduling and the Number of Males Used in Each Experiment

<table>
<thead>
<tr>
<th>Series of experiment</th>
<th>Period of experiment</th>
<th>No. of males used</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>June 13-June 28</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>July 7-July 25</td>
<td>12</td>
</tr>
<tr>
<td>C</td>
<td>July 18-Aug. 2</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>Aug, 11-Aug. 30</td>
<td>12</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Reiterative mating ability in a single male

Fig. 1 shows the frequency distribution of males as represented by the number of matings repeatedly performed by respective individuals. Individuals which mated 4 and 7 times showed the greatest frequency, but the number of matings per individual greatly differed from individual to individual. Many males mated successively for several nights, but several males mated at intervals. On the one hand, there were several males which never mated during their life, but there was a male which mated 9 times. It is speculated that unmated males possessed some sort of physiological defect. In the case of males which mated at intervals, intermittent cessation of mating probably resulted from a failure of some inherent physiological condition of the female paired at that time. Therefore, it is suspected that success and failure of mating is greatly influenced by the physiological condition of the opposite sex.

In short, it is concluded that the male of this insect is able to mate several times during his life in so far as circumstance permits, but rarely mates twice during the night. OYAMA (1972) reported that males which mated on a given night did not fly to the virgin female trap that same night, but those males which mated on the preceding night actively flew to the trap the following night.
Fig. 1. Frequency distribution of males classified by number of matings during life. The symbol A, B, C and D coincide with that in Table 1, and E indicates the total of 4 replications.

Fig. 2. Relationship between the longevity of the male and the number of matings repeatedly performed by that male.

The reiterative mating ability of the male seems to have a close connection to the length of longevity in that male. In order to ascertain this point, the numbers of matings observed in males were plotted against the respective longevities of each male (Fig. 2). According to Fig. 2, it can be said that the number of matings per individual shows a tendency to increase in a long lived individual.
Daily change in the mating activity of the male

Fig. 3 shows daily change in the mating activity of the male. In this graph, daily change in mating activity is shown by the percentage of mated males to the number of survivals examined on the subsequent night following emergence, and the survival curve of the male is also shown together for reference.

According to Fig. 3, the daily change in the mating activity is shown by a curve with 2 peaks: one which appeared on the 3rd night following emergence and another on the 10th night, respectively. However, the appearance of the second peak can probably be attributed to chance, because the number of individuals examined was greatly decreased by that time owing to natural death. The number of survivors on the 9—10th night following emergence was quite small as indicated by the survival curve. From these facts, it is considered that the mating activity of this insect becomes strongest on the 3rd night after emergence, but thereafter decreases gradually.

![Graph showing daily change in mating activity and survival curve](image)

Fig. 3. Daily change in the mating activity of the male, and survival curve. Large open circles indicate the percentage of mated males to the number of survivals at each night, and small black circles the percentage of survivals.

Relationship between the order of matings performed by a single male and the fertilization of eggs in the female

MIYASHITA and FUWA (1972) reported that a single male probably possesses the same ability to fertilize a female at the time of the 2nd mating on a par with that demonstrated during the first, but they did not examine mating subsequent to the initial one.

In the present experiment, the relationship between the order of matings performed by a single male and the fertilization of eggs in the female was analyzed in detail, and the result obtained is shown in Fig. 4. It is recognized that the ability of the male to fertilize the eggs of the female was not greatly changed by the order of matings. However, the fertilization rate of eggs greatly differed from experiment to experiment. For example, the fertilization rate of eggs was very low in experiments A and D as compared with that in experiments B and C. As the experiment was repeated 4 times during the period from June to August (Table 1), weather conditions,

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1 The emergence of adults generally occurs during the period from the late afternoon to the midnight.
Egg-laying by females in relation to success and failure in mating

When females were kept in bottles for several days, egg-laying occurred even in the virgin females. Table 2 shows a comparison between the number of eggs laid by females which mated successfully and that by females which failed to mate. In this table, the longevity of females is also shown for reference. According to Table 2, the mated female laid 1423.1 eggs on the average, while the unmated female laid only 761.0 eggs, although the eggs produced by the latter were, of course, unfertilized. There was no difference in longevity between females which mated successfully and those which failed to mate.

Fig. 5 shows the daily change in the egg production by mated and unmated females. The number of eggs laid by mated females reached a peak on the next night following mating, and then decreased sharply thereafter. Some individuals produced 1 The egg-laying generally occurs during the night.
Table 2. **Average Numbers of Eggs Laid by Females which Mated Successfully or Unsuccessfully, and Average Longevities in These Females**

<table>
<thead>
<tr>
<th>Series of experiments</th>
<th>No. of eggs/♀</th>
<th>Longevity in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mated</td>
<td>Unmated</td>
</tr>
<tr>
<td>A</td>
<td>1315.9</td>
<td>662.4</td>
</tr>
<tr>
<td>B</td>
<td>1388.2</td>
<td>687.5</td>
</tr>
<tr>
<td>C</td>
<td>1524.3</td>
<td>942.0</td>
</tr>
<tr>
<td>D</td>
<td>1463.9</td>
<td>751.9</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>1423.1</td>
<td>761.0</td>
</tr>
</tbody>
</table>

Fig. 5. Daily change in number of eggs laid by mated and unmated females. Small black circles indicate the average values for 4 replications, large open circles the grand averages, and histograms the average numbers of fertilized eggs contained in total number of eggs produced.
only fertilized eggs, but others produced both fertilized and unfertilized eggs in mix. On the average, however, the daily change in the production of fertilized eggs followed about the same tendency as the daily change in the total egg production of fertilized and unfertilized ones. According to Miyashita and Fuwa (1972), about 50% of the females mated under field conditions were polyandric. Therefore, it is probable that the production of unfertilized eggs is prevented by multiple mating.

On the other hand, in the case of females which failed to mate, the number of eggs laid was greatest on the 5th night following the start of experiment, but the peak was not as conspicuous as that observed in mated female. In addition, the period of egg-laying was somewhat prolonged. Philippe (1971) reported the same phenomenon in Chrysopa perla. Benz (1969) and LeCato and Piebkowski (1972) revealed that egg-laying in Zeiraphera diniana and Hypera postica females was strongly stimulated by the presence of sperm in the spermatophore or spermatheca. According to Benz and Schmid (1968), egg-laying in Actias selene females was also stimulated by the presence of olfactory active sex pheromone produced by males.

REFERENCES


APPENDIX

Table 3. Prescription of the Artificial Diet Used in This Experiment

| Kidney bean (soaked in water) | 300g |
| Water | 1800ml |
| Agar powder | 36g |

After washing, the bean was boiled with water and agar powder, and the following materials were added.

| Wheat germ | 300g |
| Yeast powder | 120g |
| L-ascorbic acid | 12g |
| Formalin | 10ml |
| Methyl β-hydroxybenzoate | 8.5g |
| L-cystein monohydrochloride | 1.2g |

a This prescription was conceived by Dr. S. Kamano, National Institute of Agricultural Sciences.