CHAPTER 4

DEVELOPMENT AND DIFFERENTIATION OF ACCESSORY SEX GLANDS DURING PUPAL-ADULT METAMORPHOSIS AND A PRILIMINARY STUDY ON THE INFLUENCE OF MATING ON THE SECRETORY ACTIVITY OF THE MALE ACCESSORY SEX GLANDS
4.1 Introduction

The embryonic origin of ASGs is varied with respect to sex as they are generally mesodermal in males and ectodermal in females. Development, structure and functions of ASGs of male insects have been reviewed by Leopold (1976); Chen (1984) and Happ (1984, 1992). ASGs of most male insects arise from the terminal ampullae of the vasa deferentia which themselves originate from the coelomic cavities of the ninth or tenth abdominal segments. Though Leopold (1976) and Chapman (1998) have mentioned that the male ASGs have either an ectodermal or mesodermal origin, Chen (1984) has reported that the male ASGs of mesodermal origin are observed in many insects.

In most female insects ASGs normally originate from the invagination of the ninth abdominal segment. The development of the efferent genital system in female Lepidoptera has been studied by several authors (Jackson, 1889; Verson and Bisson 1895; Dubois, 1931; Dodson, 1937; Ammann, 1954; Brunold, 1957; Srivastava and Srivastava, 1959; Wittig, 1960; Joubert, 1964; Leckercq-Smekens, 1976; Matsuda, 1976; Sethi and Dhillon, 1981). The post-embryonic development and differentiation of the ASGs in male and female insects are regulated by the interaction of JH and ecdysteroids, the former inhibiting and the latter promoting these processes (Bodenstein and Sprague, 1959; Gillott, 1992).
In many insects ASGs play a vital role in the reproductive biology. Male ASGs are involved in the building of spermatophore for sperm transfer to the female (Viscuso, et al., 2001). In addition to this, the secretion provides nutrition, change the female reproductive behaviour and physiology after copulation (Chen, 1984; Happ, 1984; Chen et al., 1998; Herndon et al., 1997; Smid, 1997; Wolfner, 1997; Gillott, 1996; Heifetz et al., 2001; Wolfner, 2002). Fecundity enhancing and receptivity-inhibiting substances are reported in the secretion of ASGs (Gillott, 2003). Studies of Hosken (1999) show that in female Scathophaga stercoraria, ASGs are involved in egg laying as well as in lubrication during copulation. Callahan and Cascio (1963) suggest that secretions of ASGs in noctuid moths act as a lubricant to aid the movement of the sperm from the spermathecal duct. The present study deals mostly with the development and differentiation of ASGs of S. mauritia during pupal-adult metamorphosis. A few studies on the changes in the protein profile of male ASGs during mating have also been conducted.

4.2 Materials and Methods

4.2.1 Dissections

Pupae of all ages and day 0 adult insects (10 Nos.) were segregated from the basic stock culture and mildly anesthetized in diethyl ether. Their ASGs were carefully dissected out in insect Ringer solution and transferred to separate glass slides. These slides were placed over a graph paper kept under
the dissection microscope and measured the lengths of ASGs. The mean length was taken as the length of ASGs of a particular age.

4.2.2 Mating Experiments

Twenty adult male and female insects (Day 0) were segregated from respective stock culture (maintained separately for both sexes to avoid mating) and kept for mating. Each pair consisting of a virgin male insect and a virgin female insect were kept for 24 h in glass beakers for mating. Same number of males (Day 0) deprived of females were kept as controls. ASGs of mated and virgin males (8 Nos.) were dissected out free of fat body on day 2 and transferred to microscopic slides, weighed and processed for estimation of total proteins and electrophoretic studies. Completion of mating was ensured by the positioning of hind regions of insects and confirmed by the hatchability of eggs.

4.2.3 Preparation of samples for estimation of total proteins

ASGs of virgin and mated male insects (8 Nos.) were thoroughly cleaned in insect saline and homogenized in 50 μl of distilled water utilizing a glass homogenizer. The homogenate was centrifuged at 2000 rpm for 10 minutes. Decanted the supernatant and stored at 0 °C until use.
4.2.4 Estimation of total proteins

Proteins were estimated following the method of modified Lowry protein assay as described by Sandermann and Stromiger (1972). Took 20 and 30 µl of the above supernatant in test tubes and added 1 ml of working solution (freshly prepared by mixing 25 ml of \{2% Na₂CO₃, 0.02 % Na K tartarate, 0.1M NaOH, 1% SDS\} and 1ml of 0.5% CuSO₄. This mixture was allowed to stand for 15 minutes at room temperature. Then added 0.1 ml of 1N Folin reagent and vortexed the tubes immediately. This solution was allowed to stand for 30 minutes at room temperature and read at 650 nm against a reagent blank in a Shimadzu UV Spectrophotometer. Bovine serum albumin (Sigma Chemical Co., St. Louis, U.S.A) was used as the standard. The results are expressed per mg of tissue.

4.2.5 Preparation of samples for electrophoresis

4.2.6 Sodium dodecylsulphate- Polyacrylamide gel electrophoresis (SDS –PAGE)

Proteins were resolved by SDS–PAGE under reducing conditions in 10% acrylamide as described by Laemmli (1970). SDS –PAGE was carried out using vertical slab gel in 8 x 7 cm gel of 1 mm thickness in a mini model vertical electrophoresis unit (Bangalore Genei Pvt. Ltd., Peenyaa, Bangalore). A 3% spacer gel was layered over the separating gel. Gel containing 3% and 10% acrylamide were prepared from stock solution of 30% by weight of
acrylamide and 0.8% by weight of N, N’-methylene-bis acrylamide. Separating gel of 10% acrylamide concentration was prepared by mixing:

(a) 6 ml Acrylamide (stock solution)

(b) 11 ml buffer (0.614M Tris- Base adjusted pH 8.8 with HCl containing 0.164 % SDS w/v).

(c) 0.9 ml Ammonium persulphate (15mg/ml) and

(d) 0.02 ml TEMED

The spacer gel was prepared by mixing:

(a) 1 ml Acrylamide (stock solution)

(b) 8.5 ml buffer (0.147 M Tris, pH adjusted 6.8 with HCl, containing 0.108 % SDS w/v)

(c) 0.5 ml ammonium per sulphate (15 mg/ml) and

(d) 0.01 ml TEMED.

Protein sample containing 1% SDS, 10% Glycerol and 5% β- mercaptomethanol was heated in a boiling water bath for two minutes and cooled before loading onto the gel. Equal amount of tissue was loaded in all the wells. Bromophenol blue was used as a tracking dye.
Electrophoresis was carried out using a buffer system (Chamber buffer, pH 8.3) containing 0.025 M Tris, 0.192 M glycine and 0.1% SDS at a constant current of 25 mA/gel. After electrophoresis the proteins in the gel were fixed in 50% methanol containing 0.075% formaldehyde for 45 minutes. The gels were stained to visualize the separated proteins with 0.06% Coomassie brilliant blue (R-250) staining solution (44 ml methanol, 44 ml distilled water, 12 ml glacial acetic acid and 875 ml distilled water). Gels were destained in a destaining solution (Methanol: 50 ml, glacial acetic acid: 75 ml, distilled water: 875 ml). The net staining intensities of protein bands were measured in a Bio Rad Gel Documentation System.

The molecular weight standards used were Myosin, Rabbit Muscle (205 kDa), Phosphorylase b (97.4 kDa), Bovine Serum Albumin (66 kDa), Ovalbumin (43 kDa), Carbonic Anhydrase (29 kDa), Soyabeen Trypsin Inhibitor (20 kDa), Lysozyme (14.3 kDa), Aprotinin (6.5 kDa) and Insulin (3 kDa). Mobility of sample protein was compared to the mobility of standards in 10% acrylamide. Molecular weight was calculated from a plot of log molecular weight versus the relative mobility of the standards.

4.3 Results

4.3.1 Development of ASGs

ASGs appeared as small discrete structures in the early pupal phase of male and female of S. mauritia. The glands underwent a sequential and
continuous growth in the pupal phase and their morphogenesis was complete by the time of adult emergence. In male pupae from day 1 to day 4 ASGs were seen as a fused pair of glands extending from ductus ejaculatorius duplex (Pl. XVI: Figs. 42, 43, 44, 45). Though the fused glands began to separate in early pupal days, the glands looked like distinct pairs from day 5 onwards (Pl. XVII: Figs. 46, 47). They showed morphological differentiation into three regions: a thick translucent anterior region (proximal), a middle long and thin opaque region (mid region) and a thick posterior translucent region (distal region). In adults the gland morphology did not change very much except an increase in the volume of the gland (Pl. XVII: Fig. 48). The linear length of the glands measured 9 ± 1.364 mm on day 1 pupa, 15 ± 2.582 mm on day 2, 20 ± 3.162 mm on day 3, 33 ± 1.944 mm on day 4, 36 ± 1.940 mm on day 5, 50 ± 3.162 mm on day 6, 58 ± 3.560 mm on day 7 and 77 ± 2.944 mm in adult (Table 1, Fig. 49).

In day 1 female pupae ASGs were found as small pair of glands of length 2 ± 0.849 mm beneath the spermatheca (Pl. XVIII: Fig. 50). The basal end of each gland was dilated into reservoir like structure. Subsequently the glands increased in length and the reservoirs became bulbous (Pl. XVIII: Figs. 51, 52; Pl. XIX: 53). A rapid increase in length of the gland was observed in day 3 pupa. On other days the elongation process was gradual and steady. The linear length of the glands measured 3 ± 0.812 mm on day 2, 7 ± 1.374 mm on day 3, 10 ± 1.541 mm on day 4, 12 ± 1.214 mm on day 5, 12 ± 0.817 mm
on day 6 and 13 ± 0.879 mm in adults (Table 1, Fig. 49). In adults the gland morphology remained same though an overall increase in volume of the reservoir is noted (Pl. XIX: Fig. 54).

4.3.2 Changes in the protein content in virgin and mated male ASGs

Amount of protein in virgin males was found to be 15.237 ± 0.675 µg/ mg of tissue whereas the quantity of protein in mated males was found to be significantly less. It measured 10 ± 0.673 µg / mg of tissue (Table 2). At 0.05 levels these values are significantly different.

4.3.3 Electrophoretic profile of ASG proteins in virgin and mated male insects

The aqueous extracts of ASG proteins were prepared and the electrophoretic profile of ASG proteins was analyzed by SDS PAGE.

4.3.3.1 SDS-PAGE pattern of Proteins in ASGs of virgin male Insects

Figure 55 represents the electrophorogram of ASGs of males. The predominant proteins of ASGs expressed in the virgin males (Lane 1, Figure 55) were ASG1, ASG 2, ASG 3, ASG 4, ASG 5, ASG 6, ASG 7, ASG 8, ASG 9, ASG 10, ASG 11, ASG 12, ASG 13, ASG 14, ASG 15, ASG 16, ASG 17, ASG 18, ASG 19, ASG 20, ASG 21, ASG 22, ASG 23 and ASG 24 of molecular weights 138 kDa, 104.7 kDa, 100 kDa, 95.50 kDa, 87.10 kDa, 77.62 kDa, 72.44 kDa, 69.18 kDa, 58.88 kDa, 54.95 kDa, 51.29 kDa, 47.86 kDa, 44.67 kDa, 41.69 kDa, 38.02 kDa, 33.11 kDa, 30.20 kDa, 29.51 kDa,
26.92 kDa, 25.12 kDa, 22.91 kDa, 20.42 kDa, 18.20 kDa, 15.14 kDa respectively. The major peptides were ASG 6, ASG 9, ASG 14, ASG 16 and ASG 19. The peptides ASG 1 and ASG 2 appear as very faint and thin bands. The other peptides appeared as medium intense bands.

4.3.3.2 SDS-PAGE pattern of Proteins of ASGs of mated male Insects

ASGs from mated male insects were separated and the proteins were resolved by SDS-PAGE. Lane 2 (Fig.55) shows the electrophoretic profile of ASG proteins of mated male insects. In mated males the staining intensities of the most of the bands were considerably less compared to that of virgin males (Table 3). Moreover three peptides seem to be absent from the electrophorogram of mated males.

The peptides ASG 1 and ASG 2 appear as very faint and thin bands as in virgin insects. ASG 4, ASG 7 and ASG 15 are absent in mated males. The staining intensities of peptides ASG 6, ASG 8, ASG 9, ASG 10, ASG 11, ASG 12, ASG 13, ASG 16, ASG 17, ASG 18, ASG 20, ASG 21, ASG 22, ASG 23 and ASG 24 were less in mated males. The peptides ASG 5, ASG 14 and ASG 19 appear in mated males as equally intense bands as in virgin males.
4.4 Discussion

Development of male ASGs

According to the studies of Noirot and Quennedey (1974, 1991) classification of insect exocrine glands is based on the presence of a basic element, the cuticular element. The ectodermal glands develop from epidermal invagination and therefore are always associated with cuticular components (Quennedey, 1998). This theory provides explanation for the embryonic origin of ASGs in many insects. Mesodermal origin of ASGs are reported in many lepidopterans like *Antheraea mylitta* (Pendum and Tembhare, 2005) and *H. olivacerus* (Gundevia and Ramamurthy, 1977). In *Spodoptera mauritia* male ASGs seem to have a mesodermal origin since cuticular component is not observed in their ultrastructure as detailed in Chapter 3. ASGs of mesodermal origin are reported in other male insects like *Drosophila* (Bairati, 1968) and *Apis mellifera* (Moors, 2005).

Various studies show that in endopterygotes, organogenesis begins in prepupal stages and continues into the pupal stage. In lepidopterans like *Heliothis virescens* and in Coleoptera ASGs grow (increase in length) steadily throughout the pupal stage (Met Calfe, 1932; Elliott, 1964; Loeb, 1991). Organogenesis of ASGs occurs before pupation in *T. molitor* whereas development and differentiation takes place during pupal stage and in the first 5-6 days of adulthood (Huet, 1966). In *Spodoptera mauritia* ASGs appear in
early pupal stage and undergoes development throughout the pupal phase and development is completed at the time of adult emergence. Since ASGs commenced its elongation and develop from day 1 pupae onwards, the organogenesis might have begun in the prepupal stage as observed in other endopterygotes. Studies of Mariamma (1989) show that in last instar larvae of *Oryctes rhinoceros* the male reproductive system appears rudimentary and differentiation of each part occurs when the pupa is 1-8 day old. Structurally the reproductive system of newly moulted adult is not different from that of a late pupa. In *T. molitor* differentiation is not completed until several days after adult emergence. In *Spodoptera mauritia* linear development of ASGs is completed by the time of adult emergence.

Studies show that development and differentiation of ASGs of insects are under the influence of hormones. In lepidopteran metamorphosis during pupal stage the marked decline in the level of circulating JH and one or more surges in hemolymph ecdysteroid titre permit the expression of adult characters i.e. differentiation of both external and the internal organs (Steel and Davey, 1985).

The studies on *T. Molitor* provide a clear picture of involvement of ecdysteroids on development and differentiation of ASGs. These studies show that the ASGs increase in size due to cell division and undergo a change in shape due to differential mitotic rates in different regions. (Grimes and
Happ, 1980). The study has provided useful indices for scoring the progressive differentiation of the bean shaped glands of *T. molitor*. The development of ASGs requires cell multiplication, acquisition of competence to make adult specific proteins and increases in cell size that accompany rapid synthesis of secretory proteins (Happ, 1990). In *T. molitor* development of ASGs is characterized by growth of the glands (a 10 fold increase in volume) largely due to two bouts of mitosis: the first on days 1-2 and the second on days 4-5 of pupal stage (Grimes and Happ, 1980; Happ and Happ 1982; Happ et al., 1985). The second bout coincides with the peak of ecdysteroid in the pupal stage (Delbecque et al., 1978). Subsequent *in vitro* studies showed that the first bout of mitosis is not ecdysteroid dependent; the second required the addition of physiological amounts of ecdysterone. It showed that hormone promoted the flow of cells from the G₂ into the G₁ and S phases (Yaginuma et al., 1988). Studies of Dorn and Schneider (1986) show that in *Oncopeltus fasciatus*, maturation of the ASGs coincides with the rise of the ecdysteroid titre. The pupal ecdysteroid peak is required for ongoing growth and differentiation in the mesodermal bean-shaped and tubular ASGs of *Tenebrio molitor* (Happ and Happ, 1982).

Studies of Loeb and Hakim (1991) showed that differentiation of the ASGs in the lepidopteran *Heliothis virescens* from the germinal imaginal discs required the presence of both a sufficient titre of ecdysterone and fat body or testis sheath factors. It seems that in *S. mauritia* ecdysteroids have caused
mitotic bouts which would have subsequently led to the growth and differentiation of the ASGs from the germinal to imaginal tissues. Ecdysteroid peak has been reported in *S. mauritia* in day 2 pupae (Mona 2001, unpublished observation). This might be the reason for the sudden increase in the length of ASGs in day 3.

**Development of female ASGs**

In female *Spodoptera mauritia* ultrastructural studies of ASGs clearly reveal the presence of a cuticular component and therefore ASGs of female *S. mauritia* appear to have an ectodermal origin. Ectodermal origin of female ASGs is reported in *Heliothis zea* (Callahan and Cascio, 1963) and in *P. americana* (Mercer and Brunet, 1959).

In *Spodoptera mauritia* the development of female ASGs take place in a steady and continuous manner. ASGs make their appearance in early pupal stage and grow in length throughout the pupal phase. In *Papilio demoleus*, ASG rudiments differentiate to adult form and size by day 4 of the pupal stage whereas ASG rudiments differentiate into distinct parts like reservoir duct, reservoir and ASGs by 72 h of pupal stage (Sendi *et al.*, 1993). In *Spodoptera mauritia* ASGs got differentiated into reservoir and gland in day 1 pupa itself.

According to earlier studies hormones regulate the differentiation of ASGs in female insects just as in the case of male insects. Specifically JH
inhibits and moulting hormones ecdysteroids promotes the development and differentiation of ASGs (Bodenstein and Sprague, 1959). Bownes and Rembold (1987) report that generally pupae have high ecdysteroid titre and low JH titre. The ecdysteroid levels drop during late metamorphosis and continue to do so in the newly eclosed adult whilst the JH levels rapidly increase after eclosion. Thus during the complex period at and just after eclosion when all the events such as vitellogenesis, mating etc are initiated, a period of rapidly changing ratios of the two hormones are observed. Our early studies report high ecdysteroid titre in the pupae of *S. mauritia* (Mona, 2001, unpublished observations). The reason for the development of ASGs to take place in the pupal phase of *S. mauritia* might be due to high ecdysteroid and low JH seen just as in other lepidopteran pupae. Similar findings have been reported in other insects like *Aedes aegypt* (Margam et al., 2006).

In the adults of *S. mauritia* even though the length of ASGs did not increase further an increase in the size of the gland reservoir is noted. Evidently this is due to the accumulation of secretion. Marked enlargement of the ASGs due to accumulation of glue like substances are reported in adult silkworm *Bombyx mori* (Jin et al., 2006). In *Schistocerca gregaria* the ASGs become increasingly swollen with secretion until they attain their maximum size just before oviposition (Szopa, 1982). Morphometric studies of ASGs of female *Teleogryllus commodus* Walker yield evidence that the glands are subjected to a significant growth during peak differentiation starting
immediately after the adult moult (Sturm, 2008). Studies of Herman et al., (1975) show that in monarch butterflies rapid post eclosion growth of ASGs are associated with high JH titres.

**Changes in the protein content in ASGs of virgin and mated male *S.mauritia***

Electrophoretic studies of proteins in ASGs of virgin insects show 24 protein components differing in their molecular weights and charge properties. The changes in staining intensity of the protein bands reflect alteration in the apparent concentration of these components. The major peptide components are ASG 6, ASG 9, ASG 14, ASG 16 and ASG 19 having molecular weights 77.62 kDa, 58.88 kDa, 41.69 kDa, 33.11 kDa and 26.92 kDa respectively. The total protein content of ASG is found to significantly decrease in mated males. In addition the peptides designated in virgin males as ASG 4, ASG 7 and ASG 15 are absent in the ASGs of mated males. Moreover the staining intensities of most of the peptides are lower in mated males. These results show mating causes a reduction in the amount of protein in ASGs of male *S. mauritia*.

Studies of Koene and Maat (2001) show that during close bodily contact, during mating males of many species transfer substances that influence the behaviour or physiology of conspecifics. Transfer of male ASG materials into the female mates have been reported by many workers.
Duportets *et al.*, (1998) has illustrated by total protein content analysis followed by gel electrophoresis that the protein content of ASGs in male moth *Agrotes ipsilon* decreased after mating. Monsma and Wolfner (1988) and Wolfner (2002) reported that during mating, male *Drosophila* transfer seminal proteins and peptides along with sperm to their mates. Studies of Lay *et al.*, (2004) show that white secretions from the tubules of the male ASGs of *Locusta migratoria* composed of peptides and proteins are transferred during mating to the female's spermatheca. Studies of Ottiger *et al.*, (2000) show that in *D. melanogaster* sex peptide (sp) and ductus ejaculatorius peptide (DUP 99B) are male pheromones transferred in the seminal fluid to the female during copulation.

In *S.mauritia* male ASG peptides constitute the main source of materials that are transported to the female during mating. In *S. litura* protein profile of male ASGs reveal the presence of 23 proteins whose molecular weights ranged from 163 to 3.8 kDa (Izadi and Subrahmanyam, 2005). In *S. mauritia* the molecular weights of ASG peptides fall more or less in this range. Studies show that the molecular weights of peptides found in secretion of ASGs in *S. litura* ranged from 100 to 3.8 kDa (Izadi and Subrahmanyam, 2005). In *S. mauritia* molecular weights of peptides showing either less staining intensity or disappearing from the electrophorogram of ASG proteins in mated males fall more or less in similar range of molecular weights of secretory peptides of ASGs as reported in *S. litura*. 

Evidences for the influence of male ASG secretions on female behaviour and physiology have been reported by many workers (Webb et al., 1999; Baer et al., 2000; Kubli, 2003; Chapman, 2003; Ram et al., 2005; Izadi and Subrahmanyam, 2005). Studies of Chen (1996) in Drosophila show that ASG protein play a key role in reproductive success of the fruitfly by changing female sexual behaviour, supporting sperm transfer, storage and displacement. He has opined that genes encoding these ASG proteins are apparently under strong evolutionary selection. ASGs of males make the seminal fluid proteins as well as the structural elements of the complex spermatophore (Khalifa, 1949). Protein variants in the ASGs of adult males of Drosophila probably have specific functions such as degradation to amino acids for either general protein synthesis (Bownes and Partridge, 1987) or for use as energy substrate during sperm transfer and storage (Chen and Oechslin, 1976). Markow and Ankney (1984) reported that in D. mafosvensis female flies utilize amino acids derived from male ejaculate for protein synthesis in ovarian oocytes and somatic tissues. Studies of Colonello and Hartfelder (2003) show that ASG products of male bees are a means of transport for sperm. Further these secretions can form a mating plug and also have specific compounds that can modify the behaviour and physiology of mated females. Callahan and Cascio (1963) suggest that secretions of female ASGs in noctuid moths act as a lubricant to aid the movement of the sperm from the spermathecal duct.
The other functions of ASG peptides in the female body have been investigated by many workers and generalized that the peptides reduce male receptivity in females. In Lepidoptera substances transferred from the male to female during copulation are usually considered to trigger a refractory behaviour in females which reduce the ability of females to elicit a sexual response in other males (Raina, 1989 and Giebultowicz et al., 1990; Gillott, 2003). In *S. mauritia* the exact role of ASG peptides in the female body is unknown but these peptides might be engaged in similar functions. Though replenishment of ASGs with further secretion has reported in many insects including lepidopterans such replenishment of ASGs with secretion is not observed in *S. mauritia*.

4.5 Summary

1. ASGs appeared as small discrete structures in the early pupal phase of male and female of *Spodoptera mauritia*. The ASGs underwent a sequential and continuous growth in the pupal phase and their morphogenesis was complete except an increase in the volume by the time of adult emergence.

2. In male pupae from day 1 to day 4, ASGs were seen as a fused pair of glands extending from ductus ejaculatorius duplex. ASGs looked like distinct pairs from day 5 onwards. They showed morphological differentiation into three regions: a thick translucent anterior region (proximal), a middle long and thin opaque region (mid region) and a thick posterior translucent region (distal region).

3. The ASGs showed a gradual and steady growth throughout the pupal phase and the linear length of ASGs measured 9 ± 1.364 mm on day 1 pupa, 15 ± 2.582 mm on day 2, 20 ± 3.162 mm on day 3, 33 ± 1.944 mm on day 4, 36 ± 1.94 mm on day 5, 50 ± 3.162 mm on day 6, 58 ± 3.56 mm on day 7 and 77 ± 2.944 mm in adults.
4. In females ASGs are seen as a pair of glands beneath the spermatheca. The basal end of each gland was dilated into reservoir like structure.

5. During pupal-adult metamorphosis the ASGs measured 2 ±0.849 mm on day 1 pupa, 3 ± 0.812 mm on day 2, 7 ± 1.374 mm on day 3, 10 ± 1.541 mm on day 4, 12 ± 1.214 mm on day 5, 12 ± 0.817 mm on day 6 and 13 ± 0.879 mm in adults.

6. Amount of protein in ASGs of virgin males was found to be 15.237 ± 0.675 µg/mg of tissue whereas the amount of protein in mated males significantly decreased to 10 ± 0.673 µg /mg of tissue.

7. SDS- PAGE profile of proteins in ASGs of virgin and mated male insects were analyzed. It was found that staining intensity of some peptides of mated insects considerably less compared to that of virgin males. Moreover 3 peptides were absent in the electrophorogram of mated males.
Fig 55: SDS-PAGE Profile of ASG Proteins in virgin and mated males

Lane 1: Virgin
Lane 2: Mated
Lane 3: Molecular weight markers