CHAPTER III
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POST-EMBRYONIC DEVELOPMENT OF THE ACCESSORY REPRODUCTIVE GLAND IN THE MALE CRICKET GRYLLODES SIGILLATUS

Introduction

In insects, the male internal reproductive organs comprise testes, vasa deferentia, seminal vesicle, ejaculatory duct and in most species accessory glands of varied complexity. Gillott (1988) have been emphasized in detail that the forms and functions of the accessory glands are the diversity of external form and habits of insects.

The development of male and female insect genitalia and associated organs have attracted many investigators and their investigations were pertained to several groups of insects because the origin and development of insect genitalia and associated structures have remained controversial. Else (1933) has investigated that the accessory reproductive glands in the males of Melanoplus differentialis develop from the mesoderm and he emphasised that the ampulla at the proximal portion of each of the appendages on the 10th segment is the
rudiment of the accessory gland in the first instar. Roonwall (1937) in *Locusta migratoria migratorioides* shown that the male accessory reproductive glands arise as evaginations on the walls of the ampullae of the 10th abdominal segment. Quadri (1940) in his review on various orthopterans has reported that the vasa deferentia and their terminal ampullae which gave rise to the male accessory reproductive gland and the tubules of the gland develop from the walls of the ampulla only in the last instar which are of mesodermal origin. Johanson and Butt (1941) in their review in orthoptera have been reviewed that in case of male insect the embryonic vasa deferentia end posteriorly with hollow terminal enlargement or ampulla inserted into the appendage rudiments of the 10th abdominal somite. Van Wyk (1952) has shown that the accessory glands of *Leucophaea maderae* are of mesodermal origin. Morere (1965) has shown in *Clitumnus extradentatus* that the gradual development and differentiation of the accessory glands from the ampulla at the terminal ends of the vasa deferentia are of mesodermal origin. Kaulenas *et al.*, (1975) have reported that the accessory reproductive gland rudiment in *Acheta domesticus* consists of a rounded mass of tissue in the earlier instar but during the last instar tubules develop from the analage and the full
compliment of the tubules is formed just prior to the imaginal moult. On the other hand, in coleopteran insects *Sitodrepa panices* (L), *Gastrodia polygoni* (L), *Rhagium bifasciatus* (Metcalfe, 1933), in Homopterans, *Idiocerus atkinsoni* and *Idiocerus populi* (Singh Pruthi, 1925) and in Lepidopterans *Pieris rapae* (L), *Hepialus lupinus* (L), *Earias fabia* (Stål, 1971) and *Bombyx mori*. (L) (Mehta, 1934) have shown that the accessory reproductive glands are ectodermal in origin.

Although, Quadri (1940) has described about accessory reproductive glands in Gryllids but he has not been given the cytological and histological details involved in the development of accessory gland for any species. Therefore, as there is no published data on the post-embryonic development of the accessory gland in house cricket. *G. sigillatus*, the present investigation has been planned to study in detail the histological and cytological changes of the male accessory reproductive gland during the post-embryonic development.

Materials and Methods

To facilitate the study on the post-embryonic development of the accessory reproductive gland in male *Gryllodes sigillatus* just emerged different instar nymphs were
isolated from the parent colony and reared in a separate rearing jars. The dated instars were sacrificed for further regular intervals of time for histological studies commencing from their analage stage in the first instar. Instars and their accessory gland analages were fixed in Bouin's fluid and Zenker's fluid with acetic acid. Both the fixative gave excellent results for histological studies.

As it was not possible to dissect out the accessory anlage of the first instar due to the minute size of the nymph, the nymphs were immersed in toto in the fixative. Penetration of the fixative was then effected under vaccum for hours. The instars after dehydration in alcoholic series and clearing in xylol were embedded in paraffin. The blocks were trimmed in such a manner that only the part containing the accessory gland analage was retained. The analages of the accessory glands from second to the seventh instars were exposed by vivisecting the nymphs in the insect Ringer (Ephrussi and Beadle, 1936), then the Ringer was replaced by the choosen fixative. Paraffin blocks of the tissues were made following the usual procedure and sections 6 μ thickness were cut. The parafffinised sections were stained with Delafield's haematoxylin or Heidenhain's haematoxylin. Of the two stains used the latter proved
satisfactory as histological details of the gland were very clear.

OBSERVATIONS

First instar

At this stage, in just emerged first instar *G. sigillatus* the vasa deferentia extend to the posterior end of the ninth sternum. Each vas deferens ends in a hollow ampulla. The two ampullae oppose each other at the middle of the junction between the ninth and tenth sternum. The wall of the ampulla is composed of several tiers of cells. The cells of the ampulla become confluent, thus forming a single large ampulla. This structural form forms the analage of the male accessory reproductive gland (MARG) and it is discernible during the later stages. The ampulla measures 72.42±2.47 μ in width 89.42±1.63 μ in length and situated ventral to the last abdominal ganglion. The nerves arising from the last abdominal ganglion innervate the anal cerci and the ampulla from both sides. This accessory gland is situated dorso-ventrally and its broader end is oriented towards the dorsal region while its narrow end faces the ventral region. The accessory reproductive analage being composed of several tiers of cells. Hence, the
anterior, posterior and ventral regions appear thicker, while the dorsal region appears relatively thinner. The cells are round and occur closely packed but cell boundaries are not distinct (Fig. 7).

**Second Instar**

The accessory gland analage of male *G. sigillatus* in the second nymphal instar was observed and it resembles the late first instar excepting for a little increase in the size. The analage is dorso-ventrally oriented and it measures $85.71 \pm 1.79 \mu$ in width and $93.85 \pm 0.55 \mu$ in length (Fig. 8).

**Third Instar**

As the accessory reproductive analage is dorso-ventrally oriented its broader end is positioned towards the dorsal region while its narrow side faces the ventral region. Concomitant with the increase in size of the analage the ventral cavity also enlarged. At this stage, the egg shaped analage measures $114.28 \pm 0.68 \mu$ in width between the insertions of the vasa deferentia and $143.00 \pm 2.93 \mu$ in length ($F_{1,9}$).
Fourth instar

An overall increase in the size of the gland analage is evident. This rudiment of MARG lies close to the ventral surface of the last abdominal ganglion. The analage is an egg shaped and its dorso-ventral axis appears broader towards the dorsal surface. It measures $191.66 \pm 4.75 \mu$ in length surface and $173.33 \pm 6.63 \mu$ in width (Fig.10). There is no deviation from the histological picture. However the analage picture presented the densely arranged round or oval cells on the uppermost tiers. (Fig.10).

Fifth instar

A general increase in the size of the analage is pronounced along the antero-posterior axis. It measures $213.33 \pm 6.38 \mu$ in width and $261.66 \pm 5.98 \mu$ in length at the antero-posterior axis. The cytological features of the cells are alike to those seen in the fourth instar analage but cells appear either spherical or oval. The rudiment of the ductus ejaculatorious has now developed a central lumen. Thus converting it into a tube but the lumen is not confluent with cavity in the analage (Fig.11).
Sixth instar

An increase in the size of the reproductive analage is noticed. The analage now measures 340.00±4.75 µ in width and 480.00±7.08 µ in length (Fig. 6). In this stage, the cells appear either oval or spherical in shape and several of them exhibit mitotic phases. The lumen within the analage and that of the ejaculatory duct are separated at the posterio-ventral region (Fig. 12).

Seventh instar

The specimens examined in the first day of seventh instar an overall general increase in the size of the MARG is noticed. It measures 887.5±8.5 µ in width and 731.66±7.00 µ in length (Fig. 13). From the ventral wall of the analage a mass of cells get implanted into the cavity and the diameter of the lumen at the posterior side is small, while it is large at the anterior side. The lumen of the ejaculatory duct opens into the cavity through the posterio-ventral wall. The specimens observed on the 2nd, 3rd, 4th, 5th, and 6th day nymphal stages, an overall increase of the MARG is noticed. On 7th day, when examined, the inpushing of cells from the lateral side has now formed into a full wall. At the lateral regions the cells
lining the cavity are heaped to form numerous small evaginations. These constitute the rudiments of the tubules and tubules have been developed from this wall (Fig.14). The tubular out growth from the anterio-lateral region of the MARG is noticed from the 7th day of the 7th instar. The development of follicles are evident in this region that by bursting open the epithelial lining of the accessory analage. The tubules arising at the anterio-lateral region are few and are not uniform in their size and show their appearance like buds. The length of the shortest tubules is 10 \( \mu \) while longest tubule is 45 \( \mu \) (Fig. 14). The specimens examined on the 8th day show the presence of tubules present in the lateral and posterior region having length of 102.5±0.79 \( \mu \) and the tubules situated at the anteriodorsal region appear shorter known as utriculi minoris. The lumen in the utriculi majores of the MARG has narrowed down anterio-posteriorly. The posterior region of the analage cavity communicates with the ejaculatory duct posterio-ventrally giving the impression that the lumen is continuous with the lumen of the ductus ejaculatorious (Fig.20). The specimens when examined on the 10th days, an increase in length of the tubules appear like a long tentacle like. They measure in 237.5±0.90 \( \mu \) length and 2 to 5 \( \mu \) in width. (Fig.17).
Table 5: Progressive changes in the length and width of the analages of the male accessory reproductive gland in the cricket, *Gryllodes sigillatus* Walker.

<table>
<thead>
<tr>
<th>Instars</th>
<th>Length (μm)</th>
<th>Width (μm)</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>First instar</td>
<td>89.42 ± 1.63</td>
<td>72.42 ± 2.47</td>
</tr>
<tr>
<td>Second instar</td>
<td>93.85 ± 0.55</td>
<td>85.71 ± 1.79</td>
</tr>
<tr>
<td>Third instar</td>
<td>143.00 ± 2.93</td>
<td>114.28 ± 0.68</td>
</tr>
<tr>
<td>Fourth instar</td>
<td>191.66 ± 4.75</td>
<td>173.33 ± 6.63</td>
</tr>
<tr>
<td>Fifth instar</td>
<td>261.66 ± 5.98</td>
<td>213.33 ± 6.38</td>
</tr>
<tr>
<td>Sixth instar</td>
<td>480.00 ± 7.08</td>
<td>340.00 ± 4.75</td>
</tr>
<tr>
<td>Seventh instar</td>
<td>731.66 ± 7.00</td>
<td>887.50 ± 8.50</td>
</tr>
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The specimens when observed on the imaginal ecdysis, i.e. on 12th day, a general increase in the size of the analage is evident along with the lateral axis. It measure 1295.16 μ in width and 915.83 μ in length. An overall increase in the size of tubules is evident. The tubular extensions have grown so big that they occupy a greater area within the visceral cavity of the animal. The tubules present at the anteriodorsal region, the utriculi minoris measure 10 to 20 μ in length and those tubules present in the lateral and posterior regions, the utriculi majores which measure 335.0±10.0 μ in length (Fig. 18).

Discussion

It has been reported by Highnam and Hill (1969) that in insects the ejacutory duct and phallus are ectodermal in origin while the vasa deferentia is of mesodermal origin. While, as early as Wheeler (1898) has established that the vasa deferens is mesodermal in origin and it terminates into a hollow ampulla. Quadri (1940) reported while studying the development of the genitalia and their ducts in several species of Orthopteroides Blatta sp. Periplaneta sp., Gryllus domesticus, Gymnogryllus erythrocephalus, Schistocerca gregaria and Locusta migratoria migratorioides - has shown that the
ejaculatory duct represents the ectodermal part of the male efferent system whereas the vasa deferentia and their terminal ampullae give rise to the accessory reproductive gland are mesodermal in origin. Similarly, the Blattoidea, and in particular *Leucophaea maderae*, the accessory reproductive gland which arises from the ampulla at the posterior tip of the vas deferens is mesodermal in origin (Van Wyk, 1952) and in *P. guttiventis* (Ranganathan, 1971). In the current investigation the development of the male accessory reproductive gland in *Gryllodes sigillatus* is in conformity with the findings of Quadri (1940), Van Wyk (1959) and Ranganathan (1971) since the accessory reproductive gland develops from the hollow ampulla at the terminal end of the vas deferens. On the other hand, in Coleopterans - *Sitodrepa panicea* (L), *Gastroidea polygoni* (L), *Anthonomus pomorum* (L) and *Rhagium bifasciatum*; homopterans - *Idiocerus populi* and *Idiocerus atkinsoni* and lepidopterans *Pieris rupae* (L) *Hepialus lupinus* (L), *Earias fabia* (Stall) and *Bombyx mori* (L) it has been shown that the accessory gland develop from the ejaculatory duct is ectodermal in origin (Metcalfe, 1933; Singh Pruthi, 1925 and Mehta, 1934).

Quadri (1940) has been reported that in the first instar the vasa deferentia terminate into a hollow ampulla
resting on the penis valve of its side. Further, in the succeeding instars, he found an asymmetry, the left side of the analage becoming longer than the right and the two ampullae closely approximate each other. He has also noticed that in the last instar the accessory reproductive gland makes its appearance in the form of a number of buds on the walls of the ampulla. The observations made in *Leucophaea maderae* (Van Wyk, 1952), *Clitumnus extradentatus* (Morere, 1965), *Melanoplus differentialis* (Else, 1933) and *Locusta migratoria migratorioides* (Roonwall, 1937) that the ampulla which give rise to the accessory gland and develops from the end of the vasa deferentia has been well documented. It has been investigated earlier in this study that the male accessory reproductive gland develops from the analage inconspicuous, the ampulla, which is a hollow bulbus termination of the vasa deferentia found at the 9th sternum. The analage, inconspicuous in the first instar which shown a progressive increase in its size in the subsequent nymphal instars but the tubules appear only in the late penultimate instar. The post-embryonic development of the accessory reproductive gland noticed in the male cricket *Gryllodes sigillatus* falls in line with that of Quadri's (1940) observation on the development of genitalia in
the cricket *Gryllus domesticus* and *Gymnogryllus erythrocephalus*.

Accessory analage differentiation

It is observed in the cricket *G. sigillatus* that the ampulla remain as an inconspicuous analage in the early nymphal instars and a gradual increase in the size of the analage with subsequent ec dysis. During the later stages of the penultimate instar the follicles appear from the walls of the ampulla and the adult form is attained just 24 to 36 hours before imaginal ec dysis. Similarly, it has been observed in *Xiphidium* (Wheeler, 1898), *Melanoplus differentialis* (Else, 1933) and *Blatta* sp., *Periplaneta americana*, *Gryllus domesticus*, *Gymnogryllus erythrocephalus*, *Schistocerca* and *Locusta* (Quadri, 1940) that there is a gradual increase in the size of the analage of the accessory gland with successive moult as they herald towards the adult stage. Else (1933) has further investigated that evaginations appear from the walls of the ampulla during the second instar. The evaginations are the rudiments of the tubules which compose the convoluted mass forming accessory gland of the adult. Bodenstein and Sprague (1969) have noticed in the development of the accessory gland in the female
cockerel Periplaneta americana that the colleterial glands remain rudimentary throughout the early stages of the nymphal life. The tremendous growth of the tubules occur towards the end of the last nymphal stage and continues until the nymph moults into an adult. Moree (1965), while studying the post-embryonic development of the male genitalia of Clitumnus extradentatus has observed that there is an enlargement of the ampullae up to the third instar. In the fourth instar the tubules begin to appear and the accessory gland attains the adult form in the last instar.

Similarly, Ranganathan (1971) has observed in the field cricket, P.guttiventris that the ampulla remain as an inconspicuous analage in the early stages and thus is a gradual increase in the size of the analage with successive moults. The tubules appear from the walls of ampulla only during the later stages of final instar. It is obvious that the development and differentiation of the accessory reproductive analage and tubules of the gland observed in the male house cricket of G.sigillatus is in accordance with the investigations made by various authors mentioned above on different groups of orthopteroid insects.
The post-embryonic development of the male and female genitalia has been studied in a few groups of insects including some gryllids. But details involving the activity of cells, the migration of cells during the development and differentiation of the follicles of the accessory gland has not been recorded. Morphological changes have been observed (Else, 1933; Roonwall, 1937; Quadri, 1940; Van Wyk, 1952 and Morere, 1965) during the post-embryonic development of the accessory gland but these above authors have not reported the cytological changes like cellular movements and mitosis etc. While, Ranganathan (1971) during the study of post-embryonic development of accessory gland in the field cricket, *P. guttiventris* observed that with the growth and differentiation of the accessory gland occur with successive moults. The tubule formation is seen in the fourth instar as bud like evaginations from the wall of the ampulla. Further, he observed that the mitotic activity is more abundant in the region of the tubule formation. In the current observations on *G. sigillatus* it has been shown that the growth and development of the accessory gland is noticed up to the 6th instar. Changes indicative of tubule formation is seen in the 7th nymphal instar as a bud like evaginations from the wall of the ampulla.
Mitotic activity is more abundant in the region of the tubule formation. Development and differentiation of the tubules require cells and the demand is met with by the increased instances of mitosis noticed in the differentiating region rather than in the surrounding areas. Presumably, this is the reason why mitotic activity is evident till 36 hours before the imaginal ecdysis in the developing analage of the accessory reproductive gland. Further, also Lococo and Huebner (1980) have investigated in their studies on the development of the female accessory gland in *Rhodnius prolixus* that feeding assists the first phase in the growth of the gland by proliferative mitosis, while movements of the ductule and its secretory cell differentiation is brought about by the second phase of morphogenetic cell division. In the present observation on *G. sigillatus*, is in accordance with those reported by Ranganathan (1971) in *P. guttiventris* and by Berrill and Karp (1976), in that once cytodifferentiation is complete and the analage tissue assumes its function and mitosis ceases in that tissue.
EXPLANATION TO FIGURES

Fig. 7: T.S. of first instar nymph, C.S. passing through accessory reproductive gland of *G. sigillatus* showing CA, Cells of ampulla R, Rectum, X 400.

Fig. 8: Whole mount of second instar MARG of *G. sigillatus* showing A, ampulla; VD, Vas deferens ED, ejaculatory duct; X 400.

Fig. 9: Whole mount of third instar MARG of *G. sigillatus* showing A, ampulla; ED, ejaculatory duct; VD, vasa deferens; X 400.
EXPLANATION TO FIGURES

Fig. 10: Whole mount of fourth instar accessory reproductive gland of *G. sigillatus* showing VD, vasa deferens, ED, ejaculatory duct; AD, Anterio-dorsal position; X 100.

Fig. 11: Whole mount of fifth instar male accessory reproductive gland of *G. sigillatus* showing VD, vasa deferens; ED, Ejaculatory duct; AD, Anterio-dorsal position, X 100.

Fig. 12: Whole mount of sixth instar male accessory reproductive gland of *G. sigillatus* showing VD, vasa deferens; ED, Ejaculatory duct, X 400.
EXPLANATION TO FIGURES

Fig. 13: The whole mount of seventh instar male accessory reproductive gland of *G. sigillatus*, showing VD; vasa deferens; ADP, antero-dorsal position; ED, ejaculatory duct; X 100.

Fig. 14: The whole mount of seventh instar MARG of *G. sigillatus* showing ADP, antero-dorsal position; ED, ejaculatory duct; VD, vasa deferens; B, buds; X 100.

Fig. 15: The whole mount of 7th instar 8th day MARG of *G. sigillatus* showing ADP, antero-dorsal position; T, tubules at the lateral region; X 100.
EXPLANATION TO FIGURES

Fig. 16: Whole mount of the 7th instar 9th day MARG of G. sigillatus showing ADP, anterio-dorsal position; T, tubules at the lateral region; X 100.

Fig. 17: Whole mount of the 7th instar 10th day MARG of G. sigillatus showing ADP, anterio-dorsal position; F, follicles at the anterior-lateral and posterior lateral region; X 100.

Fig. 18: Whole mount of the 7th instar 12 day MARG of G. sigillatus showing VLT, ventro-lateral tubules; UMI-utriculi minoris; UMA, utriculi majores; X 100.
EXPLANATION TO FIGURES

Fig. 19: T.S. of the seventh instar seventh day MARG of G. sigillatus passing through anterio-dorsal region, showing FF, formation of follicles; MA, Mitotic apparatus; UMF, utriculi minoris follicles; X 400.

Fig. 20: T.S. of the seventh instar twelveth day MARG of G. sigillatus passing through the anterio-dorsal region showing UMI, utriculi minoris follicles; VLM, ventro-lateral follicles (majores); L, lumen of the gland; X 400.