CHAPTER II
CHAPTER - II

SEXUAL MATURATION AND COURTSHIP BEHAVIOUR OF THE MALE CRICKET,
GRYLODES SIGILLATUS IN RELATION TO THE ACCESSORY
REPRODUCTIVE GLAND

A. Sexual Maturation:

Mating leads to enhanced synthesis of accessory gland
secretion has been shown in Rhodnius prolixus (Barker and
Davey, 1981), Drosophila funebris (Baumann, 1974) and in
Melanoplus sanguinipes (Cheeseman and Gillott, 1989) and the
copulation enhanced protein synthesis in Drosophila
melanogaster (Schmidt et al., 1985). Engelmann (1970) elucidated
that most orthopterans do not mate immediately after imaginal
moult into adult, despite of a variety of exogenous and
endogenous factors influence the reproduction. In insects the
time interval between the imaginal moult into adult and mating
could be termed as maturation period. In the early age groups
ability and willingness to mate successfully varies from one
individual to another. Royer (1966) has investigated the
maturation period for Gryllous bimaculatus 7.00 days. The
maturation period recorded for the cricket Acheta domesticus was
11.4 day by McFarlane (1962). Similarly, Bentur (1975) has
investigated the maturation period for male and female field cricket *P. guttiventris* as 8.7±0.4 and 10.1±0.6 days respectively. Biradar (1984) observed the maturation period for female cricket *Gryllodes sigillatus* was 7.5±0.3 days. In the present investigation it will provide the functional background for ensuing chapters that deal with different aspects of the MARG and it will focus on the growth and development of the male accessory reproductive gland. Hence, the maturation of male cricket, *Gryllodes sigillatus* was investigated.

**Adult age**

A successful mating or insemination required a large number of virile sperms. Gryllides mate repeatedly due to the smallness of spermatophore insufficient to inseminate a female fully (Alexander and Otte, 1967). The description of mating behaviour by Alexander and Thomas (1951a) and Royer (1966), studied in detail, most insect species can determine a potential sexual partner is male or female until one is initiated. Hence, the evaluation of maturation and courtship behaviour appears to be rather unique characteristic for any of the cricket. As it was observed that very young males of *Gryllodes sigillatus* after imaginal moult into adult
never engage themselves in mating activities. They showed negative response for more than 72 h. These males clearly indicated that they were immature. Hence, it is relevant to investigate the youngest male to mate for the first time successfully and is termed as the maturation period of male cricket *G. sigillatus*.

**Experimental procedure and results**

Just hatched adult crickets after the imaginal ecdysis into adult were isolated from the parent colony and dated. For the present experiment 1 to 10 day old adult males and more aged receptive females of *G. sigillatus* were selected from the stock culture. A total of 150 males and 250 females - not less than 10 insects for each group were tested separately for their maturation period. In our observation, the period for each group of individual adult was lasted for about 120 minutes during the scotophase of 12L:12D condition. Average maturation time, percent mating and duration for mating were recorded.

Results are incorporated in Table 1. From the data it was noticed that the youngest male cricket *G. sigillatus* to mate successfully for the first time was 4-day-old. The average age of the adult for maturation time was $8.2 \pm 0.8$ days and time
Table 1: Maturation period of male cricket *Gryllodes sigillatus* (Walker) after imaginal ecdysis into adult.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Adult males</th>
<th>Age of Adult males</th>
<th>Maturation period (Time taken for mating (minutes)) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21</td>
<td>1 day old</td>
<td>Did not mate</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>2 day old</td>
<td>Did not mate</td>
</tr>
<tr>
<td>C</td>
<td>15</td>
<td>3 day old</td>
<td>171.16 ± 13.52</td>
</tr>
<tr>
<td>D</td>
<td>15</td>
<td>4 day old</td>
<td>30.16 ± 1.62</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>5 day old</td>
<td>19.33 ± 0.84</td>
</tr>
<tr>
<td>F</td>
<td>12</td>
<td>6 day old</td>
<td>15.66 ± 0.92</td>
</tr>
<tr>
<td>G</td>
<td>12</td>
<td>7 day old</td>
<td>11.75 ± 1.30</td>
</tr>
<tr>
<td>H</td>
<td>15</td>
<td>8 day old</td>
<td>6.5 ± 2.51</td>
</tr>
<tr>
<td>I</td>
<td>12</td>
<td>9 day old</td>
<td>4.75 ± 0.25</td>
</tr>
<tr>
<td>J</td>
<td>10</td>
<td>12 day old</td>
<td>5.65 ± 0.35</td>
</tr>
<tr>
<td>K</td>
<td>12</td>
<td>20 day old</td>
<td>6.5 ± 1.15</td>
</tr>
</tbody>
</table>
Table 2: Statistical analysis and comparison of the results presented in the Table 1.

<table>
<thead>
<tr>
<th>Treatment V/S Treatment</th>
<th>t-values</th>
<th>Degrees of Freedom</th>
<th>Probability 0.05</th>
<th>Significance Probability 0.001</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C v/s D</td>
<td>9.34</td>
<td>29</td>
<td>2.05</td>
<td>NOT</td>
<td>3.66</td>
</tr>
<tr>
<td>D v/s E</td>
<td>4.402439</td>
<td>24</td>
<td>2.06</td>
<td>NOT</td>
<td>3.75</td>
</tr>
<tr>
<td>E v/s F</td>
<td>2.0852272</td>
<td>21</td>
<td>2.08</td>
<td>NOT</td>
<td>3.83</td>
</tr>
<tr>
<td>F v/s G</td>
<td>1.77772727</td>
<td>23</td>
<td>2.07</td>
<td>YES</td>
<td>3.71</td>
</tr>
<tr>
<td>G v/s H</td>
<td>1.3779527</td>
<td>26</td>
<td>2.06</td>
<td>YES</td>
<td>3.71</td>
</tr>
<tr>
<td>H v/s I</td>
<td>0.6340579</td>
<td>26</td>
<td>2.06</td>
<td>YES</td>
<td>3.71</td>
</tr>
<tr>
<td>I v/s J</td>
<td>1.50</td>
<td>21</td>
<td>2.08</td>
<td>YES</td>
<td>3.83</td>
</tr>
<tr>
<td>J v/s K</td>
<td>0.566666</td>
<td>21</td>
<td>2.08</td>
<td>YES</td>
<td>3.83</td>
</tr>
</tbody>
</table>
A — Time taken for mating
B — Per cent mating.
taken for mating was 6.5 ± 2.5 minutes. Further, results showed that as the age advanced the adult crickets displayed increasing mating and more percent of them mated (Table 2).

Discussion

Male crickets of *G. sigillatus* started producing chirping sound, after 48 h of imaginal moult into adult, with a high frequency but it was not a mating sound signal. Lohar and Edson (1973) observed that some female crickets of *T. commodus* exhibited mating within 24 h after their imaginal moult into adult and showed no behavioural maturation. McFarlane (1968) noticed that the appearance of first spermatophore was 11.4 days in the male crickets of *A. domesticus* and he determined the same as the maturation period. While, Shuvalov and Popov (1972) studied on the receptivity response to sound signals in cricket, *A. domesticus* that the young females less than 5-days old exhibited negative response to male song. Such negative response was noticed in males which failed to mate in less than 3 day old male. In these the response was the behavioural maturation. Similarly, Bentur
(1975) observed the behavioural maturation for the cricket *P.guttiventris* and Biradar (1984) noticed the behavioural maturation in female cricket, *G.sigillatus*. In the current experimental investigation it was observed that the males of *G.sigillatus* did not mate within 72 h. after emergence into adult. The percent mating in the middle aged crickets and with the aged male crickets for mating was not correlated (Fig. 3). The progressive and gradual increase of maturation and courtship behaviour indicated certain regulating mechanisms associated with the secretory activity. The accessory reproductive gland or the testes or release of endocrine substance from endocrine glands may be regulating the maturation and mating behaviour in this cricket. Considerable developmental changes took place during the post-nymphal development and the mechanisms were experimentally investigated.

B. COURTSHIP BEHAVIOUR IN THE MALE CRICKET *GRYLLODES SIGILLATUS* WALKER

Entomological literature on various aspects of courtship behaviour is voluminous. The act of mating in
insects involves complex patterns of behaviour. Records on cricket mating behaviour revealed an elaborate pattern which has been worked out by many authors (Houghton, 1909; Jenson, 1909; Gurhardt, 1913; Khalifa, 1950a; Huber, 1955; Alexander, 1957, 1961a, 1962; Alexander and Thomas, 1959; Leroy, 1964; Royer, 1966; Bentur, 1975; Biradar, 1984).

The normal act of courtship behaviour implicated sequential steps. To obtain information in the cricket *G. sigillatus* male and female insects isolated from the laboratory stock colony after the emergence into adult and selected about 15 days old crickets. Observations on the courtship behaviour were made in housing them in the experimental jars of 500 cc capacity Ogale glass jars. Each of the experimental groups of males were placed with equal number of females in each jar. Observations were continued for six hours daily commencing from 6 p.m. for a period of 10 days. The mating patterns and courtship behaviour have been studied as a footstep for the further investigation in the studies of accessory reproductive gland of male *G. sigillatus*. The following were considered as courtship behaviour in the male house cricket of *G. sigillatus* (a) Location of sexual partner by antennal contact, (b) Normal chirping and surrounding.
(c) Mating call i.e. with low chirping and backward jerking movement, and (d) Copulation and spermatophore transfer.

Observations

A very young male within 24 h after the imaginal moult into adult sung a song but not a mating song. In such chirping male crickets the tegmina were raised at an angle of 90°, and their chirping sound was of high frequency. Such young male crickets showed a negative response to courtship. The normal male crickets, when placed in an experimental jar with female were shown by the characteristic indication of antennal contact. If there is a female cricket, male approached closely and antennated. It tapped on abdominal tip and antennae of female. Like both established mutual contact indicating sexual response. Then the male, turned about 180° after spotting the female started producing courtship song by lifting tegmen at an angle of 30-45° from the thorax. It was a courtship song with low frequency. It (Male) moved backwardly and provoking the female to mount. The receptive female excited and mounted the male from behind. As soon as the female mounted the male stopped its chirping song and started rubbing and raising the abdominal tip towards that
of female bursa copulatrix for perfect position of clasping. Then the male insect lastly flexed its tip of the abdomen to clasp the female genitalia. The clasped couple remained motionless until the transfer of spermatophore by the male. At the same time the clasping male insect continued to beat or flicker its antenna touching the antenna of clasping female. The courted and excited female cricket lifted its ovipositor at an angle of about 90° so that the bursa copulatrix projected out. Lastly the male cricket lifted the abdominal tip so as to enable the sperm tube to contact the bursa copulatrix of female insect. About 2 to 3 minutes were required to transfer the spermatophore. Thereafter the jelly like portion of the spermatophore was eaten away by the copulated female cricket soon after separation. But the capsule containing the spermatozoan ampulla was hanging from the female genitalia about more than 30 minutes.

The spermatophore of *G. sigillatus* consists of a gelatinous bulb like ampulla, a narrow connecting neck, a flattened attachment plate and a long sperm tube. There is a capsule containing spermatozoa within and it is surrounded by the gelatinous ampulla. A duct leads from capsule through the neck and attachment plate into the sperm tube (Fig. 4). When a
freshly deposited spermatophore is observed it was containing abundant sperms. On the other hand, the spermatophore after mating event when dropped from the female genitalia did not contain any sperm or sometimes contains a few sperms.

C. RELATIONSHIP OF TESTES, ACCESSORY REPRODUCTIVE GLAND AND ENDOCRINE GLAND IN MATURATION AND COURTSHIP BEHAVIOUR OF THE MALE CRICKET G. SIGILLATUS

Several authors have investigated the relationship of sexual organs in some Orthoptera. Extirpation of gonads in males and females of G. compestris had no effect and they behaved normally in their mating behaviour (Regen, 1909, 1910). Removal of primary sexual organs had no effect on sexual behaviour or copulation in male and female of S. gregaria (Husain and Baweja, 1936; Husain and Mathur, 1945; Loher, 1960; Odhiambo, 1966). Similarly in grasshoppers, Chorthipus parallelus and Omocetus viridulus (Hoskell, 1960) and Gomphocercus rufus (Loher and Huber, 1964). Whereas either castration or extirpation of accessory reproductive glands was ineffective in the sexual maturation of grasshopper, S. gregaria (Loher, 1960; Odhiambo, 1966) and the field cricket, P. guttiventris (Bentur, 1975). In the present study an attempt has been made to determine the role of testis, accessory
reproductive gland and endocrine glands in maturation and courtship of male *G. sigillatus*.

Experimental procedure and results

Surgical procedure

For the present experiment 20 crickets of each, for testectomy, extirpation of the accessory reproductive gland and allatectomy, were chosen from synchronous batch of one day old males. For the extirpation of the testes, crickets were mild etherised. Two longitudinal incisions were made on the dorso-lateral region on either side of the mid-dorsal line of 4th to 6th tergal plates. Then exposing the testis it was pulled out through incision, after snapping the connection with the vasadeferens with the help of a pair of ethanol sterilised fine forceps, the testis on the other side was also removed.

The extirpation of male accessory reproductive glands, similarly incisions were made in the pleural region of 7th and 8th abdominal segments and pulled out the accessory reproductive glands through the incision, with the help of ethanol sterilised fine pair of forceps. The wound was sealed with the molten paraffin wax after sprinkling with antibiotics.
For allatectomy the male crickets were anaesthetised
under mild ether anaesthesia. Allatectomy was performed within
one hour after imaginal ecdysis. The anaesthetised crickets
were fixed on a wax platform. The head of the insect was
stretched anteriorly and placed over a raised ridge of the wax
platform. A small piece of cuticle was cut from the posterior
end of the head with the help of a pair of forceps. The
membrane covering the neck was pierced and ice-cold Singer was
poured over this region. The forceps were now gently probed
anteriorly through the head muscles and corpora cardiaca was
first located. Later, the corpora allata, lying on the
dorsolateral side of the oesophagus and attached to the corpora
cardiaca were pulled out by holding the base of nervi corporis
allati. The wound was closed with low melting wax and
few crystals of streptomycin sulphate were placed on the wound.
There was about 20% mortality. Few male crickets were also
maintained as sham-operated to serve as control.

Results are incorporated in Table 3. The extirpation
of the testes and the accessory reproductive gland did not
impair the courtship behaviour of the male cricket
G.sigillatus. The testectomised
males mated readily. Testectomised male crickets average
Table 3: Sexual maturation period of normal adult, testes extirpated and MARG removed males of *Gryllodes sigillatus* (Walker).

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of adults</th>
<th>Treatments</th>
<th>Sexual maturation period (days)</th>
<th>Time taken for mating (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>A</td>
<td>50</td>
<td>Normal adult males</td>
<td>8.2 ± 0.8</td>
<td>6.5 ± 2.5</td>
</tr>
<tr>
<td>B</td>
<td>47</td>
<td>Testes extirpated males</td>
<td>8.51 ± 0.7</td>
<td>42.32 ± 11.25</td>
</tr>
<tr>
<td>C</td>
<td>53</td>
<td>MARG removed males</td>
<td>8.3 ± 1.2</td>
<td>38.71 ± 12.76</td>
</tr>
</tbody>
</table>
Table 4: Statistical analysis and comparison of the results presented in the Table 3.

<table>
<thead>
<tr>
<th>Treatment V/S</th>
<th>t-values</th>
<th>Degrees of Freedom</th>
<th>Probability 0.05</th>
<th>Significance 0.05</th>
<th>Probability 0.001</th>
<th>Significance 0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>A v/s B</td>
<td>0.2066666</td>
<td>96</td>
<td>1.96</td>
<td>YES</td>
<td>3.29</td>
<td>YES</td>
</tr>
<tr>
<td>A v/s B</td>
<td>0.1105263</td>
<td>96</td>
<td>1.96</td>
<td>YES</td>
<td>3.29</td>
<td>YES</td>
</tr>
<tr>
<td>B v/s C</td>
<td>2.6043636</td>
<td>99</td>
<td>1.96</td>
<td>NOT</td>
<td>3.29</td>
<td>YES</td>
</tr>
<tr>
<td>B v/s C</td>
<td>0.1499375</td>
<td>99</td>
<td>1.96</td>
<td>YES</td>
<td>3.29</td>
<td>YES</td>
</tr>
</tbody>
</table>
maturation period was 8.5±0.7 days and these males deposited and transferred their spermatophore like normal insects. But there were no sperms in the spermatophore. The accessory reproductive gland removed male crickets did not form spermatophores but attempted to copulate frequently. In such males clasping of genitalia lasted for a brief time and they showed their maturation period 8.3±1.2 days (Table 3). These values did not significantly differ when compared with those of sham-operated ones. The allatectomised male crickets were slightly paler in colour than controls. These did not show any type of sexual behaviour but chirped when left with females. The histological pictures of the corpus allatum of just emerged males was smaller and densely packed with nuclei and sparse cytoplasm (Fig.5). On the other hand the males exhibited courtship behaviour, on the 4th day after the imaginal ecdysis, the corpus allatum was large, with loosely arranged nuclei and abundant cytoplasmic granules (Fig.6). cells are circular in nature.

Discussion

Castration in adult males of G.compestria did not affect their behavioural nature towards females and sang like normal ones (Regen, 1909, 1910). Males of S.gregaria
mated readily as normal ones (Husain and Baweja, 1936). More frequent copulation with testectomised males of locust *S. gregaria* was noticed by Husain and Mathur (1945). Whereas, testectomised male crickets of *P. guttiventris* mated readily (Bentur, 1975). Similarly, testectomised males of *G. sigillatus* displayed instinctive behaviour of mating towards females and mated readily indicating normal maturation time as in locust, *S. gregaria* (Odhiambo, 1966).

The extirpation of accessory reproductive gland in male crickets of *G. sigillatus* did not affect the normal maturation period and they courted but failed to transfer spermatophore to females. The results are in agreement with findings for locust *S. gregaria* (Loher, 1960; Odhiambo, 1966). Huber (1955) investigated that the severance of ventral nerve chord in *G. campestris* resulted in inhibition of mating. Hoskell (1961) reported that the crickets displayed normal mating behaviour and mated only when a spermatophore was formed. The investigations of Hoskell (1960) in male grasshoppers of *C. parallelus* and *C. viridulus* are in agreement with the present findings for the male cricket *G. sigillatus*. Contrary to this, male crickets of *P. guttiventris* either testectomised or extirpated of accessory reproductive gland took almost the same
duration for mating as the normal insects (Bentur, 1975). Ovarectomisation in cricket, *G. campestris* did not affect the mating abilities (Regen, 1910), *S. gregaria* (Hussain and Baweja, 1966) and *G. sigillatus* (Biradar, 1984). Similarly, the present findings, for the male cricket *G. sigillatus* were in accordance with the above investigations. The testes and accessory reproductive glands have no direct influence on either inhibitory or acceleratory effect on maturation. Probably, they might have produced certain endogenous stimuli (Engelmann, 1970) to enhance the courtship behaviour. Therefore, testec-tomisation or extirpation of accessory reproductive gland in *G. sigillatus* failed to inhibit or accelerate the ability of maturation time and courtship behaviour.

The endocrine gland, the *corpus allatum* has a significant role in maturation and sexual behaviour of insects. The *corpora allata* have been shown to control sexual behaviour in *Schistocerca gregaria* (Loher, 1961; Pener, 1965, 1967; Odhiambo, 1966b; Cantacuzene, 1967b) and in *Nomadalis septumfasciata* (Pener, 1968). Girardie and Vogel (1966) did not observe sexual behaviour in alletectomised males of *Locusta migratoria cinerascens* but Cantacuzene (1967c) and Strong (1968) observed that the alletectomised male locusts maintained
courtship behaviour. On the other hand, in the males of grasshopper *Gomphocercus rufus* (Loher, 1962) and cockroach *Byrsotria* (Barth, 1962) the corpora allata do not seem to exercise a control over the sexual behaviour. Wajc and Pener (1969) opined that the relationship between the sexual behaviour and corpora allata in the males of *Locusta migratoria* is controversial. In the present findings, the loss of sexual behaviour in allatetomised male crickets of *G. sigillatus*, showed synchrony with the sexual behaviour of 4th day imaginal adult. The histological changes of the corpus allatum suggested a significant role in the control over the courtship behaviour of male *Gryllodes sigillatus*.

The mating behaviour was primarily initiated by sound production in several crickets studied by Jenson (1909), Gerhart (1913) and Khalifa (1950a). The mating pattern and courtship behaviour have been studied in some crickets namely *O. fasciatus* (Jonson, 1909), *L. campestris* (Gerhardt, 1913), *G. domesticus* (Khalifa, 1950a), *G. compestris* (Huber, 19550, *P. guttiventris* (Bentur, 1975) and *G. sigillatus* (Biradar, 1984). The courtship behaviour of the male *G. sigillatus* can be compared with those of above mentioned investigations. The mating behaviour of *O. fasciatus* corresponded with antennal
contact and courtship song in *G. sigillatus*. While, antennal contact and courtship song for the cricket *G. bimaculatus* (Royer, 1966) and *P. guttiventris* (Bentur, 1975) were similar to the present investigations. The mating behavioural pattern in crickets as observed by Jenson (1909), Gerhardt (1913) and Khalifa (1950a) involved courtship song as an initial step. But in the cricket *G. sigillatus* when compared with the findings of Alexander and Thomas (1959) and Bentur (1975) the courtship song did not initiate the mating behavioural pattern.

Tactile, auditory and visual sense organs are in the order of importance in mating and help in recognising the partner of opposite sex as well as in mounting and appreciating the sexual receptivity in female *G. sigillatus* (Biradar, 1984). The mating behaviour for the cricket *G. sigillatus* constituted the basic pattern to evaluate quantitative courtship behaviour displayed by the adult male. Courtship behaviour, though progressed stepwise which exhibited by any paired groups of adults it can stop at any time depending upon the receptivity
or willingness of the partners. Such a break indicated the maturity and receptivity of the male crickets. Therefore, the mean of maximum points attained by the individual adult male cricket in a given length of time revealed the average receptive state of the male insect. The mean can be compared with that for another group of adult male crickets tested under similar conditions. The mating behaviour (Alexander and Thomas, 1959; and Royer, 1966), described the mounting and clasping. Hence, study of adult insects receptivity is a rather unique characteristic for any of the insects courtship behavioural patterns.
EXPLANATION TO FIGURES

Fig. 4: Freshly deposited spermatophore of *Gryllodes sigillatus* showing A. ampulla; C, capsule; ST, sperm tube; X 35.

Fig. 5: T.S. of corpus allatum just after ecdysis into adult male cricket, *G. sigillatus* showing SN, smaller nuclei; SC, sparse cytoplasm; X 1000.

Fig. 6: T.S. of corpus allatum of fourth day, after imaginal ecdysis into adult male *G. sigillatus* showing LAN, loosely arranged nuclei; AC, abundant cytoplasm X 1000.