STUDIES ON THE ACCESSORY SEX GLANDS OF *SPODOPTERA MAURITIA* BOISD. (LEPIDOPTERA: NOCTUIDAE)

Thesis submitted
to the University of Calicut
in partial fulfilment of the requirements for the
Degree of
DOCTOR OF PHILOSPHY
in Zoology

By

Thanuja A. Mathew, M.Sc., M. Phil.

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT
KERALA, 673 635, INDIA

2008 September

DEPARTMENT OF ZOOLOGY

UNIVERSITY OF CALICUT

Tel: Office : (0494) 2401144 ext. 419
HOD : (0494) 2401144 ext. 420
Fax : 0494 2400 269
Calicut University P.O., 673 635, Kerala

Dr. V.S.K. Nair, Ph.D.
Professor (Rtd.)

Date: .....

CERTIFICATE

This is to certify that this is an authentic record of the research work carried out by **Mrs.Thanuja A. Mathew**, **M.Sc., M.Phil.**, from September 2004 to September 2008 as a full-time student in partial fulfilment of the requirements for the Degree of **DOCTOR OF PHILOSOPHY** in the Faculty of Science of the University of Calicut under my supervision and guidance. No part of this thesis has been presented before for any other degree. I also certify that she has passed the M.Phil Degree examination of the University of Kerala in 1997 and has registered for Ph.D under M.Phil./Ph.D Integrated course of Calicut University in 2004.

V.S. KRISHNAN NAIR

DECLARATION

I do hereby declare that the present work is original and it has not previously formed the basis for the award of any degree or diploma.

Calicut University,
September, 2008

Thanuja A. Mathew

ACKNOWLEDGEMENTS

I wish to express my heartfelt gratitude and indebtedness to Dr. V.S. Krishnan Nair, Professor & Head (Retd.), Department of Zoology, University of Calicut for his supervision and valuable guidance throughout the period of my work. I am extremely indebted to him for the efforts taken by him for the correction of this manuscript.

The funding of this work has been met from the major research project sanctioned to me by Department of Science and Technology (DST), Govt. of India, New Delhi under Women Scientist Scheme -'A' (WOS-'A'). The genesis of the research problem as well as the preparation of the project proposal was done under the supervision of Dr. V.S. Krishnan Nair for which I am thankful to him and I express my gratitude to DST, New Delhi. I thank Dr. Gayathri, Assistant Professor, NIMHANS for permitting me to utilize the Electron Microscope facilities. I also thank DR. Chandrasekhar Sagar (Assistant Professor, NIMHANS), Mrs. B.N. Hemavathy (Senior Technician, NIMHANS) and Mr. Ramesh (Technician, NIMHANS) for the technical assistance received from them.

I would like to express my sincere thanks to Dr. Gokuldas, Professor and Head of the Department of Zoology and Dr. K. P. Janardanan, Professor and former Head of the Department of Zoology, University of Calicut for providing the necessary lab facilities. I am immensely thankful to Dr. Kannan, V.M, Reader, Department of Zoology for the valuable help extended to me in Electrophoretic studies and my colleague, Mrs. Sakunthala, C (Research Fellow, FIP) for the support and help extended to me in times of need.

I express my thanks to Mr. V. Lohithakshan for the help in the rearing of the insect colony, Mr. K.Balu, Mr. Vimesh and Mr. Rajesh, Bina Photostat & DTP for the help received for the

preparation of the manuscript.

I am greatly obliged to my parents, husband and other family members for their care and co-operation extended to me throughout my work and especially thankful to my children Rachel and Ruth for their love and understanding though they had a hard time during my period of work. Above all I owe to Almighty Lord for showing the right path throughout my life.

Thanuja A. Mathew

CONTENTS

		Page
CHAPTER 1	**GENERAL INTRODUCTION**	
1.1	Insect Reproductive System	1
1.2	Reproductive structures involved in secretory processes	2
1.3	Reproductive structures involved in secretion in male insects	3
1.3.1	Gonadal glands	3
1.3.2	Ductal glands	4
1.3.3	Seminal Glands	6
1.3.4	Collateral glands	8
1.4	Reproductive structures involved in secretion in female insects	8
1.4.1	Gonadal Glands	8
1.4.2	Ductal glands	8
1.4.3	Spermathecal Glands	9
1.4.4	Collateral glands	9
1.5	Accessory sex glands: An Overview	9
1.6	Role of hormones in the development and differentiation of ASGs	15
1.7	Insect Growth Regulators	16
1.8	IGRs based on hormones	17
1.9	Objectives of the investigation	23
CHAPTER 2	**PEST STATUS, REARING AND BIOLOGY OF *SPODOPTERA MAURITIA* BOISD. (LEPIDOPTERA: NOCTUIDAE)**	
2.1	Pest Status of *S. mauritia*	26
2.2	Rearing and maintenance of the larvae of *S. mauritia*	27
2.3	Biology of *S. mauritia*	27
2.4	Experimental animals	31
CHAPTER 3	**HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES ON THE ACCESSORY SEX GLANDS OF *SPODOPTERA MAURITIA***	
3.1	Introduction	33
3.2	Materials and Methods	34
3.3	Results	38
3.3.1	Histomorphology of male ASGs	38
3.3.2	Histomorphology of female ASGs	39
3.3.3	Ultrastructural studies of ASGs	40
3.3.3.1	Ultrastructural studies of male ASGs	41
3.3.3.2	Ultrastructural studies of female ASGs	45
3.4	Discussion	47
3.5	Summary	68

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 71

4.2 Materials and Methods 72

4.3 Results 76

4.3.1 Development of ASGs 76

4.3.2 Changes in the protein content in virgin and mated male ASGs 78

4.3.3. Electrophoretic profile of ASG proteins in virgin and mated male insects 78

4.3.3.1 SDS-PAGE pattern of proteins in ASGs of virgin male insects 78

4.3.3.2 SDS-PAGE pattern of proteins of ASGs of mated male insects 79

4.4 Discussion 80

4.5 Summary 88

CHAPTER 5 ROLE OF HORMONES AND EFFECTS OF HORMONE AGONISTS ON DEVELOPMENT AND DIFFERENTIATION OF ACCESSORY SEX GLANDS

5.1 Introduction 90

5.2 Materials and Methods 91

5.3 Results 94

5.3.1 Effects of PPN on the pupal- adult metamorphosis 94

5.3.2 Effects of PPN on development and differentiation of male ASGs 94

5.3.3 Ultrastructural studies on male ASGs of PPN treated insects 96

5.3.4 Effects of PPN on development and differentiation of female ASGs 97

5.3.5 Ultrastructural studies on female ASGs of PPN treated insects 98

5.3.6 Effects of PPN on newly ecdysed adult males 99

5.3.7 Changes in the protein content in treated male ASGs 99

5.3.8 Electrophoretic profile of proteins in the ASGs of PPN treated male insects 100

5.3.8.1 SDS-PAGE pattern of proteins in ASGs of 0.1 µg PPN treated insects 100

5.3.8.2 SDS-PAGE pattern of proteins in ASGs of 20 µg PPN treated insects 100

5.3.9 Effects of methoxyfenozide on the morphogenesis of ASGs 101

5.3.9.1 Effects of 3 µg methoxyfenozide on the ASGs of day 0 (tanned) pupae 101
5.3.9.2 Effects of 30 µg methoxyfenozide on the ASGs of day 0 (tanned) pupae 102
5.4 Discussion 102
5.5 Summary 114

REFERENCES 117

CHAPTER 1

GENERAL INTRODUCTION

Abounding almost every where and feeding on plants many insect pests transform millions of tons of valuable plant matter into animal matter and wastes. One important reason for the domination of insect pests in this biosphere has been attributed to their remarkable ability for successful insemination and subsequent ovipositional activities that are primarily controlled by accessory sex glands (ASGs). ASGs occur as secondary structures associated with the primary reproductive organs to ensure the reproductive success of the species either by facilitating the transfer or by protecting the gametes.

1.1 Insect Reproductive System

The male reproductive organs typically consist of a pair of testes connected to a pair of seminal vesicles and a median ejaculatory duct. In some Lepidoptera the two testes are fused completely to form a median structure. The testes are composed of a number of follicles. Each testis follicle is connected to tubular vas deferens by means of a short vas efferens. The vas deferens runs backwards to lead into the distal end of the ejaculatory duct. In many insects the seminal vesicles in which the sperms are stored before transfer to the female are dilations of the vasa deferentia. The ejaculatory duct extend inwards as an unpaired duct. This is called simplex. Its anterior end

known as duplex is dilated and bifurcated. This is connected with the ASGs and vasa deferentia. In Lepidoptera sperms are temporally stored in the expanded regions of the vasa deferentia and then are transferred to duplex.

Among the various insect orders, the external shape and morphology of organs in the female genital tract are variable. In female insects the reproductive system consists of a pair of ovaries. Each ovary is composed of several ovarioles that lie loose in the body cavity. The ovarioles are joined at their distal end by an apical filament that is connected to the body wall, and proximally by the lateral oviducts. The number of ovarioles per ovary is species specific. Each ovariole has a germarium at its distal tip where oogonia undergo meiosis to produce oocytes. Each oocyte becomes surrounded by a single layer of follicle cells that in the early stages of oocyte growth controls the incorporation of vitellogenin, and later secretes the egg shell around the mature egg. The lateral oviducts merge to form a median oviduct opening posteriorly into a genital chamber. Sometimes the genital chamber forms a tube, the vagina and this is often developed to form a bursa copulatrix for reception of aedeagus from the males. Associated with the median oviduct a single spermatheca with spermathecal gland and paired ASGs are seen.

1.2 Reproductive structures involved in secretory processes

Within the class Insecta the form and relative function of various glands that participate in the reproductive process are almost as diverse as the

insects themselves. In many species of insects the structures associated with the reproductive system are involved in different kinds of secretory processes. They include:

(1) Gonadal glands comprising glands of testes and glands of ovaries.

(2) Ductal glands including glands of the vasa deferentia, ejaculatory duct, lateral oviduct, common oviduct and vagina including bursa copulatrix and spermatophoral receptacle.

(3) Seminal glands

(4) Spermathecal glands

(5) Collateral glands

1.3 Reproductive structures involved in secretion in male insects

1.3.1 Gonadal glands

The principal function of the glandular cells in the testes is to supply nutrients to the differentiating germ cells. In the testes of many insects including Lepidopterans a large cell (Verson's cell) in the apex of germarium assumed to have a nutritive function has been found. In *Dielphia euphorbiae* at the time of maturation of spermatids in the late larval and pupal period a maximum number of granules and droplets are observed in the cells of the inner lining of testicular sheaths (Buder, 1917). Nutritive function for

Verson's cell of testes is reported in *Bombyx mori* (Omura, 1938). Nurse cells of testes are involved in the secretion of a mucoprotein which forms the caps in the sperm heads (Szollosi, 1974). Testes are also found to secrete chemicals during insemination.

1.3.2 Ductal glands

They include glands of vasa deferentia and ejaculatory duct. They vary from primitive secretory epithelial cells to compact glands in nature. Secretory cells of the ejaculatory duct may have either ectodermal or mesodermal origin. Ectodermal origin of ejaculatory duct is reported from *Locusta migratoria* (Gregory, 1965); *Drosophila melanogaster* (Bairati, 1968); *Melolontha melolontha* (Landa, 1960) and *Chironomus plumoses* (Wensler and Rempel, 1962). When the ejaculatory duct includes a mesodermal component, the epithelial cells of this component are almost always secretory. Species with mesodermal secretory cells are found in the ejaculatory duct of *Diadromus pulchellus* (Rojas Rousse, 1972), *Plecia nearctica* (Trimble, 1974) and in all Lepidoptera (Norris, 1932; Musgrave, 1937; Omura, 1938; Callahan and Cascio, 1963; Reimann and Thorson, 1976, 1979; Lai-Fook, 1982). In Lepidoptera the mesodermal ejaculatory duct component includes the paired 'duplexes' and a median noncuticular simplex. The simplex is divisible into distinct regions on the basis of the secretions it contains and the histology of the cells. In

ejaculatorius duplex of *Spodoptera litura* apocrine mode of secretion with occasional merocrine or holocrine secretion is observed (Amaldose, 1987). Leopold (1970) concludes that the nature of ejaculatory duct secretions in *Musca domestica* is proteinaceous and contain a high proportion of dibasic aminoacids. Thibout (1971) demonstrated the presence of proteins, mucopolysacharide and lipid glycoproteins in *Acreolepia assectella*.

Various functions have been ascribed to the ejaculatory duct secretions of insects. Wensler and Rempel (1962) have reported that in insects like *Chironomus* where the collateral glands are absent, the ejaculatory duct secretes the components of seminal fluid. Sheehan *et al.*, (1979) and Stein *et al*., (1984) reports the presence of carboxylesterase enzyme in the anterior ejaculatory duct of *D. melanogaster* which appears to be involved in the sperm motility perhaps by facilitating metabolism of lipids in the ejaculate (Gilbert, 1981).

In Lepidoptera secretions of the noncuticular simplex contribute along with those from collateral glands for the formation of the spermatophore. Several authors have shown that the non-cuticular simplex of Lepidoptera secretes a sperm activator (Omura, 1938; Shepherd, 1974; Herman and Peng, 1976). The ejaculatory duct of *Musca domestica* produces a secretion which inhibits receptivity in females (Reimann *et al*., 1967).

Secretory cells of vas deferens vary considerably in shape, size and

stainability among species or in different parts of the duct in the same species. The biochemical nature of secretory product of the vas deferens is not well understood. Cantacuzene (1968, 1971) and Rojas- Rousse (1972) have identified the secretion as a mucopolysacharide. Gerber *et al*., (1971) on the other hand state that the secretion is a carbohydrate-protein complex, but does not include glycogen or chitin.

Landa (1959) have observed that in *Melolontha melolontha* the secretion of vas deferens is used for growth of the cyst cells which are later transferred to the female ducts along with the spermatophore. Bouix (1966), Rojas –Rousse (1972) and Gerber *et al*., (1978) speculate that the secretion of vas deferens is used to nourish sperm while they are being retained in the male genital tract. Involvement of the vas deferens in spermatophore production has been clearly demonstrated in some Trichoptera (Khalifa, 1949) and in *Lytta nuttali* (Gerber *et al*., 1971). In Tettigonoids (Orthoptera) the material which binds sperm together as a spermatodesma is secreted by cells of the intratesticular region of the vas deferens.

1.3.3 Seminal Glands

Seminal glands are glandular structures occurring in the sperm storage organs of male insects called the seminal vesicles. They are absent in Trichoptera. The seminal vesicles are simply dilations of the vas deferens in Thysanura, Ephemeroptera, most Hemiptera, Neuroptera and in some

Hymenoptera. While in other Hymenoptera and nematocerous Diptera, they are dilations of the ejaculatory duct. Seminal glands are not secretory in all species. In most species they are of mesodermal origin. Ectodermal origin of seminal glands is reported in nematocerous Diptera. In Lepidoptera, there are two sites of storage. With in each vas deferens is a swollen region which is normally referred to as the true seminal vesicles. In *Anagasta kuhniella,* the upper part of each branch of the the ductus ejaculatorius duplex act as seminal vesicles (Reimann and Thorson, 1976).

The histology of the seminal vesicles is basically similar to that of other parts of efferent duct. In almost all species examined to date the epithelium of the seminal vesicles apparently functions as a store for sperm. In most species however the epithelium is secretory, at least temporarily and is therefore columnar, though when the seminal vesicles replete with sperms cells often take a more flattened appearance. The nature of seminal vesicle secretion in *Schistocerca gregaria*, studied by Cantacuzene (1967) showed that it primarily contains proteinaceous granules which are later replaced by acidic mucoplysacharides. The seminal plasma of *Periplaneta americana* contains much glycogen and phospholipids, other unidentified PAS positive substances and a small amount of proteins (Vijayalakshmi and Adiyodi, 1973).

1.3.4 Collateral glands

Collateral glands are paired glandular structures which in most species release their product into the common genital tract at its anterior end or at some point along its length. They include ASGs, prostate glands, the mushroom shaped glands and the congoblate glands of cockroach.

1.4 Reproductive structures involved in secretion in female insects

1.4.1 Gonadal Glands

Glandular cells involved in secretory process include nurse cells and follicular epithelium of ovaries. They help in the nourishment of the developing gametes, in the production of yolk components, secretion of chorion and in the formation of vitelline membrane. The follicular cells secrete, at different times throughout oocyte development, a variety of material. In *Nepidae* the follicular epithelium secrete a cementing substance for gluing the eggs to the substratum (Hinton, 1961).

1.4.2 Ductal glands

It includes glands of the lateral oviduct, common oviduct and vagina. The epithelial cells of lateral and common oviduct have a secretory function. Lateral oviduct secretions have several functions including ootheca formation, lubrication of eggs passing through the genital tract, cementing the eggs to each other and to the substrate as observed in *Lytta* (Sweeny *et al.*, 1968;

Gerber *et al*., 1971). In *Acrididae* the common oviduct secretion forms an extra chorion around the egg (Hartley, 1961). Although the vagina in most species is nonsecretory, the diverticulum at the anterior end of vagina called 'Bursa copulatrix' has a secretory function. Spermatophore is digested and absorbed in the bursa in *Melolontha melolontha* (Landa, 1960) and in *Lytta nuttali* (Gerber *et al*., 1971). Khalifa (1949) observed that a bursal gland might provide nourishment for the sperm in the absence of spermatophore.

1.4.3 Spermathecal Glands

In many insects, the spermatheca serves both as a sperm storage structure and as a secretory organ. In many species, storage and glandular functions are physically separated through the development of one or more spermathecal glands. Secretion of spermatheca provides nutrients for the sperm.

1.4.4 Collateral glands

Collateral glands in female insects include ASGs, colleterial (cement) glands of cockroaches and Milk glands of tsetse flies.

1.5 Accessory sex glands: An Overview

The classification of ASGs in insects is essentially based on anatomical and ontogenic relationships. In different groups of insects these glands vary considerably in size, shape, number, anatomical placements and

embryological origin (Blain and Dixon, 1973; Ramalingam, 1974; Adiyodi and Adiyodi, 1975; Leopold, 1976; Happ, 1984; Couche and Gillott, 1990; Chapman, 1998; Ferreira *et al*., 2004). They may occur as heterogeneous, unpaired structures as in Dictyoptera, as multiple paired structures as in the Thysanoptera (Shaaya, 1933) and Coleoptera (Escherich, 1894) or just as paired structures which is most common. ASGs are primitively absent in Thysanura, Ephemeroptera, Plecoptera, Dermaptera and in most Odonata but in many higher Diptera they are secondarily lost.

ASGs vary from a simple tube, identical to other conductive channels of the reproductive tract to histologically complex tubes with regional differentiation as occurs in most lepidopterans (Riemann and Thorson, 1979; Lai-Fook, 1982). Anatomically ASGs of most male insects possess a single glandular epithelium surrounding a lumen filled with secretion. Outer to the epithelium either a single or a double layer of muscle layer is seen (Adiyodi and Adiyodi, 1974; Lai-Fook, 1982; Couche and Gillott, 1990; Fernandez and Cruz-Landim, 2005; Cruz-Landim and Dallacqua, 2005).

ASGs show remarkable uniformity in terms of both their embryonic origin and their general cytology. In males ASGs are of mesodermal in origin and are described as mesadenia. Specifically they arise from the terminal ampullae of the vasa deferentia which themselves are derived from the coelomic cavities of the ninth or tenth abdominal segment. Throughout the

larval period the mesadenial anlagen remains in an embryonic condition i.e., small hollow vesicles attached to the mesodermal cords which later become the vasa deferentia. Ectadenia which opens into the ejaculatory duct are found in Orthoptera and in many other insects. In some species of Heteroptera and Coleoptera, both ectadenia and mesadenia are present. In endopterygotes organogenesis takes place during the pupal stage. In *Bombyx mori* ASGs are fully differentiated during eclosion whereas in *Tenebrio molitor* differentiation is not completed until several days after adult emergence (Gillott and Gaines, 1992).

Numerous studies have demonstrated that ASGs play an essential role in reproduction. In most insects ASGs become functional in adults. Several functions have been attributed to the secretion produced by ASGs. The functions of ASGs can be classified as structural, biochemical, behavioural and physiological (Fernandez and Cruz-Landim, 2005).

Early histochemical studies showed that the ASG secretion is a complex mixture of proteins, often conjugated with lipid or carbohydrate moieties, free lipids, carbohydrates, prostaglandins, amines and cGMP, uric acid aminopeptidases, free amino acids and hydrolytic enzymes like esterases, amidases etc (Roth, 1967; Cmelik *et al*., 1969; Leopold, 1981; Federer and Chen, 1982; Judd *et al*., 1983; Sevala and Davey, 1991; Muse and Balogun 1992; Smid *et al*., 1992).

The ASGs produce secretions with a variety of functions, including contribution to the seminal fluid and activation of the spermatozoa (Davey, 1985; Chen, 1984). The primary function of the secretion produced by the male ASGs is spermatophore formation. ASGs are involved in the building of spermatophore for sperm transfer to the female (Viscuso, *et al*., 2001). Spermatophore of Lepidoptera is formed wholly within the female ducts after the start of copulation. The secretion from ASGs form the outer matrix of spermatophore and form the spermatophragma which blocks the duct to the female's bursa copulatrix (Osanai *et al*., 1987; Fanger and Naumann, 1993). Male insects often transfer a number of auxiliary substances to females during copulation. Male *Drosophila* transfer seminal fluids which, among other things, stimulate egg laying (Kubli, 1996). Males may also transfer nutrients to females which are subsequently incorporated into somatic maintenance or reproductive output (ova) (e.g. Simmons, 1995), and for some Diptera it has also been suggested that spermatozoa additionally function as nutrient provisioning (Pitnick and Markow, 1994). Importantly however, many theoretical and empirical studies indicate that it is the interactions between male and female characteristics which determine the outcome of many reproductive processes (Knowlton and Greenwell, 1984; Rice, 1996; Zeh, 1997; Otronen *et al*., 1997; Wilson *et al*., 1997; Holland and Rice, 1998; Hosken and Stockley, 1998). The secretion changes the female reproductive behaviour and physiology after copulation. (Chen, 1984; Happ, 1984; Gillott,

1996; Herndon *et al*., 1997; Smid, 1997; Wolfner, 1997; Chen *et al*., 1998; Heifetz *et al*., 2001). Fecundity enhancing and receptivity-inhibiting substances have also been reported in the secretion of ASGs (Gillott, 2003).

In females, ASGs are found in Thysanura, Odonata, many Orthopteroid insects, Thysanoptera, Homoptera and most endopterygotes. Unlike male insects they are absent in Orthoptera, Psocoptera, Heteroptera and in most Coleoptera (Mustuda, 1976). In most insects they are paired structures and join the common genital tract at a point behind the opening of spermathecal duct. The glands normally originate from an invagination on the ninth abdominal sternum. The anlagen remain small throughout most of the larval period with organogenesis beginning during the last juvenile stadium.

Though the ASGs differ in form among female insects, their histology is quite uniform and includes from inside to outside, a chitinous intima, one or two layers of cells, and a basement membrane. A layer of muscle outside the basement membrane may or may not be present. The presence of chitinous intima explains the ectodermal origin of ASGs as reported earlier (Gillott, 1988; Kaulenas, 1992). Though the morphology of ASGs vary among different insect orders, and also within a limited group of insect species, their internal structure depends upon the function of the organs (Brunet 1952; Gillott 1988; Kaulenas 1992). Synthesis of proteins, lipids and glycogenous polysaccharides are reported from the ASGs of female lepidopterans

(Salkeld and Potter, 1953; Beament and Lal, 1957; Grayson and Berry, 1974; Geetha, 2003).

The function of ASGs of female insects varies (Davey, 1985). ASGs produce oviposition pheromone, secretions which coat and fasten eggs to laying substrates, silk to form egg cocoon, provide lubrication, egg protection, dissolve spermatophores and provide nutrition for the young larvae. In *Musca domestica* ASG secretions are moved with spermatozoa to the fertilization chamber where they aid micropyle cap removal, allowing fertilization to take place (Leopold and Degrugillier, 1973; Leopold *et al*., 1978). Furthermore, female ASG secretions trigger the acrosome reaction when present with micropylar cap substance and, in higher concentrations, cause degradation of spermatozoa (Degrugillier, 1985). However in some insects female ASGs are typically adhesive-producing (Lococo and Huebner, 1980). The other functions of the secretions are relatively unknown, especially when compared with male ASGs and their secretions (Chapman *et al*., 1995; Fernandez and Klowden, 1995; Kubli, 1996; Rice, 1996; Soller *et al*., 1997; Tram and Wolfner, 1998). 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.

1.6 Role of hormones in the development and differentiation of ASGs

Previous studies show that in most insects including lepidopterans the post-embryonic development and differentiation of ASGs are regulated by the interplay of two major insect hormones; juvenile hormone (JH) and ecdysteroids, the former inhibiting and the latter promoting these processes. Growth and protein synthesis in the ASGs are regulated by both ecdysteroids and juvenile hormone (JH), with development and differentiation being under the control of ecdysteroids and protein secretion being regulated by JH in Lepidoptera (Herman, 1973; Herman and Bennett, 1975; Herman and Dallmann, 1981). In males of *Bombyx mori* and *Tenebrio molitor* ecdysteroids were found stimulating the development of ASGs during the pupal period but acting antagonistically during the adult stage (Shinbo and Happ, 1989; Yaginuma and Happ, 1989). In the Lepidopteran *Heliothois virescens* (F), the differentiation of ASGs from the genital imaginal discs requires the presence of both a sufficient titer of ecdysteroids and testis sheath factors (Loeb, 1991).

In contrast to this, post- eclosion activity (i.e. production of secretion) of ASGs for most species is regulated by JH. In male moths of *Ephestia cautella* ecdysteroid titres are relatively low throughout their adult life (Shaaya *et al*., 1991). The allatectomy inhibit post-eclosion growth of ASGs in *Danaus Plexippus* L. (Herman, 1975, 1975). The differences in JH titres affect reproductive output (Trumbo and Robinson, 2004). A rapid increase in

the JH titre in the newly eclosed adults is reported in *Drosophila melanogaster* and is a probable key feature in the maturation of gametes and testes (Bownes and Rembold, 1986).

Many authors have reported hormonal regulation of ASG secretory activity in female insects (Ejeze and Davey, 1974, 1976, 1977; Koepp *et al*., 1985; Davey, 1985). Specifically JH inhibits and ecdysteroids promote differentiation of ASGs (Bodenstein and Sprague, 1959). With regard to the endocrine control of collateral gland secretion in Lepidoptera. Several authors have reported that allatectomy or head/neck ligation prevented normal development of the female glands (Herman, 1975; Herman and Bennet, 1975; Herman and Dallmann (1981) and Lessman *et al*., (1982). Herman and Barker (1976) reported that a single large dose of ecdysterone stimulated gland development in monarch butterflies.

1.7 Insect Growth Regulators

Insect growth regulators (IGRs) are insecticides that mimic the action of hormones on the growth and development of insects. The influence of hormones in an insect's life cycle and reproduction is the centre point in the development of IGRs which act as hormone agonists or antagonists. These compounds induce a disruption of the normal growth and reproduction of insects. IGRs with their reduced toxicity to the environment and target specificity are highly advantageous when compared to conventional

insecticides in integrated pest control strategies. They have a good margin of safety to man and domestic animals and to other most non target biota including invertebrates, fish, birds and other wild life. The effectiveness and selectivity of IGRs provide new tools in Integrated Pest Management (IPM) Programmes (Oberlander and Silhacek, 1998).

There are three categories of IGRs

(1) Compounds which directly or indirectly influence the hormones which regulate post embryonic development, metamorphosis and reproduction of insects e. g. Juvenile hormone (JH) analogues and anti-JH agents

(2) Compounds which inhibit cuticle formation through an effect on cuticle synthesis e.g. benzoyl phenyl ureas

(3) Compounds with miscellaneous modes of action e.g. azadirachtin.

IGRs based on insect hormones have great significance as pesticides of the future and also as excellent chemical probes to elucidate the role of hormones in the basic physiological processes of insects.

1.8 IGRs based on hormones

There are three major categories of insect hormones: neurohormones secreted by the neurosecretory cells of brain and segmental ganglia, Juvenile hormone (JH) secreted by corpora allata (CA) and ecdysteroids secreted by

the prothoracic glands and other tissues. Ecdysteroids and JH regulate many physiological events throughout the insect life cycle including moulting, metamorphosis, ecdysis, diapause, reproduction and behaviour (Gelman *et al*., 2007). A critical titre of hormones in body fluids is a prime requirement in different physiological processes of insects. Any interference in the biosynthesis and degradation of hormones will disrupt the hormone dependent physiological processes of insects. Further the regulation of secretion, transportation from the secretory to the target site, binding to the membrane receptors, degradation, excretion and feed back control are all biochemical steps vulnerable to manipulation for insect control purposes. Such manipulation of the hormonal levels in the haemolymph will cause a derangement of hormone dependent processes of morphogenesis and reproduction. Based on this concept many hormone analogues and antihormones have already been developed.

(i) IGRs based on neurohormones

Peptide hormones produced and released from the neurons play diverse functional roles in insects as chemical messengers controlling growth and development in insects. A number of neuropeptides are synthesized in the median and lateral neurosecretory cells of brain. IGRs based on neuropeptides for insect pest control is not well advanced since many neurohormones in insects have not been fully characterized. The diversity and complexity of

neurohormones however offer a lot of possibilities for design and development of neurohormone analogues.

(ii) IGRs based on ecdysteroids

Ecdysteroids are the steroid hormones of insects. Ecdysteroids control insect development, being known primarily as regulators of moulting and metamorphosis, but they have also been implicated in the control of many other physiological and developmental processes e.g. reproduction and embryogenesis (Koolman, 1989). Ecdysone mimics or ecdysoids are compounds which are structurally similar to ecdysteroids and possess moulting hormone activity in insects. They are classified into four groups. Zooecdysteroids, phytoecdysoids (extracted from plants), synthetic ecdysoids (steroids with moulting hormone activity) and nonsteroidal agonists. There have been a number of studies on the effect of ecdysone analogues/agonists on the reproduction of important pest species (Carpenter and Chandler, 1994; Smagghe and Deghlee, 1994; Biddinger and Hull, 1999; Knight, 2000). The ecdysone analogues/agonists are highly specific to lepidopteran larvae and their effectiveness against many economically important horticultural, agronomic and forest pests have been reported (Chandler *et al*., 1992; Charmillot *et al*., 1994; Retnakaran *et al*., 1997; Trisyono and Chippendale, 1997, 1998). Wing (1988) has suggested that the ecdysteroid analogues/ agonists would interact with the ecdysteroid receptor complex and thereby

induce their effects. The first bisacylhydrazine ecdysteroid agonist was discovered by Rom and Hass Company in 1983. Subsequent chemical modification of this compound led soon to the discovery of a slightly more potent analogue, RH- 5849 (Wing, 1988). Treatment of insects with minute doses of RH- 5849 interferes with normal feeding activity in larval lepidopterans and insects belonging to other orders, by forcing a lethal, premature moult (Wing *et al*., 1988; Sakunthala and Nair, 1995). Later another non-steroidal ecdysone mimic RH- 5992 (tebufenozide) was discovered and this compound was more potent than RH-5849 in lepidopteran larvae.

Methoxyfenozide (RH-2485) belongs to the novel class of IGRs, (bisacylhydrazine ecdysteroid agonists) mimicking natural ecdysteroids. They have same mode of action as the endogenous 20-hydroxyecdysone (20-H) but the effects are long lasting (Retnakaran *et al*., 1995). Dhadialla *et al*., (1998) have reported that RH-2485 has a selective action on lepidopteran insects. The other important ecdysteroid agonists or analogues are RH- 5849, Tebufenozide (RH-5992) and Halofenozide (RH-0345). N- tert – Butyl, N, N’ dibenzoylhydrazine and its analogues are nonsteroidal ecdysone agonists that exhibit insect moulting hormonal and larvicidal activities (Minackuchi *et al*., 2003).

(iii) Anti ecdysteroid Agents

Since ecdysteroids play a critical role in insect development, reproduction and embryogenesis, anti ecdysteroid agents which alter ecdysteroid titre have great potential as insecticides. The normal growth and development of *Manduca sexta* larvae can be inhibited by two vertebrate hypocholesterolaemic agents, triparanol and 22, 25 di-azacholesterol, by blocking the conversion of β-sitosterol to cholesterol which is a precursor of ecdysone synthesis (Svoboda *et al*., 1972).

(iv) IGRS based on JH

The major role of JH in insects is to modify the action of ecdysteroids and prevent the switch in the commitment of epidermal cells from larval to imaginal type. In the presence of JH, ecdysteroids are unable to promote the current program of gene expression. JH promotes sexual maturation and behaviour in mature insects. Williams (1967) was the first to suggest that this hormone or its analogues could be used as specific insect control agents. This led to the discovery of JH analogues or juvenoids with great potential in IPM programmes. Juvenoids functionally resembles JH but may or may not be similar in structure. Synthetic JH and JH analogues/ agonists (JHAs) have been shown to have sterilizing and toxic activities against many insects (White and Lamb, 1968; Lim and Yap, 1996; Parkman and Frank, 1998;

Liu and Chen, 2001; Rajapakse *et al*., 2002; Abo-Elghar *et al*., 2004; Lim and Leu, 2005; Ouchi, 2005; Liu and Trumble, 2005; Darriet and Corbel, 2006).

The well known juvenoids include Epofenonane (Hangartner *et al*., 1976) Methoprene, Hydroprene, Kinoprene (Henrick *et al*., 1976) Phenoxy phenoxy carbamate (Peleg, 1982) Fenoxycarb and Pyriproxyfen (PPN). They are highly effective IGRs that cause a wide range of developmental derangements in susceptible insect species affecting embryogenesis, larval development, metamorphosis and reproduction.

PPN (2-[1-methyl -2-{4-phenoxy phenoxy} ethoxyl] pyridine) is a potent JH agonist that is active in a wide range of arthropods including ants (Vail and Williams, 1995; Vail *et al*., 1996); fleas (Bull and Meola,1993); white flies (Ishaaya *et al*., 1994; Ishaaya and Horowitz, 1995), scale insects (Peleg, 1988); cockroaches (Koehler and Patterson, 1991); and lepidopterans (Smagghe and Deghlee, 1994). It is a relatively stable JHA with low mammalian toxicity (Yokoyama and Miller, 1991; Higbee *et al*., 1995; Abdallahi *et al.*, 2000). It was first registered in Japan in 1991 for controlling public health pests (Miyamoto *et al*., 1993). As seen with other JH agonists multiple effects were induced in a single species. The compound interferes with embryogenesis, oocyte production, emergence, metamorphic moult and causes morphological deformities ((Miller, 1989; Hatakoshi, 1992; Bull and Meola, 1993; Miller and Miller, 1994; Vennard *et al*., 1998). It has limited

bioaccumulative ability (Sahaefer *et al*., 1988; Sahaefer and Murba, 1990) and at present PPN is among the most frequently used pesticides.

(iv) Antijuvenile hormone agents

The limited scope of JHAs as insect control agents necessitated the discovery of compounds with anti JH activity. Anti JH agents disrupts the normal development of early larval instars and inhibits JH dependent reproductive activities (Sam Mathai and Nair, 1984a; Santha and Nair, 1986, 1988, 1991; Santha *et al*., 1987; Nair, 1993). Some well known examples of anti JH agents are Precocenes, Fluromevalonolactone (FMev, ETB, EMD, Compactin, Piperonyl butoxide, Allylic alcohols, Bisthiolcarbamate etc. Anti JH agent, precocene causes JH deficiency in treated insects by selectively destroying the parenchymal cells of corpora allata (Unnithan *et al*., 1977).

1.9 Objectives of the investigation

From the foregoing review it is evident that ASGs play a crucial and critical role in the reproductive biology of insects. The development and differentiation of ASGs takes place in the pupal-adult metamorphosis under hormonal regulation. ASGs have been subjected to extensive investigations in many insect orders. However studies of ASGs of lepidopteran insects are comparatively few. Hence it was thought worthwhile to have an extensive analysis of ASGs of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) at morphological, histological and ultrastructural levels. Further studies on the

development and differentiation of ASGs during pupal-adult metamorphosis of *S. mauritia* have been carried out. Also a few studies on the influence of mating on the secretory activity of ASGs have been looked into. In addition an elaborate study on the effects of two IGRs with hormonal activity on the development, differentiation and secretory activity of ASGs has also been analyzed.

S. mauritia is a pest of paddy in Kerala. This species is chosen for the present study due to the availability of a sizable background data from this laboratory on the effects of insect growth regulators with hormonal and antihormonal activity on larval development, metamorphosis and reproduction of this insect (Nair, 1981, 1993; Sam Mathai and Nair, 1983, 1984a,b; Santha and Nair, 1986, 1987, 1988; Santha *et al*., 1987; Nair and Rajaleksmi, 1989; Pradeep and Nair, 1989; Balamani and Nair, 1989a,b, 1991, 1992; Jagannadh and Nair, 1992, 1993; Sakunthala and Nair, 1995; Venugopalan *et al*., 1994; Benny and Nair, 1999; Safarulla *et al*., 2003; Sindhu and Nair, 2004; Pradeep and Nair, 2005).

The effects of treatments of IGRs on the histomorphogenesis of ASGs, development and differentiation of ASGs and the secretory activity of ASGs of adult male *S. mauritia* are dealt with the present study. It is hoped that the results of this investigation will lead to a better understanding of endocrine regulation of development and differentiation of ASGs as well as will provide

valuable information concerning the potential of IGRs in pest control strategies.

Chapter 1 deals with a detailed review of ASGs, their classification, structure, function and hormonal regulation

Chapter 2 provides basic information on the pest status and a detailed account of the rearing and maintenance of *S. mauritia* Boisd. (Lepidoptera: Noctuidae) under laboratory conditions.

Chapter 3 has given emphasis on the structural details of ASGs of adult male and female *S. mauritia* utilizing histological and ultrastructural techniques.

Chapter 4 deals with the development and differentiation of ASGs during pupal- adult metamorphosis and preliminary studies on how mating influences the secretory activity of male ASGs utilizing biochemical procedures.

Chapter 5 examines the effect of two IGRs on the histomorphogenesis, ultrastructure and the secretory activity of *S. mauritia.*

HAPTER 2

PEST STATUS, REARING AND BIOLOGY OF *SPODOPTERA MAURITIA* BOISD. (LEPIDOPTERA: NOCTUIDAE)

2.1 Pest Status of *S. mauritia*

Spodoptera mauritia Boisd. (Lepidoptera: Noctuidae) popularly known as the paddy swarming caterpillar or paddy army worm is a major pest of *Oryza sativa* and is distributed all over India. The insect usually occurs on rice from July to September. In general, nursery and early growing stages of paddy are attacked by this pest. The larval stages are seen to migrate from one field to another. Appearing in swarm of thousands, they destroy whole seedlings of a particular field and then march to the next field. Hence the pest is referred to as the 'army worm'. The paddy fields destroyed by this pest appear as though grazed by cattle. The larvae feed on leaves, leaving behind only mid ribs. If the outbreak is severe, serious damage results, leading to a heavy loss of crop. It is estimated that the loss in yield caused by larval infestation of *S. mauritia* range from 10 to 20%. The status of the pest is further complicated by their ability to migrate to alternate host plants like *Ischaemum aristatum* during off- season periods, at the end of which they make a full scale come back to the nursery stages of paddy. *Spodoptera* sp. has been widely utilized in physiological, biochemical and endocrinological studies and its position being tenth in the group of insect species often used

for research.

2.2 Rearing and maintenance of the larvae of *S. mauritia*

The female adult moths are usually attracted to fluorescent lamps during night. They are collected using an insect sweep net. The moths are then brought to the laboratory and kept in glass chimneys closed at both ends with muslin cloth. They are fed with 10% solution of honey soaked in cotton swabs. The females laid eggs on the cloth (Pl. I: Fig. 1). The eggs hatched after three days of incubation.

The larvae were fed daily with fresh, tender leaves of grass, *Ischaemum aristatum* collected from the paddy fields. The containers were kept clean and kept away from wind and intense light. The larvae were maintained at room temperature, RH 90 $\pm$ 3% and 12:12 light: dark photoperiod regime. When the larvae became larger in size, they were transferred to plastic troughs. During dry season, the cloth covering the trough was moistened occasionally. The pupae were segregated and kept in separate glass beakers for adult emergence.

2.3 Biology of *S. mauritia*

The *S. mauritia* larvae under laboratory conditions underwent six larval instars and developed at a uniform rate.

1. First instar larva

The first instar larvae descended by means of silken thread to the base of the container. They were fed with tender leaves of fresh grass. The first instar larvae were characterized by the presence of large black head capsule and light green coloured body. They had small wart like tubercles throughout the body. These tubercles possessed small setae. They descended by means of silken threads to the tender leaves supplied for feeding. The larvae fed only the green part of the leaves leaving behind the veins. The larvae moved in a characteristic leaping manner. The larvae measured 1 mm in length and 0.5 mm in width. Duration of the instar was 2-3 days.

2. Second instar larva

Second instar larvae were pale green in colour and were characterized by three white longitudinal lines on the dorsal surface of the body extending from prothorax to the last abdominal segment. Two pairs of white lines were also present on the sides of the body. Second instar larvae also retained the wart like tubercles throughout the body. These larvae also utilized silken threads for descending as in the case of first instar larvae. Body was about 2.5 mm in length and 0.5 mm in width. Duration of the second instar was about 2-3 days.

3. Third instar larva

The third instar larvae were pale green in colour and possessed three white longitudinal lines, one on the dorsal side and the other two on the lateral sides. The third instar larvae possessed characteristic dark reddish superspiracular lines extending from anterior to posterior end. The wart like tubercles present in the second instar disappeared and the larvae no longer used silken threads to descend. Fully grown larvae showed an average length of 6 mm and a width of about 1 mm. The duration of the instar was about 2-3 days.

4. Fourth instar larva

Fourth instar larvae were greyish-black in colour. The three longitudinal lines became dull white in colour. The two lateral reddish black lines were still visible. Black intermittent dots on each segment arranged dorso-laterally along the length of the body. The dorsum of the larvae was paler than the supraspiracular area. Fully grown larvae showed an average length of about 15 mm and width of 2 mm. The larval period was about 2-3 days.

5. Fifth instar larva

The fifth instar larvae were greyish-black in colour (Pl. I: Fig. 2). The larvae were characterized by double rows of prominent black triangular

markings on the dorso-lateral side bordered with narrow white stripes. The supraspiracular stripes became pinkish in colour. The total length of the fully grown larvae was about 20 mm and width was about 4 mm. The larvae fed voraciously. The fifth instar larval period was about 3 days.

6. Sixth instar larva

The sixth instar larvae were greyish black in colour. The triangular markings became wider and darker than those of the fifth instar larvae. Sixth instar larvae fed voraciously during the first three days. Fully grown larvae had an average length of 37mm and width of 6 mm (Pl. I: Fig. 3). The larvae stopped feeding, emptied their gut (Gut purge) and transformed into wandering stage by day 4. Wandering larvae showed average length of about 26 mm and a width of 4.5 mm (Pl. I: Fig. 4a). The wandering larvae transformed into prepupae by day 5 (Pl. I: Fig. 4b). The prepupal stage was characterized by a highly wrinkled body. Prepupae underwent pupation within 20-24 h. The body of prepupae measured 20 mm in length and 5 mm in width. Sixth instar larval period extended up to 6 days.

7. Pupal instar

Pupae were of obtect type and were dark brown in colour. The pupal body measured about 17 mm and had a width of 5 mm (Pl. I. Fig. 5). Adults emerged within 7- 8 days after pupation. Female pupae took 7 days, whereas males took one more day for eclosion to adults.

8. Adults

Adults were medium sized moths with a conspicuous spot on the forewings, which have wavy pattern on the fringe. The insects measured about 15 mm in length and had a wing span of 30-35 mm. Adult moths exhibited sexual dimorphism in their morphological characteristics. Males were dark greyish with white markings on forewings (Pl. I. Fig. 6b). They possessed large tufts of hair on the forelegs. Females lacked both the white markings and the tufts of hairs (Pl. I. Fig. 6a). Mating took place at night within 24 h after emergence. Eggs were laid 24 h after mating. Eggs were laid in masses of 100-500 each and were covered with buff coloured silken hairs. On the whole embryonic period lasted 2-3 days, larval period 19-23 days and pupal period 7-8 days.

2.4 Experimental animals

The pupae and adults used for various experiments were obtained from the laboratory stock culture, reared and maintained as described above. Insects were sexed in the fifth instar larval stage and reared separately. Experimental insects were reared in a similar manner as is the case with the laboratory stock culture. Prepupae were separated from the stock culture to obtain newly ecdysed pupae. Newly ecdyped pupae were characterized by the pale green colour. Soon the pupae changed in colour to light brown and finally dark brown which occurred due to tanning of the pupal cuticle. Adults

emerged after 7-8 days. The age of the pupae was abbreviated to day *n* where day 0 indicates the day of ecdysis to this stage. Newly ecdysed pupae were treated as day 0, pupae 24 h after moulting was considered as those of day 1 and so on.

CHAPTER 3

HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES ON THE ACCESSORY SEX GLANDS OF

SPODOPTERA MAURITIA

3.1 Introduction

In insects ASGs elicit a crucial and critical function in the reproductive biology through their secretion. Since the secretory function of ASGs is closely correlated to their structural peculiarities, detailed studies of ASGs of various insect species have been carried out at both light microscopic and electron microscopic levels (Musgrave, 1937; Omura, 1938 ; Anderson, 1950; Callahan and Cascio, 1963; Gregory, 1965; Odhiambo, 1966, 1969, 1970, 1971; Cantacuzene, 1967; Bairati, 1968; Tongu *et al*., 1972; Adiyodi and Adiyodi, 1974; Gerber, 1976; Adlakha *et al*., 1976; Gerber *et al.*, 1978; Ramalingam and Craig, 1978; Brits, 1978, 1979; Riemann and Thorson, 1979; Federer and Chen, 1980, 1982; Happ and Happ, 1982; Lai-Fook, 1982; Couch and Gillott, 1990; El-Zoheiry,1999; Marchini, 2003; Fernandez and Cruz-Landim, 2005; Dallai, 2008).

Based on the histological studies undertaken in various insect species, it is found that ASGs in male and female insects display more or less similar structure irrespective of their varied origin and morphological variation. Most

often male ASGs consist of a single glandular epithelium surrounding a fluid filled lumen (Adiyodi and Adiyodi, 1974; Lai-Fook, 1982; El-Zoheiry, 1999a,b; Fernandez and Cruz-Landim, 2005). Various authors have reported that in most female insects ASGs are composed of a single layer of glandular epithelium, cuticle secreting cells and a chitinous intima lining a fluid filled lumen (Brunet, 1952; Berry, 1968; Tobe *et al*., 1973; Bonnan fant-Jais, 1974; Ma and Denlinger, 1974; Szopa, 1982; Dallai, 2008).

According to earlier studies, the glandular epithelial cells of ASGs of both sexes are well equipped with elaborate protein synthetic machinery and are secretory in nature (Adiyodi and Adiyodi, 1974; Ramalingam and Craig, 1978; Lai-Fook, 1982; Federer and Chen, 1982; Szopa, 1982; Fernandez and Cruz-Landim, 2005). In order to analyse the functional dynamics of ASGs, a detailed histological and ultrastructural studies on the ASGs of both male and female *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) were undertaken.

3.2 Materials and Methods

Animals

Development and differentiation of ASGs of *S. mauritia* take place during the pupal-adult transformation and in the adults which possessed fully differentiated ASGs. In the present study ASGs of adult males and females of *Spodoptera mauritia* were utilized. Pupae belonging to both sexes were

obtained from laboratory stock culture (Chapter 2) and kept in separate containers for adult emergence.

Dissections

Adult insects were mildly anaesthetized in specimen tubes having a wad of cotton soaked in ether at the bottom. The insects on becoming immobile were taken out of these specimen tubes and pinned dorsal side up in a wax-lined petri dish filled with Insect Ringer solution (Ephrussi and Beadle, 1936). A longitudinal cut was made on the dorsal abdominal region. The left and right cuticular flaps were then pinned laterally on to the wax tray. The alimentary canal was detached from both ends and removed to expose the reproductive system. In male insects ASGs were seen as an extension of ductus ejaculatorius duplex of reproductive system with their distal ends floating freely in the body cavity. The proximal end of ASGs is cut from the ductus ejaculatorius duplex and transferred to a watch glass using a fine forceps. In female insects ASGs were seen as a pair of thin elongated glands below the spermatheca of the reproductive system. ASGs of female insects were detached from the posterior region of median oviduct using fine forceps and transferred to a watch glass.

Histological techniques

The ASGs were washed thoroughly in insect Ringer solution and fixed overnight in Bouin’s fluid. The glands were then washed for a long time in distilled water, dehydrated in grades of ethanol, cleared in methyl benzoate infiltrated and embedded in paraffin wax. Five micron thick sections were cut

using a Reichert Precision Rotary Microtome. Sections were stained in Harris haematoxylin-eosin, observed and photographed under Carl Zeiss Research Microscope provided with Axiovision software for image analysis.

Electron Microscopy

The ASGs of male and female adult insects were dissected in insect Ringer solution and transferred immediately into 3% gluteraldehyde fixative in 0.1 M phosphate buffer (pH between 7.2 and 7.4). The fixation was pursued in the cold (4ºC) and continued overnight. The glands were then washed thoroughly in 0.1 M phosphate buffer (pH 7.4), post- fixed in 1% Osmium tetroxide for 1-2 hrs at 4ºC, washed thoroughly in 0.1 M phosphate buffer and dehydrated in graded alcohols. Glands were en- bloc stained by immersing in a 2% solution of Uranyl acetate in 95% ethanol at 4ºC for 1 h. The glands were cleared in Propylene oxide and then left in a mixture of Propylene oxide and Araldite mixture (1:1) overnight in a rotator at room temperature. The Araldite medium contained Araldite (CY 212-10g), Dodecenyl succinic anhydride (DDSA-10g) as hardener, Dibutylphthalate (1g) as plasticizer and N Benzyl dimethyl amine (BDMA- 0.4g) as accelerator. The glands were then transferred into fresh Araldite mixture for infiltration at room temperature and kept in the rotator for 6 h. Finally the tissues were embedded in the Araldite mixture in beam capsules and kept for polymerization at 60 ºC for 48 h.

After polymerization semi thin and ultra thin sections were cut with a glass knife using a Leica U6 ultramicrotome. Semi thin sections of 1 micron thickness were transferred from the trough fitted to the glass knife's edge to a clean glass slide. Optimum drying was obtained by placing the slide on a hot plate at about 80ºC for 30 seconds to 1 minute. This procedure also promoted a vigorous adherence of section to the slide. These semi thin sections were stained with a solution of Toluidene blue (1g/100ml) in distilled water containing 0.5% Sodium carbonate. The staining was performed by adding few drops of stain on top of sections and the slides were kept at 80ºC for 20 sec. The excess stain was drained; the slides were washed in running water and dried. The stained sections were observed under the light microscope. After selecting suitable area of interest for ultra thin sectioning, blocks were further trimmed. Ultra thin (70 nm thick) sections were collected on meshed grids by placing the grids underneath the sections floating in the trough. The grids were then slowly raised to avoid folding of the sections. These sections were stained first in a saturated solution of uranyl acetate in 50 % methanol for 1 - 2 h by immersing the grids in the staining solution. After washing well in distilled water the sections were stained in a 2% Lead citrate solution. Sections were then washed thoroughly and dried. The stained sections were examined under Bio- Twin Transmission Electron Microscope operating at 80 kv and electron micrographs were taken to analyse fine structural details.

3.3 Results

3.3.1 Histomorphology of male ASGs

The internal reproductive system of adult male *Spodoptera mauritia* consists of a single median testis, a pair of seminal vesicles, vasa deferentia, ductus ejaculatorius duplex, ASGs, ductus ejaculatorius simplex and aedeagus (Fig. 7). The seminal vesicles are swollen enlargements of the vasa deferentia. The vasa deferentia of *S. mauritia* are paired narrow tubes. Each vas deferens extends from the posterior end of its respective seminal vesicles to a region midway on one of the branches of ductus ejaculatorius duplex. In *S. mauritia* the paired vasa deferentia cross each other before they open into the ductus ejaculatorius duplex. The duplex is a paired, short, glandular and 'S' shaped structure for storing the secretion of ASGs and sperm bundle leaving vas deferens. ASGs arise from the anterior end of the duplex. The ductus ejaculatorius simplex is a simple long duct extending from the posterior portion of the duplex to the aedeagus.

ASGs are seen as enormously coiled structures around the testis extending from the duplex from which they are separated by a constriction. When uncoiled they are found to be a pair of elongated tubes lying close to each other with an approximate length of 77 mm. ASGs are longer than all the other parts of the reproductive system except ductus ejaculatorius simplex. In freshly dissected insects the glands show distinct morphological

differentiation into three regions: a thick translucent anterior region (proximal), a long, thin and opaque middle region (mid region) and a thick posterior translucent region (distal region).

Light microscopic studies of transverse sections of adult glands show that the proximal and distal regions of ASGs possess similar basic structure i.e. a single layer of epithelial cells resting on a basement membrane surrounded by muscle layer. The cells of proximal region are comparatively larger with small-centralized nuclei and they possess dense cytoplasm. The lumen of the gland is found to be filled with immense secretion (Pl. II: Fig. 9). The mid region is composed of 2-3 layers of closely packed small cuboidal cells with small nuclei. Their cytoplasm appears to be less dense. The lumen contained secretion (Pl. II: Fig. 10). In the distal region, the cells possess large and central nuclei with dense cytoplasm containing granules.

3.3.2 Histomorphology of female ASGs

The female reproductive system of *S. mauritia* consist of a pair of ovaries, oviduct, bursa copulatrix, spermatheca with its associated spermathecal gland, paired ASGs and vagina. Each pair of ovary comprises four ovarioles and they are continuous with the lateral oviducts by means of a short pedicel. The paired lateral oviducts fuse to form the median oviduct. Bursa copualtrix is a pouch like complex structure constituting the ostium bursae and the seminal duct. The ostium bursae is moderately sclerotised. The

ostium bursae leads to the ductus bursae, the ductus bursae opens into the corpus bursae. The corpus bursae is simple and sac like structure. The seminal duct arises from the anterior end of the ductus bursae and is almost continuous with the point of attachment of the spermathecal duct (Fig. 8).

ASGs are found beneath the spermatheca as a pair of elongated tubular glands measuring about 13 mm in length. The basal end of each gland was dilated into reservoir like structure. The paired ASGs open through a common duct into the posterior region of the median oviduct. Light microscopic studies of transverse sections of adult ASGs show that they are composed of a single layer of tall columnar epithelial cells with large centralized nuclei resting on a basement membrane and granular cytoplasm surrounded by a connective tissue layer. Inner to this, a single layer of small epithelial cells called chitinogenous cells are observed. In addition a chitinous intima lining the lumen is seen (Pl. II: Fig. 11). The lateral reservoirs exhibited similar cellular features but their columnar cells are taller than that of the glands (Pl. II: Fig.12) and the lumen contain secretion as seen in the glands.

3.3.3 Ultrastructural Studies of ASGs

Ultrastructural studies were conducted on ASGs of adult male and female insects of *Spodoptera mauritia*.

3.3.3.1 Ultrastructural Studies of Male ASGs

Epithelial cells of all the three regions i.e., proximal, mid and distal region of the ASGs of *S. mauritia* have an ultrastructure characteristic of protein secreting cells.

Proximal region

(1) Basement membrane

The epithelium of the proximal region of ASGs of *S. mauritia* is single layered resting on a thick continuous basal lamina or basement membrane overlapped by a continuous latticework of connective tissue made of outer circular and inner longitudinal muscles (Pl. III: Fig. 13). In between the muscle layers, tracheoles and a few mitochondria are present. The basement membrane is not always seen to be overlaid by uniform layers of musculature. In some regions only a single layer of circular muscles is seen above the basement membrane. But at the junction where single and double layers of muscles meet quite a bit of tracheoles are seen (Pl. III: Fig. 14).

(2) Epithelial cells

The proximal region of ASGs is composed of a single layer of columnar epithelial cells resting on a basement membrane. Nucleus is located towards the mid – basal part of the cells. The nucleus is large and oval in shape with conspicuous nucleoli and irregularly distributed heterochromatin

bodies. Heterochromatin is clumped into large masses in many regions of the nucleus. (Pl. IV: Fig. 15). Many small spherical mitochondria and Golgi apparatus with associated secretory vesicles are seen in the cytoplasm. Cytoplasm in the vicinity of the nucleus is enriched with a fine and elaborate network of Rough Endoplasmic Reticulum (RER) (Pl. IV: Fig. 16). At many regions parallel arrays of RER are seen (Pl. V: Fig. 17).

(3) Lumen

The lumen is filled with different sized globules of secretory bodies and multivesicular bodies. The secretory bodies in the lumen have no uniform appearance. They consist of small electron dense clots and large globules of varying electron density filled with an amorphous material or granular material. Most of the large secretory globules have a less electron dense central core with electron dense patches on the periphery (Pl. V: Fig. 18). Presumably large globules are formed by the coalescence of small secretory globules. Secretory granules are seen to be assembled near the periphery of the lumen from where they are channeled into the lumen. The apical membrane of the cells appears intact.

Mid region

(1) Basement membrane

The mid region of ASGs of *S. mauritia* consists of a compact layer of 3-4 layers of epithelial cells, which rest on a moderately thick continuous basal lamina or basement membrane. (Pl. VI: Fig. 19). The basement membrane is overlaid by two discontinuous muscle layers; outer longitudinal and inner circular muscles (Pl. VI: Fig. 20).

(2) Epithelial cells

The epithelial cells contain comparatively smaller and circular nuclei with very few heterochromatin patches. The outer wall of the nuclear envelope is irregular and uneven. Prominent nucleoli are lacking. Cytoplasm is abundant with dilated or vesicular RER. Some times intracisternal granules are seen inside the vesiculated RER (Pl.VI: Fig. 21). Many free ribosomes are seen scattered among the cytoplasm and interdigitation of intercellular membrane is found among the epithelial cells. Another remarkable feature is the presence of mitochondria rich and mitochondria less cells among the glandular epithelium. Clustering of mitochondria is seen in the cytoplasm of mitochondria rich cells (Pl. VII: Figs. 22, 23).

(3) Lumen

The lumen contains secretion. The presence of secretory bodies in the lumen is less when compared to that of the proximal region. The apical border of the cells towards the lumen is infolded and modified to villi like structures. The secretory vesicles are seen to be released into the lumen through these villi (Pl. VIII: Fig. 24).

Distal region

(1) Basement membrane

Ultrastructural studies of distal region showed that epithelial cells rest on a very thick basement membrane. Two layers of muscles; outer longitudinal and inner circular muscles are seen overlying the epithelium (Pl. VIII: Fig. 25). In some regions of epithelium the double layer of muscles is replaced by a single layer. In this case a single layer of longitudinal muscle layer is seen above the epithelium.

(2) Epithelial cells

A single layer of columnar epithelial cells constitute the glandular epithelium of distal region of ASGs of *S. mauritia.* They rest on a basement membrane. Cytoplasm is enriched with a fine and elaborate network of RER (Pl. VIII: Fig. 26). Large round or oval nucleus is seen with indented nuclear membrane and irregularly distributed heterochromatin bodies (Pl. IX: Fig. 27).

Heterochromatin is clumped into large masses in many regions of the nucleus. Mitochondria are numerous along with other cell organelles and many are found clustered near the apical projections of the cell (Pl. IX: Fig. 28). Golgi bodies are seen in the cytoplasm. Secretory granules or secretory bodies are few in the cytoplasm. The apical surface of the epithelial cells towards the lumen is produced into many small projections.

(3) Lumen

The secretion in the lumen is less compared to the proximal region of the ASG. Apart from the secretory bodies the lumen contains other cytoplasmic debris also. Though comparatively smaller than the secretory bodies of proximal region, different sized secretory globules and secretory granules are present. It seems that the secretory bodies are released into the lumen by the sloughing off the apical projections of the epithelial cells (Pl. IX: Fig. 29).

3.3.3.2 Ultrastructural Studies of female ASGs

(1) Basement membrane

The epithelium of the ASGs of the female *S. mauritia* is single layered and rest on a continuous basal lamina or basement membrane. At certain places basal infoldings are seen in the basal membrane. (Pl. X: Fig. 30). A thin connective tissue layer lies over the glandular epithelium (Pl. X: Fig. 31).

(2) Columnar epithelial cells

A single layer of tall columnar epithelial cells form the epithelium of the gland. The columnar cells rest on the basement membrane. The epithelial cells are not tightly linked among themselves. Intercellular channels are seen in between the cells. The cellular architecture shows a large round or oval shaped nucleus with associated nucleolus and clumps of heterochromatin (Pl. XI: Fig. 32). The nuclear membrane is folded or indented and studded with ribosomes. The cytoplasm is massively proliferated with vesiculated RER. The cisternae of RER are distended and contain intracisternal granules (Pl. XI: Fig. 33).

Two morphological variations of mitochondria are seen in the epithelial cells. Large and elongated mitochondria are frequent throughout the cytoplasm. (Pl. XII: Fig. 34). Small circular forms of mitochondria are also seen. Golgi bodies are seen in the tissue. Among the columnar cells some specialized structures are seen in the vicinity of nucleus towards lumen. It consists of a central core bordered with electron dense materials and a number of radiating processes (6-8 nos.) (Pl. XII: Fig. 35). These structures are found to be in contact with intracellular channels. Electron dense granules are found near these structures (Pl. XIII: Fig. 36). In between the radiating processes a felt work of structures are seen and these areas are devoid of other cellular organelles except mitochondria. Many small circular mitochondria are seen to

be gathered near the processes (Pl. XIII: Fig. 37). Vacuoles containing fibrous materials, secretory vesicles and granules are seen in the cytoplasm.

(3) Chitinogenous cells

These are a single layer of small epithelial cells thought to be secreting the chitinous intima. Their nucleus is large and contains large masses of condensed heterochromatin (Pl. XIV: Fig. 38). The nucleus occupies most of the volume of the cell. The apical portion of the plasma membrane is looped and septate desmosomes occur at certain points in the lateral margins (Pl. XIV: Fig. 39, Pl. XV: Fig. 41). The apical cytoplasm is invaginated into small pockets and contains mitochondria and a few free ribosomes. Microtubules are also seen scattered in this area.

(4) Lumen

The lumen is lined by highly irregular chitinous intima, which forms infoldings at certain regions. At certain points the intima appears to be thinner or porous by nature. Lumen contains fine and uniform droplets of secretion along with other many small electron dense particles (Pl. XV: Fig. 40).

3.4 Discussion

3.4.1 Histological studies of male ASGs

Like in many other lepidopteran insects such as *Heliothis zea* (Callahan and Cascio, 1963); *Anagasta kuhniella* (Riemann and Thorson, 1979) and *Calpodes ethlius* (Lai-Fook, 1982) the internal reproductive system

of *S. mauritia* consists of a single median testis, a pair of seminal vesicles, vasa deferentia, ductus ejaculatorius duplex, ASGs, ductus ejaculatorius simplex and aedeagus. In *S. mauritia* ASGs extend from the duplex from which they are separated by a constriction and show distinct morphological and histological differentiation into three regions. Studies of Musgrave (1937) show that in *Ephestia kuhniella*, ASGs are differentiated into four different histological regions. In *Choristoneura fumiferana* (Outram, 1970) ASGs are differentiated into three different histological regions. In *Anagasta kuhniella* (Riemann and Thorson, 1979) five regions are identified. Lai Fook (1982) recognized six regions in the ASGs of *Calpodes ethlius*.

Earlier studies show that ASGs of lepidopterans like *Calpodes ethlius* (Lai- Fook, 1982); *Achoria grisella* (Fernandez and Cruz-Landim, 2005) and *Antheraea mylitta* (Pendum and Tembhare, 2005) possess similar basic structure i.e., a single layer of epithelial cells resting on a basement membrane surrounded by muscle layer. Light microscopic studies of transverse sections of ASGs of *S.mauritia* show that similar arrangement is seen in the proximal and distal regions of ASGs whereas the mid region of ASGs is composed of multilayer of small cuboidal cells. In gland 4 in the collateral gland complex of *Schistocerca gregaria* the glandular epithelium is 2 or 3 cells thick (Odhiambo, 1969). Presence of musculature outside the epithelium is reported in *Calpodes ethlius* (Lai- Fook, 1982) and in *Achoria grisella* (Fernandez and Cruz-Landim, 2005).

3.4.2 Histological studies of female ASGs

Like many other adult lepidopteran females the reproductive system of *S. mauritia* includes a pair of ovaries, oviduct, bursa copulatrix, spermatheca with its associated spermathecal gland, paired ASGs and vagina. ASGs are found beneath the spermatheca as a pair of elongated tubular glands and the basal end of each gland has dilated into reservoir like structure.

Light microscopic studies on the transverse sections of ASGs show that the glands possessed an inner chitinous intima, chitinogenous cells, a single layer of long columnar epithelial cells resting on a basement membrane and an outer connective tissue layer. This is consistent with the arrangement seen in lepidopterans like *Heliothis zea* (Callahan and Cascio, 1963) and *Hyalophora cecropia* (Berry, 1968). Similar arrangement is reported from the ASGs of *Tenebrio molitor* (Hyun and Moon, 2003) and Dufour glands of *Melipona bicolor* (Abdalla and Cruz- Landim, 2003).

3.4.3 Ultrastructural Studies of male ASGs

(1) Basement membrane

The term ‘basement membrane’ is applied to the outer sheath of ASGs which are non contractile and present between the base of the cells and the smooth muscle (Callahan and Cascio, 1963). In ASGs of male *S. mauritia* the single layered glandular epithelium rests on a continuous basal lamina or

basement membrane as seen in the ASGs of *Calpodes ethlius* (Lai- Fook, 1982). Similar arrangement is reported from *Culex pipiens pallens* (Tongu *et al*., 1972); *Periplaneta americana* (Adiyodi and Adiyodi, 1974); *Aedes triseriatus* (Ramalingam and Craig, 1978) and in med fly *Ceratitis capitata* (Marchini, 2003).

Outer to the epithelium of ASGs lie a continuous network of muscle layers. In the proximal region, circular muscles are present outer to longitudinal muscles while in mid and distal regions the arrangement is reversed so as to have inner circular and outer longitudinal muscles. Muscle layer outer to the epithelium of ASGs has also been reported from other insects from Lepidoptera. In *Calpodes ethlius* two layers of muscles, an inner less robust circular and outer more robust longitudinal muscles surround all regions of ASGs and the muscles are penetrated by tracheoles and nerve endings (Lai- Fook, 1982). In *Achoria grisella* the ASGs contain a visceral muscle layer (Fernandez and Cruz- Landim, 2005). The presence of muscle layer surrounding the epithelium of ASGs has been reported from other insects like *Culex pipiens* (Tongu *et al*., 1972); *Tenebrio molitor* (Gadzama *et al*., 1974); *Aedes triseriatus* (Ramalingam and Craig, 1978); *Aenictus gracilis* (Shymalanath and Forbes, 1984) and *Blaps polychresta* (El- Zoheiry, 1999a,b).

In *S. mauritia* the connective tissue of ASGs might be functioning as a

mechanical support. Also the contraction of the muscle fibres would be propelling the contents of the glands up into the ductus ejaculatorius duplex. Longitudinal muscles are known for their greater extensibility and they can tolerate remarkable lengthening (Rose *et al*., 2001). So this arrangement would be helping the mid region, the longest segment of ASG as well as the distal ends to push their contents more efficiently to the anterior ends. At the same time the basement membrane would assist in anchoring the surrounding nerve fibres, muscle fibres and tracheoles to their proper positions. All the molecules that pass between the epithelial cells and the haemolymph have to negotiate with the basement membrane and other connective tissue and it is known from the vertebrates that they act as charged molecular sieves.

(2) Epithelial cells

Regional differentiation of ASGs into many regions is reported from the lepidopterans like *Ephestia* (Musgrave, 1937); *Acrolepia* (Thibout, 1971); *Phthorimaea* (Brits, 1978); *Anagasta* (Riemann and Thorson, 1979) and *Calpodes ethlius* (Lai- Fook, 1982). Studies of Lai- Fook (1982) show that all the regions of ASGs consist of a single layer of epithelial cells in *Calpodes ethlius.* In contrast in *S. mauritia* a single layer of columnar epithelial cells occupies the proximal and distal regions of the ASGs whereas mid region of the ASGs is composed of multilayer of cuboidal cells.

Ultrastructural studies of ASGs of *S. mauritia* show that the epithelial

cells of all regions possess nuclei located towards the mid-basal part. The nucleus is large, oval and lobbed with distinct nucleoli and large masses of heterochromatin. Lobbed condition of the nuclei is seen in the epithelial cells of ASGs of *Anagasta kuehniella* (Riemann and Thorson, 1979). Studies of Fernandez and Cruz-Landim (2005) show that the nuclei in the epithelial cells of ASGs in *Achoris grisella* contain irregularly distributed chromatin. Adiyodi and Adiyodi (1974) have reported that the condensed heterochromatin distributed in the nucleus of epithelium of ASGs in *Periplaneta americana* represents a metabolically inert condition.

Studies show that RER is especially well developed in cells actively engaged in protein synthesis as well as in protein export. In *S. mauritia* the cytoplasm of epithelial cells is enriched with fine and elaborate network of parallel arrays of RER. The abundance of RER in the cytoplasm is an indication of high synthetic activity of a cell. Particle studded membranes are elaborately developed and form a reticulum which is remarkably similar in extent and organization to that noted in the cells of the mammalian pancreas (Sjostrand and Hanson, 1954; Palade, 1955). Federer and Chen (1982) have related the appearance of organelles like RER, Golgi complex and the large number of secretory granules with various inclusions to the high synthetic activity of the cell.

The size of Endoplasmic Reticulum (ER) varies considerably in

different cell types in the ASGs of *S. mauritia* and is related to their function. Early studies show that ER is often small and relatively undeveloped in undifferentiated cells but they increase in size and complexity with differentiation. Based on the presence of an elaborate network of RER in the cytoplasm of epithelial cells - the most important feature of the protein synthesizing cells, the epithelial cells of *S. mauritia* seem to be equipped with well-versed protein synthetic machinery as seen in the epithelial cells of ASGs of *Achoris grisella* (Fernandez and Cruz-Landim, 2005).

Morphological variations of RER are seen in different regions of the ASGs of *S. mauritia*. In the proximal and distal region of the ASGs, RER attains cisternal or labyrinth form. Elaborate whorls of RER might act as a store of intracellular membrane with associated ribosomes in which associate mRNA is protected from degradation (Gooding, 1973; Hecker and Rudin, 1979; Felix *et al*., 1991). RER of the mid region attains the vesiculated or dilated form. In *Achoris grisella* the morphology of RER shows regional variation intracellularly i.e., cisternal form is seen in the basal and vesicular form in the apical regions of the same cell (Fernandez and Cruz-Landim, 2005). RER consist of flattened and swollen cisternae in parallel arrays and occur throughout the cell except in region close to the lumen in the mushroom shaped male ASGs of *Periplaneta americana* (Adiyodi and Adiyodi, 1974). Ramalingam and Craig (1978) have observed that RER containing swollen cisternae in *A. triseriatus* indicate a heavy intracisternal accumulation of

secretory materials. RER is associated with storage of lysosomal and secretory vesicles (Andries, 1977; Billingsley and Downe, 1986). Gillott (1988) has pointed that the extremely dilated RER may represent intracellular storage of material. These studies suggest that due to the presence of vesiculated RER, in *S. mauritia* mid region of ASGs might also be functioning as a site of storage of the secretory materials.

Small oval shaped mitochondrial profiles with oblique and transverse crystae are evenly distributed in proximal and distal regions of ASGs of *S. mauritia* whereas clustering of mitochondria occurs in the epithelial cells of mid region. As mitochondria are invariably associated with energy utilizing processes, the clouding of these organelles in the epithelium of mid region evidently shows that longer segment of the ASGs might be pushing forth its contents ahead utilizing the energy provided by the mitochondria. In addition to this, mitochondria might be acidifying the luminal contents by the modulation of proton secretion to allow the normal movement of sperm as done in the epididymys and vas deferens of many vertebrates and invertebrates. Numerous mitochondria are seen in the epithelial cells of ASGs of *Achoris grisella* (Fernandez and Cruz-Landim, 2005). Lai Fook (1982) has opined that mitochondria may play a role in providing energy to the sperm in *Calpodes ethlius*. Accumulation of mitochondria in the epithelial cells of ASGs of *Culex pipiens pallens* (Tongu *et al*., 1972) and *Blaps polychrystae* (El-Zoheiry, 1999a) have been reported.

In the epithelial cells of ASGs of *S. mauritia*, Golgi apparatus with associated secretory vesicles are seen in the vicinity of nucleus and RER. In the epithelial cells of proximal region of the ASGs, the secretory vesicles appear to be channeled into the lumen from the secretory region of Golgi bodies. In the ASGs of *Calpodes ethlius*, the epithelial cells contain numerous Golgi bodies with associated dense-cored vesicles (Lai-Fook, 1982).

Early studies show that secretory proteins follow the classical route of synthesis in the RER, processing in the Golgi apparatus, holding in the intermediate vesicles and releasing from secretory vesicles (Lehane, 1976, 1989; Jordao *et al.*, 1996). The epithelial cells in *S. mauritia* seem to be responsible for the synthesis of secretion and release of accumulated secretory material, usually in the form of secretory vesicles, into the lumen upon proper induction.

(3) Lumen

Early studies show that the lumen of ASGs of sexually mature male insects are filled with a fine granular secretion. (Adiyodi and Adiyodi, 1975; Gerber, 1978; Riemann and Thorson, 1979; Federer and Chen, 1982; Lai-Fook, 1982; Marchini *et al.*, 2003; Moor, 2003; Fernandez and Cruz-Landim, 2005). In *S. mauritia*, in all the regions of ASGs, lumen is filled with secretory structures which differ in size, shape and electron density ranging from darkly stained small granules to large secretory globules with

less electron dense central core. Different granules of differing electron density are reported in the ASGs of lepidopteran insects like *Anagasta kuehniella* (Riemann and Thorson, 1979); *Calpodes ethlius* (Lai-Fook, 1982) and *Achoris grisella* (Fernandez and Cruz-Landim, 2005).

Riemann and Thorson (1979) have reported that in ASGs of *Anagasta kuehniella* secretion is primarily *via* Golgi-derived vesicles while apocrine mode of secretion by the sloughing off of the apical cytoplasm is seen in the posterior region. In *S. mauritia* secretory granules are assembled near the periphery of the lumen of the proximal region of ASGs from where they are channeled off into the lumen. In the distal region they seem to be sloughed off along with some apical cell membrane into the lumen.

In the mid region of ASG of *S. mauritia* the apical border of the cells towards the lumen is highly infolded and modified to villi like structures. The secretory globules are seen to exit into the lumen through the bases of the villi. A few microvilli involved in the partial resorption of the substances eliminated during the secretion are reported from the ASGs of *Achoris grisella* (Fernandez and Cruz-Landim, 2005).

In the ASGs of *S. mauritia* the size and appearance of the secretory globules are different in different regions of ASGs. In proximal region the secretory globules are larger when compared to those of the other regions. Small secretory vesicles are also seen intermingled with large globules. In

mid region large but a few secretory globules are seen in the lumen. The presence of secretory globules in the lumen of distal region is less compared to that of the proximal region and the lumen contains much cytoplasmic debris also. The nature of secretory globules in proximal and distal regions of ASGs are seemed to be different. Whether this difference is due to the different modes of secretion as Lai-Fook (1982) have reported or due to the difference in their biochemical composition is not clear.

Musgrave (1937) has reported that the various sized globules found along the length of the glands represent the physiological change occurring in the secretion. In ASGs of *Anagasta kuehniella* secretory granules form quite different aggregates in different levels of the lumen. But there is little corresponding variation in the morphology (Riemann and Thorson, 1979). In *Achoris grisella* the secretion in the lumen of the distal extremity of the gland consist of small globules while the granules increase in size near the junction of the gland with the seminal vesicles. Proximally the granules fuse to each other and form amorphous masses (Fernandez and Cruz-Landim, 2005). In ASGs of *Calpodes ethlius* the region adjacent to the constriction with the duplex, the major secretion consists of small, very dense spherical globules and a few larger granules of lesser density (Lai-Fook, 1982). Large secretory granules are reported from the anterior regions of the ASGs from other insects also (Tongu *et al.*, 1972). Lai-Fook (1982) has stated that the luminal contents of distal portions of the ASGs of *Calpodes ethlius* consist mostly of

apical portions of apocrine cells intermingled with dense granules.

In *S. mauritia* ASGs appear to synthesize proteinaceous materials which are secreted into the gland lumen and transported upwards to ductus ejaculatorius duplex. Proximal and distal regions have abundant RER and major synthesis and secretion appear to takes place in these regions as their cells are enriched with both abundant protein synthesizing and secretory apparatus. Though mode of secretion and nature of secretory globules are different in the proximal and distal region, secretion in the gland lumen seemed to be primarily proteinaceous. Presence of an elaborate network of RER in the glandular epithelium of proximal and distal regions supports this thought. The secretion produced in the distal region might be propelled up to the mid region by the contraction of the muscle fibres especially by the outer longitudinal muscles. Mid region appears to be concerned with the transport / propulsion of the secretory materials as evidenced by having a robust outer longitudinal muscles and numerous energy providing mitochondria in the cells. The remarkable extensibility of the longitudinal muscles which is present in the outer region of epithelial cells of mid region might be helping in the propulsion of the secretory materials into the proximal region. By taking into consideration the size and nature of the secretory bodies, the proximal region appears to hold storage function also. Numerous functions like formation of spermatophore, accompanying the sperms at copulation, furnishing a medium for the transfer of sperm during ejaculation and

modulation of reproductive physiology of females are assigned to the secretions of ASGs (Clements, 1963; Lum, 1961; Gillott, 2003). In *S. mauritia* also the secretion stored in the proximal region of ASGs would be performing the same functions as those described for other insects.

3.4.4 Ultrastructural studies of female ASGs

1) Basement membrane

The epithelium of the ASGs of female *S. mauritia* is single layered and rest on a continuous basal lamina or basement membrane as observed in other lepidopterous insects like *Heliothis zea* (Callahan and Cascio, 1962). Also a few basal infoldings are seen in the secretory epithelium. Studies of Fawcett (1967) show that basal infoldings are usually associated with the uptake of materials. In *S .gregaria* (Szopa, 1982) and *Teleogryllus commodus* (Sturn, 2002) the infolds of the basal cell surface ensure an increased uptake of the secretory precursors from the haemolymph. In the Dufour glands of *Melipona bicolor Lepeletier*, invaginations of plasma membrane are present in the apical and basal portions, which vary in depth according to the state of glandular activity state (Abdalla and Cruz- Landim, 2003). In *S. mauritia* since the ASGs are secretory in function, the infoldings in the epithelial cells might be helping in the intake of materials from the haemolymph. It is not clear whether extraglandular synthesis of protein is occurring necessitating the presence of these basal infoldings. Extra glandular synthesis of proteins has

been found to occur in the ASGs of *Melanoplus ssanguinipes* (Friedel and Gillott, 1976).

Studies of Szopa (1982) show that in *S.gregaria* the ASGs are covered by a connective tissue layer. In the Dufour glands of *Melipona bicolor Lepeletier*, muscle fibres are present outer to epithelium (Abdalla and Cruz-Landim, 2003). In *S. mauritia* ASGs are enveloped by a thin layer of connective tissue.

(2) Columnar epithelial cells

A single layer of columnar epithelium constitute the glandular epithelium of ASGs in *S. maurita*. In addition to this, a layer of chitinogenous cells and a cuticular intima which lines the lumen are present. Similar structures are observed in the ASGs of other insects. In *Chrysomya putoria* the secretory region is composed of three layers; a cuticular intima lining the lumen followed by a layer of small cells and then a layer of large secretory cells (Trione and Avicini, 1997). In *Orchesella villosa*, a thick cuticular layer lines the epithelial cells of the ASGs (Dallai *et al*., 2008). In *Heliothis zea* the ASGs are composed of large cuboidal epithelium surrounding a lumen (Callahan and Cascio, 1963). The ASGs of *Tenebrio molitor* is composed of two kinds of cells: secretory epithelial cells and duct forming cells (Dallai, 2008). In *S. gregaria* the epithelium becomes reorganized into a unicellular epithelium towards the 5th stadium, which persists in adult stages

(Szopa, 1982). The Dufour glands in *Melipona bicolor Lepeletier* are composed of a single layer of epithelial cells which is lined by a thin cuticle at the luminal surface. Here the glandular cells vary from cubic to columnar (Abdalla and Cruz -Landim, 2003).

In *S. mauritia* the epithelial cells do not appear to be tightly apposed to each other. Intercellular channels are seen between the cells. In the ASGs of *S. gregaria* where adjacent epithelial cells of adult insects are not linked to each other they have short stretches of desmosomes. Intercellular channels occur in the remaining regions often widening into intercellular gaps (Szopa, 1982).

The protein secreting cells of colleterial glands of *Hyalophora cecropia* have many features associated with pancreatic acinae like extensive RER and elaborate Golgi complex and a modest number of mitochondria (Berry, 1968). In the Dufour glands in *Melipona bicolor Lepeletier*, the nucleus of the columnar cells are located on the basal region and usually with irregular contour showing sometimes more than one nucleolus (Abdalla and Cruz- Landim, 2003). In left colleterial gland of *Periplaneta americana* one or more granular nucleoli occur in the nucleus (Mercer and Brunet, 1959).

In *S. mauritia* the epithelial cells have a large round or oval shaped nuclei located in the basal portion of the cells and have granulated nucleolus and clumps of heterochromatin. The nuclear membrane is folded or indented

and studded with ribosomes. The cytoplasm is massively proliferated with vesiculated RER. The cisternae of RER are distended and contain intracisternal granules. Studies of Szopa (1982) show that during reproductive stage, RER attains a vesicular form and the cisternae of the RER become progressively more distended and contain moderately electron dense materials. The presence of elaborate RER suggest that the extensive synthesis of proteins is occurring.

Many large mitochondria with lamellar crystae are scattered throughout the cytoplasm of ASGs of *S. mauritia*. In some mitochondria intramitochondrial granules are visible. Golgi bodies are not very frequently seen in the tissue. In *S. gregaria* epithelial cells of ASGs contain a large number of free ribosomes and randomly distributed mitochondria with dense matrices. Rudimentary Golgi complexes are found occasionally (Szopa, 1982). In the ASGs of *Teleogryllus commodus* high production of site specific macromolecules during peak differentiation of the glands is indicated by up to fourfold increase in the volume of mitochondria per cell along with enlarged nucleus, nucleolus and highly propagated RER (Sturm, 2008). Presence of large mitochondria and other amplified synthetic machinery in the ASGs of *S. mauritia* show that the epithelial cells of the glands are in highly differentiated state to carry out the secretory function. Also presence of secretion in the lumen prove that the glandular epithelium is in the secretory phase.

Vacuoles containing a fibrous network of material occur in the cytoplasm of gland epithelium of ASGs of *S. gregaria* whereas no definite secretory vesicles are seen in the cytoplasm or in close proximity of the apical membrane at any stage (Szopa, 1982). In the present study also vacuoles are seen in the cytoplasm. In *Heliothis zea,* cytoplasm is highly vacuolated and the secretory edge of the cells is highly irregular due to the protrusion of the secretory globules into the lumen (Callahan and Cascio, 1963). In ASGs of *S. mauritia* no vacuoles or secretory vesicles are seen either near the apical membrane or near the lumen of the epithelial cells of ASGs.

Earlier studies prove that ASGs of female insects have some specialized structures concerned with the secretion, storage and transport of synthesized materials. The protein secreting cells of colleterial glands of *Hyalophora cecropia* contain a unique secretory apparatus which is not commonly found in the protein secreting cells. It consisted of a cuticular tubule inserted into microvilli –lined cavity at the apical end of the cell (Berry, 1968). In *T. molitor* each secretory epithelial cells has its own peculiar end apparatus and the glandular secretion of the epithelial secretory cells is drained into the lumen through the end apparatus. This type of glandular secretion in insects is called type III. In *Orchesella villosa,* cells involved in fluid secretion having an extracellular cistern filled with an electron-transparent material are intermingled with the secretory cells. Their secretory product opens into the cistern (Dallai *et al*., 2008). In the ASGs of *Chrysomya*

putoria the glandular secretion is synthesized in the cytoplasm of the secretory epithelial cells, stored or modified in the reservoir and then drained to the lumen through an end apparatus seen in the apical region of the cell (Trione, 1997). In *S. gregaria* the secretion is released by microapocrine method. It seems that very fine droplets of secretion were released and these coalesced in the lumen (Szopa, 1982).

The secretory system called the end apparatus found in the apical portion of the type III epithelial cells of left colleterial glands of *Periplaneta americana* consist of a funnel like involution of the thin cuticle lining the lumen of the tubule. The free secreting surface consists of a relatively coarse, closely packed radially directed tubular cell processes with a felt work of material found beyond the tips of these processes. Occasionally the contents of the cell may be pressed forward in to the funnel and penetrate almost into the lumen (Mercer and Brunet, 1959). In *S. mauritia* among the columnar epithelial cells of ASGs some specialized structures are seen in the vicinity of nucleus towards lumen. Each structure consists of a central core bordered with electron dense materials and a meshwork of radiating processes (6-8 Nos.) containing many electron dense granules. An elaborate feltwork like structures are seen in between these radiating processes and these processes are seen to be in contact with the intracellular channels.

Many small circular mitochondria are seen to be gathered near the

arms and these areas are devoid of other cellular organelles. Electron dense granules are seen near these structures. In *S. mauritia* though these specialized structures are not homologous with the secretory apparatus of the ASGs of other insects these structures may be considered to possess similar mechanics as that of the end apparatus found in the type III epithelial cells of the left colleterial gland of *Periplaneta americana* (Mercer and Brunet, 1959). In *S. mauritia* the radiating processes of these structures contain many electron dense secretory materials as found in the end apparatus of type III epithelial cells of the *Periplaneta americana.* Based on these observations it may be assumed that these specialized structures found in the epithelial cells of the ASGs of *S. mauritia* might be functioning as a secretory or storage apparatus from where secretory materials are drained into the lumen through the intercellular canals. This might be the reason for not observing any secretory globules in the apical region of the cells as seen in male ASGs.

(3) Chitinogenous cells

Chitinogenous cells which are believed to secrete chitinous intima are commonly seen in the ASGs of many insect species. In the left colleterial gland of the *Periplaneta americana* a single layer of small flattened cells called chitinogenous cells secreting chitinous intima are seen surrounding the lumen (Mercer and Brunet, 1959). Cuticulogenous cells secreting the cuticle are seen in the ASGs of *Hyalophora cecropia* (Berry, 1968); *Galleria*

mellonella (Barbier, 1975); *Rhodnius prolixus* (Lococo and Huebner, 1980) and *Chrysomya putoria* (Trione and Avancini, 1997). According to Szopa (1982) in ectodermal glands, one cell type is required for the secretion of the cuticle and the other cell type produces another type of secretion. In *S. mauritia* in addition to the secretory epithelial cells, a single layer of chitinogenous cells is present. It seems these cells produce the chitinous intima. Chitinogenous cells possess large nucleus with huge masses of condensed heterochromatin. In the left colleterial gland of *Periplaneta americana* the cytoplasm of the chitinogenous cells contain smooth ER (Mercer and Brunet 1959). In contrast to these findings, the cytoplasm of the chitinogenous cells in *S. mauritia* has abundant RER. Chitinogenous cells with distinct and specific type of ultrastructure are found associated with the secretory cells in *Hyalophora cecropia*. This cell type is attached to the cuticular elements of the gland and the main features of its cytoplasm are extensive bundles of microtubules, which presumably serve as supportive elements for the secretory cells (Berry, 1968). In *S. mauritia* chitinogenous cells are found in close association of chitinous intima. Microtubules are also seen in the apical cytoplasm of chitinogenous cells.

In *S. mauritia* the plasma membrane of the apical portion of cytoplasm near the lumen is looped and septate desmosomes occur at certain points in the lateral margins with many fragmented or incomplete lengths of separate desmosomes. When lumen is formed, in transitional stages of development of

glands, previously connected cells got separated and as a result fragmented desmosomes are formed. In *S. gregaria* the lateral plasma membrane of apical cytoplasm of epithelial cells near the lumen are often looped and occasionally circles of cytoplasm appear to be enclosed in membrane (Szopa, 1982). In *S. mauritia* also looping of plasma membrane is observed.

(4) Lumen

From the earlier studies it is clear that ASGs of many insect species secrete materials into the lumen. In the left colleterial gland of *Periplaneta americana* the lumen is filled with vacuolated droplets or masses of droplets of various sizes (Mercer and Brunet, 1959). The lumen is lined by a thin cuticle and filled with secretory materials in *Tenebrio molitor* (Hyun and Moon, 2003). In *Orchesella villosa* the lumen is filled with a dense secretion after the reproductive phase and the ASG secretion play a protective role towards the eggs (Dallai *et al*., 2008). In *Heliothis zea* secretory globules are seen protruding into the lumen (Callahan and Cascio, 1962). ASGs of *S. mauritia* also contain secretion within the lumen. In many insect species the secretion of colleterial glands secrete cement like substance for sticking the eggs to the substratum. Studies of Callahan and Cascio (1963) on some noctuid moths suggest that in Lepidoptera the secretions of the colleterial glands act as a lubricant to aid movement of the sperm into the spermathecal duct. The secretion in the ASGs of *S. mauritia* might be performing in similar

manner. i.e., to lubricate the pathway of sperms as well as aid in the attachment of the eggs to the under surface of leaf blade. According to Clements (1963) the ASG secretion nourishes the sperms provided that some sperms are transferred to spermatheca. There is also evidence that the secretion may stimulate the mechanism of oviposition.

3.5 Summary

1. Histomorphological investigations were conducted on the ASGs of adult male and female *Spodoptera mauritia* to study the morphology and anatomy of the glands.
2. The present study show that male ASGs are a pair of elongated tubes lying close to each other arising from the anterior end of the ductus ejaculatorius duplex on male reproductive system showing distinct morphological differentiation into three regions: proximal, mid and distal regions.
3. Light microscopic studies of male ASGs showed that the proximal and distal regions are composed of a single layer of columnar epithelial cells resting on a basement membrane surrounded by muscle layer whereas the mid region is composed of 2-3 layers of closely packed small cuboidal cells. The lumen of all the regions contains secretion.
4. Female ASGs are a pair of elongated tubular glands found beneath the spermatheca of the reproductive system. The basal end of each gland got dilated into reservoir like structure.
5. Light microscopic studies of female ASGs showed that these are composed of an inner chitinous intima lining the lumen, chitinogenous cells, columnar epithelial cells resting on a basement membrane and an outer connective tissue layer.
6. Ultrastructural studies show that the epithelium of the proximal region of male ASGs of *S. mauritia* is single layered resting on a thick, continuous basal lamina or basement membrane overlapped by a continuous latticework of connective tissue made of outer circular and inner longitudinal muscles.
7. In the proximal region of male ASGs the nuclei of the epithelial cells

are large and oval in shape with conspicuous nucleoli and irregularly distributed heterochromatin bodies. Heterochromatin is clumped into large masses in many regions of the nucleus. Cytoplasm in the vicinity of the nucleus is enriched with a fine and elaborate network of RER. Many spherical mitochondria are evenly distributed throughout the cell and Golgi bodies with associated secretory vescicles are also seen in the cytoplasm.

8. Secretory granules are seen to be assembled near the periphery of the lumen of proximal region from where they are channeled into the lumen. The lumen of the proximal region is filled with different sized globules of secretory bodies of varying electron density and multivesicular bodies. Coalescence of secretory globules are often observed in lumen.

9. The mid region of male ASGs of *S. mauritia* consists of a compact layer of 3-4 layers of epithelial cells resting on a moderately thick continuous basal lamina or basement membrane. The basement membrane is overlaid by two discontinuous muscle layers; outer longitudinal and inner circular muscles.

10. The epithelial cells of mid region contain smaller and circular nuclei with very few heterochromatin patches. Prominent nucleoli are lacking. Cytoplasm is abundant with dilated or vesicular RER. Intracisternal granules are seen inside the vesiculated RER and many free ribosomes are seen scattered among the cytoplasm.

11. Mitochondria rich and mitochondria less cells are observed among the glandular epithelium of mid region. Clustering of mitochondria is seen in the cytoplasm of mitochondria rich cells.

12. The lumen of mid region contains less secretion compared to that of the proximal region. The apical border of the cells towards the lumen is infolded and modified to villi like structures. The secretory vesicles are seen to be released into the lumen through these villi.

13. Ultrastructural studies of distal region of male ASGs showed that epithelial cells rest on a very thick basement membrane. Two layers of muscles; outer longitudinal and inner circular muscles are seen overlying the epithelium. In some regions of epithelium a single layer of longitudinal muscle layer is seen above the epithelium.

14. A single layer of columnar epithelial cells constitute the glandular epithelium of distal region of ASGs of *S. mauritia* resting on a basement membrane.

15. In epithelial cells of distal region of ASGs, cytoplasm is enriched with a fine and elaborate network of RER. Large round or oval nucleus is seen heterochromatin clumped into large masses. Mitochondria are numerous along with other cell organelles and many are found clustered near the apical projections of the cell. Golgi bodies are seen in the cytoplasm.The apical surface of the epithelial cells towards the lumen is produced into many small projections.

16. The lumen of distal region of ASGs contains different sized secretory globules and cytoplasmic debris. secretory globules are seemed to be released into the lumen by the sloughing off the apical projections of the epithelial cells.

17. Epithelial cells of proximal, mid and distal regions of male ASGs of *S.mauritia* are well equipped with protein synthetic machinery and they exhibit secretory activity while mid region is also concerned with storage and transportation of the luminal contents.

18. Ultrastructural studies of female ASGs reveals that the glandular epithelium is enriched with abundant protein synthetic machinery. Amplification of organelles indicates that ASGs are highly differentiated to carry out the secretory activity.

19. Among the columnar epithelial cells some specialized structures thought to be involved in intracellular storage of secretion before it is being drained into the lumen are seen in the vicinity of nucleus towards lumen. This structure consists of a central core bordered with electron dense materials and a meshwork of radiating processes (6-8 nos.) containing many electron dense granules. An elaborate felt work like structures are seen in between these radiating processes and these processes are seen to be in contact with the intracellular channels.

20. The lumen contains uniform and fine secretory globules along with other electron dense granules.

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 ºC 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% Na_2CO_3, 0.02 % Na K tartarate, 0.1M NaOH, 1% SDS} and 1ml of 0.5% $CuSO_4$. 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% ß- 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), Soyabean 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 ASG1 and ASG 2 appear as very faint and thin bands. The other peptides appeared as medium intense bands.

4.3.3.2SDS-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 G_2 into the G_1 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 aegept* (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 s*ex 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. mafovensis* 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 Giebultowiez *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.

CHAPTER 5

ROLE OF HORMONES AND EFFECTS OF HORMONE AGONISTS ON DEVELOPMENT AND DIFFERENTIATION OF ACCESSORY SEX GLANDS

5.1 Introduction

Insects possess a well developed neuroendocrine system consisting of the neurosecretory cells located in the brain, suboesophageal ganglion and ganglia of the ventral nerve cord (Nayar, 1973). Other important endocrine glands in insects include the corpora allata, corpora cardiaca, prothoracic glands and the midgut tissue (Prabhu and Sreekumar, 1994; Chapman, 1998). The endocrine system of insects is clearly the best studied and characterized among invertebrates. Evidently this is due to the economic and ecological significance of insects in this biosphere. Further there is urgent need to control insects which are pests of agriculture and those which serve as vectors of disease causing germs.

The recent discovery of compounds with hormonal and antihormonal activities have greatly facilitated studies on Insect Endocrinology. These compounds which induce hormone deficiency or hormone excess in treated insects are excellent probes to analyze the role of hormones in insect metamorphosis and reproduction. Further these compounds have greater potential in Integrated Pest management (IPM) programmes as insect control

agents (Nair, 1993; Oetken *et al*., 2000). These compounds are designated as Insect Growth Regulators (IGRs). IGRs acting as ecdysone agonists/ antagonists or JH analogues/ anti JH agents disrupt the endocrine and reproductive physiology of a number of insects to aid in their control.

The insect reproductive system is often looked upon as an excellent target to analyze the effects of IGRs in IPM programs. The development and differentiation, synthesis and release of secretions of ASGs are under the control of endocrine system in insects. Critical titer of ecdysteroids secreted by prothoracic glands and juvenile hormone (JH) secreted by corpora allata (CA) are necessary for the normal development of ASGs (Gillott and Gaines, 1992). Wigglesworth (1936) first suggested that the corpora allata regulate ASG activity, following his observation that decapitation of male *Rhodnius prolixus* Stal. caused ASGs to remain attenuated and empty. Since this pioneering work, many authors have shown that by allatectomy followed by implantation of active CA or topical application of JH, restore the secretory activity of ASGs. Many authors have shown that JH regulate protein synthesis in the ASGs (Blaine and Dixon, 1973; Gillott and Freidel, 1976; Venkatesh and Gillott, 1983; Ogiso and Takabashi, 1984; Gillott and Gaines, 1992). The present study deals with the effect of Pyriproxyfen (PPN), a JH agonist and Methoxyfenozide, an ecdysteroid agonist on the development, differentiation and the secretory activity of ASGs.

5.2 Materials and Methods

5.2.1 Experimental animals

The pupae and adults of the required age used for various experimental studies were obtained from laboratory stock culture reared and maintained as described previously (Chapter 3). In all experiments developmentally synchronous insects were used.

5.2.2 Chemicals

Juvenile hormone agonist (JHA)

Pyriproxyfen (PPN), an agonist of JH was obtained as a research sample from M/s Valent Corp., USA. PPN was dissolved in acetone to prepare different concentrations. For treatment procedures, measured quantities of these compounds were used using a 10 µl Hamilton microsyringe.

Ecdysteroid agonist

The ecdysteroid hormone agonist, Methoxyfenozide was obtained as a gift from Dr.Unnithan, G.C, University of Arizona, U.S.A. The compound was dissolved and diluted in distilled water to obtain different concentrations.

5.2.3 Treatments

(1) Treatment of PPN on newly ecdysed day 0 pupae

PPN was dissolved and diluted in acetone to obtain two different concentrations namely 0.1µg/µl and 1µg/µl. The different doses of PPN were applied topically on the abdominal region of newly ecdysed day 0 pupae (100 Nos.) using a 10µl Hamilton microsyringe. Same number of pupae kept as controls received an equivalent volume of acetone only. Experimental and control pupae were kept in separate beakers covered with muslin cloth. The

pupae were checked daily for mortality and morphological abnormalities. To study the morphogenetic changes ASGs were dissected out of pupae on each post treatment day. Further ASGs of adults and adultoids were processed for histological, ultrastructural and electrophoretic studies.

(2) Treatment of PPN on day 0 adult males

Ten newly emerged (day 0) adult males of *S. mauritia* were treated topically with a single dose of 20 µg/µl of PPN. Same number of adult insects kept as controls were treated with an equivalent volume of acetone. Experimental and control insects were kept in separate beakers covered with muslin cloth. The insects were checked daily for mortality and morphological abnormalities. ASGs were removed from PPN treated insects on day 1, total protein content was estimated and further subjected to electrophoretic studies.

(3) Treatment of Methoxyfenozide on day 0 (tanned) pupae

Methoxyfenozide was dissolved and diluted in distilled water to obtain two different concentrations like 3µg /µl and 30µg / µl and applied topically on the abdominal region of day 0 (tanned) pupae using a 10 µl Hamilton microsyringe. 100 pupae were used for each experimental study. Same number of pupae were kept as control and the control pupae received an equivalent volume of distilled water. Experimental and control pupae were kept in separate beakers covered with muslin cloth and they were checked

daily for mortality and morphological abnormalities. ASGs were dissected out on in day 7 and day 8 and studied their morphogenetic changes.

5.2.4 Histological techniques (see Chapter 3).

5.2.5 Electron microscopy techniques (see Chapter 3).

5.2.6 Protein estimation (see Chapter 4)

5.2.7 Sodium dodecylsulphate- Polyacrylamide gel electrophoresis (SDS –PAGE) (see Chapter 4)

5.3 Results

5.3.1 Effects of PPN on the pupal- adult metamorphosis

In 1µg PPN treated pupae 100% mortality was observed by day 4 whereas 61% mortality was observed by day 4 in 0.1µg/µl PPN treated pupae. The survivors did not emerge as adults. These pupae when dissected on day 8 were found to contain adultoids with unstretched wings inside the pupal cases. The control pupae emerged normally and showed normal imaginal differentiation.

5.3.2 Effects of PPN on development and differentiation of male ASGs

This study was undertaken to investigate the effects of PPN on ASGs of *S. mauritia*. ASGs did not develop in pupae treated with 1µg PPN and 100% mortality was observed in these pupae by day 4. In 0.1µg PPN treated insects ASGs appeared as small buds on day 4. They didn't elongate much on

subsequent pupal days and they were seen as a fused pair of blind tubes without any regional differentiation. In control pupae ASGs showed normal development as seen in normal insects. ASGs of adultoids had a length of 4± 0.245 mm whereas those of control adults measured 77 ± 0.812 mm (Pl. XX: Figs. 56, 57). In treated pupae length of ASGs were 1 ± 0.082 mm on day 4, 2.5 ± 0.217 mm on day 5, 3 ± 0.18 mm on day 6 and 4 ± 0.163 mm on day 7 (Table 4, Fig. 60). In control pupae length of ASGs were 9.03 ± 0.209 mm on day 1, 15.1 ± 0.1 mm on day 2, 20.03 ± 0.057 mm on day 3, 33.1 ± 0.1 mm on day 4, 35.93 ± 0.115 mm on day 5, 50.13 ± 0.058 mm on day 6 and 57.73 ± 0.152 mm on day 7.

One important feature noted in the histological studies of ASGs of PPN treated insects was the retention of pupal histology i.e., the glands of adultoids consisted of an undifferentiated mass of multi layered cells along the entire length of the glands similar to those seen in pupal glands. Strikingly in PPN treated pupae fused nature of the glands was observed. The cell and nuclear volumes were very much reduced and the lumen contained no secretion (Pl. XX: Figs. 58, 59). The histological features of ASGs of control insects were consistent with the histological features of the ASGs of normal insects. The proximal and distal regions of ASGs possessed a single layer of columnar epithelial cells resting on a basement membrane surrounded by muscle layer. The cells of proximal and distal regions were comparatively larger with centralized nuclei and dense cytoplasm while the mid region was composed of

2-3 layers of closely packed small cuboidal cells with small nuclei and less denser cytoplasm. In all the regions the lumen of the gland was found to be filled with secretion.

5.3.3 Ultrastructural studies on male ASGs of PPN treated insects

The electron micrographs of ASGs of adultoids showed that most cells of ASGs were abnormal and nonfunctional without any well developed cellular organelles. In control insects, ultrastructural studies of ASGs revealed a well equipped protein synthetic and secretory machinery in their epithelial cells as seen in the ASGs of normal insects (Pl. XXI: Figs. 61, 62). Other feature observed in ASGs of PPN treated insects was crumpled intercellular membranes (Pl. XXII: Fig. 63). Moreover many cells of ASGs appeared necrotic and degenerating with smaller or shrunken nuclei. The nuclei showed disintegration in many cells. (Pl. XXII: Fig. 64). In advanced stages of degeneration nuclei had lost much of the internal contents and within these irregularly shaped nuclei condensed chromatin clumps appeared suggesting a pycnotic process (Pl. XXIII: Fig. 65). In some cells dense granules were found inside the nuclei. Nucleoli were seen to be totally disorganized. Even though the cell boundaries were maintained by the cell membranes an overall shrinkage of the cells was quite often observed along with shrinkage of nuclei. (Pl. XXIII: Fig. 66). The basal lamina was not evident. Another notable feature was the complete absence of the muscle layers. A few mitochondria

were seen in the cytoplasm. Large empty spaces were seen in between the cells (Pl. XXIV: Fig. 67). Though clumping of chromatin was seen to occur in some nuclei there was an apparent reduction of distribution aggregates. Some nuclei were found to be totally devoid of chromatin clumps (Pl. XXIV: Fig. 68). The RER which was highly developed in the epithelial cells of ASGs of control insects were hardly distinguishable in the epithelial cells of ASGs of adultoids. The lumen of the glands was empty without any secretion. (Pl. XXIV: Fig. 68). From the fine structure of ASGs of PPN treated insects it is evidently seen that they are incapable of synthesizing and secreting protein as done by ASGs in control/ normal insects.

5.3.4 Effects of PPN on development and differentiation of female ASGs

This study was undertaken to investigate the effects of PPN on ASGs of *S. mauritia*. ASGs did not develop in pupae treated with 1µg PPN and 100% mortality was observed in these pupae by day 3. In 0.1µg PPN treated insects ASGs appeared as small buds on day 3. In females ASGs showed an inhibition of growth under the effect of PPN. Reservoirs were hardly distinguishable from the glands (Pl. XXV: Figs. 69, 70). In PPN treated insects ASGs measured a length of 1 ± 0.0816 mm on day 3. They measured 2 ± 0.141 mm on day 4, 2 ± 0.05 mm on day 5 and 3 ± 0.812 mm on day 6. In adultoids, ASGs measured only 4 ± 0.216 mm whereas ASGs of control adult insects measured 13.26 ± 0.723 mm. In control insects the length of ASGs

measured was 2 ± 0.849 on day 1, 3.1 ± 0.1 mm on day 2, 7.13 ± 0.152 mm on day 3, 10 ± 0.1 mm on day 4, 12.13 ± 0.577 mm on day 5 and 12.03 ± 0.115 mm on day 6 (Table 5, Fig. 72).

Histological studies showed conspicuously small columnar cells having small nuclei. The lumen was devoid of secretion (Pl. XXV: Fig. 71). These studies clearly demonstrate that PPN treatments not only affected pupal adult morphogenesis but also affected development and differentiation of ASGs.

5.3.5 Ultrastructural studies on female ASGs of PPN treated insects

Many abnormal features were observed in the ultrastructure of ASGs of PPN treated insects. ASGs of female adultoids contained small and undifferentiated epithelial cells (Pl. XXVI: Fig. 73). The connective tissue layer was degenerated. Basal lamina was not evidently seen. Another striking feature was the shrinkage of nuclei of the epithelial cells. Nuclei contained a few heterochromatin. The cytoplasm contained very few RER and abnormal mitochondria (Pl. XXVI: Fig. 74). Large sized mitochondria were seen in epithelial cells of ASGs of control insects. Many empty spaces were seen among the epithelial cells (Pl. XXVII: Fig. 75). Another notable feature was the appearance of too many lysosomes around the RER (Pl. XXVII: Fig. 76). The chitinogenous cells appeared ruptured and vacuolated. The chitinous intima was totally absent. Cytoplasmic vacuolation was more prominent towards lumen (Pl. XXVIII: Fig. 77) and no secretion was found in the

lumen of the glands. Most striking feature was the absence of specialized secretory or storage apparatus which were very well developed in control insects (Pl. XXVIII: Fig. 78). Ultrastructure of ASGs of control insects revealed a well differentiated glandular epithelium. All the features were same as that of the normal insects.

5.3.6 Effects of 20 µg PPN on newly ecdysed adult male

This study was undertaken to investigate the effect of a high dose of PPN on the electrophoretic profile of ASG proteins of adult *S. mauritia*. The adult insects did not show any mortality and they were quite healthy and active after they recovered from a short period of anesthetic effect. When they were dissected on day 1, ASGs looked slightly swollen evidently due to the accumulation of secretion.

5.3.7 Changes in the protein content in treated male ASGs

Amount of protein was estimated in ASGs of day 0 control insects was found to be 9.28 ± 0.876µg / mg of tissue whereas the amount of protein in the ASGs of adultoids was considerably decreased to 1.9 ± 0.141 µg / mg of tissue (Table 6, Fig. 79). At 0.05 levels these values are significantly different. Amount of protein in ASGs of day 1 control insects was found to be 13.63 ± 0.410 µg / mg of tissue whereas the amount of protein in the ASGs of day 1 males treated with 20 µg PPN immediately after eclosion on day 0 had increased to 42.75 ± 1.343 µg/mg of tissue (Table 6, Fig. 80). At 0.05 levels these values are significantly different.

5.3.8 Electrophoretic profile of proteins in the ASGs of PPN treated male insects

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

5.3.8.1SDS-PAGE pattern of proteins in 0.1 µg PPN treated insects

ASGs from adultoids and the day 0 adult males emerged from control pupae were separated and the proteins were resolved by SDS- PAGE. Fig. 81, Lane 3 shows the electrophoretic profile of ASG proteins of adultoids. The staining intensities of the most of the bands was considerably less when compared to that of control (Fig. 81, Lane 4).

5.3.8.2 SDS-PAGE pattern of proteins in ASGs of 20 µg PPN treated insects

Fig. 81, Lane 2 shows the electrophoretic profile of ASG proteins of day 1 adults males treated with 20µg/µl of PPN immediately after eclosion on day 0. In the electrophorogram of treated males neither bands were found to disappear nor were new bands appeared. The staining intensities of most of the peptides of treated males were considerably more when compared to that of control.

5.3.9 Effects of methoxyfenozide on the morphogenesis of ASGs

This study was undertaken to investigate the effects of methoxyfenozide, an ecdysone agonist on the morphogenesis of ASGs of *S. mauritia*.

5.3.9.1 Effects of 3 µg methoxyfenozide on the ASGs of day 0 (tanned) pupae

The treated pupae showed 33% mortality by day 5. The survivors emerged into normal looking adults. ASGs were dissected out of the experimental and control insects and observed for morphogenetic changes.

In treated males ASGs were found to be slightly voluminous and abnormally entangled at many regions (Pl. XXIX: Fig. 82). The distal region of ASGs was very short and it measured 3 ± 0.096 mm while distal region of ASGs of control insects measured 16 ± 0.15 mm in length. Pairing was not distinctly seen at many places of mid region. The overall length of ASGs was found to be 37.17 ± 0.153 mm while ASGs of controls measured 77.13 ± 0.058 mm.

In treated females ASGs dissected out of emerged female adults was slightly thicker and they looked normal but the reservoirs were looking abnormal (Pl. XXIX: Fig. 84).

5.3.9.2 Effects of 30 µg methoxyfenozide on the ASGs of day 0 (tanned) Pupae

This study was undertaken to investigate the effect of a high dose of methoxyfenozide on ASGs of *S. mauritia*. Mortality was 50 % in treated pupae and a failure of emergence was exhibited by the survivors.

ASGs which were dissected out of the male adultoids appeared malformed and defective (Pl. XXIX: Fig. 83). They measured only 20 ± 0.153 mm in length whereas ASGs of control insects measured 77.13 ± 0.58 mm in length (Pl. XXIX: Fig. 86).

ASGs dissected out of female adultoids were abnormally thicker and shorter (Pl. XXIX: Fig. 85). The overall morphology of the reproductive system was distorted whereas the ASGs of control insects appeared normal (Pl. XXIX: Fig. 87).

5.4 Discussion

Effects of PPN on pupal- adult metamorphosis

The sequential events of pupal-adult metamorphosis in insects have been demonstrated to be under the control of ecdysteroids, juvenile hormones (JH) and brain hormones (Gilbert *et al*., 1996). JH may either inhibit the secretion of prothacicotropic hormone involved in the activation of moulting glands to produce and release ecdysteroid (Sehnal, 1985) or directly inhibit the action of ecdysteroids at the organ / tissue level. Earlier studies show that

in Lepidoptera JH is absent in the pupal stage. On the other hand ecdysteroids increase to a major peak in the haemolymph during pharate adult stage and this increase in the ecdysteroids titre promote pupal-adult metamorphosis. In normal conditions ecdysteroid titer drop to a negligible level towards the end of pupal phase for the eclosion hormone to be released. (Truman, 1971; Riddiford, 1985).

Studies of Negeshi *et al*., (1976) show that insect JH or its analogues (JHAs) when applied during a vulnerable period of physiological development of the insect disrupt the normal growth and developmental patterns of insects. In most families of Lepidoptera, treatment of JHAs affect pupal-adult transformation leading to the formation of a second pupa or pupal-adult intermediate (Srivastava and Gilbert, 1968; Daoud and Sehnal, 1974; Srivastava and Prasad, 1982; Maruthiram *et al*., 1987; Santha and Nair, 1988). The toxicity of JH is usually correlated with the concentration of the unbound hormone (Orth *et al*., 2003). Beckage (1999) explains that if the JH titer is maintained at too high a level due to administration of a JH agonist in the larval or nymphal stage then moulting to an intermediate form is induced. Studies of Richardson and Lagos (2007) reveal that PPN causes direct mortality, reduces longevity and inhibits the progeny production in *Aphis glycines*. In *S. mauritia* PPN treatments induced various abnormalities comprising toxicity, failure of emergence and the formation of adultoids. Many authors have cited that JH analogues/agonists stimulate ecdysteroids

production. Our earlier studies show treatments of newly ecdysed pupae of *S. mauritia* with JHA considerably increase endogenous ecdysteroid titres (Mona, 2001,unpublished observation). So there is a possibility of production of abnormally high endogenous ecdysteroid levels which may eventually produce many toxic effects by the disruption of the endogenous ecdysteroid titre. But studies of Zufelato *et al*., (2000) show that in *Apis mellifera* application of PPN led to a delay of ecdysteroid peak by 4 days while a decrease in ecdysteroid titre was reported when 0.1µg and 0.2 µg of PPN were applied topically on newly ecdysed pupae of *T. molitor* (Aribi *et al*., 2001). Unlike other JHAs PPN might be exerting a different effect in *S. mauritia* . In *S. mauritia* PPN application may have shifted ecdysteroid peak towards the late pupal phase. Sustained release of the endogenous ecdysteroids above the threshold level towards the later stage of pupal-adult transformations might block the release of the eclosion hormone. This might explain failure of eclosion in the PPN treated pupae of *S. mauritia* and the production of adultoids. Adultoids resulting from JHA treatment have been reported in O*ryctes rhinoceros* (Dhondt *et al*., 1976). Also inhibition of emergence is reported in PPN treated pupae of *Polypedilum nubifer* (Tarayles *et al*., 1994) and *Plutella xylostella* (Oouchi, 2005).

Effects of PPN on development and differentiation of ASGs

Previous studies show that ASG development takes place in pupal period in holometabolous insects (Happ, 1984). In *Locusta migratoria* the committance for the terminal differentiation of ASGs is triggered during the critical period when there is a decrease in the JH titer (Baehr *et al*., 1979). A significant amount of retardation is noted in the development and differentiation of ASGs. However a complete inhibition of the glandular differentiation is not observed. An inhibitory action of JH analogues on the development and differentiation of ASGs were reported by several authors (Landa and Metwally, 1974; Ramalingam and Craig, 1977; Gelbic and metwally, 1981; Roychoudhury and Chakravorty, 1987). The important feature noted in the histological studies of ASGs of treated males was the retention of pupal histology i.e., instead of having a single layer of columnar cells, the glands of adultoids consisted of an undifferentiated mass of multi layered cells similar to those seen in pupal glands. Moreover in PPN treated males ASGs were fused as seen in the early pupal stage of normal/control insects. Similar observations were reported by other workers (Cantacuzene, 1968; Szollosi, 1975). The presence of multiple layers of cells may be a primitive condition before rearrangement into a single layer during maturation into adult glands.

In the present study degenerative, irregular and inhibitory effects on

development of ASGs are due to the high titre of JH circulating in the haemolymph due to treatment. In *S. mauritia* the inhibitory effect of PPN is more conspicuous as this JH agonist was applied to the pupae when the endogenous titre of JH was minimum or absent. Studies of Mariamma (1989) in *Oryctes rhinoceros* show a dose dependent effect of JHA with regard to differentiation of ASGs.

Previous studies show that development and differentiation of ASGs involve cell multiplication, increases in cell size and acquisition of competence to make adult specific proteins accompanying rapid synthesis of secretory proteins According to Highnam and Hill (1969) in insects cell division and differentiation are intrinsic properties of the cell, residing in the genes and that the particular expression at any stage is controlled by JH.

In *T. molitor* development and differentiation of ASGs occurring in pupal stage are characterized by two bouts of mitosis (Grimes and Happ, 1980; Happ and Happ 1982; Happ *et al*., 1985). The first bout of mitosis is not ecdysteroid dependent while the second required the addition of physiological amounts of ecdysterone. The second bout of mitosis coincides with the peak of ecdysteroid in the pupal stage (Delbecque *et al*., 1978). Yaginuma *et al*., (1988) describe that ecdysteroids promote the flow of cells from the G_2 into the G_1 and S phases of cell cycle.

An excess of JH or its analogues in the haemolymph might inhibit the ASG development in two different ways. It might directly block the cell division and differentiation of ASG cells. It is assumed that in *S. mauritia* the cells of ASGs might have undergone the first bout of mitosis as evidenced by the presence of a proliferated mass of cells. At a later stage cell proliferation got arrested possibly due to the high titre of JH in the haemolymph. Alternatively the second bout of mitosis might not have occurred possibly due to a failure of an ecdysteroid peak or a decrease in the ecdysteroid titer as explained by earlier workers (Zufelato *et al.*, 2000; Aribi *et al*., 2001). Many authors have opined that degradation of JH mimics takes place rapidly (Gilbert *et al*., 2000 and Kamita *et al*., 2003) but Edwards *et al*., (1993) suggest that the action of PPN have a delay of approximately 24 h. (Mona, 2001, unpublished observation) show that in *S. mauritia* the ecdysteroid peak reaches maximum in day 2 pupae and then diminishes in subsequent days. This means that even though PPN is applied on day 0 pupae it may induce a delayed effect to block the ecdysteroids peak. A disruption in the differentiation of the cells might have affected the maturation of the cells which explains why secretory material is not observed in the lumen of ASG of treated insects.

Effects of PPN on the ultrastructure of male ASGs

The electron micrographs of ASGs of treated males show that the most of the cells appear abnormal and nonfunctional. It is clear that treatments of PPN caused degeneration and necrosis of epithelial cells of ASGs. These degenerative and inhibitory effects on development of ASGs are due to a high titre of JH circulating in haemolymph. In *S. mauritia* the inhibitory effects of PPN treatment is more severe as the endogenous JH titre is low during pupal stage in natural situations. An excess of JH or its analogues/agonists might block the cell division and differentiation of cells. This is why the epithelial cells do not exhibit any signs of synthetic or secretory activity in treated insects.

It seems that PPN have blocked the differentiation of cells of ASGs in a similar way as its well known inhibitory effects on morphogenesis. The reduction of cytoplasm is a sign of necrotic cells. Nucleus with scanty clumps of heterochromatin and disintegrated nucleoli show that the nucleus is undergoing pycnotic process. Appearance of large empty spaces among the cells show an advanced phase of degeneration. Most of the cellular organelles are underdeveloped or rather undifferentiated due to the influence of PPN. Muscular outer wall of ASGs shows complete degeneration. Degeneration of muscle fibrils in flight muscles of *Ips paraconfusus* in response to JHA treatment was reported (Unnithan and Nair, 1977). From the present study it

is very clear that the PPN treatments have deleteriously affected the synthetic and secretory activity of the ASGs. Absence of secretion in the lumen is also an evidence of this.

In treated females the epithelial cells are smaller with small nucleus containing a few heterochromatin. The connective tissue layer is lacking. The cells appeared nonfunctional and nonsecretory. The specialized secretory apparatus which appeared to be involved in the secretion of ASGs of control insects was totally absent in treated insects. Vacuolation is prominent towards lumen. A few vesiculated RER are seen. Another remarkable feature is scarcity of mitochondria which were abundant and elongated in ASGs of control insects. Degenerated and abnormal mitochondria in response to JHA treatment were reported in *Ips paraconfusus* (Unnithan and Nair, 1977). Other striking features are absence of chitinous intima and vacuolation of chitinogenous cells. As PPN treatments produced vacuolation in the chitinogenous cells, development of the chitinous intima is disrupted. In *Culex pipiens* the cuticle of 4th-stage larvae treated with PPN exhibited an amorphous cuticular region instead of normal lamellate cuticle with vacuolation (El-Shazly and Refaie, 2002). This evidence indicates that PPN interferes with the deposition of normal cuticle, Although PPN is a juvenile hormone mimic, its effect on the cuticle somewhat resembles the effect of chitin synthesis inhibitors such as diflubenzuron. As expected from the abnormal internal milieu of the cells the secretory function is impaired and the

lumen of the glands is empty.

Changes in the protein content and SDS-PAGE pattern in treated male ASGs

In *S. mauritia* PPN exerts different effects in the protein synthesis of ASGs when applied in different phases of life cycles. Amount of protein decreases significantly in ASGs when PPN is treated in newly ecdysed pupae whereas the amount of protein significantly increase in the ASGs of when PPN is treated in newly eclosed adults. Figure 81, Lane 3 represents the electrophoretic profile of ASG proteins of adultoids. Here not only the staining intensity of the most of the bands was considerably less compared to that of control (Fig. 81, Lane 4) but also some bands have disappeared. Fig. 81, Lane 2 shows the electrophoretic profile of ASG proteins of day 1 adult males treated with 20 µg PPN immediately after eclosion. The staining intensities of the most of the bands were more compared to that of control. These results show dual effects of PPN in two different phases of the life cycle of *S. mauritia*. These antagonistic effects of PPN could be explained based on the physiological role of JH in natural conditions. JH is a unique developmental hormone in several aspects. During metamorphosis JH blocks the expression of subset of genes that specifies the imaginal phenotype and during adult reproductive stage it activates the expression of subset of genes that are necessary for reproduction (Jones *et al*., 1993).

In insects, the titre of JH is usually controlled by JH-specific esterases and some other mechanisms (Gade *et al*., 1997; Gilbert *et al*., 2000; Kamita *et al*., 2003). However treatments of PPN may disrupt the regulatory mechanisms resulting in the presence of detrimentally high JH titres. This will disrupt not only the endocrine physiology but also might cause pharmacological effects (Webb *et al*., 1999; Wilson, 2004). In *Locusta migratoria* PPN repressed the synthesis of two proteins and stimulate the synthesis of another high molecular weight protein (de Kort and Koopmanschap, 1991). It is assumed that PPN have impaired protein synthesis in the ASGs of 0.1 µg treated insects. In *Aedes aegypti* JHA impair the capacity of fat bodies of pupae to synthesize proteins resulting in a lowered concentration of fat body proteins (Gordon and Burford, 1984). There are evidences that JH affect the post transcriptional processing or translation processes. The ability of JH to prevent translation of the transcripts for the basic hexameric proteins of *Trichoplusia* after it no longer affected their gene transcripts support the possible role at this level (Jones *et al*., 1993) Another possibility is that PPN might have hindered the synthesis of protein products by inhibiting the transcription of many genes or by promoting the degradation of RNAs.

Previous studies show that the secretory function of ASGs in adults (reproductive stage) is under the influence of JH. In many insect species including *Drosophila melanogaster* JH has been shown to influence post-

ecdysial maturation of male ASGs (Yamamoto, 1989). A rapid increase in the JH titer in the newly eclosed adults is reported in *D.melanogaster* and is a probable key feature in the maturation of gametes and testes (Bownes and Rembold, 1986). In male moths of *Ephestia cautella* ecdysteroid titres are relatively low throughout their adult life (Shaaya *et al*., 1991). Studies of Gillott and Friedel (1976) show that treatment of synthetic JH in adult *Melanoplus sanguinipes* results in an increase in the protein content of ASGs. They further suggest that synthesis of proteins take place in fat body under the stimulatory effect of the JH analogue and these proteins are incorporated into the secretions of ASGs. In *S. mauritia* also the increase in the staining intensity of the peptides is thought to be correlated with the stimulatory effect of PPN on the enhancement of ASG proteins in adult insects.

Effects of methoxyfenozide on morphogenesis of ASGs

During pupal-adult metamorphosis ecdysteroids increase to a major peak during the first half of pupae i.e. pharate adult development which then declines to promote pupal-adult ecdysis (Dean *et al*., 1980; Sehnal *et al*., 1986; Shaaya *et al*., 1986; Spindler-Barth *et al*., 1986; Warren *et al*., 1986; Shaaya *et al*., 1993). Our previous studies show that in *S. mauritia* haemolymph ecdysteroid titres increase on day 1 and form a significant peak on day 2 and thereafter decrease to minimum (Mona, 2001, unpublished observation). Pupal ecdysteroid peak is required for the ongoing growth and

differentiation of ASGs in monarch butterflies (Herman and Barker, 1976); *Tenebrio molitor* (Happ and Happ, 1982; Grimes and Happ, 1987). In *S. mauritia* also ASG development takes place in pupal period as observed in other insects. In the present study the treatment of male pupae with 3µg methoxyfenozide which exhibits strong ecdysone–like activity caused a slight increase in the size of the glands. Here the size of cells might have increased so as to produce a hypertrophic effect. However the overall length of ASGs was highly reduced. The female ASGs are also affected by the treatment. Happ (1990) suggested that 20-hydroxyecdysone promote the growth of ASGs by accelerating the cell cycle and there by transforming the imaginal tissues to adult ASGs. The present findings demonstrate that though methoxyfenozide had acted to promote the growth of ASGs to a certain extent this effect could not be sustained. This could be related to the time of application of the methoxyfenozide as it is important for the ecdysteroid analoues/agonists as explained by Pszczolkowski *et al*., (1998). Treatment of pupae with 30 µg of methoxyfenozide induced development of adultoids in *S. mauritia.* This might be due to the maintenance of high titre of endogenous ecdysteroid titres since it is well accepted that eclosion hormone could be released only after a drop in ecdysteroid level (Truman, 1971; Riddiford, 1985). Treatment of male pupae with the high dose of methoxyfenozide caused an inhibitory effect on the development and differentiation of ASGs whereas no severe inhibition is observed in females though their ASGs show

some retardation. In holometabolous insects development and differentiation of ASGs take place during pupal phase depending on a critical titre of ecdysteroids. Hence treatment of pupae with high dose of methoxyfenozide might have resulted hyperecdysonism and many of the coordinated processes in cell differentiation might have got disrupted. Inhibitory effects of ecdysteroids in high doses are reported. In experiments using *Drosophila* cell lines Wyss (1976) reported that low levels of ecdysone stimulated cell divisions and cell differentiation whereas high levels had an inhibitory effect.

5.5 Summary

1. Hundred percent mortality was observed by day 4 when newly ecdysed day 0 pupae were treated with 1µg PPN whereas 61% mortality was observed by day 4 in 0.1µg/µl PPN treated pupae but the survivors showed failure of emergence. When dissected on day 8 they were found to contain adultoids with unstretched wings inside the pupal cases. The control pupae emerged normally and showed normal imaginal differentiation.

2. In 0.1µg PPN treated male insects ASGs appeared as small buds on day 4. The length of ASGs of adultoids measured 4 ± 0.245 mm whereas those of control adults measured 77 ± 0.812 mm. The length of ASGs of treated pupae measured 1 ± 0.082 mm on day 4, 2.5 ± 0.217 mm on day 5, 3 ± 0.18 mm on day 6 and 4 ± 0.163 mm on day 7. In control pupae, length of ASGs measured 9.03 ± 0.209 mm on day 1, 15.1 ± 0.1 mm on day 2, 20.03 ± 0.057 mm on day 3, 33.1 ± 0.1 mm on day 4, 35.93 ± 0.115 mm on day 5, 50.13 ± 0.058 mm on day 6 and 57.73 ± 0.152 mm on day 7.

3. Male ASGs of PPN treated insects retained pupal histology. They consisted of an undifferentiated mass of multi layered cells along the entire length of the gland. The cell and nuclear volumes were very much reduced and the lumen contained no secretion.

4. In control males, the proximal and distal regions of ASGs possessed a single layer of columnar epithelial cells resting on a basement

membrane surrounded by muscle layer while the mid region was composed of 2-3 layers of closely packed small cuboidal cells. In all the regions the lumen of the gland was filled with secretion.

5. The ultrastructure of ASGs of male adultoids showed that their ASGs are incapable of synthesizing and secreting protein. Most of the cellular organelles were undifferentiated. Many cells appeared necrotic and degenerating with large empty spaces and pycnotic nuclei. Some nuclei were devoid of chromatin clumps. Nucleoli were totally disorganized and an overall shrinkage of the cells was observed. Musculature was lacking. RER were hardly distinguishable and the lumen of the glands was empty without any secretion whereas ultrastructural studies of ASGs of control insects revealed well equipped protein synthetic and secretory machinery in their epithelial cells.

6. ASGs did not develop in female pupae treated with 1µg PPN and 100% mortality was observed in these pupae by day 3. In 0.1µg PPN treated insects ASGs appeared as small buds on day 3. Reservoirs were hardly distinguishable from the glands.

7. Histology of female adultoids showed conspicuously small columnar cells having small nuclei. The lumen was devoid of secretion.

8. Ultrastructure of ASGs of female adultoids showed degeneration of the connective tissue, many empty spaces among the epithelial cells and shrinkage of nuclei of the epithelial cells. The cytoplasm contained very few RER, abnormal mitochondria and too many lysosomes. Cytoplasmic vacuolation was more prominent towards lumen. The chitinogenous cells appeared ruptured and vacuolated, chitinous intima and specialized secretory apparatus were totally absent. No secretion was found in the lumen of the glands.

9. Ultrastructure of ASGs of control insects revealed a well differentiated glandular epithelium as seen in normal insects.

10. Amount of protein in ASGs of adultoids was found to be 1.9 ± 0.141 µg/mg of tissue whereas the amount of protein in the ASGs of control insects was found to be $9.28 \pm 0.0.141$µg/mg of tissue. Amount of protein in ASGs of 20 µg PPN treated insects was 42.75 ± 1.343 µg/mg of tissue whereas the amount of protein in the ASGs of control insects was 13.63 ± 0.410 µg / mg of tissue.

11. SDS- PAGE profile of ASG proteins of adultoids showed that a few peptide bands were absent band and the staining intensities of the most of the bands were considerably less when compared to that of control.

12. Electrophoretic profile of ASG proteins of day 1 adult males treated with 20µg/µl of PPN on day 0 showed that the staining intensities of most of the bands were considerably more when compared to those of control.

13. Day 0 tanned pupae treated with 3µg methoxyfenozide showed 33% mortality by day 5 and the ASGs of emerged adults males were found to be slightly voluminous and abnormally entangled at many regions. Pairing was not distinctly seen at many places of mid region. The distal region of ASGs was very short and measured 3 ± 0.096 mm. The overall length of ASGs got reduced to 37.17 ± 0.153 mm. ASGs of emerged adult females looked normal and slightly tranluscent.

14. ASGs of adult males and females kept as controls showed normal development.

15. The day 0 tanned pupae treated with 30µg methoxyfenozide showed 50% mortality and a failure of emergence. ASGs of the male adultoids appeared malformed and defective. They measured only 20 ± 0.153 mm in length. ASGs of female adultoids were thicker and abnormally shorter and the overall morphology of the reproductive system was distorted.

16. ASGs of adult males and females kept as controls showed normal development.

17.

REFERENCES

Abdalla, F.C and Cruz-Landim. (2003). Occurrence, morphology and ultrastructure of the Dufour gland in *Melipona bicolor lepeletier*. (Hymenoptera: Meliponini). *Revista Brasileira de Entomologia.,* **48** (1), 9-19.

Abdallahi, E., Adam, A., Vifimela, E.(2000). Studies on activity of Pyriproxyfen and fipronil on *Opius concolor* Szeplligeti (Hymenoptera: Braconodae) and its substitution host *Ceratitis capitata* (Widelmann) (Diptera:Tephitidae) *Boletin SanidadVegetal Plagus* **26,** 503-511 (In Spanish)

Abo-Elghar, G.E., El-Sheikh, A.E., El-Sayed, F.M., El-Maghraby, H.M., El-Zun, H.M. (2004). Persistence and residual activity of an organophosphate, pirimiphos-methyl and three IGRs, hexaflumuron, teflubenzuron and pyriproxyfen, against the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Pest Manag. Sci.,* **60** (1), 95-102.

Adiyodi, K.G., and Adiyodi, R.G. (1974). Ultrastructure of the utriculi majores in the mushroom shaped male accessory gland of *Periplaneta americana. Z. Zellforsch .*, **47**, 433-440

Adiyodi, K.G., and Adiyodi, R.G. (1975). Morphology and cytology of the accessory sex glands in invertebrates. *Int. Rev. Cytol.*, **43**, 353-398.

Adlakha, V., Bhargava S., and Pillai, M.K.K. (1976). Histological and histochemical studies on the male accessory glands of Culicine mosquitoes *Aedes aegypti. Entomon*., **1**,101-110.

Amaldose, G. (1987). Ultrastructure and physiology of Ductus ejaculatorius duplex and seminal vesicles of male *Spodoptera litura* (Lepidoptera: Noctuidae). *Phytophaga.*, **1**(1), 93-103.

Ammann, H. (1994). Die postembryonale Entwicklung derweiblichen Geschlechtsorgane in der Raupe von Solenebia triquetrella F.R. (Lep.) erganzenden Bemerkungen uberdientwicklung des mannilichen Geschechtsapparates. *Zool. Jahrb. Anat*., **73**, 337-394

Anderson, J. M. (1950). A cytological and cytochemical study of the male accessory reproductive glands in the Japanese beetle *Popillia japonica.* New man. *Biol. Bull*., **99**, 49-64.

Andries, J.C. (1977). Specialization of the endoplasmic reticulum in the apex of the midgut cells of *Aeshna cyanea* (Insecte: Odonate). *Gen. Comp. Endocr*., **39**,174-191

Aribi, N., Lakbar, S., Smagghe, G., and Soltani, N. (2001). Comparative action of RH-0345 and Pyriproxyfen on molting hormone production and protein analysis in mealworm pupae. *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet*., **66** (2a), 445-54

Baehr, J.C., Porcheson P., Papillon, M., and Dray, F. (1979). Haemolymph levels of juvenile hormone, ecdysteroids and proteins during the last two larval instars

of *Locusta migratoria.* *J. Insect Physiol.,* **25**, 415-421.

Baer, B., Maile, R., Schmid-Hempel, P., Morgan, E.D., and Lones, G.R. (2000) Chemistry of a Mating Plug in Bumble bees. *J. Chemical Ecolgy.,* **26** (8), 1869-1875.

Bairati, A. (1968). Structure and ultrastructure of the male reproductive system of *Drosophila melanogaster* Meig. 2. The genital duct and accessory glands. *Monit. Zool. Ital.*, **2**, 105–182.

Balamani, E., and Nair, V.S.K. (1989a). Effects of antijuvenile hormone agents and a juvenile hormone analogue on neck ligated post feeding last instar larvae of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *Insect Sci. Appl.*, **10**, 583-589.

Balamani, E., and. Nair, V.S.K (1992). Inhibitory effects of a juvenile hormone analogue on prothoracic gland activity in the penultimate and last instar larval development of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *Zoologischer Anzeiger.,* **228**, 182-187.

Balamani, E., and. Nair, V.S.K. (1989b). Prothoracicotropic action of juvenile hormone analogue on neck ligated last instar larvae of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) *Entomon*., **14**, 283-288.

Balamani, E., and. Nair, V.S.K. (1991). On the morphogenetic role of juvenile hormone in the prepupal stage of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *Invertebrate Reprod. Dev.,* **30**, 175-179.

Barbier, R. (1975). Differentiation de structures ciliairis et mise en place des canaux au cover de 1 organogenese des glandes colleteriques de *Galleria mellonella* L. (Lepidoptera, Pyralidae). *J. Microsc. Biol*. *Cell*., **24**, 315-326.

Beament, J.W.L and Lal, R. (1957). Penetration through the egg shell of *Pieris brassicae* (L). *Bull. Ent. Res*., **48**, 109-125.

Beckage, N.E. (1999). Insect growth regulators. In: *Biological and Biotechnological Control of Insect Pests* (Eds. J.E. Rechcigl and N.A. Rechcigl)., pp. 123-137 (CRC Press).

Benny, T. M., and Nair, V.S.K. (1999). Involvement of ecdysteroids in the fusion of testes lobes of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *J. Entomol*. *Res*., **23**(4), 343-345.

Berry, S. J. (1968). The fine structure of the colleterial glands of *Hyalophora cecropia* (Lepidoptera) *J. Morphology*., **125** (3), 259-279.

Biddinger, D. J., and Hull, L.A. (1999). Sublethal effects of selected insecticides on growth and reproduction of a laboratory susceptible strain of tufted apple bud moth (Lepidoptera: Tortricidae). *J. Econ. Ent*., **92**, 314-324.

Billingsley, P.F., and Downe, A.E.R. (1986). Nondigestive cell types in the midgut epithelium of *Rhodnius prolixus* (Hemiptera: Reduviidae). *J. Med. Entomol*., **23,** 212-216.

Blain, W. D., and Dixon S. E. (1973). The effect of juvenile hormone on the function of the accessory gland of the adult male cockroach *Periplaneta*

americana (Orthoptera: Blattidae). *Can. Ent*., **105**, 1275-1280.

Bodenstein, D., and Sprague I. B. (1959). The developmental capacities of the accessory sex glands in *Periplaneta americana. J. Exp. Zool*., **142**, 177-202.

Bonnan Sant-Jais, M.L. (1974). Morphologie de la glande lactee d'une Glossine, *Glossina austeni* Newst, au cours du cycle de gestation. I. Aspects ultrastructuraux en periode de gestation. *J. Microsc*. (Paris) **19**, 265-284.

Bouix, G. (1966). Particularites structurales et fonctionnelles de l' appareil reproducteur male des Coleopteres Carabidae. *Bull. Soc. Zool. Fr*., **91**, 353-365.

Bownes, M., and **Partridge**, L. (1987). Transfer of molecules from ejaculate to females in *Drosophila pseudoobscura*. *J. Insect. Physiol.,* **33**, 941-947.

Bownes, M., and Rembold, H. (1987). The titre of juvenile hormone during the pupal and adult stages of the life cycle of *Drosophila melanogaster*. *Eur. J. Biochem*., **164**, 709-712.

Brits, J. A. (1978). The structure and physiology of the male reproductive system of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). J. *Ent. Soc. S. Adr*., **41**, 285-296.

Brits, J. A. (1979). The development of the male reproductive system of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *J. Ent. Soc. S. Adr*., **42**, 383-393.

Bruder, J.E. (1917). Die spermatogenese Von *Dielphia euphorbiae* L. *Arch. Zellforsch.*, **14**, 26-78.

Brunet, P.C.J. (1952). The formation of the ootheca by *Periplaneta americana*. The structure and function of the left colleterial gland. *Q. J. Micro. Sci.,* **93**, 47-69.

Brunold, E. (1957). Die Entwicklung des weiblichen Genitalapparates von *Solenobia triquetrella* F.R. (Lep:Psychidae) Wahrend des Puppen stadiums. *Zool. Jahrb. Anat.*, **75**, 581-614.

Bull, D.L., and Meola, R.W. (1993). Effect and fate of growth regulator pyriproxyfen after application to the horn fly (Diptera: Muscidae). *J. Econ. Ent.,* **86**,1754-1760.

Callahan, P.S., and Cascio, T. (1963). Histology of the reproductive tract and transmission of sperm in the corn earworm, *Heliothis zea. Ent. Soc. Amer*., **56**, 535-56.

Callahan, P.S., and Cascio, T. (1962). Histology of the reproductive tract and transmission of sperm in the corn earworm, *Heliothis zea Ann. Ent. Soc.Amer.,* **51**, 413-428.

Cantacuzene, A. M. (1971). Origine et caracteres ultrastructuraux des cellules spermiophages ducriquet migrateur *Locusta migratoria J. Microsc*., (Paris) **10**, 179-190.

Cantacuzene, A. M.(1968). Recherches morphologiques et physiologiques sur les glandes annexes males des orthopteres 111. Modes d'association des spermatozoides d' Orthopteres, *Z- Zellforsch*., 113-126.

Cantacuzene, A.M. (1967). Histologie des glandes annexes males de *Schistocerca gregaria* F. (Orthopteres). Effect de allatectomie sur leve structure et leur activite C.R. *Acad. Sci., Paris.* **264**, 93-96.

Carpenter, J. E., and Chandler, L.D. (1994). Effects of sublethal doses of two insect growth regulators on *Helicovera zea* (Lepidoptera: Noctuidae) reproduction. *J. Entomol. Sci.,* **29,** 425-435.

Chandler, L.D., Pair, S.D., and Harrison, W.E. (1992). RH- 5992, a new insect growth regulator active against corn earworm and fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Ent*., **85**, 1099-1103.

Chapman, R. F. (1998). The insects: structure and function, Cambridge University press. Cambridge, 770.

Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, MF., Smith, HK., and Patridge, L**.(2003).** The sex peptide of *Drosophila melanogaster*: investigation of post-mating responses of females using RNA interference. *Proc. Natl. Acad. Sci.,* **100**, 9923-9928.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., and Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* female sis mediated by male accessory gland products. *Nature.,* **373**, 241-44.

Charmillot, P.J., Pasquier, D., Alipaz, N. (1994). Le tebufenozide, un nouveau produit selectif de lutte contre le carpocapse *Cydia pomonella* L.et la tordeuse de la pelure Adoxophyes orana F.V.R. *Revue Suisse Viticulture, Arboriculture et Horticulture.,* **26**, 123-129.

Chen P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., and Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behaviour of female *D. melanogaster*. *Cell*., **54** (3), 291-8.

Chen, P.S. (1984). The functional morphology and histochemistry of insect male accessory glands and their secretion. *Ann. Rev. Entomol.*, **29**, 233-255.

Chen, P.S. (1996). The accessory gland proteins in *Drosophila*: structural, reproductive and evolutionary aspects. *Experientia.,* **52**, 503-10.

Chen, P.S., and Oechslin A. (1976). Accumulation of glutamic acid in the paragonial gland of *Drosophila nigromelanica. J. Insect Physiol*., **22**, 1237-1243.

Clements, A. N. (1963). The physiology of mosquitoes. p.164, Macmillan Co., New York.

Cmelik, S.H.W., Bursell, E., and Slack, E. (1969). Composition of the gut contents of third instar testse larvae (*Glossina morsitans* Westwood) *Comp. Biochem. Physiol*., **29**, 447-453.

Colonello, N. A., and Hartfelder, K. (2003). Protein content and pattern during mucus gland maturation and its control in honey bee drones. *Apidologie.,* **34**, 257-267.

Couche, G.A., and Gillott, C. (1990). Structure of the accessory reproductive glands of the male migratory grasshopper, *Melanoplus sanguinipes* *J. Morphol.*, **203** (2), 219-245.

Cruz-Landim, C ., and Dallacqua, R.P**Dallacqua, R.P** ., (2005**).**Morphology and protein patterns of honey**.***Genet. Mol. Res.* **34**(3),473-81.

Cusson, M., Mc Neil, J.N., and Tobe, S.S. (1990). In Vitro biosynthesis of juvenile hormone by corpora allata of *Pseudeletia unipuncta*. *J. Insect physiol*., **40**, 129-136.

Dallai, R., Zizzari, Z.V., and Fanciulli, P.P. (2008). Fine structure of the spermatheca and of the accessory glands in *Orchesella villosa* (Collembola: Hexapoda). *J. Morphol*., **269** (4), 464-78.

Daoud, D.S., and Sehnal, F. (1974). Effects of juvenoids on the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) *Bull. Entomol Res*., **64**, 643-651.

Darriet, E., and Korbel, V. (2006). Laboratory evaluation of pyriproxyfen and spinosad, alone and in combination, against *Aedes aegypti* larvae. *J. Med. Entomol***., 43**(6), 1190-1194.

Davey, K.G. (1985). The male reproductive tract. pp 1-14 In: Kerkut, G.A and L.I Gilbert (Eds)., *Comprehensive insect physiol., Biochem. Pharmacol.*, Vol.**1,** 487**,** Pergamon Press, Oxford.

De Kort, C. A. D., and Koopmanscha, A.B. (1991). A juvenile hormone analogue affects the protein pattern of the haemolymph in last-instar larvae of *Locusta migratoria* *J. Insect Physiol*., **37**(2), 87-93.

Dean, R., Bollenbacher, W.E ., Locke, M., Smith, S. L., and Gilbert, L.I. (1980). Haemolymph ecdysteroid levels and cellular events in the intermoult/ moult sequence of *Calpodes ethlius* *J. Insect Physiol*., **26**, 267-280.

Degrugillier, M.E. (1985). *In vitro* release of house fly *Musca domestica* L. (Diptera: Muscidae), acrosome material after treatment with secretion of female accessory gland and micropyle cap substance. *Int. J. Insect Morphol.Embryol.*,**14**,381–391.

Delbecque, J. P., Hirn, M., Delachambre, J., De Reggi. M. (1978), Cuticular cycle and molting hormone levels during the metamorphosis of *Tenebrio molitor* (Insecta: Coleoptera). *Dev Biol.*, **64**, 11–30.

Dhadialla, T.S., Carlson, G. R., and Le, D.P. (1998). New insecticides with ecdysteroidal and juvenile hormone activity. *Annu. Rev. Entomol.*, **43**, 545-569.

Dhondt, A.A., Mc Govern, T.P., and Beroza, B.M. (1976) *J. Econ. Entomol*., **69**,165.

Dodson, M. (1937). Development of the female genital ducts in *Zygaena* (Lepidoptera). *Proc. Roy. Entomol*. *Soc*. London (A)., **12**, 61-68.

Dorn, A., and Schneider, G. S. (1986). Development of the male genital system of *Oncopeltus fasciatus* (Insecta: Heteroptera) and its endocrine regulation- a review. *General Endocrinol*., (Life Sci. Adv)., **5**, 1-10.

Du bois, A. M. (1931). Morphologische Untersuchengen uber die Entwicklung des Kopulationsapparates der intersexuellen Weibchen von *Lymantria dispar* L. Wilh. Roux *Archiv f. Entwick. Organ*., **124,** 93-137.

Duportets, L., Dufour, M.C., Couillaud, F., and Gadenne, C. (1998). Biosynthetic activity of corpora allata, growth of accessory sex glands and mating in the male moth *Agrotis ipsilon* (Hufnagel) *J. of Exp. Biol.,* **201**(16), 2425-2432.

Edwards, G.C., Braun, R.P., and Wyatt, G.R. (1993). Induction of vitellogenin synthesis in *Locusta migratoria* by the juvenile hormone analog, Pyriproxyfen. *J. Insect Physiol.,* **39** (7), 609-614.

Ejezie G.C., and Davey K.G. (1977). Some effects of mating in female tsetse *Glossina austeni*. *J. Exp. Zool.,* **200**, 303-310.

Ejezie, G.C., and Davey, K.G. (1976). Some effects of allatectomy in the female tsetse, *Glossina austeni J. Insect Physiol*. **22,** 1743-1749.

Ejezie, G.C., and Davey, K.G. (1974). Changes in the neurosecretory cells, corpus cardiacum and corpus allatum during pregnancy in *Glossina austeni* Newst. *Bull. Ent. Res*., **64,** 247-256.

Elliot, R. (1964). Recognition of the sexes in some Scarabeid larvae *N.Z.J.SC.*, **17,**181-187.

El-Shazly, M.M., and Refaie, B.M (2002) Larvicidal effect of the juvenile hormone mimic pyriproxyfen on *Culex pipiens. American Mosquito Control Association*., **18**(4), 321-328.

El-Zoheiry, A..H. (1999a). Histological studies on the bean shaped accessory glands in the male beetle *Blaps polychresta, J. Egypt. Ger. Soc. Zool.*, **28(**C), 241-258.

El-Zoheiry, A..H. (1999b). Histological studies on the tubular accessory glands in the male beetle *Blaps polychresta*. *J. Egypt. Ger. Soc-Zool*., **28** (C), 259-274.

Ephrussi, B., and Beadle, G.W. (1936). A technique of transplantation for *Drosophila*. *Amer. Naturalis* ., **70**, 218-225.

Escherich, K. (1894). Anatomische studien uberdes mannliche Genitalsystem der Coleopteran. *Z. Wiss. Zool. Abt*., **57**, 620- 41.

Fanger, H., and Naumann, C.M. (1993). Correlation between the mesodermal male genital ducts and the spermatophore structure in a Ditrysian moth, *Zygaena trifolii* (Esper, 1783). (Insecta, Lepidoptera:Zygaenidae) *Acta zool.,* **74,** 239-246.

Fawcet, D.W (1981). *The cell.,* Philadelphia, W.B. Saunders Company. 862.

Federer, H., and Chen, P.S. (1980). Ultrastructure and Funktion der Pasagonien von *Drosophila funebris. Rev. Suisse. Zool*., **87**, 875-880.

Federrer, H., and Chen, P.S.(1982). Ultrastructure and nature of secretory proteins in the male accessory gland of *Drosophila funebris. J. Insect Physiol.,* **28**, 743-751.

Felix, C.R., Betschart, B., Billingsley, P.F., and Freyvogel, T.A., (1991). Post

feeding induction of trypsin in the midgut of *Aedes aegypti* L. (Diptera: Culicidae) is separable into two cellular phases. *Insect Biochem*., **21**, 197-203.

Fernandez N.M., and Klowden, M.J. (1995). Male accessory gland substances modify the host seeking behaviour of gravid *Aedes aegypti* mosquitoes. *J. Insect Physiol*., **41**, 965–970.

Fernandez and Cruz-Landim (2005). Morphology and ultrastructure of the male accessory glands of *Achoria grisella* (Fabricius).(Lepidoptera: Pyralidae). *Braz. J. Morphol. Sci*., **22**(3), 129-136.

Ferreira A., Abdalla F.C., Ker, W. E., and Cruz-Landim, C. (2004) Comparative anatomy of the male reproductive internal organs of 51 species of bees. *Neotrop. Entomol*., **33**, 569-576.

Friedel, T., and Gillottt, C.(1976). Extraglandular synthesis of accessory reproductive gland components in male *Melanoplus sanguinipes*. *J. Insect. Physiol*., **22**, 1309-1314.

Gade, G., Hoffmann, K.H., and. Spring, J.H (1997). Hormonal regulation in insects: facts, gaps and future directions. *Physiol. Rev.*, **77,** 963-1032.

Gadzama, N. M., and Happ, G. M. (1974). The structure and evacuation of the spermatophore of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Tiissue and Cell.*, **6**, 95-108.

Geetha, P.R. (2003). Chemical nature of female accessory reproductive glands secretions in *Opisinia arenosella* Walker (Lepidoptera:Cryptophasidae). *Entomon*., **28** (4), 373-377.

Gelbic, I., and Metwally, M. M. (1981). Changes in the development of male germinal cells in *Spodoptera littoralis* caused by the effects of juvenoids (Lepidoptera: Noctuidae). *Acta ent. bohemoslov*., **78**, 10-17.

Gelman, D.B., Pszczolkowski, A., Blackburn, M.B and Ramaswamy, S.B (2007). Ecdysteroids and juvenile hormones of whiteflies, important insect vectors for plant viruses. *J. Insect. Physiol.*, **53***(3)*, 274-84.

Gerber, G.H. (1976). Reproductive behaviour and physiology of *Tenebrio molitor*. (Coleoptera: Tenebrionedae). III. Histogenetic changes in the internal genitalia, mesentron and cuticle during sexual maturation *Can. J. Zool*., **54**, 990-1002.

Gerber, G.H., Church, N.S., and Rempel, J.G. (1971) The anatomy, histology and physiology of the reproductive systems of *Lytta nuttali* (Coleoptera: Meloidae). *Can. J. Zool*., **49,** 523-533.

Gerber, G.H., Neil G.B., and Westdal, P.H. (1978). The anatomy and histology of the internal reproductive organs of the sunflower beetle, *Zygogramma exclamations* (Coleoptera: Cheysomelidae). *Can. J. Zool*., **56**, 2542-2553.

Giebultowicz, J. M., Feldlaufer, M., and Gelman, D.B. (1990). Role of ecdysteroids in the regulation of sperm release from the testis of the gypsi moth

Lymantria dispar. J. Insect Physiol., **36**, 567-571.

Gilbert, D.G. (1981). Ejaculate esterase 6 and initial sperm use by female *Drosophila melanogaster J. Insect. Physiol*., **27**, 641-650.

Gilbert, L. I., Rybczynski, R., and Tobe, S.S. (1996). Endocrine cascade in metamorphosis . In " Metamorphosis: Post embryonic reprogramming of Gene Expression in Amphibian and insect cells" (Eds. Gilbert, L.I and Tata, J.R and Atkinson, B.G).pp. 59-107 Academic Press. San Diego.

Gilbert, L.I., Granger, N.A., and Roe, R.M. (2000). The juvenile hormones: historical facts and speculations on future research directions. *Insect Biochem. Mol. Biol*., **30**, 617-644.

Gillott, C. (1988). Arthropoda- Insecta .pp.319-471 In: Adiyodi K.G and R.G Adiyodi (Eds.). *Reproductive Biology of Invertebrates., Vol III* (Aceessory Sex Glands) Oxford and IBH, New Delhi 518pp

Gillott, C. (1996). Male insect accessory glands: functions and control of secretory activity. *Invertebr. Reprod . Dev.,* **30,** 199-205.

Gillott, C. (2003). Male accessory gland secretion: Modulators of female Reproductive physiology and behaviour. *Annu. Rev. Entomol*., **48**, 163-84

Gillott, C., and Fred, T. (1976). Development of the accessory reproductive glands and its control by the corpus allatum in adult male *Melanoplus sanguinipes J. Insect Physiol*., **22**, 365-372.

Gillott, C., and Gaines S. B. (1992). Endocrine regulation of male accessory gland development and activity. *Can. Ent.*, **124**, 871-886

Gooding, R.H. (1973). The digestive processes of haematophagous insects IV. Secretion of trypsin by *Aedes aegypti* (Diptera:Culicidae). *Can. Entomol*., **105**, 599-603

Gordon, R. and Burford, I. R. (1984). Effects of methoprene, a juvenile hormone analogue, on the larval and pupal stages of the yellow fever mosquito, *Aedes aegypti. J. Insect Physiol*., **30** (4), 279-286.

Grayson, S., and Berry, S.J. (1974). Synthesis and intracellular transport of protein by the colleteral gland of the *Cecropia* silkmoth. *Dev. Biol*., **38**, 150-156.

Gregory, G.E. (1965). The formation and fate of spermatophore in the African migratory locust, *Locusta migratoria migratorioides* Reiche and Fairmaire . *Trans. Roy. Ent Soc. London.,* **117**, 33-66.

Grimes, M.J. and Happ G.M. (1980). Fine structure of the bean-shaped accessory gland in the male pupa of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Int. J. Insect Morphol. Embryol*., **9**, 281-296.

Grimnes, K.A. and Happ G.M. (1987). Ecdysteroids *invitro* promote differentiation in the accessory glands of male mealworms beetles. *Experientia*., **43**, 906-907.

Gundevia, H.S., and RamaMurthy, P.S. (1977). The male accessory reproductive glands and spermatophore in *Hydrophetus olivaceus* (Polyphaga-

Coleoptera). *Z. Mikrosk. Anat. Forsch.*, **91**, 475-492.

Hangartner, W.W., Suchy, M., Wipf, H.K., and Zurflueh, R.C. (1976). Synthesis and laboratory and field evaluation of a new highly active and stable insect growth regulator. *J. Agric, Food Chem.*, **24**, 169-175.

Happ, G.M. (1984). Structure and development of male accessory glands in insects. In: *Insect Ultrastructure* Vol. **2**. (Eds. R.C. King and H. Akai), Plenum Press, New York, pp. 365-396.

Happ, G.M. (1990). Ecdysteroids and the development of male accessory glands of insects. *Adv. Invert. Reprod*., **5**, 181-18.

Happ, G.M. (1992). Maturation of the male reproductive system and its endocrine regulation. *Annu. Rev. Entomol*., **37**, 303-20.

Happ, G.M. and Happ, C.M. (1982). Cytodifferentiation in the accessory glands of *Tenebrio molitor* X. Ultrastructure of the tubular gland in the male pupa . *J. Morphol.*, **172**, 97-112.

Happ, G. M., Mac Leold, B.J., Szopa, T.M., Bricker, C.S., Lowell, T.C., Sankel, J.H and Yunker, C. (1985). Cell cycles in the male accessory glands of mealworm pupae. *Dev. Biol*., **107**, 314-324.

Hartley, J.C. (1961). The shell of acridid eggs. *Q. JI microsc. Sci*., **102**, 249-255.

Hatakoshi, M. (1992) An inhibitory mechanism over oviposition in the tobacco cutworm *Spodoptera litura* by juvenile hormone analogue Pyriproxyfen. *J. insect Physiol.*, **38**, 793-801.

Hecker, H., and Rudin, W. (1979) Normal versus α amanitin induced cellular dynamics of the midgut epithelium in female *Aedes aegypti* L. (Insecta: Diptera) imagines. *Acta Trop*., **28**, 80-104.

Heifetz, Y., Tram, U., and Wolfner, M. F. (2001). Male contributions to egg production: the role of accessory glands and sperm in *Drosophila melanogaster*. *Proc. R. Soc. Ser. B-Biol. Sci.*, **268**, 175-180.

Henrick, C.A., Willy, W. E., and Staal, G.B. (1976). Insect juvenile hormone activity of alkyl (2E, 4E)- 3, 7, 11- trimethyl-2, 4-dodecadienoates. Variations in the ester function and the carbon chain. *J. Agric. Food. Chem.*, **24**, 207-218.

Herman, W. S. (1975). Endocrine regulation of post-eclosion enlargement of the male and female reproductive glands in monarch butterflies. *Gen. comp. Endocrinol.*, **26**, 534-40.

Herman, W. S., and Bennet, D.C. (1975). Regulation of oogenesis, female specific protein production, and male and female reproductive gland development by juvenile hormone in the butterfly, *Nymphalis antiopa.* *J. Comp. Physiol.*, **99**, 331-338.

Herman, W. S., and Dallmann, S.H. (1981). Endocrine biology of the painted lady butterfly *Vanessa cardui. J. Insect Physiol*., **27**, 163-168.

Herman, W.S. (1973). The endocrine basis of reproductive inactivity in Monarch

butterflies *Gen. comp. Endocr*., **26**, 534-541.

Herman, W.S. and Peng, P. (1976). Juvenile hormone stimulation of sperm activator production in male monarch butterflies. *J. Insect Physiol***., 22**, 579-581.

Herman, W.S. and Peng, P. (1976). Juvenile hormone stimulation of sperm activator production in male monarch butterflies. *J. Insect Physiol***., 22**, 579-581.

Herman, W.S., and Barker J. F. (1976). Ecdysterone antagonism, mimicry and synergism of juvenile hormone action on the Monarch butterfly reproductive tract. *J. Insect Physiol*., **22**, 643-648.

Herndon, L.A., Chapman, T., Kalb, J.M., Lewin, S., Partridge, L., and Wolfner, M. F. (1997). Mating and hormonal triggers regulate accessory gland gene expression in male *Drosophila*. *J. Insect. Physiol.,* **43**, 1117-1123.

Higbee, B.S., Horton, D.R., Krysan, J.L. (1995). Reduction of egg hatch in pear psylla (Homoptera:Psyllidae) after contact by adults with insect growth regulators. *J. Econ.Entomol*., , 1420-1424.

Highnam, K. C., and Hill, L. (1969). In: *The comparative Endocrinology of the invertebrates*. E. Arnold Ltd. London. pp. 270.

Hinton, H.E. (1961). The structure and function of the eggshell in Nepidae (Hemiptera). *J. Insect Physiol*., **7**, 224-257.

Holland, B., and Rice, W. R. (1998). Chase-away sexual selection: Antagonistic seduction verses resistance; *Evolution.,* **52**, 1–7.

Hosken, D. J and Ward, P. I. (1999). Female accessory reproductive gland activity in the yellow dung fly *Scathophaga stercoraria* (L.) *J. Insect Physiol*., **45** (9), 809-814.

Hosken, D. J., and Stockley, S. (1998). Sperm counts. *Trends in Ecology and Evolution.,* **13**, 91–92.

Huet, C. (1966). Etude experimentale du development de l'appareil genital male de *Tenebrio molitor* (Coleoptera: Tenebrionidae) *Comptes Rendus des séances. Soc. Biol.*, (Paris) **160**, 2021-2025.

Hyun, K. T., and Moon, M. J. (2003). Fine structure of female accessory reproductive gland in the mealworm beetle, *Tenebrio molitor Entomol Res.*, **33** (3), 165-172.

Ishaaya, I., and Horowitz, A.R. (1995). Pyriproxyfen, a novel insect growth regulator for controlling whiteflies: mechanisms and resistance management. *Pesticide Sci.,* **43**, 227-232.

Ishaaya, I., De Cook, A., and Deghlee, D. (1994). Pyriproxyfen, a potent suppressor of egg hatch and adult formation of the greenhouse whitefly (Homoptera: Aleyrodidae). *J. Econ. Ent*., **87**, 1185-1189.

Izadi, H., and Subrahmanyam, B. (2005). Protein profiles of *Spodoptera litura* (F). male accessory reproductive gland and its secretions. *Commun. Agric. Appl. Biol. Sci*., **70** (4), 857-62.

Jackson, W.H. (1889). Studies in the morphology of Lepidoptera. *Trans. Linn. Soc.*

Lond. (Zool.)., **5**, 168-186.

Jagannadh, V., and Nair, V. S. K. (1993). Effects of azadirachtin, and a juvenile hormone analogue on neck ligated post feeding larvae of *Spodoptera mauritia* Boisd. *Indian J. Exp. Biol.*, **31**, 199-200.

Jagannadh, V., and Nair, V.S.K. (1992). Azadirachtin induced effects on larval-pupal transformation of *Spodoptera mauritia Physiol. Entomol.*, **17**, 56- 61.

Jin, Y., Chen, Y., Jiang, Y and Xu, M. (2006). Proteome analysis of the silkworm (*Bombyx mori* L). Colleterial gland during different developmental stages. *Arch. Insect. Biochem. Physiol*., **61**(1), 42-50.

Jones, G., Venkataraman, V., Manczark, M., and Schellung, D. (1993). Juvenile hormone action to suppress gene transcriptions and influence message stability. *Dev. Gent*., **14**, 323-332.

Jordao, B.P., Lehane, M.J., Terra, W.R., (1996a) An immunochemical investigation of trypsin secretion in the mid gut of *Stomoxys calcitrans*. *Insect Biochem. Mol.Biol*.,

Jordao, B.P., Terra, W.R., and Ribeiro, A.F.(1996b) Trypsin secretion in Musca domestica larval midguts. A biochemical and immunological study. *Insect Biochem. Mol.Biol*.,

Joubert, P.C. (1964). The reproductive system of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae). Development of the female reproductive system. *S. Afr. J. Agric. Sci*., **7**, 65-78.

Judd, S.R., Leopold, R.A. and Turner, R.B. (1983). Characteristics of aminopeptidase activity of female housefly reproductive accessory glands. *Int. J. Inv. Reprod*., **6**, 93-98.

Kamita, S.G., Hinton, A.C., Wheelock, C.E . (2003). Juvenile hormone (JH) esterase: Why are you so JH specific ? *Insect Biochem. Mol. Biol*., **33**, 1261-1273.

Kaulenas, M.S. (1992). Insect accessory reproductive structures. Function, structure, and development. *Zoophysiol*., **31**, 159-206

Khalifa, A. (1949). Spermatophore production in Trichoptera and some other insects. *Trans. Roy Ent. Soc. London.*, **100**, 449-471.

Knight, A. L. (2000). Tebufenozide targeted against codling moth (Lepidoptera: Tortricidae) adults eggs and larvae. *J. Econ. Entomol.*, **93**, 1760-1767.

Knowlton, N., and Greenwell, S. R. (1984). Male sperm competition avoidance mechanisms: the influence of female interests. In: *Sperm competition and the evolution of animal mating systems* (Smith, R.L, Ed). Orlando: Academic Press. 61–84.

Koehler, P. G., and Patterson, R.S. (1991). Incorporation of Pyriproxyfen in a German cockroach (Dictyoptera: Blatellidae) management program. *J. Econ. Entomol.*, **84** (3), 917-21.

Koen, J. M., and Matt, A.T. (2001). Allo hormones: a class of bioactive substances

favoured by sexual selection. *J. Comparative Physiology A: Neuroethology, Sensory, Neural, and behavioural Physiol.,* **187** (5), 323-326.

Koepp, J. K., Fuchs, M., Chen, T. T., Hunt, L.M., Kovalick, G.E., and Briers, T. (1985). The Role of Juvenile hormone in reproduction".*In comprehensive Insect Physiol. Biochem. Pharmacol.,* (Ed. Kerkut, G.A and Gilbert, L. I) **8**, 165-203, Pergamon Press, New York.

Koolman, J. (1989). In: *Ecdysone: from Chemistry to Mode of action*. George Thieme Verlag, Stuttgart

Kubli, E. (1996). The *Drosophila* sex peptide: a peptide pheromone involved in reproduction, In: *Advances in developmental biochemistry* (Wassarman. P. Ed.) New York, J. A. I Press **4**, 99-128.

Kubli, E. (2003). Sex peptides: Seminal peptides of the *Drosophila* male. *Cellular and molecular Life Sci..,* **60** (8), 1689-1704.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature.,* **227**, 680-685.

Lai Fook, J. (1982). Structure of the accessory glands and duplex of the internal male reproductive system of *Calpodes ethlius* (Hesperidae: Lepidoptera). *Can J. Zool*., **60**, 1202-1215.

Landa, V. (1959). The development and function of the male reproductive organs of *Melolontha melolontha L. Acta Soc. Ent. Cech*., **56**, 313-331.

Landa, V. (1960). Origin, development and function of the spermatophore in cockchafer (*Melolontha melolontha* L.) *Acta Soc. Ent. Cech*., **57**, 297-316.

Landa, V., and Metwally, M. M. (1974). Effects of conventional chemosterilants and juvenile hormone analogues on spermatogenesis in *Trogoderma granarium* (Coleoptera: Dermestidae) *Acta Ent. Bohemoslov.,* **71**, 45-152.

Lay, M., Loher, W., and Hartmann, R. (2004). Pathways and destination of some male gland secretions in female *Locusta migratoria* (R&F) after insemination. *Arch. Insect. Biochem Physiol.,* **55** (1), 1-25.

Leclercq-Smekens, M. (1976). Organogenese et differenciation des voies genitals femelles *d'Euproctis chrysorrhea* L.(Lepidoptere: Lymantriidae). *Int. J. Insect Morphol. Embryol*., **5**, 241-252.

Lehane, M. J. (1976). Digestive enzyme secretion in *Stomoxys calcitrans* (Diptera: Muscidae) *Cell and Tissue Res.,* **170**, 275-287.

Lehane, M.J. (1989). The intracellular pathway and kinetics of digestive enzyme secretion in an insect midgut cell. *Tissue and Cell*., **21**, 101-111.

Leopold, R.A. (1970). Cytological and cytochemical studies on the ejaculatory duct and accessory secretion in *Musca domestica. J. Insect physiol.,* **16**, 1859-1872.

Leopold, R.A. (1976). The role male accessory glands in insect reproduction. *A Rev. Ent*., **21**, 199-221.

Leopold, R.A. (1981). Accessory reproductive gland involvement with the sperm-egg interaction in muscoid flies In: *Advances in invertebrate Reproduction* (Eds. W.H. Clark, Jr. and T.S. Adams). Vol.2. Elsevier/ North Holland, New York, pp. 253-270.

Leopold, R.A., and Degrugillier, M. E. (1973). Sperm penetration of housefly eggs; evidence for involvement of a female accessory secretion. *Science* **181**, 555-557.

Leopold, R.A., Meola, S., Degrugillier, M.E. (1978). *The egg fertilization site within the house fly, Musca domestica (L.) (Diptera: Muscidae)The egg fertilization site within the house fly, Musca domestica (L.) (Diptera: Muscidae)* *Int. J. Insect Morphol. Embryol*. **7** (2), 111-120.

Lessman, C.A., Rollins, L., and Herman, W.S. (1982). Effects of juvenile hormone I, II and III on reproductive tract growth in male and female monarch butterflies. *Comp. Biochem. Physiol*., **71**,141-144.

Lim, J. L., and Yap, H.H. (1996). Induction of wing twisting abnormalities and sterility on German cockroaches (Dictyoptera: Blattellidae) by a juvenoid Pyriproxyfen. *J. Econ. Entomol.,* **89**, 1161-1165.

Lim, S.P., and Lee, C.Y. (2005). Effects of juvenile hormone analogs on new reproductives and colony growth of Pharaoh ant (Hymenoptera: Formicidae) *J. Econ. Entomol*., **98** (6), 2169-2175.

Liu, D.G., and Trumble, J. T (2005). Interactions of plant resistance and insecticides on the development and survival of *Bactericera cockerelli* (Sulc) (Homoptera:Psyllidae) *Crop Port*., **24**, 111-117.

Liu, T. X., and Chen, T. Y. (2001). Effects of a juvenile hormone analog, Pyriproxyfen, on the apterous form of *Lipaphis erysimi*. *Entomologia Experimentalis et Applicata.,* **98**, 295-301.

Lococo, D., and Huebner, E. (1980). The ultrastructure of the female accessory gland, the cement gland, in the insect *Rhodnius prolixus Tissue and Cell*., **12**, 557-580.

Loeb, M. J. (1991). Growth and development of spermducts of the tobacco budworm moth *Heliothis virescens in vivo* and *in vitro Invertebrate Repr. Dev.*, **19**, 97-105

Loeb, M.J., and Hakim, R. S. (1991). Development of genital imaginal discs of *Heliothis virescens* culture *in vitro* with 20-hydroxy ecdysone and fat body or testis sheaths. *Invertebrate Reproduction and Development.,* **20**, 181-191

Lum, P.T.M, (1961). The reproductive system of some Florida mosquitoes 1. The male reproductive tract. *Annals of the Ent. Soc. Amer.*, **54**, 397-401

Ma, W.C., and Denlinger, D. L. (1974). Secretory discharge and microflora of milk gland in tsetse flies. *Nature.,* London. **247**, 301-303.

Marchini, P., Del Bene, G., Cappelli, L., and Dallai, R (2003). Ultrastructure of the male reproductive accessory glands in the medfly *Ceratitis capitata* (Diptera: Tephritidae) and preliminary characterization of their secretions. *Arth. str.*

and Dev., **31**, 313-227.

Margam, M., Gelman, D.B., and Palli, S.R. (2006). Ecdysteroid titers and developmental expression of ecdysteroid regulated gene during metamorphosis of the yellow fever mosquito *Aedes aegypti* (Diptera:Culicidae) *Venu. J. Insect physiol*., **52**(6), 558-568.

Mariamma, J. (1989). Differentiation of male reproductive system in *Oryctes rhinoceros* (Coleoptera, Scarabidae). *Proc. Indian Acad. Sci. (Anim. Sci.).*, **98** (4), 233-242.

Markow, T., and Ankney, P.F. (1984). *Drosophila* males contribute to oogenesis in a multiple mating species. *Sci.*, **224**, 302-303.

Maruthiram, G., Kishen Rao, S., Thakur, S.S., Raghunatha Rao, D., and Subba Rao, L. (1987). Effect of juvenoid, Hydroprene on the pupa of sweet potato weevil *Cylas formicarius* (Fabr.) (Coleoptera: Curculionidae). *J. Anim. Morphol. Physiol*., **34** (1, 2), 45-54.

Mastuda, R. (1976). Morphology and Evolution of the insect Abdomen, Pergamon Press, Oxford.

Mercer, E. H., and Brunet, P.C.J. (1959). The electron microscopy of the left colleterial gland of the cockroach. *J. Biophy. Biochem. Cytol.* **5**, 257-261.

Met Calfe, M.E. (1932). The structure and development of the reproduction system in the reproductive system in the Coleoptera with notes on its homologies. *Q.J. Microsci*., **75**, 49-129.

Miller, R.W. (1989). Evaluation of S-31183 for fly (Diptera: Muscidae) control as a feed through compound for poultry, cattle and swine. *J. Agri. Entomol.* **6**, 77-81.

Miller, R.W., and Miller, J.A. (1994). Pyriproxyfen bolus for control of fly larvae. *J. Agri. Entomol*. **1**, 39-44.

Minakuchi, C., Nakagawa, Y., Kamimura, M., and Miyagawa, H. (2003). Binding affinity of nonsteroidal ecdysone agonists against the ecdysone receptor complex determines the strength of their molting hormonal activity. *Eur. J. Biochem*., **270 (2270 (20), 4095 – 4104.**

Miyamoto, J., Hirano, M., Takimoto, Y., and Hatakoshi, M. (1993). Insect growth regulators for pest control, with emphasis on juvenile hormone analogs: Present and future prospects In: *Pest control With enhanced Environmental Saftey* (Ed. Duke S.O., Menn J.J., Plimmer J.R). Acs Symp. Ser. Washington, D.C. Am. **524**, 144-168.

Mona, P.M. (2001). On the developmental profile of hormones in *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). Ph.D Thesis, University of Calicut .

Monsma, S.A., and Wolfner, M F. (1988). Structure and expression of a *Drosophila* male accessory gland gene whose product resembles a peptide pheromone precursor. *Genes Dev.* **2**, 1063-73.

Moors, L., Spass O., Koeniger G., and Billen J. (2005). Morphological and ultrastructural changes in the mucus glands of *Apis mellifera* drones during pupal development and sexual maturation. *Apidologie.*, **36**, 245-254.

Muse, W.A., and Balogun, R.A. (1992) Carbohydrates in the accessory reproductive gland of adult male variegated grasshopper *Zonocerus variegates* (Orthoptera: Acridoidea). *Entomon.*, **17** (3-4), 155-158.

Musgrave, A. J. (1937). Histology of the male and female reproductive organs of *Ephestia kuhniella* . *Proc. Zool. Soc*. (London)., **107**, 337-364.

Nair, V.S.K. (1981). Hormonal control of the mating behaviour in the females of *Spodoptera mauritia* (Lepidoptera: Noctuidae). *Curr. Sci*., **50**, 690-691.

Nair, V.S.K. (1993). Applied potential of insect hormone research: perspectives and prospects. In: Chemical Ecology of Phytophagous Insects. (Ed. Ananthakrishnan, T.N., and Raman, A). pp 89-104, Oxford- IBH Publ. Co., New Delhi.

Nair, V.S.K., and Rajalakshmi. (1989). Effects of fluoromevalonate on penultimate and last instar larvae of *Spodoptera mauritia* Boisd. *Ind. J. Exp. Biol.* **27**, 170-173.

Nayar, K.K. (1973). *Elements of endocrinology*, Prentice hall International, New Delhi, London, Toronto, Tokyo.

Negeshi, T., Shimada, S., and Asano, S. (1976). Effects of insect growth regulators on the tobacco cutworm, *Spodoptera litura*: Rectal prolapse. *J. Insect Physiol.*, **22**, 1523-1527.

Noirot, C., and Quennedy, A. (1974). Fine structure of insect epidermal glands. *Annu. Rev. Entomol*., **19**, 61-80.

Noirot, C., and Quennedy, A. (1991). Glands, gland cells, glandular units: some comments on terminology and classification, *Ann. Soc. Entomol*. *Fr. (N.S.).*, **27**,123-128

Norris, M.J. (1932). Contributions towards the study of insect fertility. 1. The structure and operation of the reproductive organs of the genera *Ephestia* and *Plodia*. *Proc. Zool. Soc* (London)., **102**, 595-611.

Oberlander, H., and Silhacek, D.L. (1998). Mode of action of insect growth regulators in Lepidopteran tissue culture. *Pesticide sci.,* **54**, 300-302.

Odhiambo, T.R. (1969a). The architecture of the accessory reproductive glands of the male desert locust II. Microtubular structures. *Tissue and cell.*, **1**,325-340.

Odhiambo, T.R. (1969b). The architecture of the accessory reproductive glands of the male desert locust IV. Fine structure of the glandular epithelium. *Phil Trans. Soy. Soc., London*. **256** (B), 85-114.

Odhiambo, T.R. (1970). The architecture of the accessory reproductive glands of the male desert locust II. Components of the muscular wall. *Tissue and cell.,* **2**, 233-248.

Odhiambo, T.R. (1971). The architecture of the accessory reproductive glands of the male desert locust V. Ultra structure during maturation. *Tissue and cell.,* **3**, 309-324.

Oetken, M; Bachmann, J., Schulte-Oehlmann, U. and Oehlmann. J. (2004). Evidence for endocrine disruption in invertebrates. *International review of cytology.,* **236**, 1- 44.

Ogiso, M., and Takabashi, S.Y. (1984) Trehelases from the male accessory glands of the American cockroach: Developmental changes and the hormonal regulation of the enzymes. *Gen. Comp. Endocrinol.,* **55**, 387-392.

Omura, I. (1938). Studies on the reproductive system of the male of *Bombyx mori*. Post testicular organs and post-testicular behaviour of the spermatozoa *J. Fac. Agric. Hokkaaido Imp. Univ*., **40**,129-170.

Orth, A. P., Tauchman, S.J., Doll, S.C., and Goodman, W.G. (2003). Embryonic expression of juvenile hormone binding protein and its relationship to the toxic effects of juvenile hormone in *Manduca sexta*. *Insect Biochem. Mol. Biol.,* **33**(12), 1275-84.

Osnai, M., Aigaki, T and Kasuga, H. (1987). Energy metabolism in the spermatophore of the silkmoth, *Bombyx mori,* associated with accumulation of Alanine derived from Arginine. *Insect Biochem.,* **17** (1), 71-75.

Otronen, M., Reguera, P., Ward, P.I., (1997). Sperm storage in the yellow dung fly *Scathophaga stercoraria*: Identifying the sperm of competing males in separate female spermathecae *Ethology* ., **103**, 844-854.

Ottiger, M., Soller, M., Stocker, R. F and Kubli, E. (2000). Binding sites of *Drosophila melanogaster* sex peptide pheromones. *J. Neurobiol*. **44,** 57-71

Ouchi, H. (2005). Insecticidal properties of a juvenoid, Pyriproxyfen on all stages of the Diamond back moth, *Plutella xylostella* (Lepidoptera:Yponomeutidae) *Appl. Entomol. Zool*., **40**, 145-149.

Outram, I. (1970). Morphology and histology of reproductive system of the male spruce budworm *Choristoneura fumiferana*. *Can. Entomol.,* **102**, 404-414.

Palade, G. E. (1955). A small particulate component of the cytoplasm. *J. Biophy. Biochem. Cytol.,* **1**, 59-68.

Parkman, J. P., and Frank, J.H. (1998). Development and reproduction of mole crickets (Orthoptera: Gryllotalpidae) after treatments with hydroprene and Pyriproxyfen. *J. Econ. Entomol*., **91**, 392-397

Peleg, B.A. (1988). Effect of a new phenoxy juvenile hormone analogue on California red scale (Homoptera: Diaspididae) Florida wax scale (Homoptera: Coccidae) and the ectoparasite *Aphytis holoxanthus* Debache (Hymenoptera:Aphelinidae). *J. of Econ. Entomol*., **81,** 88-92.

Pendum, V.R., and Tembhare, D.B.(2005). Histobiochemical studies on the male accessory sex glands of *Antheraea mylitta* (Dury). (Lepidoptera: Saturnidae)

and the effects of juvenile hormone and ecdysone. *Entomo*n., **30** (1), 5-12.

Pitnick, S., Markow T.A. (1994). Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm, Vol. 91. In: *Proceedings of the National Academy of Sciences* USA. pp. 277–281.

Prabhu, V.K.K., and Sreekumar, S. (1994). Endocrine regulation of feeding and digestion in insects. In: *Perspectives in Entomological research.* (Ed. By Agarwal, O.P). pp 117-135, Scientific Publishers, Jodhpur.

Pradeep, A.R., and Nair, V.S.K. (2005). Acquiring vitellogenic competence in the rice pest *Nilaparvata lugens* Stal: Effects of a juvenile hormone analogue, hydroprene. *Int. J. Indust. Entomol.,* **10**, 137-141.

Pradeep, A.R., and. Nair, V.S.K. (1989). Morphogenetic effects of precocene II in the brown plant hopper *Nilaparvata lugens* stal. (Homoptera: Delphacidae) *Proc. Indian Natn. Sci. Acad.,* B **55**, 381-386.

Pszczolkowski, M., Kuszczak, B. Smagghe, G. (1988). Endocrine background of how 20-hydroxyecdysone agonist, RH 5849, influences diurnal pattern of pupation in *Spodoptera littorali.* *Entomologia Experimentalis et ApplicataEntomologia Experimentalis et Applicata* ., **87 (387 (3), 255 – 261.**

Quennedy, A. (1998). Insect epidermal gland cells: ultrastructure and morphogenesis, in Harrison F.E., Locke M. (Eds.), *Micros. Anat. Invert.,* Wiley- liss, **11** A, 177-207.

Raina, A. K. (1989). Male-induced termination of sex pheromone production and receptivity in mated females of *Heliothis zea. J. Insect Physiol*., **35**, 821-826.

Rajapakse, C.N., Meola, R., and Readio, J. (2002). Comparative evaluation of juvenoids for control of cat fleas (Siphonoptera: Pulicidae) in top soil. *J. Med. Entomol*, **39**(6), 889-894.

Ramalingam, S. (1974). Structure and function of male accessory glands of mosquitoes. Ph.D Thesis. As cited in *J. Insect Physiol*., **24** (3), 251-259.

Ramalingam, S., and Craig, G.B. Jr. (1978). Fine structure of the male accessory glands in *Aedes triseriatus*. *J. Insect. Physiol*., **24**, 251-259.

Retnakaran, A., Mac Donald, A., Tomkins, W.L., Davis, C.N., Brownright, A.J., and Palli, S.R.(1997). Ultrastructural effects of a non-steroidal ecdysone agonist, RH-5992, on the sixth instar larvae of the spruce budworm, *Choristoneura fumiferana. J. Insect. Physiol*., **43**, 55-68.

Retnakaran, A., Hiruma, K., Palli, S. R and Riddiford, L. M. (1995). Molecular analysis of the mode of action of RH 5992, a lepidopteran specific nonsteroidal ecdysteroid agonist. *Insect Biochem. and Mol. Biol*., **25**, 109-117.

Rice, W.R. (1996). Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature*., **381**(6579), 232- 4.

Richardson, M.L., and Lagos, D.M. (2007). Effects of a juvenile hormone analogue, Pyriproxyfen on the apterous form of soybean aphid (*Aphis glycines*). *J. Appl. Entomol*., **131**(5), 297-302.

Riddiford, L. M. (1985). Hormone action at the cellular level. 37-84 in Kerkut, G.A., and Gilbert L.I (Eds.) *Compr.Insect Physiol. Biochem. Pharmacol.,*. **8**, 595pp. Pergamon Press, Oxford.

Riemann, J. G., and Thorson, B.J. (1976). Ultrastructure of the ductus ejaculatorius duplex of the Mediterranean flour moth *Anagasta kuhniella* (Zeller) (Lepidoptera: Pyrallidae). *Int. J. Insect. Morphol. Embryo*., **5**, 227-240.

Riemann, J.G., and Thorson B.J. (1979). Ultrastructure of the accessory glands of the Mediterranean flour moth. *J. morphol*., **159**, 355-392.

Riemann, J.G., Moen, P.J. and Thorson, B.J. (1967). Female monogamy and its control in houseflies. *J. Insect Physiol*., **13**, 407-418.

Rojas-Rousse, D. (1972). Description et fonctionnement de l'appareil genital interne de *Diadromus pulchellus* Wesmael (Hymenoptera: Ichneumonidae)- I Testicules, canaux deferents glandes annexes, canaux collecteurs, canal ejaculateur du male *Int. J. Insect. Morphol. Embryol*., **1**, 225-232.

Rose, U., Ferber, M. and Hustert, R. (2001). Maturation of muscle properties and its hormonal control in an adult insect. *J. of Exp. Biol*., **204**, 3531-3545.

Roth, L.M. (1967). Uricose glands in the accessory sex gland complex of male Blattaria. *Ann. Ent. Soc. Amer*., **60**, 1203-1211.

Roychoudhury, N., and Chakravorty, S., (1987). *Entomon*., **12**, 261.

Safarulla, K., Benny, T.M., and Nair, V.S.K. (2003). Macromolecular synthesis in wing discs of *Spodoptera mauritia* Boisd: effects of a juvenile hormone analogue. *Indian J. Exp. Biol.*, **41**(2), 167-170.

Sakunthala, C., and. Nair, V.S.K (1995). Effects of ecdysone agonist RH 5849 on embryonic and post embryonic development of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *Indian J. Exp. Biol.,* **33**, 690-693.

Salked, E.H., and Potter, C. (1953). The effect of age and stage of development of insect eggs on their resistance to insecticides. *Bull. Ent. Res*., **4**4, 527-580.

Sam Mathai and Nair, V.S.K. (1983). Effects of precocene II on last instar larvae of *Spodoptera mauritia* (Lepidoptera: Noctuidae). *Curr. Sci*., **52**, 376-377.

Sam Mathai and V.S.K. Nair (1984a). Treatment of larvae with repeated doses of precocene II induces precocious metamorphic changes in *Spodoptera mauritia*. (Lepidoptera: Noctuidae) *Arch. Insect. Biochem. Physiol.*, **1**, 199-203.

Sam Mathai and V.S.K. Nair (1984b). Effects of precocene II on ovarian follicle development and vitellogenesis in *Spodoptera mauritia*. (Lepidoptera: Noctuidae) *abst.XVII. Internt. Con.Entomol.*, p.21.

Sandermann and Stromiger(1972). *J. Biol.Chem*., **247**,5123-5131.

Santha, P. C., and Nair, V.S.K (1986) Age- dependent responses of last instar larvae of *Spodoptera mauritia* to precocene II. *Physiol. Entomol*., **11**, 335-341.

Santha, P. C., and Nair, V.S.K. (1987). Larval- pupal transformation of *S.*

mauritia (Lepidoptera). Effects of juvenile hormone analogue. *Zool. Anz*., **218**, 379-384.

Santha, P.C., and Nair, V.S.K. (1988). Precocene induced abnormalities in FI generation of *Spodoptera mauritia* Boisd. (Lepidoptera). *Zool. Anz.*, **220**,104-110.

Santha, P.C., and Nair,V.S.K.(1991). Precocene induced changes in the cerebral neurosecretory cells of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *Zool. Anz.*, **226**,163-173.

Santha, P.C., Sankaranarayanan, P., and Nair, V.S.K. (1987). Precocene induced effects on pupal-adult development of *Spodoptera mauritia* Boisd. *Geobios New Rep.*, **6**, 35-38.

Schaefer, C. H., and Murba, T. (1990). Chemical persistence and effects of S-31183, 2[1-methyl-2-{4-phenoxyphenoxy}ethoxy] pyridine, on aquatic organisms in field tests. *J. econ. Ent*., **83**, 1768-1776.

Schaefer, C.H., Murba, T., Dupras, E. F., Mulligan, F.S., and Wilder, W.H. (1988). Efficacy, non- target effects and chemical persistence of S-31183, a promising mosquito (Diptera: Culicidae) control agent. *J. econ. Ent.*, **81**, 1648-1655.

Sehnal, F. (1985). Growth and life cycle, pp. 1-86 in Kerkut, G.A., and L.I. (Gilbert) (Eds.), *Compr.Insect Physiol. Biochem. Pharmacol*., Vol.**2**, Pergamon Press. Oxford, 505 pp.

Sendi, J. J., Srivastava, K.P., and Singh, M.R.K. (1993). Postembryonic study on the development of the female efferent genital system in the lemon-butterfly, *Papilio demoleus* L. (Lepidoptera). *J. Res. Lepid.* **32,**162.-169.

Sethi, k., and Dhillon, S. S. (1981). Development of female genital ducts in *Earias vitella* (Fabr.) (Lepidoptera: Arctiidae). *Ind. Zool.*, **5**, 47-58.

Sevala,V.L and Davey, K.G. (1991) The transparent Accessory Reproductive gland secretes a polypeptide into the haemolymph of male Rhodnius prolixus. *Insect Biochem.*, **21** (2), 215-221.

Shaaya, E., Calderon, M., Pisarev, V., Spindler, K.D. (1991). Effect of a juvenile hormone analogue on the life span, egg laying, and ecdysteroid titer of virgin *Ephestia cautella* females. *Arch. Insect Biochem. Physiol.*, **17** (2-3), 183-188.

Sharga, U.S. (1933). On the internal anatomy of some Thysanoptera. *Trans. R. Entomol. Soc*. *London.*, **81**, 185-204.

Sheehan, K.B., Richmond, R. C., and Cochrane, B. J. (1979). Studies of esterase 6 in Drosophila melanogaster 111. The developmental pattern and tissue distribution. *Insect Biochem*., **9,** 443-450.

Shepherd, J. C. (1974). Activation of Saturnid moth sperm by a secretion of the male reproductive tract. *J. Insect Physiol.*, **20**, 2107-2122.

Shinbo, H., and Happ, G.M. (1989). Effects of Ecdysteroids on the growth of the post-testicular reproductive organs in the silkworm, *Bombyx mori.* *J. Insect Physiol*., 35: 855-864.

Shymalanath, S., and Forbes, J. (1983). Anatomy and Histology of the male reproductive system in the adult and pupa of the Doryline Ant. *Aeneictus gracilus* Emery (Hymenoptera: Formicidae). *New York Entomol. Soc*., **91**(4), 377-393

Simmons, L.W.(1995). Relative parental expenditure, potential reproductive rates, and the control of sexual selection in Katydids. *The American Naturalist,* **145**, 797–808.

Sindhu, A., and Nair,V.S.K. (2004). Influence of juvenile hormone analogue on food consumption and digestive enzyme activities in *Spodoptera mauritia* Boisd. *Indian J. Exp. Biol.,* **42**, 491-494.

Sjostrand, F. S., and Hanzon, V. (1954). Membrane structures of cytoplasm and mitochondria in exocrine cells of mouse pancreas as revealed by high resolution electron microscopy. *Exp. Cell Research.*,**7**, 393-414.

Smagghe, G., and Deghlee, D. (1994). Action of the nonsteroidal mimic RH-5849 on larval development and adult reproduction of insects of different orders. *Invert. Repr.Dev*., **25**, 227-236.

Smagghe, G., Carton, B., Wesemael, W., Ishaaya, I., and Tirry, L.(1999). Ecdysone agonists; mechanism of action and application on *Spodoptera* species. *Pesticide Science*., **55**(3), 386-389.

Smid, H. M., (1997). Transfer of male accessory gland peptide to the female during mating in *Leptinotarsa decemlineata. Invert.Repr.Dev*., **34**, 47-53.

Smid, H. M., and Schooneveld, H. (1992). Male accessory Sex Glands contain a new class of exocrine peptidergic cells in *Leptinotarsa decemblineata Invert. Repro. Dev*., **21** (2) 141-148.

Soller, M., Bownes, M., and Kubli, E., (1997). Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*. *Eur. J. Biochem*., **243**, 732–738.

Spindler-Barth, M., Turberg, A and Spinder, K.D (1991). On the action of RH 5849, a nonsteroidal ecdysteroid agonist, on a cell line from *Chironomus tentans*. *Arch.insect biochem.physiol*., **16** (1), 11-18.

Srivastava , U.S., and. Gilbert , L.I (1968). Juvenile Hormone: Effects on a higher Dipteran. ***Sci.,*** 161(3836), 61 – 62.

Srivastava, U. S., and Prasad, S.S. (1982). A study of Pupal-adult intermediates produced with juvenoid treatment of *Spodoptera litura* pupae. *Proc. Indian Acad. Sci*., **91**, 337-348.

Srivastava, U.S., and Srivastava, B. P. (1959). Observations on the postembryonic development of the female reproductive organs in *Leucinodes orbonalis* Guen. (Lepidoptera: Pyraustidae) with notes on the homology in two sexes. *J. Linn. Soc. Lond. Zool*., **44**, 302-311.

Steel, C.G.H., and Davey, K.G. (1985). Integration in the insect endocrine system. pp.1-34 in Kerkut, G.A., and Gilbert L.I (Eds.) *Compr. Insect Physiol., Biochem. Pharmacol.*, Pergamon Press, Oxford. **8**, 595pp.

Stein, S.P., Tepper, C.S., Able, N.D., and Richmond, R.C. (1984). Studies of esterase 6 in *Drosophila melanogaster* XVI. Synthesis occurs in the male reproductive tract (anterior ejaculatory duct) and is modulated by juvenile hormone. *Insect Biochem*., **14**, 527-532.

Sturm, R. (2008). Morphology and histology of the ductus receptaculi and accessory glands in the reproductive tract of the female cricket, *Teleogryllus commodus*. *J. Insect Sci*., 8, 35.

Svoboda J.A., Thompson, M. J., and Robins, W.E. (1972). Azasteroids: Potent inhibitors of insect molting and metamorphosis. *Lipids.,* **7**, 553-556.

Sweeny, P.R., Church, N.S., Rempel, J.G and Gerrity, R.G.(1968). The embryology of *Lytta viridana* Le Conte (Coleoptera: Meloidae).III. the structure of the chorion and micropyles. *Can. J. Zool.*, **46**, 213-217.

Szollosi, A. (1974). Ultrastructural study of spermatodesm of *Locusta migratoria migratorioides* (R. and F): acrosome and cap formation. *Acrida.*, **3**, 175-19.

Szollosi, A. (1975). Imaginal differentiation of the spermiduct in acridids: Effect of juvenile hormone. *Acrida* ., **4**, 205- 216.

Szopa, T.M. (1982). Development of the accessory reproductive glands and genital ducts in female *Schistocerca gregaria*. *J. Insect. Physiol*., **28**, 475-483.

Thibout, E. (1971). Description de 1'apparail genital male et formation du spermalophore chez *Acrolepia assectella* (Lepidoptera: Plutellidae). C.R. *Acad. Sci*. *Paris.*. **273**, 2546-2449.

Tobe, S. S., Davey, K.G. and Heubner, E. (1973). Nutrient transfer during the reproductive cycle of *Glossina austeni* Newst. Histoloy and histochemistry of the milk gland, fat body and oenocytes. *Tissue and Cell.,* **5**, 633-650.

Tongu, Y., Suguri, S., Sakumoto, D., Itano, K., and Inatomi, S. (1972). The ultrastructure of mosquitoes 6. male accessory gland of *Culex pipiens pallens Jap. J. Sanit. Zool*., **23**, 129-139.

Tram, U., and Wolfner, M.F. (1998). Seminal fluid regulation of female attractiveness in *Drosophila melanogaster*. *Proc. natl. Acad. Sci*., USA **95**, 4051-54.

Trayler, K.M., Pinder, A.M., and Davis, J.A. (1994). Evaluation of the juvenile hormone mimic Pyriproxyfen (S-31183) against Nulsance Chironomids (Diptera: Chironomidae), with particular emphasis on *Polypedilum nubifer* (Skuce). *J. Aust. ent. Soc*., **33**, 127-130.

Trimble, J. J. (1974). Ultrastructure of the ejaculatory duct region of the love bug, *Plecia nearctica* Hardy. (Diptera: Bibionidae). *Int. J. Insect Morphol. Embryol.*, **3**, 353-359.

Trione, G., and Avicini, R.M.P. (1997). Development of female accessory glands of *Chrysomya putoria* (Wiedemann) (Diptera:Calliphoridae) during oogenesis. *Int. J. Insect Morphl. Embryol*., **26** (1), 1-7.

Trisyono, A., and Chippendale, G.M. (1997). Effect of the nonsteroidal ecdysone agonists, methoxyfenozide and tebufenozide, on the European corn borer

(Lepidoptera : Pyralidae). *J. Econ. Ent.*, **90**, 1486-1492.

Trisyono, A., and Chippendale, G.M. (1998). Effect of the ecdysone agonists, RH-2485 and tebufenozide, on the South west corn borer *Diatraea grandiosella. J. Econ. Ent.*, **53**, 177-18.

Truman, J.W. (1971). Physiology of insect ecdysis. I. The eclosion behaviour of saturnid moths and its hormone release. *J. Exp.Biol*., **54**, 805.

Trumbo, S.T., and Robinson, G.E. (2004). Nutrition , Hormones and life history in burying beetles. *J. Insect Physiol*., **50**, 383-391.

Unnithan, G. C., and Nair, K.K. (1977). Ultrastructure of juvenile hormone-induced degenerating flight muscles in a bark beetle, *Ips paraconfusus Cell Tissue Res*., **185**(4), 481-90.

Unnithan, G. C., Nair K.K., and Bowers, W.S (1977). Precocene-induced degeneration of the corpus allatum of adult females of the bug *Oncopeltus fasciatus J. Insect. Physiol*., **23**, 1081-1094.

Vail, K.M., and Williams, D.F (1995). Pharaoh ant (Hymenoptera: Formicidae) colony development after consumption of Pyriproxyfen baits. *J. Econ. Entomol*., **88**, 1695-1702.

Vail, K.M., and Williams, D.F., and Oi, D.H. (1996). Perimeter treatments with two bait formulation of pyriproxyfen for control of Pharaoh ants (Hymenoptera: Formicidae) *J. Econ. Entomol.,* **89**, 1501-1507.

Venkatesh, K., and Gillott, C. (1983). Protein production in components of the accessory gland complex of male *Melanoplus sanguinipes* (Insecta: Orthoptera). *Int. J. Invertebrate Reproduction.,* **6**, 317-325.

Vennard,C., Nguama, B., Dillon, R.J., Ouchi, H., and Charnley, A.K. (1998). Effects of the juvenile hormone mimic pyriproxyfen on egg development, embryogenesis, larval development and metamorphosis in the desert locust *Schistocerca gregaria*. *J. of Econ. Entomol.*, **91**, 41-49.

Venugopalan, A.K., Benny, T. M., and Nair, V.S.K. (1994). Testicular development and spermatogenesis in *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). *J. Anim. Morphol. and Physiol*., **41**,125-134.

Verson, E., and Bison, E. (1895). Development postembryonaire des organs sexual accessories chez le male du *Bombyx mori. Arch. Ital. Biol.*, **24**, 135-138.

Vijayalekshmi,V.R., and Adiyodi, K.G., (1973). Accessory sex glands of male *Periplaneta americana* (L). part III- Histochemistry of the mushroom-shaped and conglobate glands. *Indian J. exp. Biol*., **11**(6), 521- 4.

Viscuso, R., Narcisi, L., Sottile, L., Brundo, M.V.(2001). Role of male accessory glands in spermatodesm reorganization in Orthoptera, Tettigonioidea. *Tissue and Cell.,* **33**, 33-39.

Warren, J.T., and Gilbert, L.I. (1986). Ecdysone metabolism and distribution during the pupal-adult development of *Manduca sexta*. *Insect Biochem.,* **16**, 65-82.

Webb, T. J., Shu, S., Ramaswami, S.B., and Dorn, S. (1999). Influence of juvenile

hormone and mating on oogenesis and oviposition in the codling moth, *Cydia pomonella Arch. Insect Biochem. physiol*., **41**,186-200.

Wensler, R.J.D., and Rempel, J.G. (1962). The morphology of the male and female reproductive systems of the midge, *Chironomus plumosus L. Can. J. Zool*., **40**, 199-229.

White, D.F., and Lamb, K.P. (1968). Effect of a synthetic juvenile hormone on adult cabbage aphids and their progeny. *J. Insect.Physiol*., **14**, 395-402.

Wiggles worth, V.B. (1936). The function of the corpus allatum in the growth and reproduction of *Rhodnius protixus* (Hemiptera). *Quart. J. .Micr. Sci*., **79,** 91-120.

Williams, C.M. (1967). The juvenile hormone: Its role in the endocrine control of moulting, pupation, and adult development in the *Cecropia* silkworm. *Biol. Bull. Woods Hole.* **12** (1), 572-585.

Wilson, N., Tubman, S.C., Eady, P.A., Robertson, G.W.(1997). Female genotype affects male success in sperm competition. *Proc. R. Soc. London.,* B **264,** 1491-1495.

Wilson, T.G. (2004). The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: How these compounds kill insects. *J. of Insect Physiology* ., **50**,111-121.

Wing, K.D (1988). RH 5849, a non steroidal ecdysone agonist: Effects on a *Drosophila* cell line. *Sci.*, **241**, 467-469.

Wolfner, M. (1997). Tokens of love: functions and regulation of *Drosophila* male accessory gland products. *Insect Biochem. Mol. Biol*.**2**7,179-92.

Wolfner, M.F. (2002). The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity*., **88**, 85-93.

Wyss, C. (1976). Juvenile hormone analogue counteracts growth stimulation and inhibition by ecdysones in clonal *Drosophila* cell line. *Experentia***., 32**, 1272-1274.

Yaginuma, T., and Happ, G.M. (1989). 20-Hydroxy ecdysone acts in the male pupa to commit accessory glands towards trehelase production in the adult mealworm beetle (*Tenebrio molitor*). *Gen. Comp. Endocrinol.*, **73**, 173-185.

Yaginuma, T., Kai, H., and Happ, G.M. (1988). 20 hydroxyecdysone accelerate the flow of cells into the G phase and the S phase in a male accessory gland of the meal worm pupa (*Tenebrio molitor*). *Dev. Biol.*, **126**,173-181.

Yokoyama, V.Y., and Miller, G.T. (1991). Potential of Pyriproxyfen as a quarantine treatment for codling moth and oriental fruit moth (Lepidoptera: Tortricidae). *J. Econ.Entomol*., **54**, 942-947.

Zeh, J. (1997). Polyandry and enhanced reproductive success in the Harlequin-beetle-riding pseudoscorpion. *Beh. Ecol. Sociobiol.*, **40**, 111–118.

Zufelato, M.S., Bitondi, M.M.G., Simoes, Z. L. P., and Hartfelder, K. (2000). The

juvenile hormone analogue Pyriproxyfen affects ecdysteroid–dependent cuticle, melanization and shifts the pupal ecdysteroid peak in the honey bee (*Apis mellifera*) *Arth. Str. Dev.*, **29** (2), 111-119.

Fig. 7: Male Reproductive System

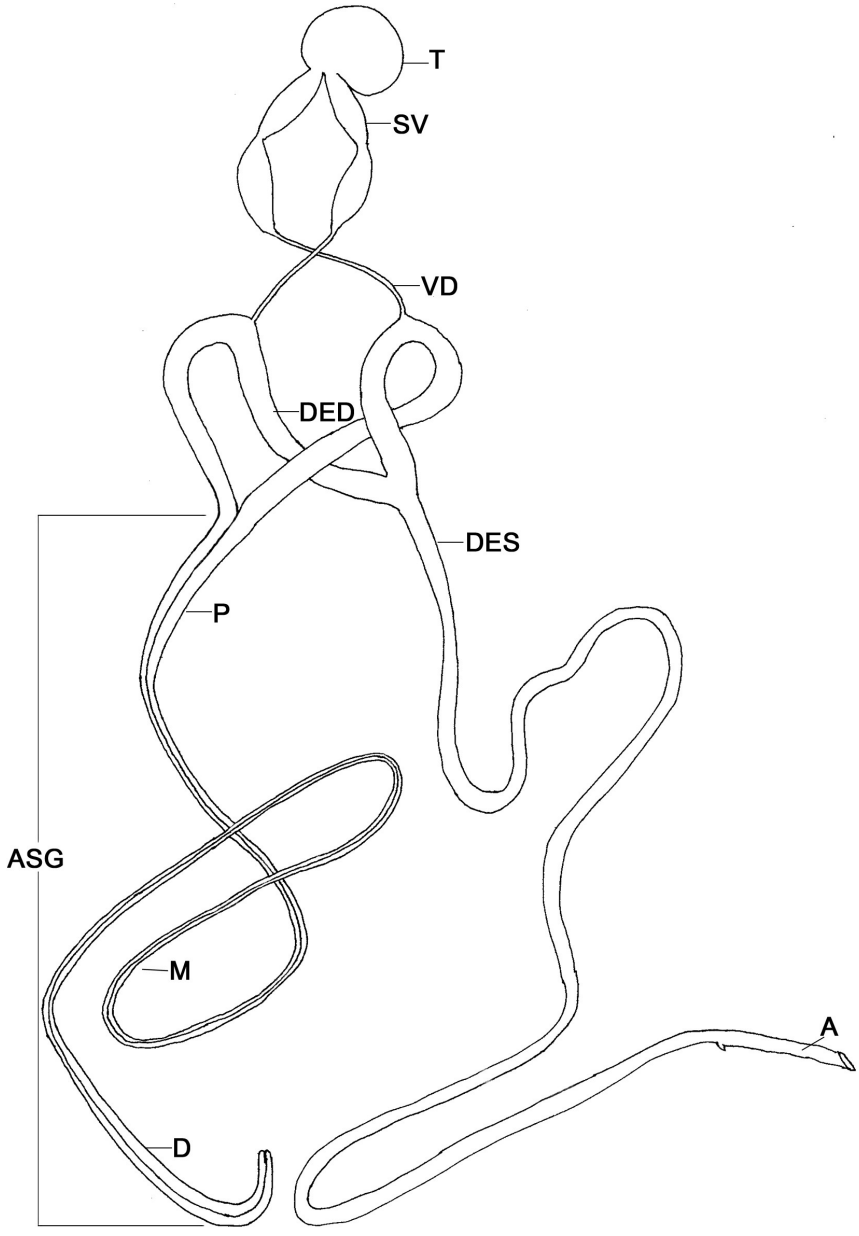


Fig. 8: Female Reproductive System

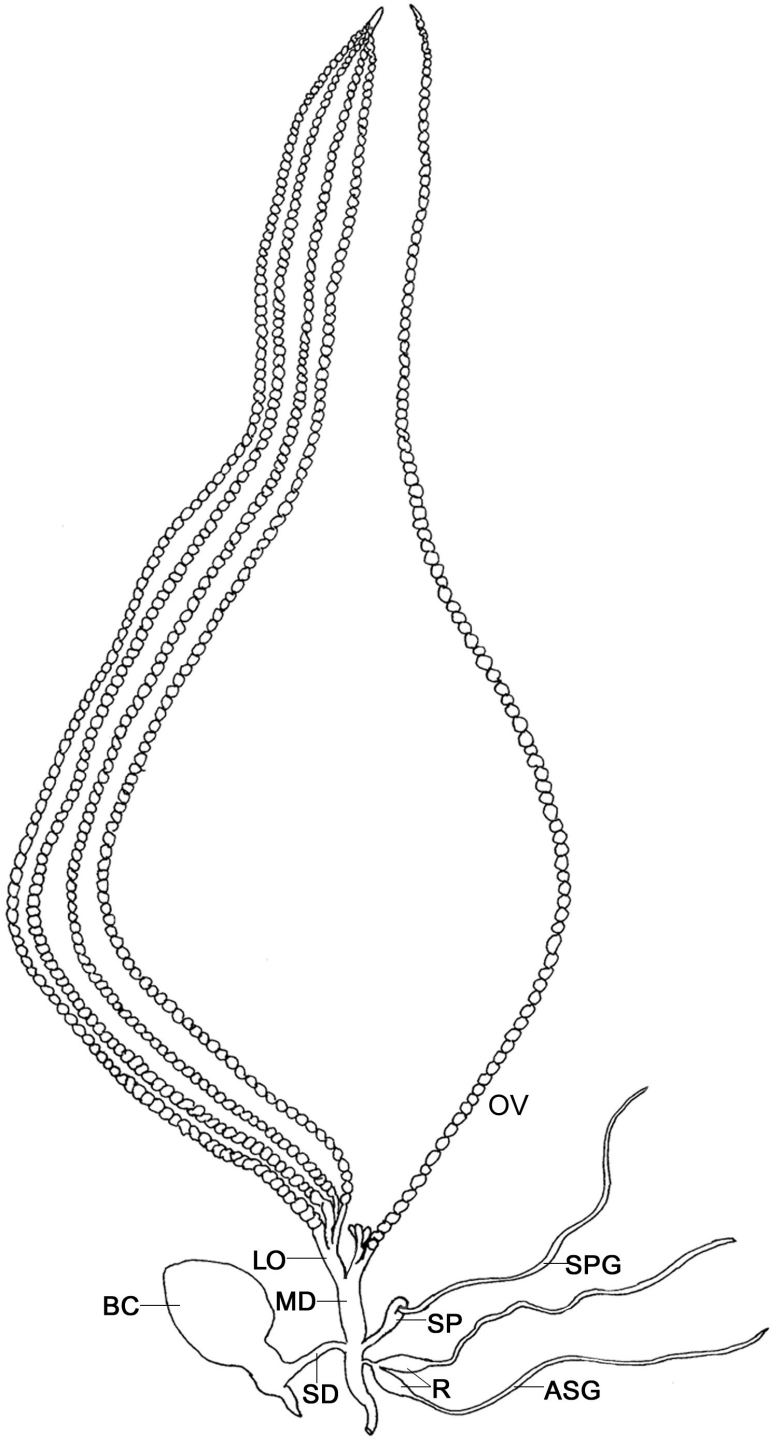
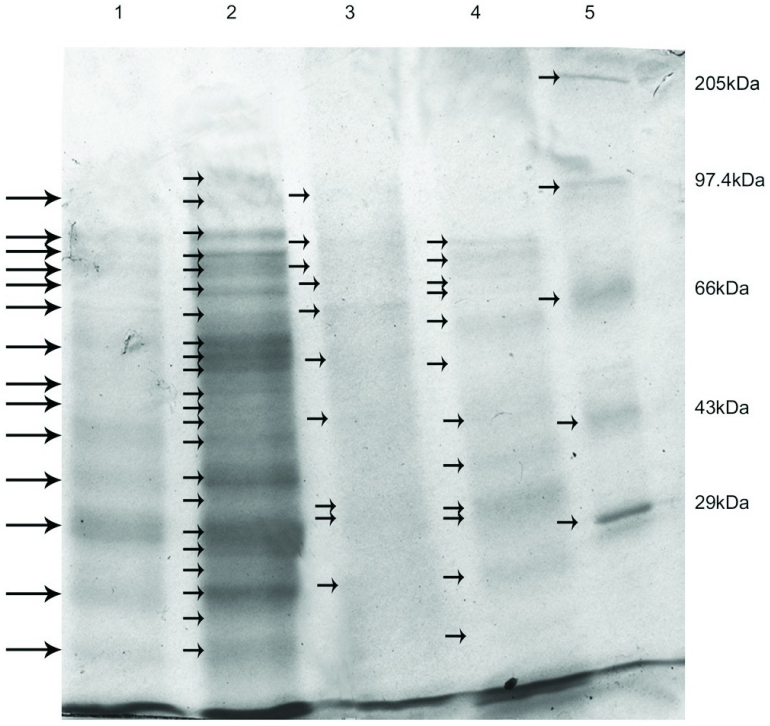


FIGURE : 81 SDS -PAGE PROFILE OF ASG PROTEINS IN TREATED MALE ***S.MAURITIA***



Lane 2: Mated
Lane 3: Molecular weight markers

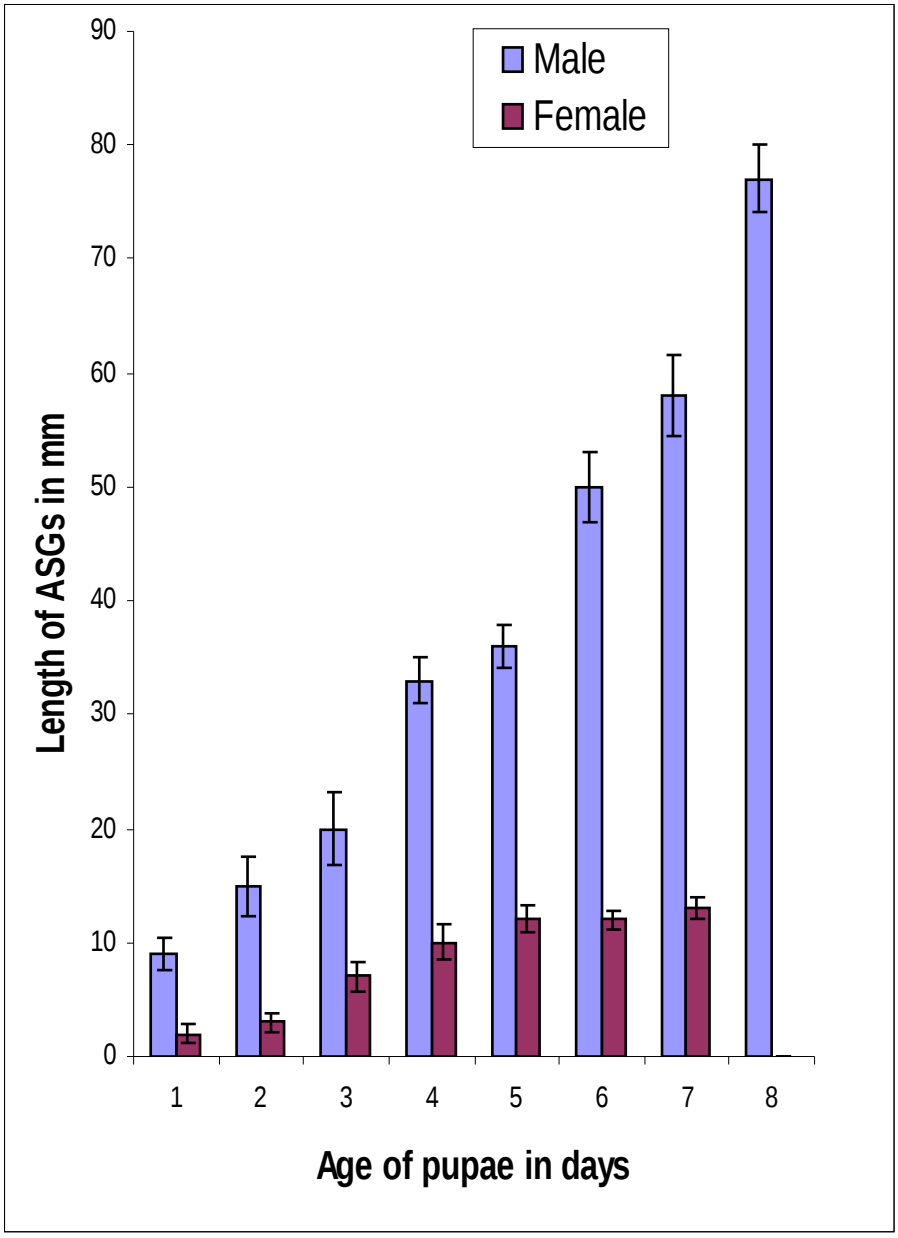


Fig. 49 Linear development of ASGs during pupal adult metamorphosis

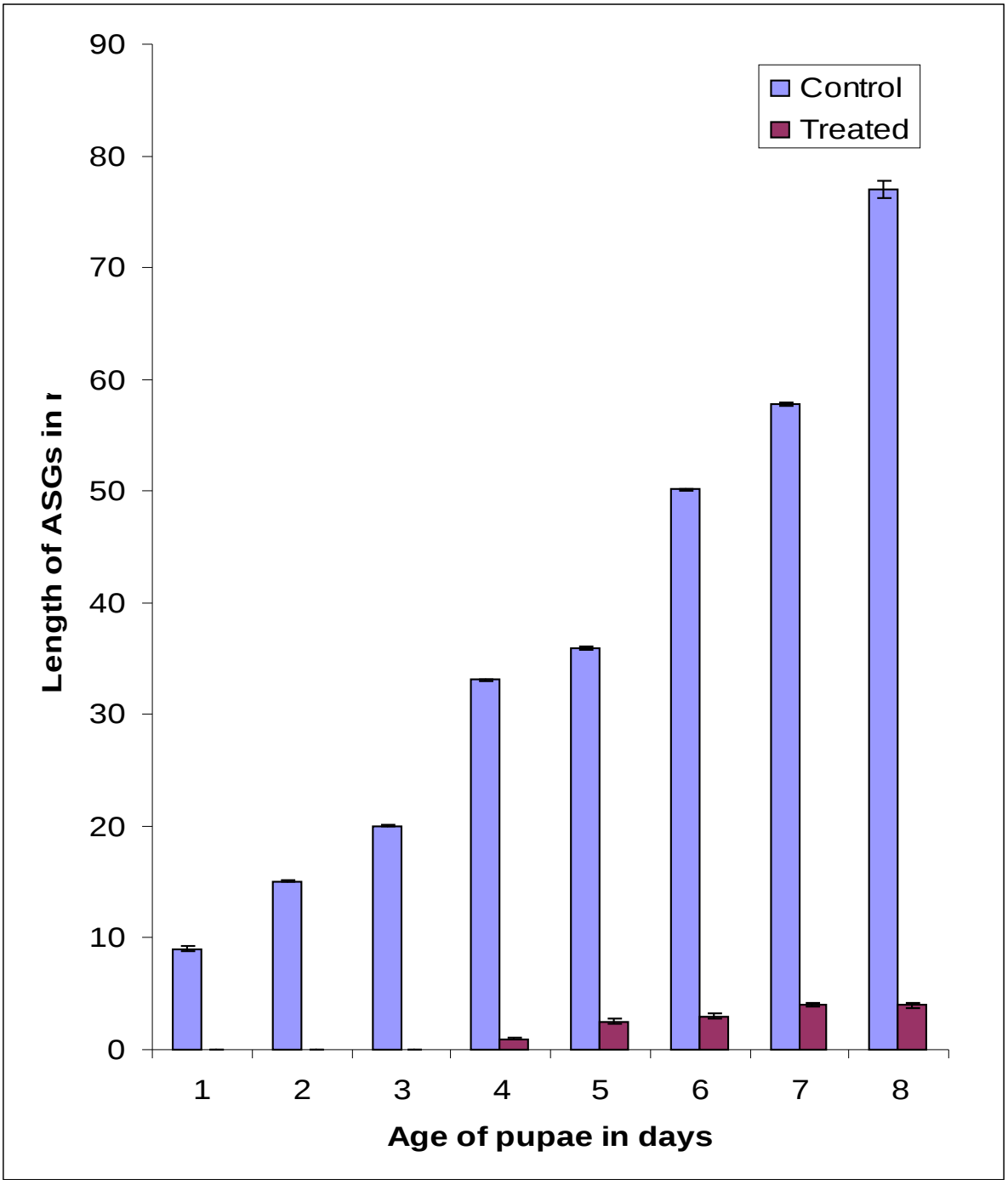


Fig. 60. Effect of PPN on the linear development of ASGs in pupal-adult metamorphosis of male *S.mauritia*

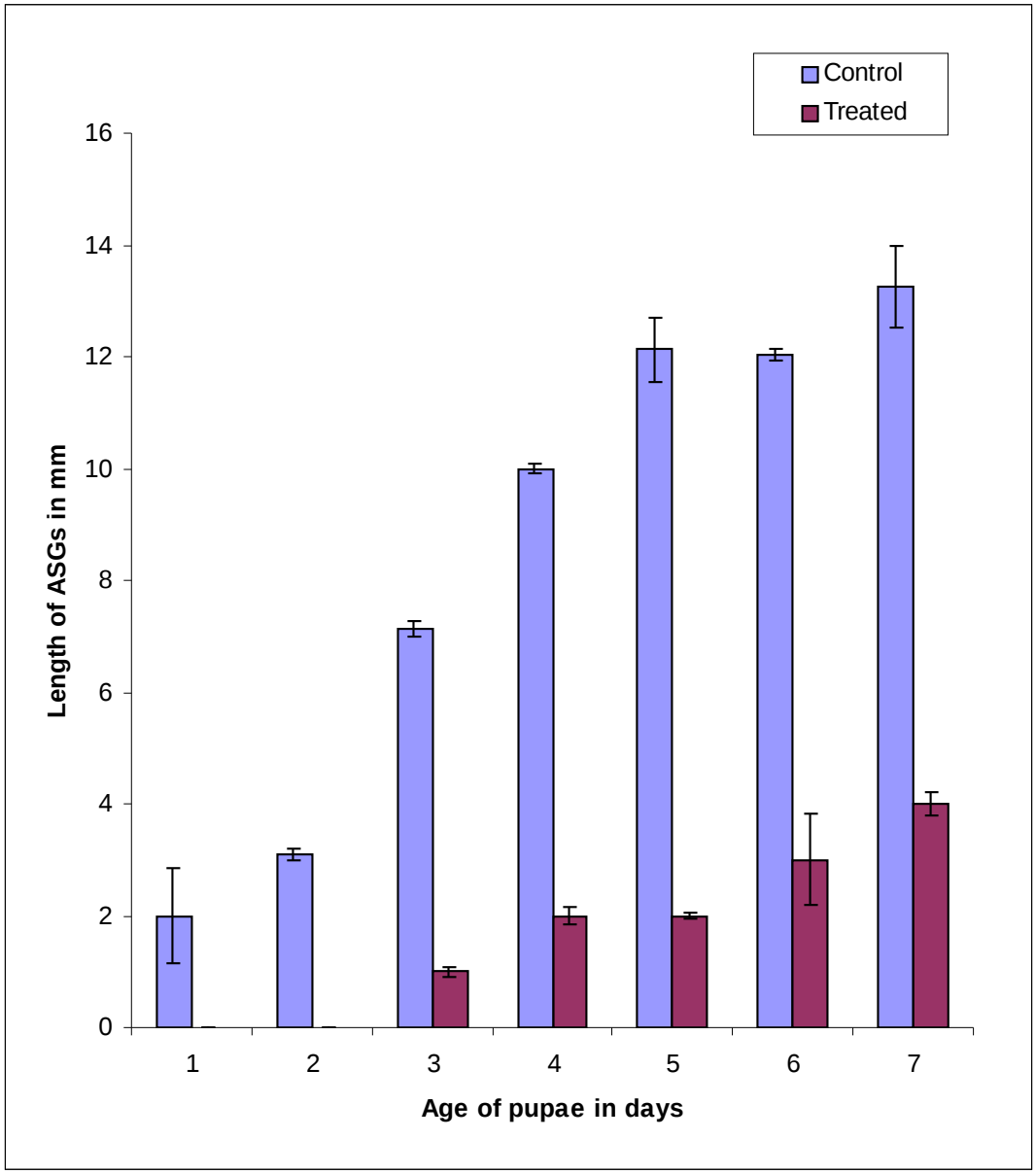


Fig. 72. Effect of PPN on the linear development of ASGs in pupal- adult metamorphosis of female *S.mauritia*

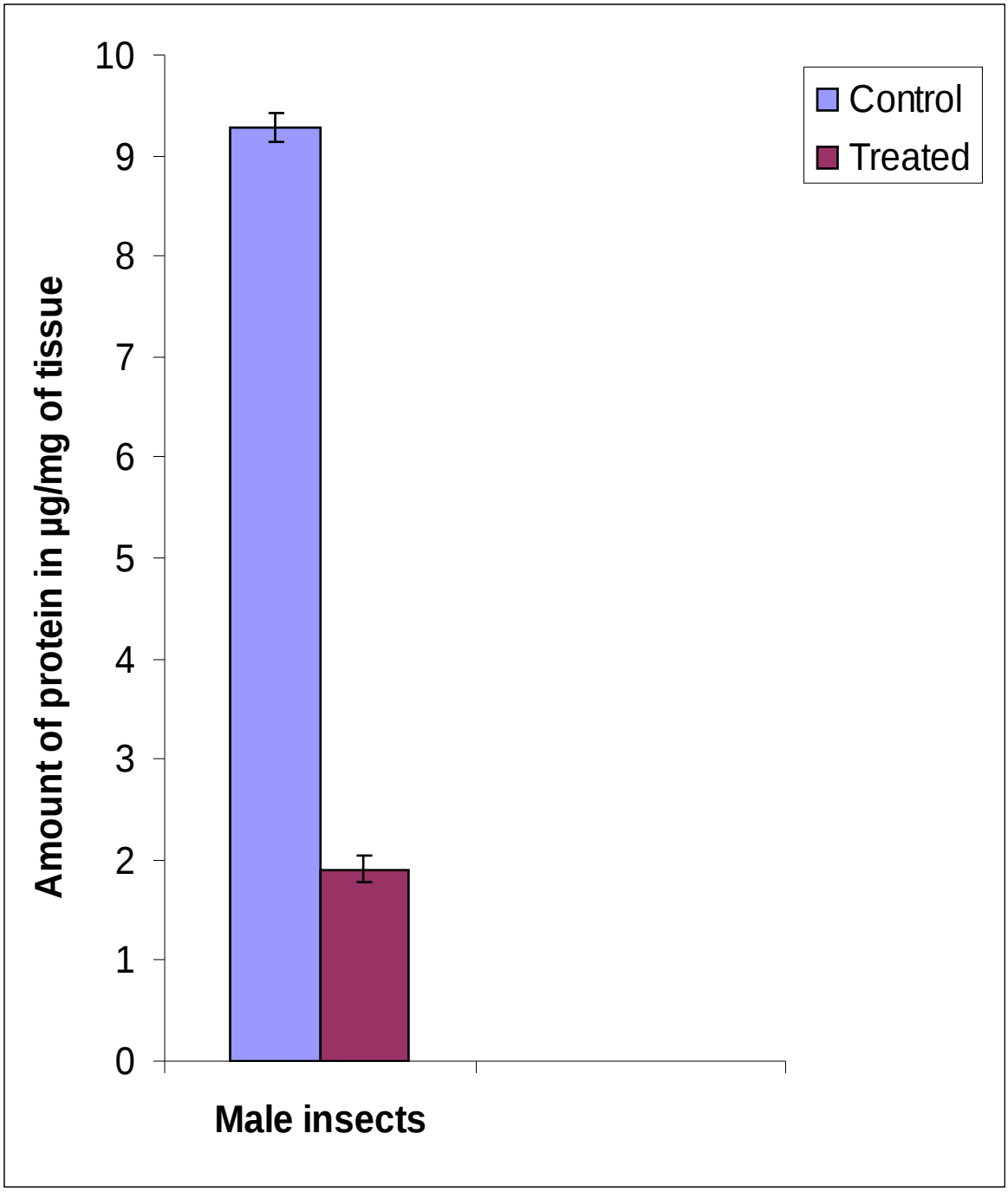


Fig. 79. Changes in the amount of protein in ASGs of male insects treated with 0.1 µg PPN on newly ecdysed day 0 pupae

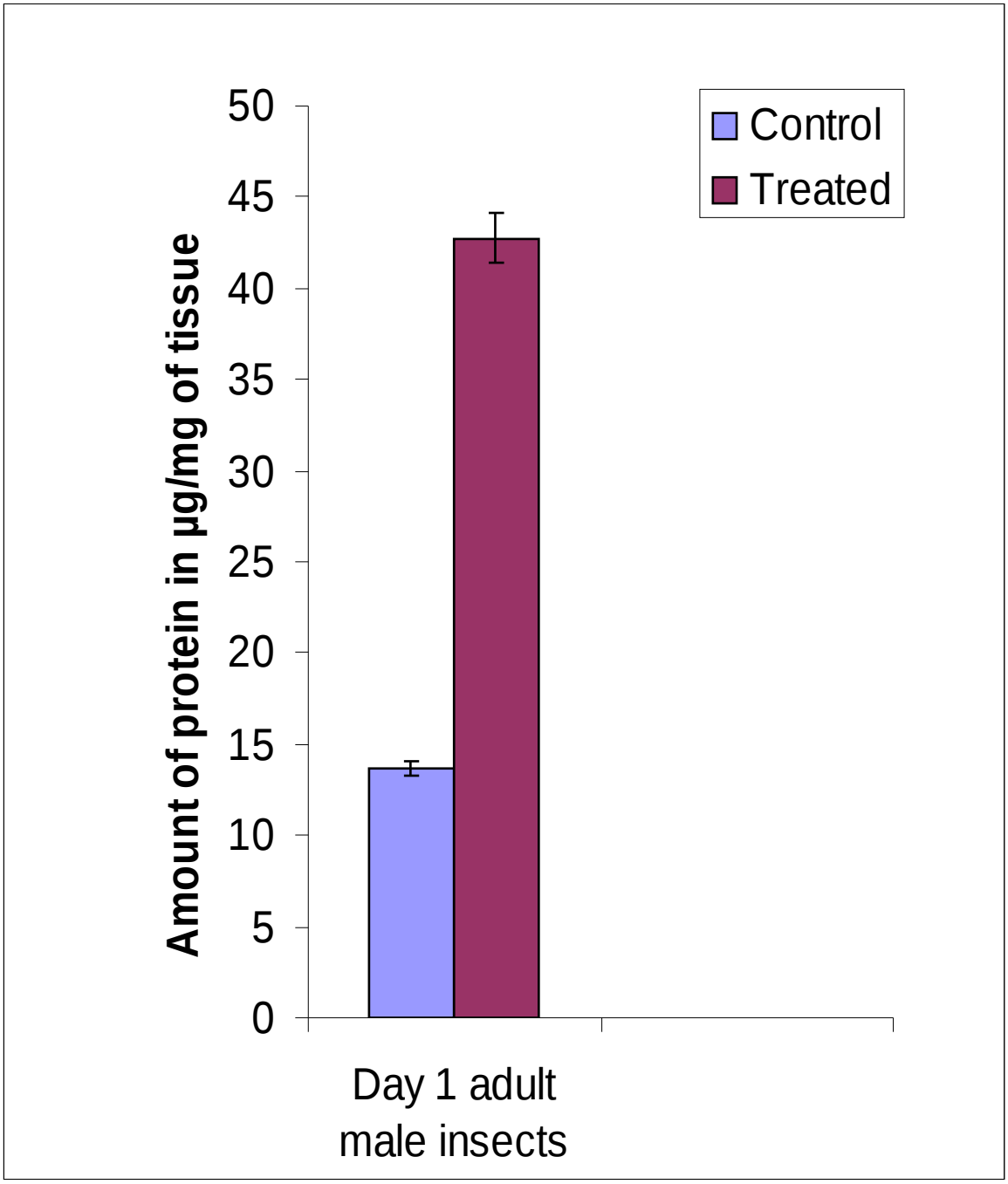


Fig. 80. Changes in the protein content in the ASGs of 20µg PPN treated adults

Fig. 7 Male Reproductive system

T	Testis
SV	Seminal vesicles
VD	vas deferens
DED	Ductus ejaculatorius duplex
DES	Ductus ejaculatorius simplex
ASG	Accessory sex glands
P	proximal region
M	Mid region
D	Distal region
A	Aedeagus

Fig. 81. SDS-PAGE profile of ASG proteins in PPN treated and control male insects

Lane 1. ASG protein profile of day 1adult controls

Lane 2. ASG protein profile of day 1 adults treated with 20 µg PPN immediately after eclosion on day 0

Lane 3. ASG protein profile of adultoids

Lane 4. ASG protein profile of day 0 adult controls

Lane 5. Molecular weight markers

Fig. 8 Female Reproductive system

OV	Ovarioles
LO	Lateral oviduct
MD	Median oviduct
BC	Bursa copulatrix
SD	Seminal duct
SP	Spermatheca
SPG	Spermathecal gland
ASG	Accessory sex glands
R	Reservoir

PLATE I

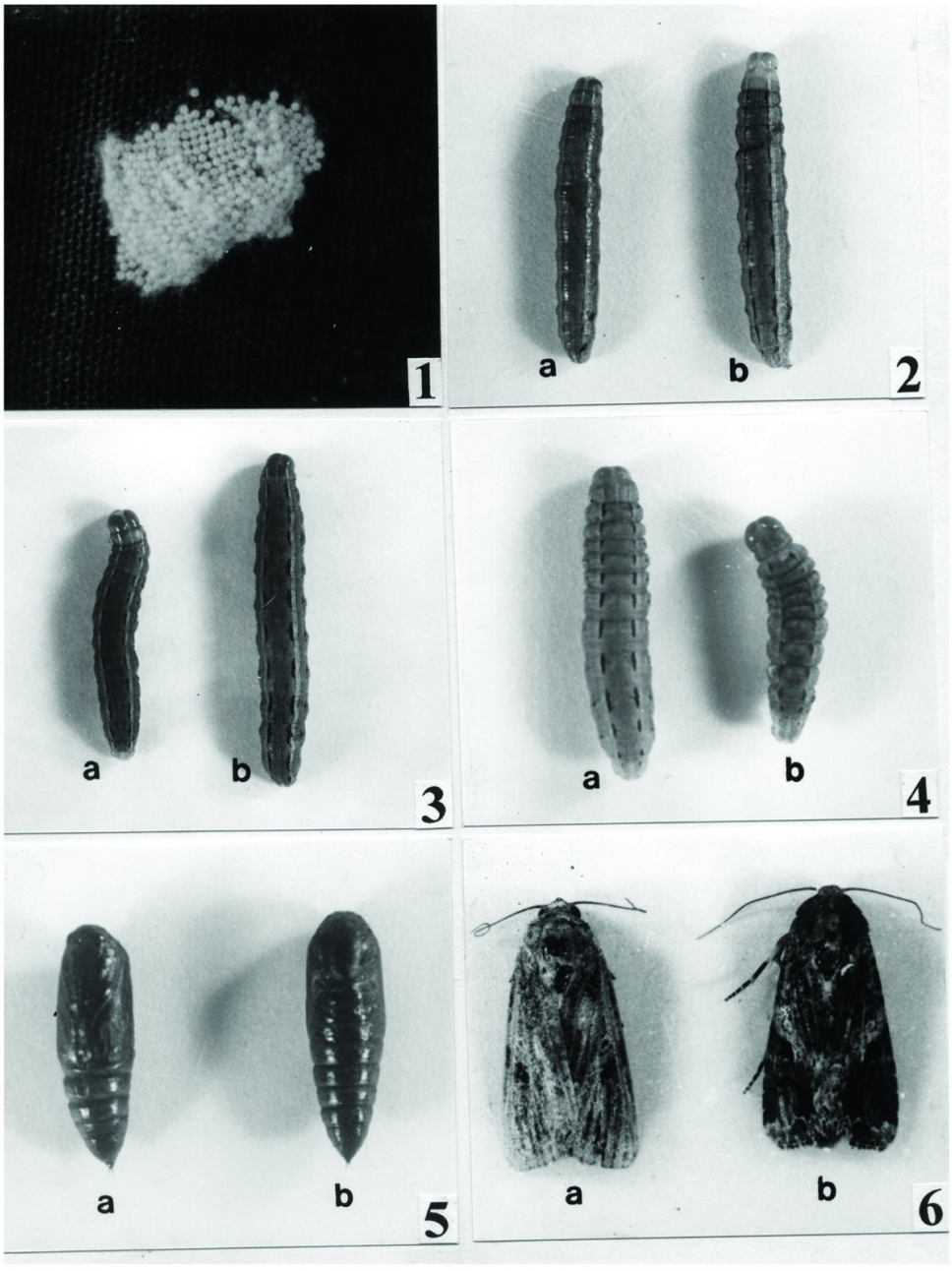
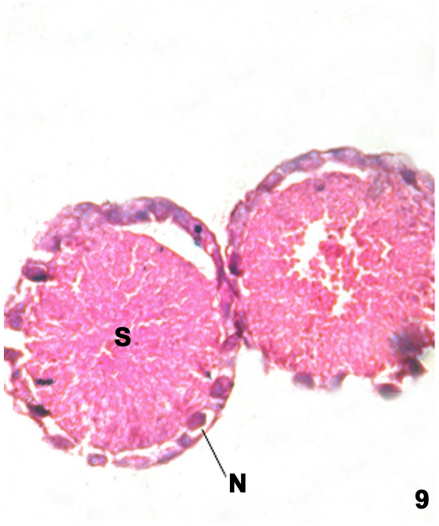
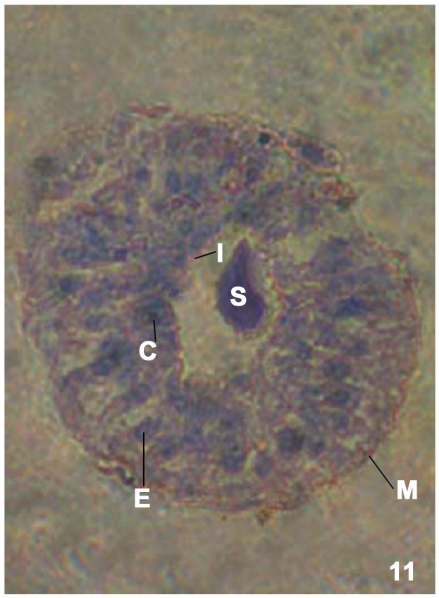


PLATE II





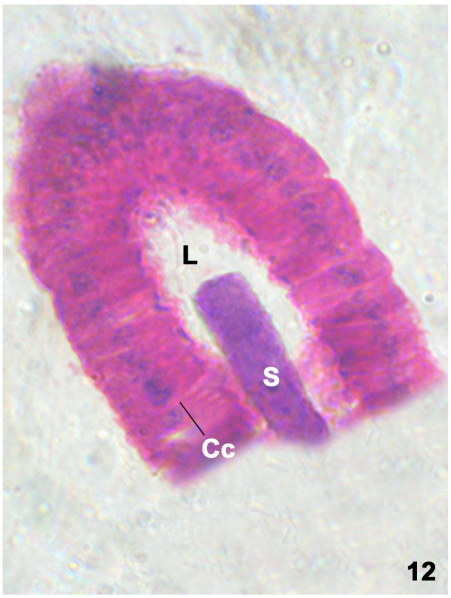
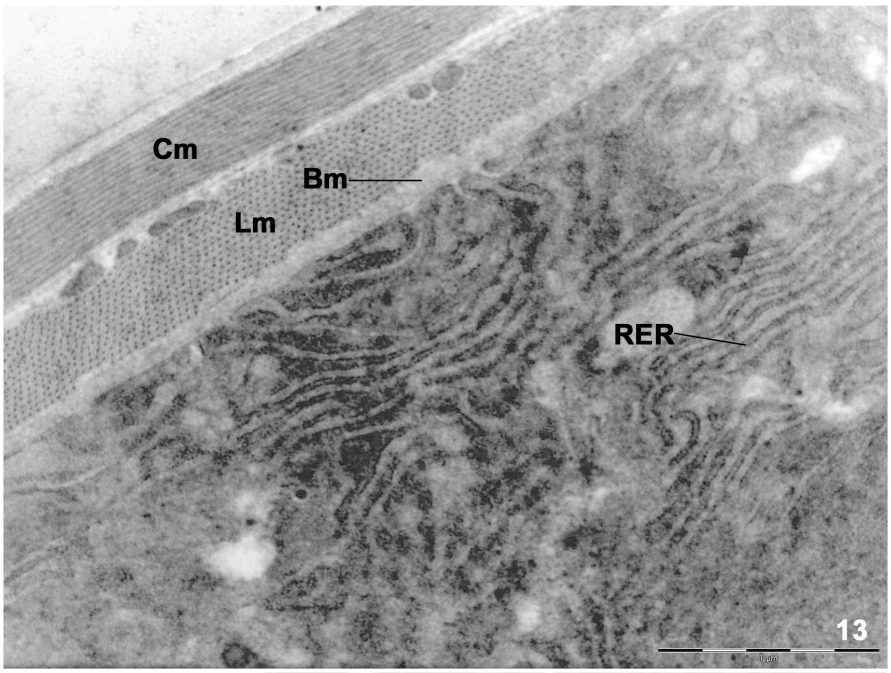


PLATE III



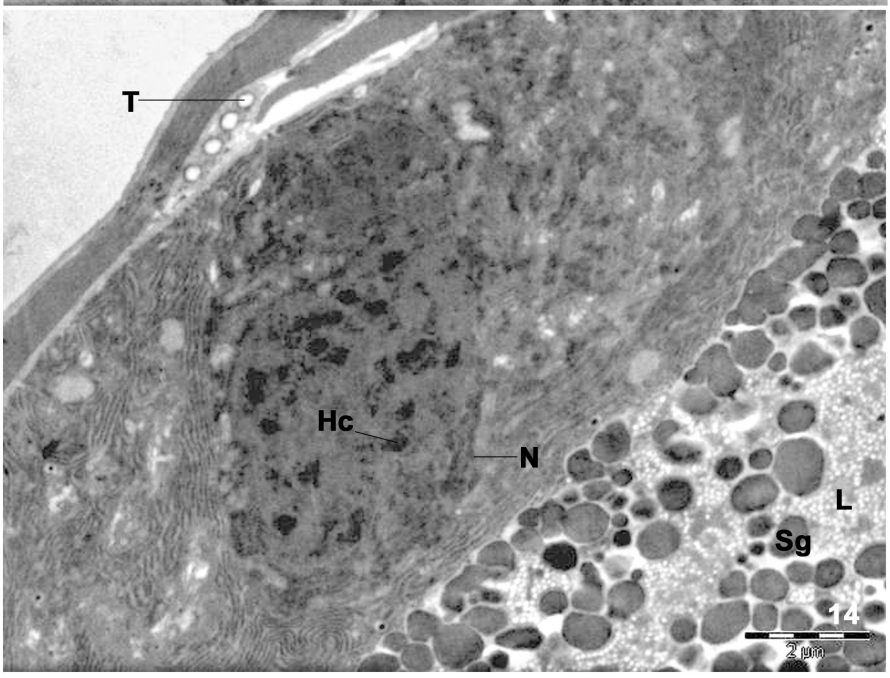


PLATE IV

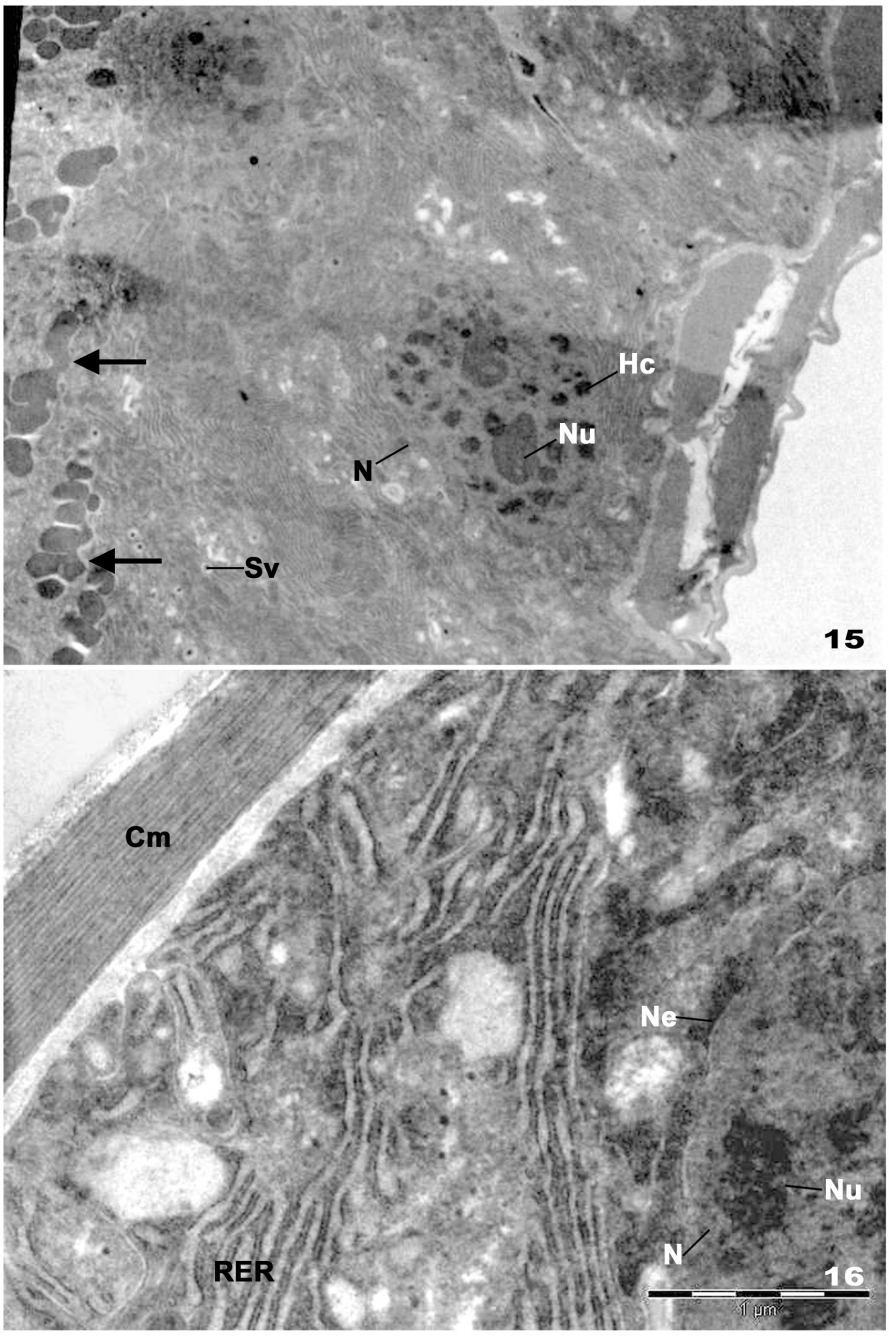


PLATE V

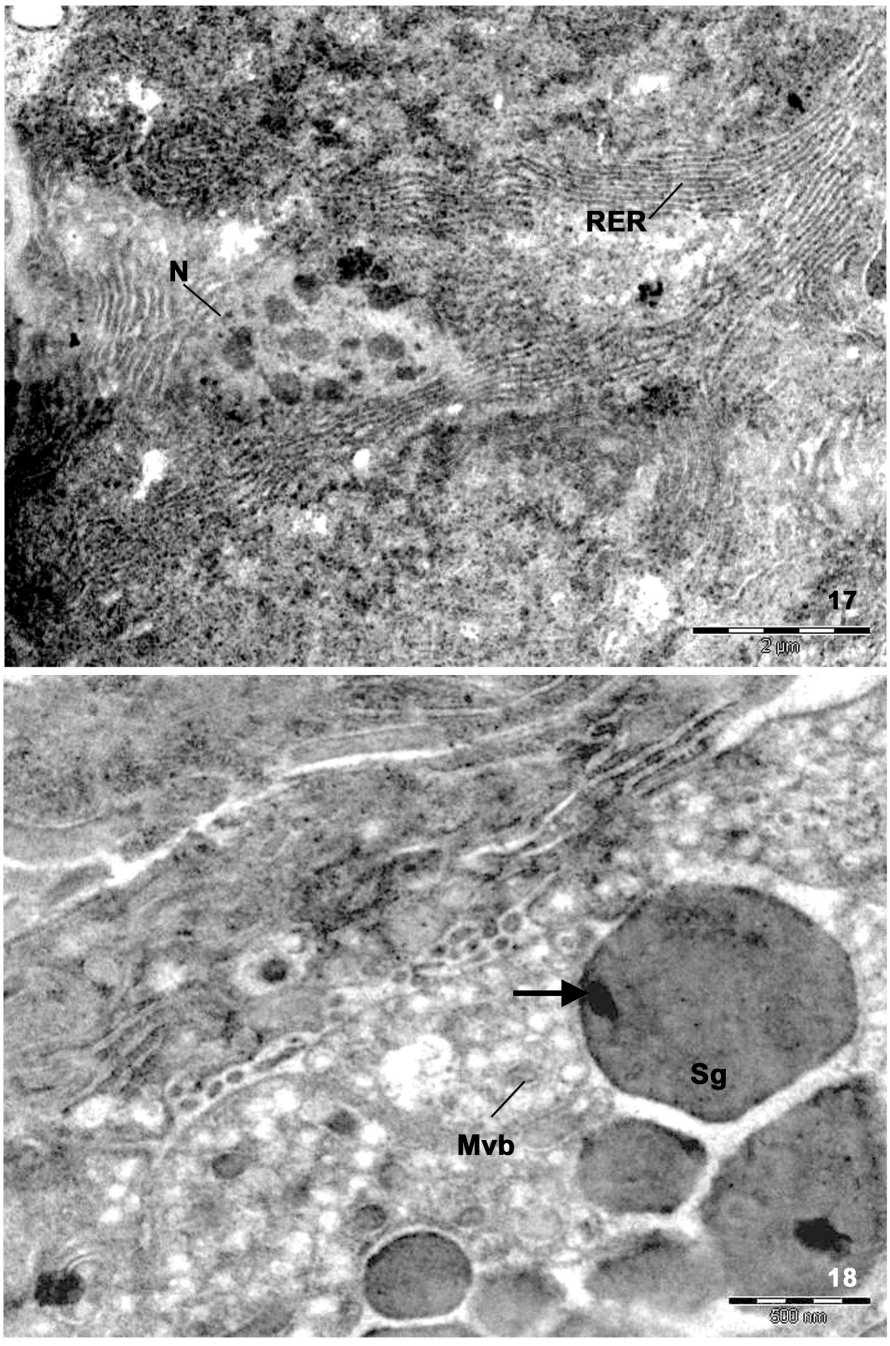


PLATE VI

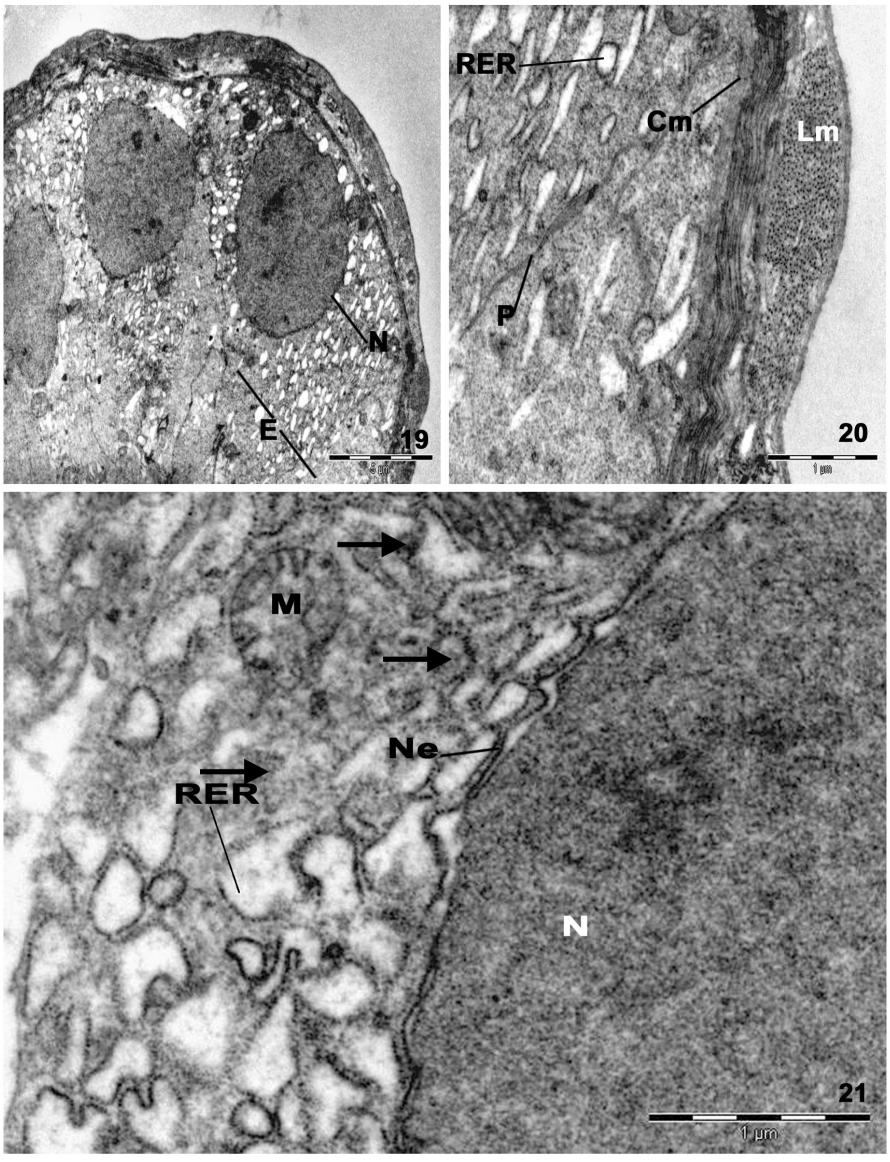
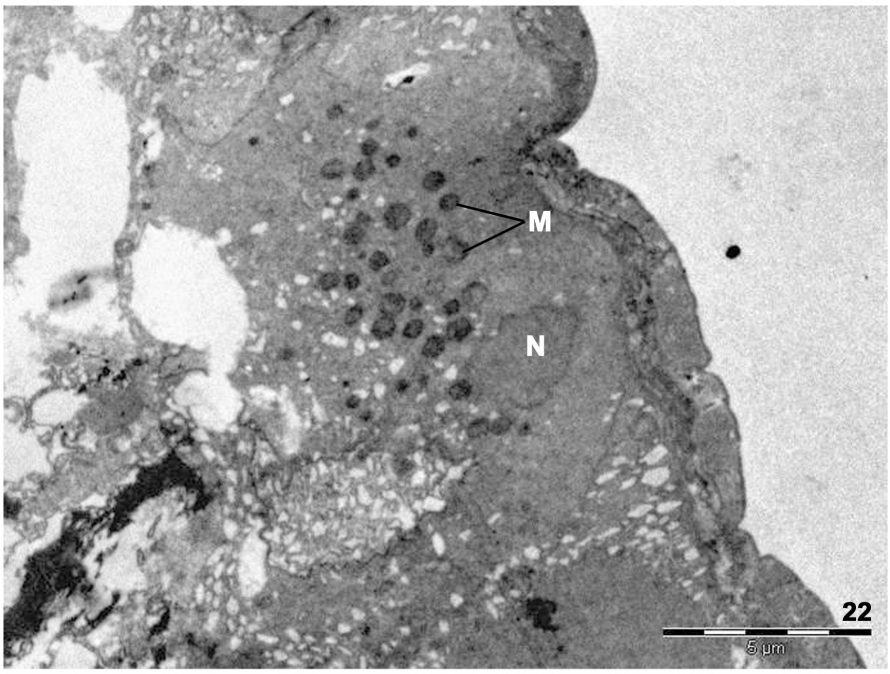


PLATE VII



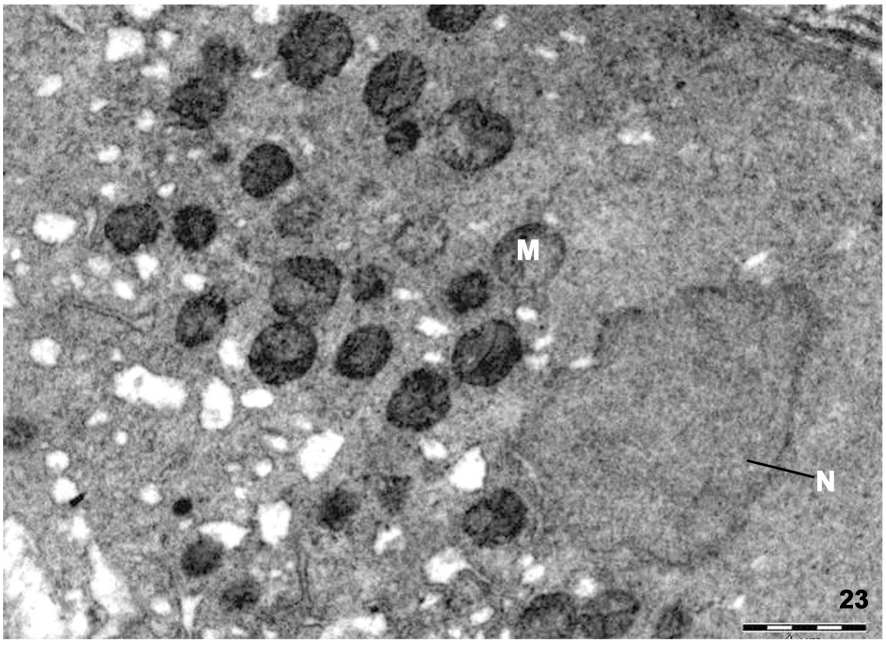


PLATE VIII

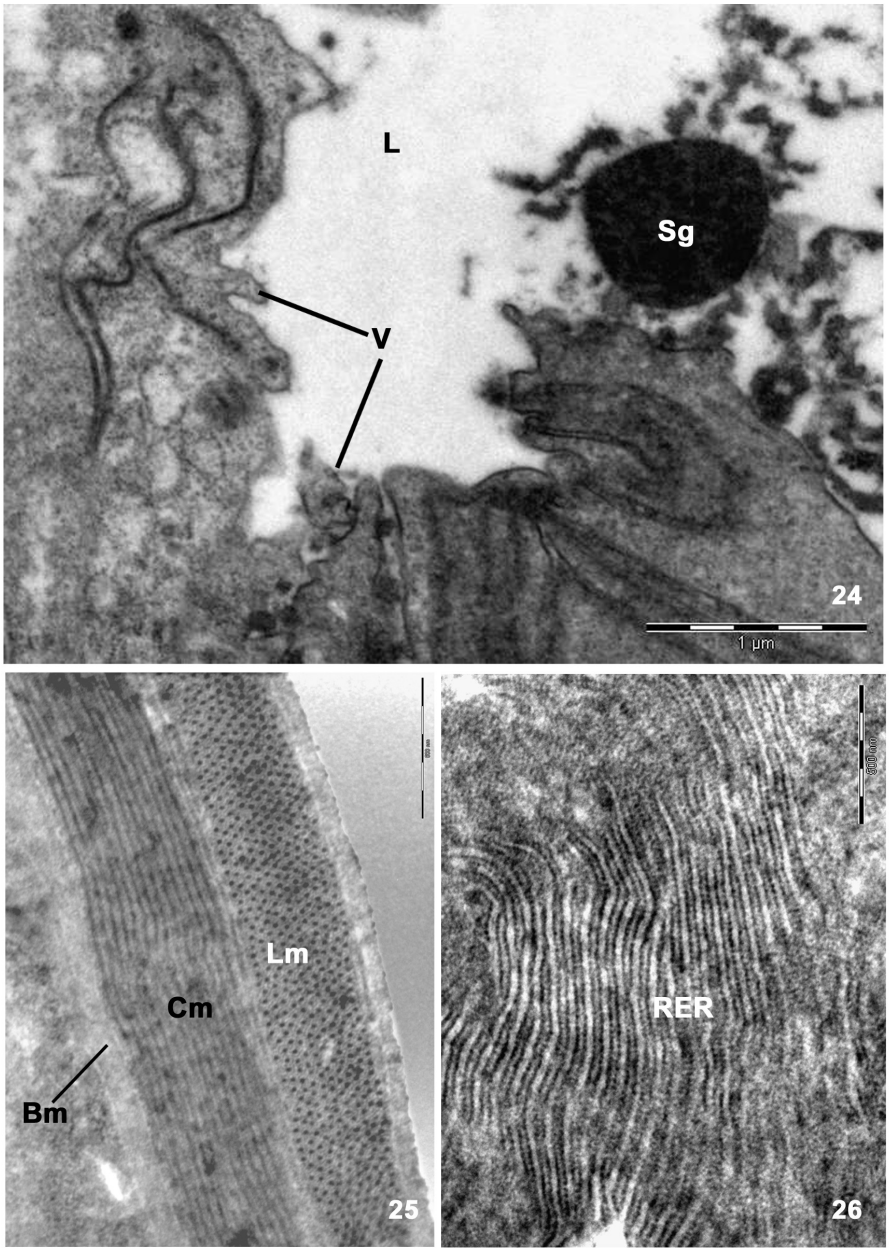
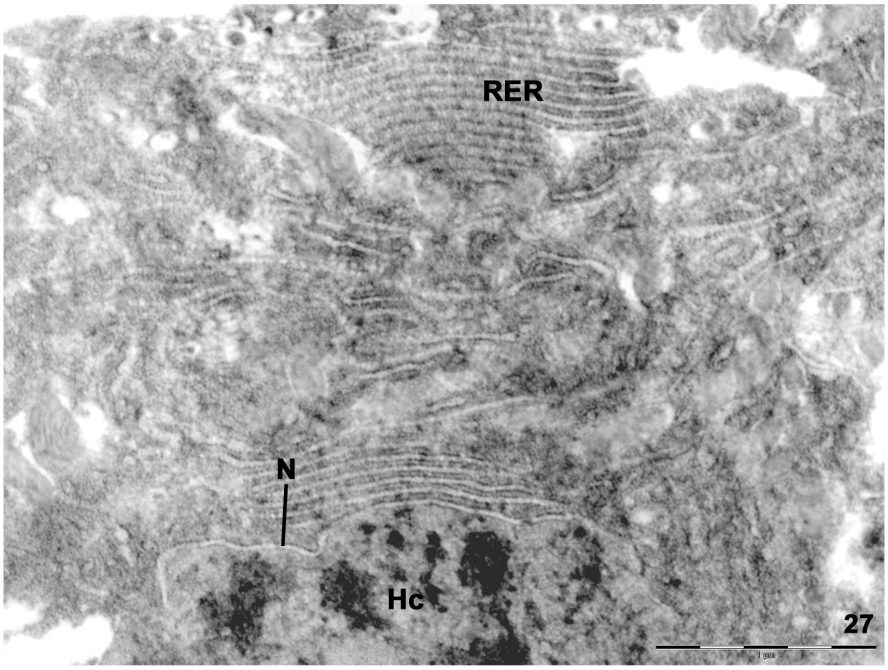
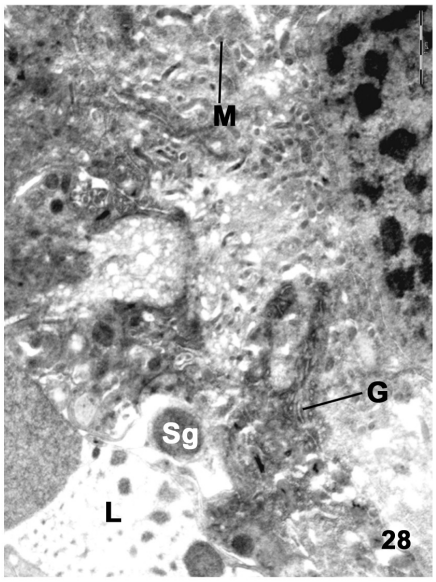


PLATE IX





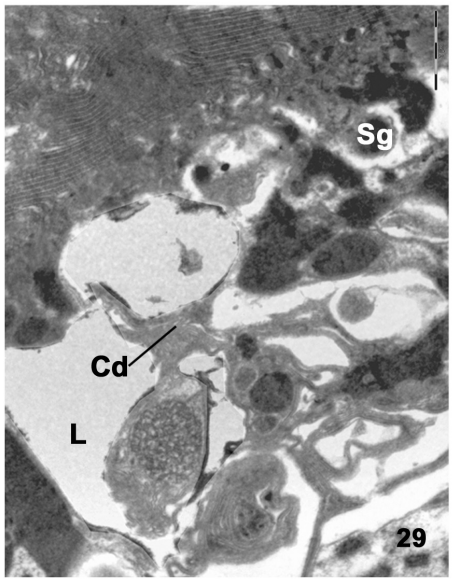
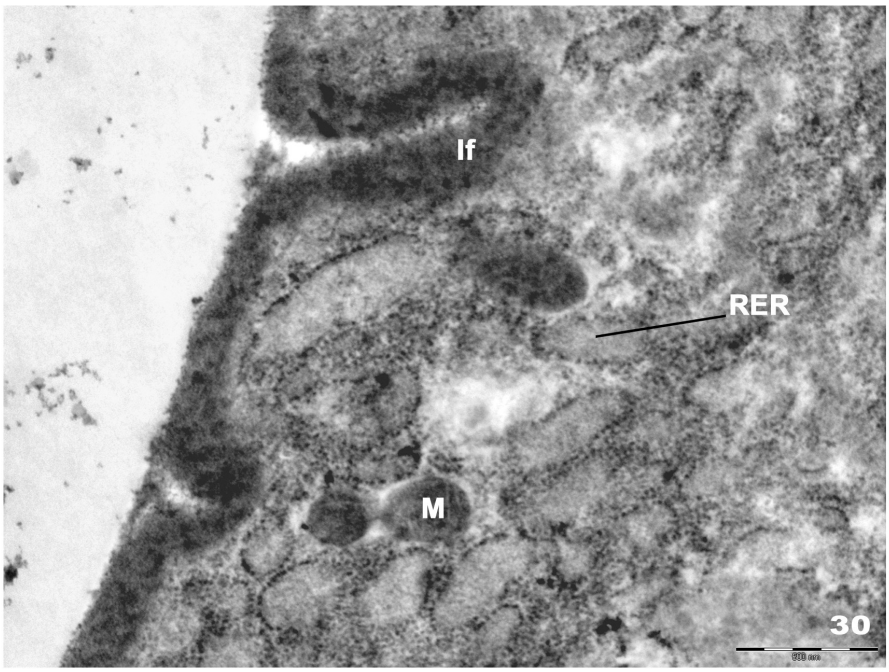


PLATE X



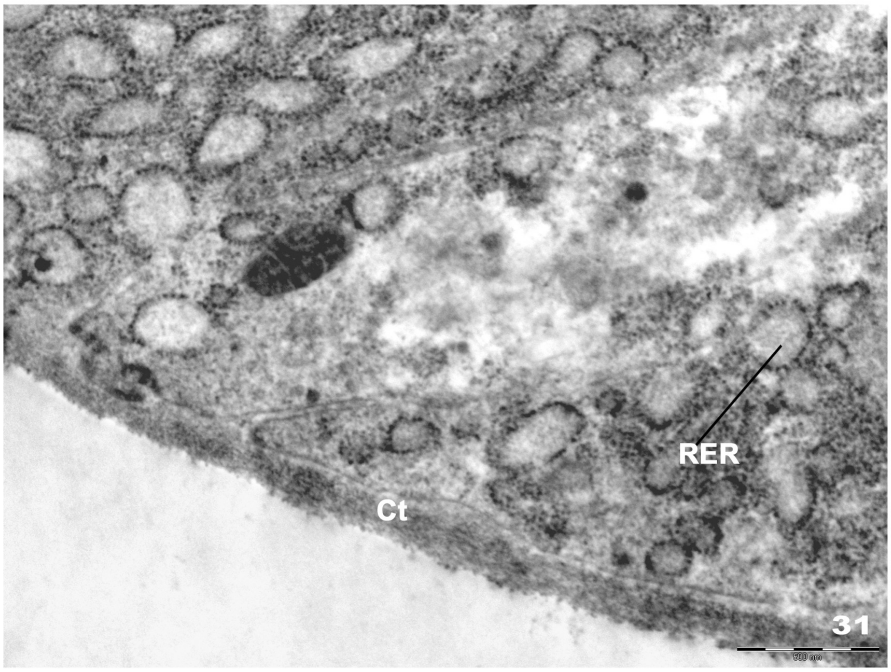
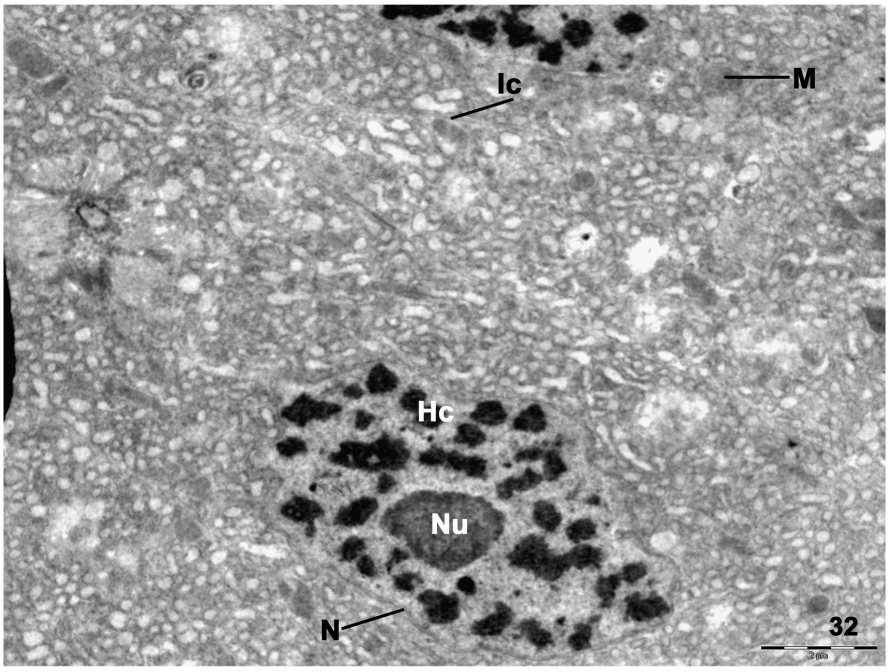


PLATE XI



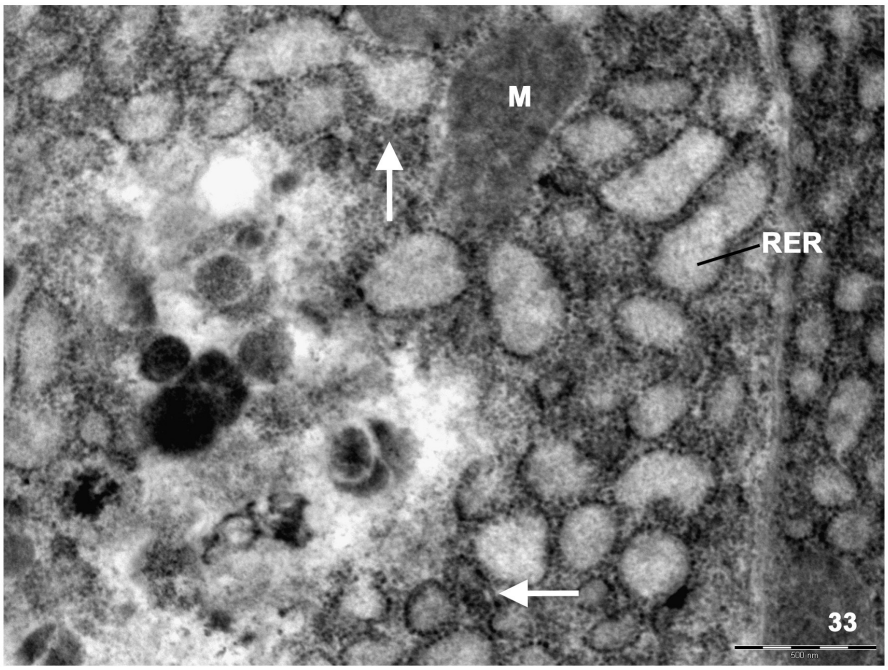
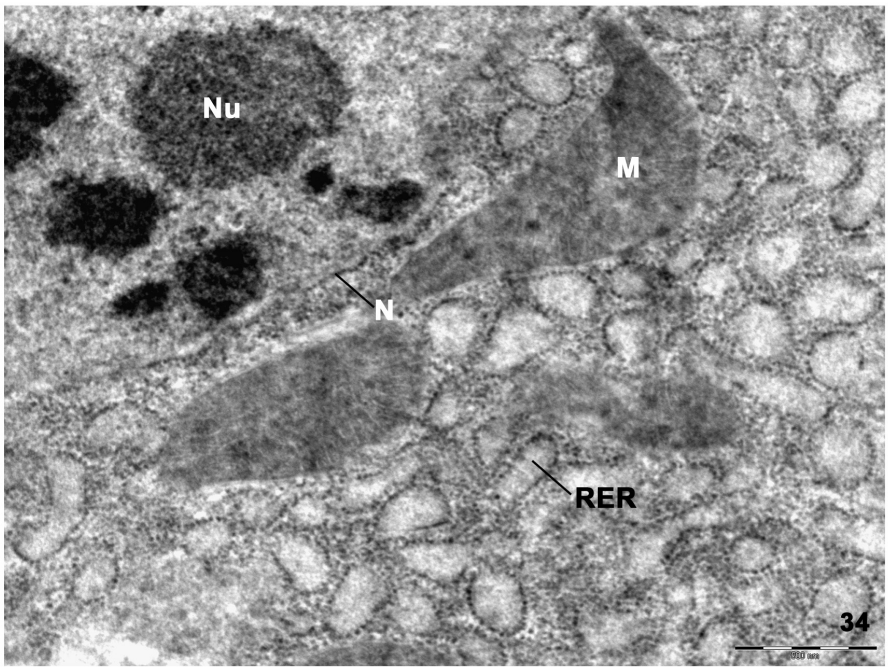


PLATE XII



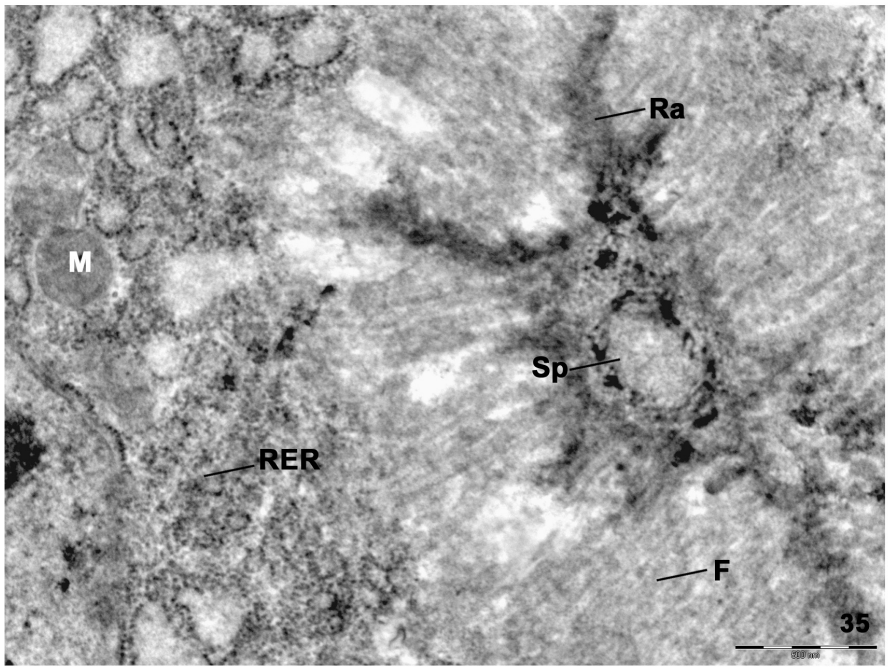
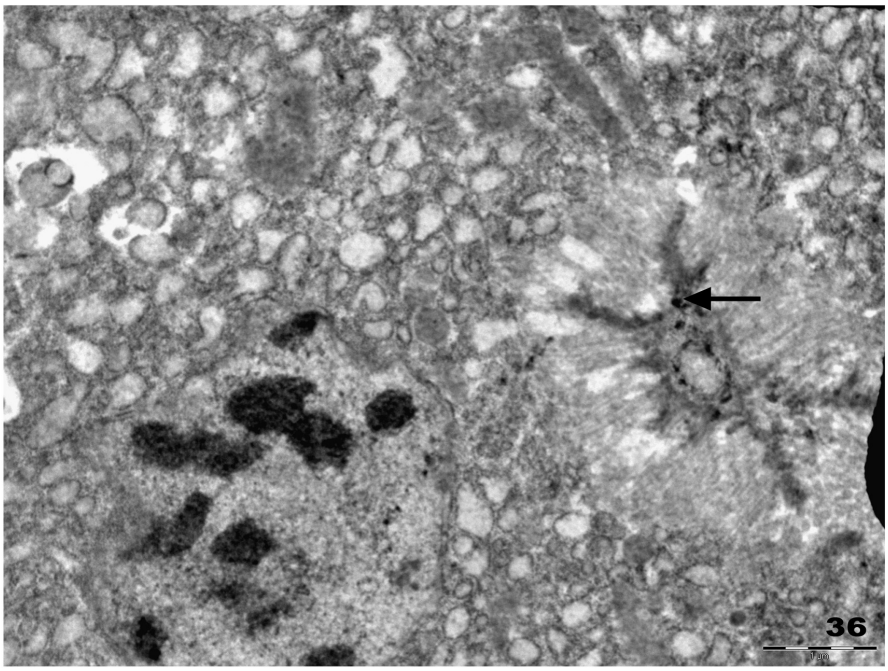


PLATE XIII



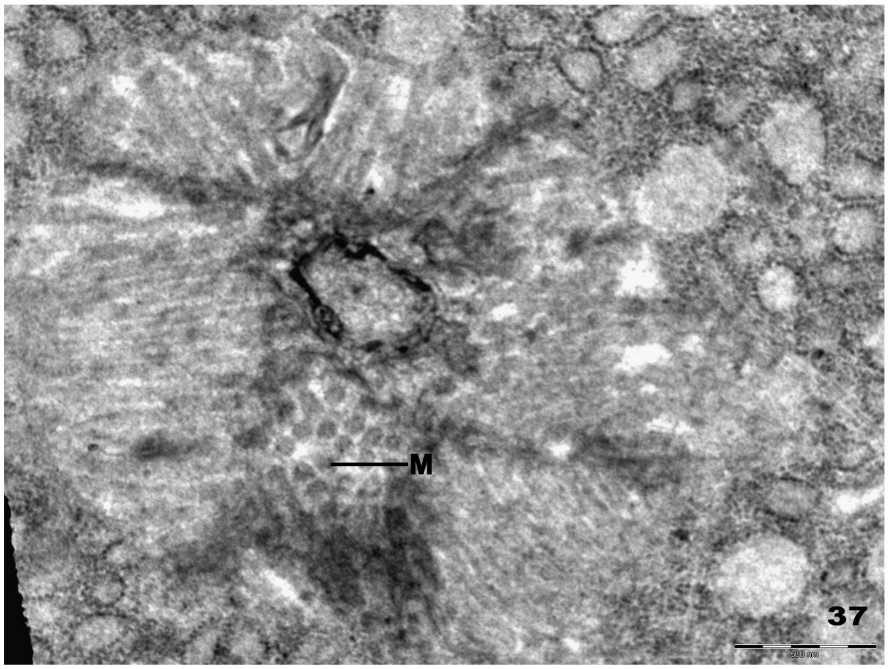


PLATE XIV

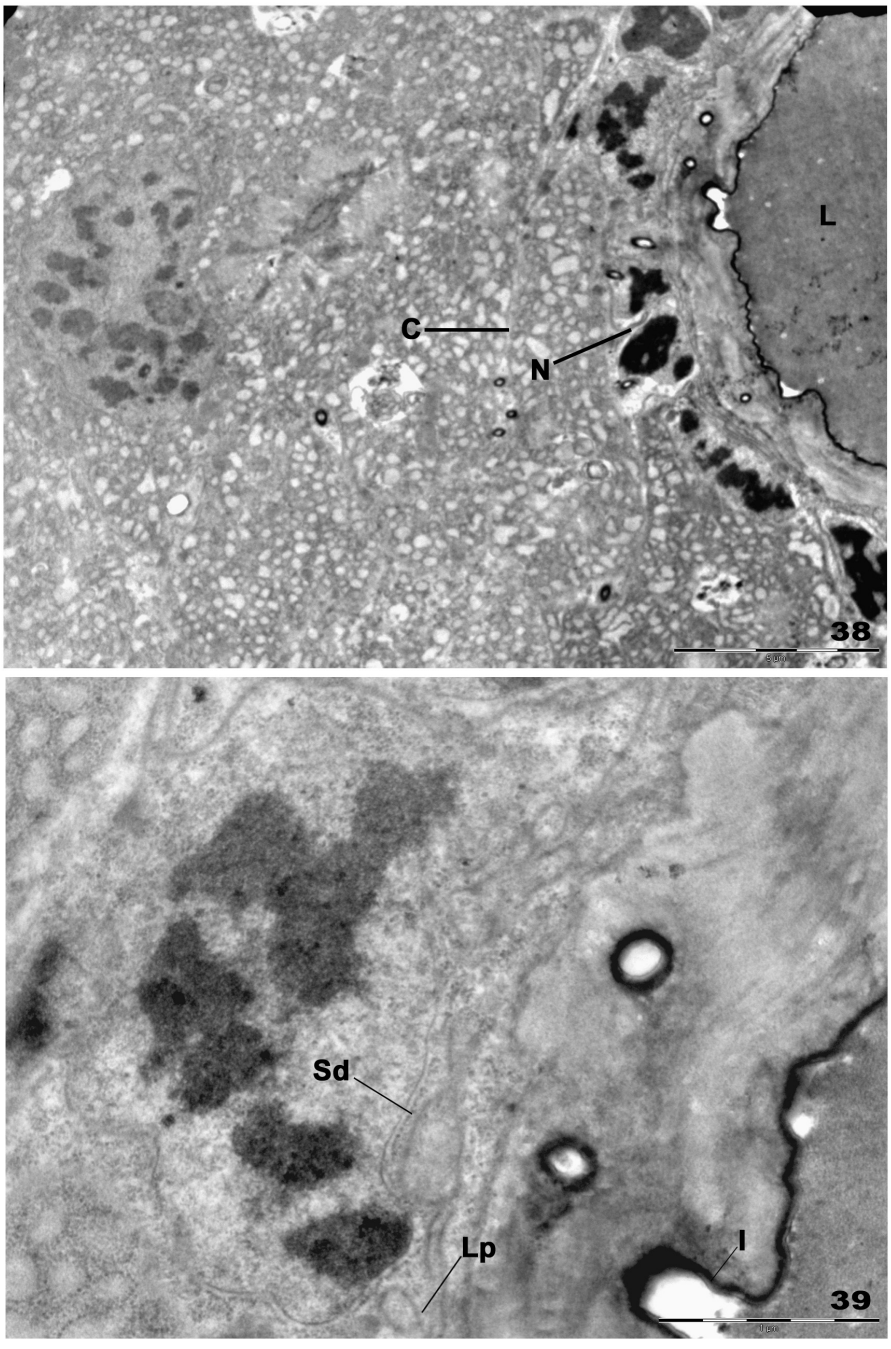


PLATE XV

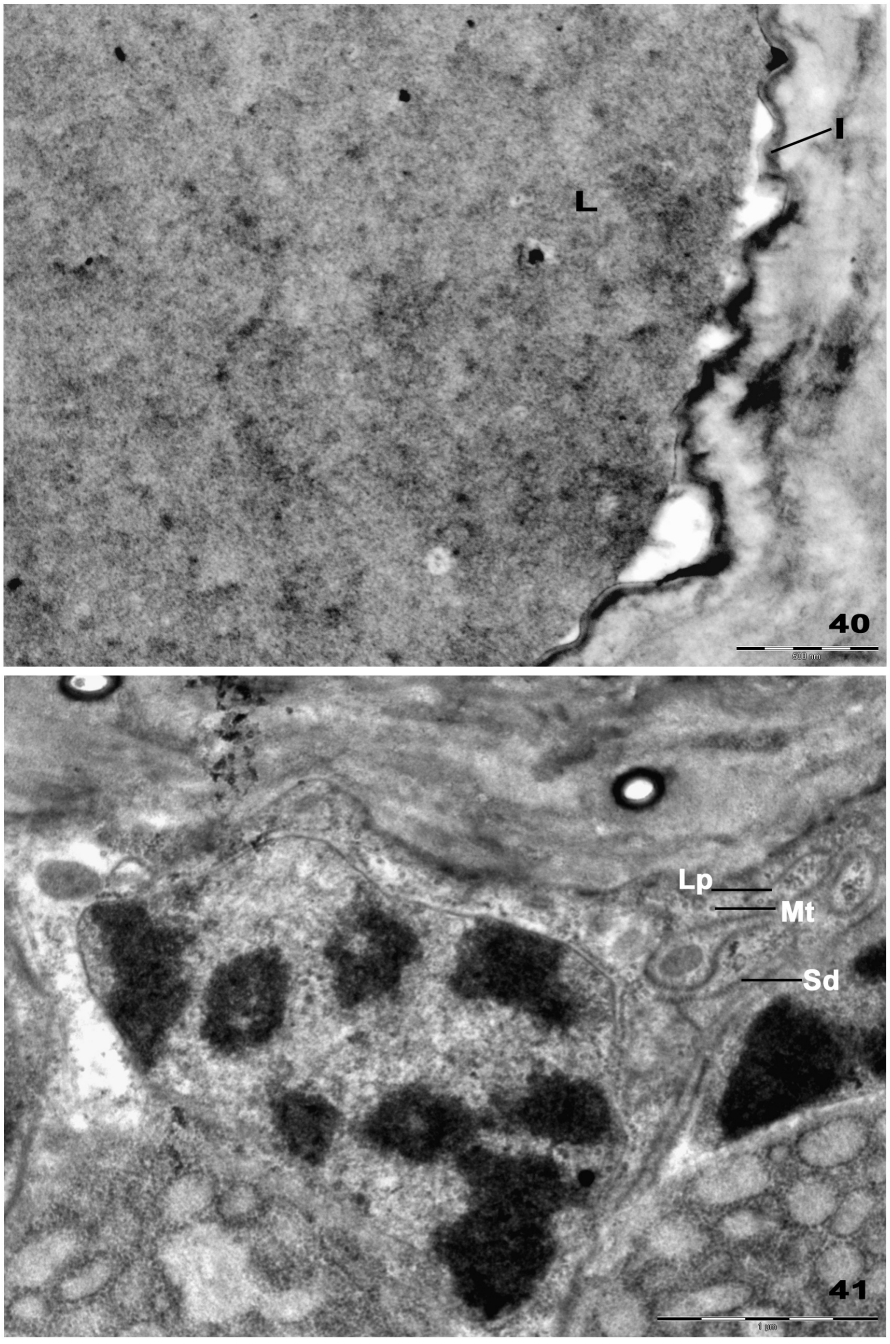


PLATE XVI

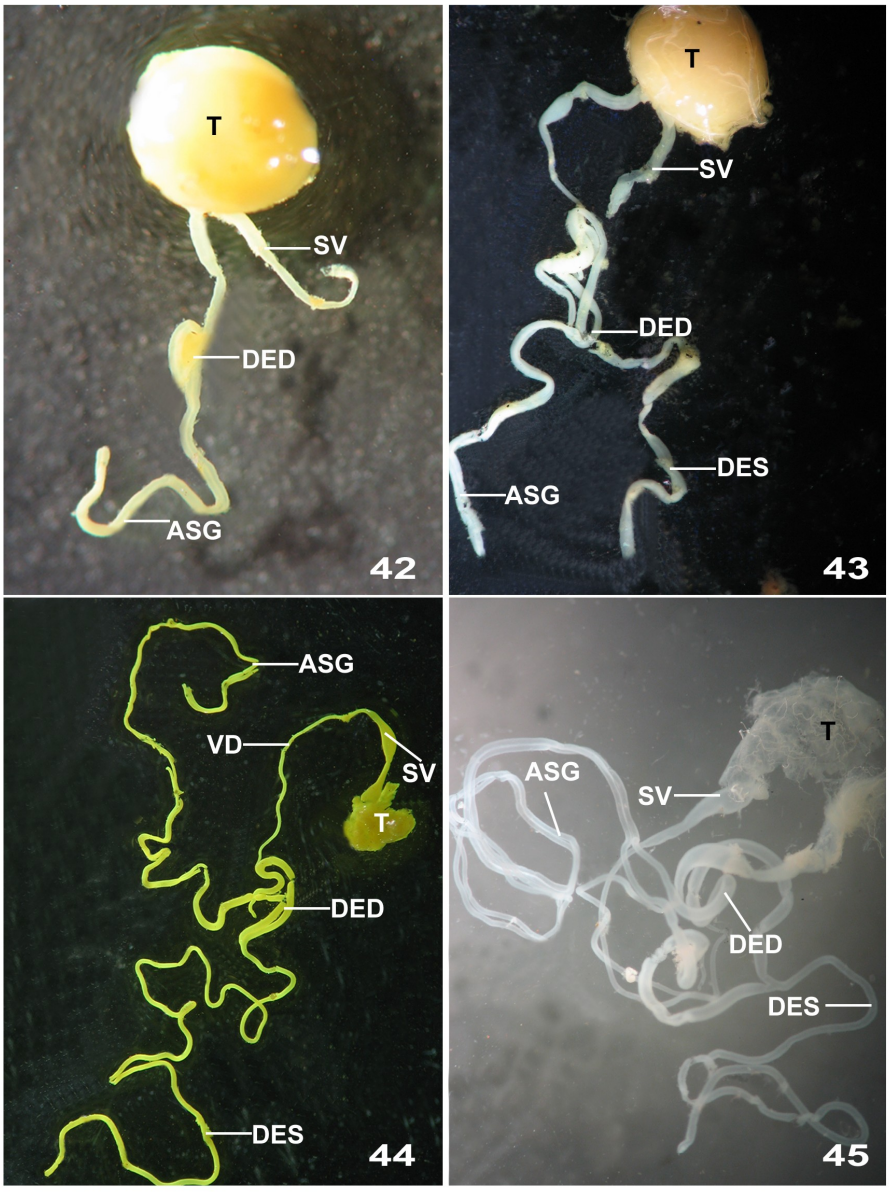
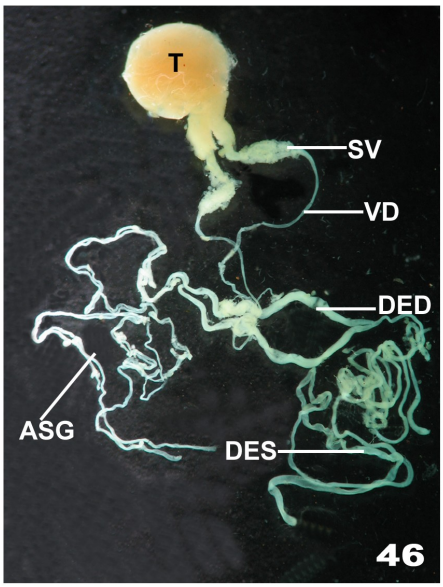
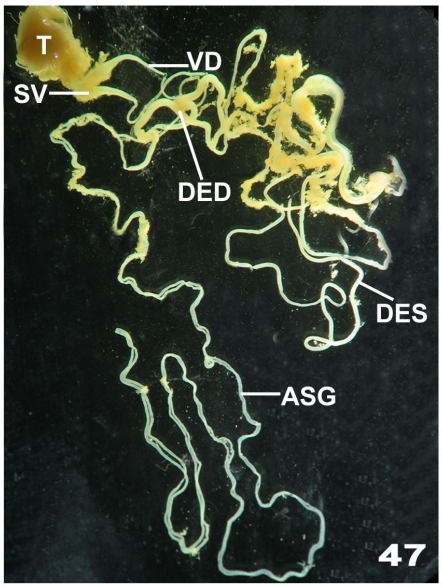

T
SV
DED
ASG
42
T
SV
DED
DES
ASG
43
ASG
VD
SV
T
DED
DES
44
T
ASG
SV
DED
DES
45

PLATE XVII


T
SV
VD
DED
ASG
DES
46


T
VD
SV
DED
DES
ASG
47

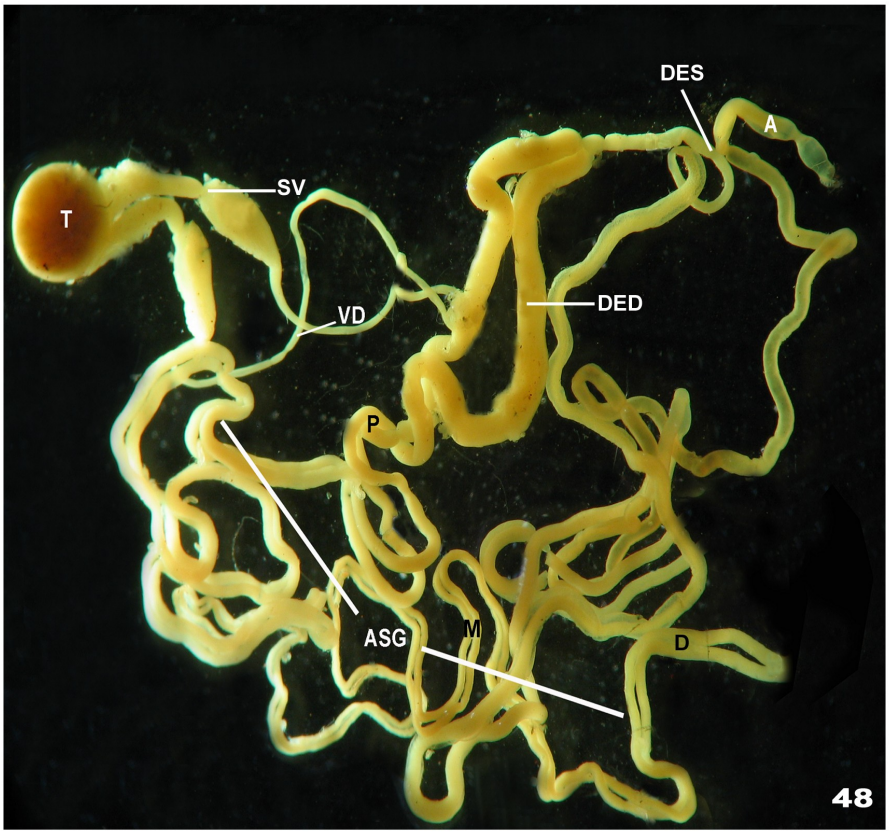

DES
A
SV
T
VD
DED
P
ASG
M
D
48

PLATE XVIII

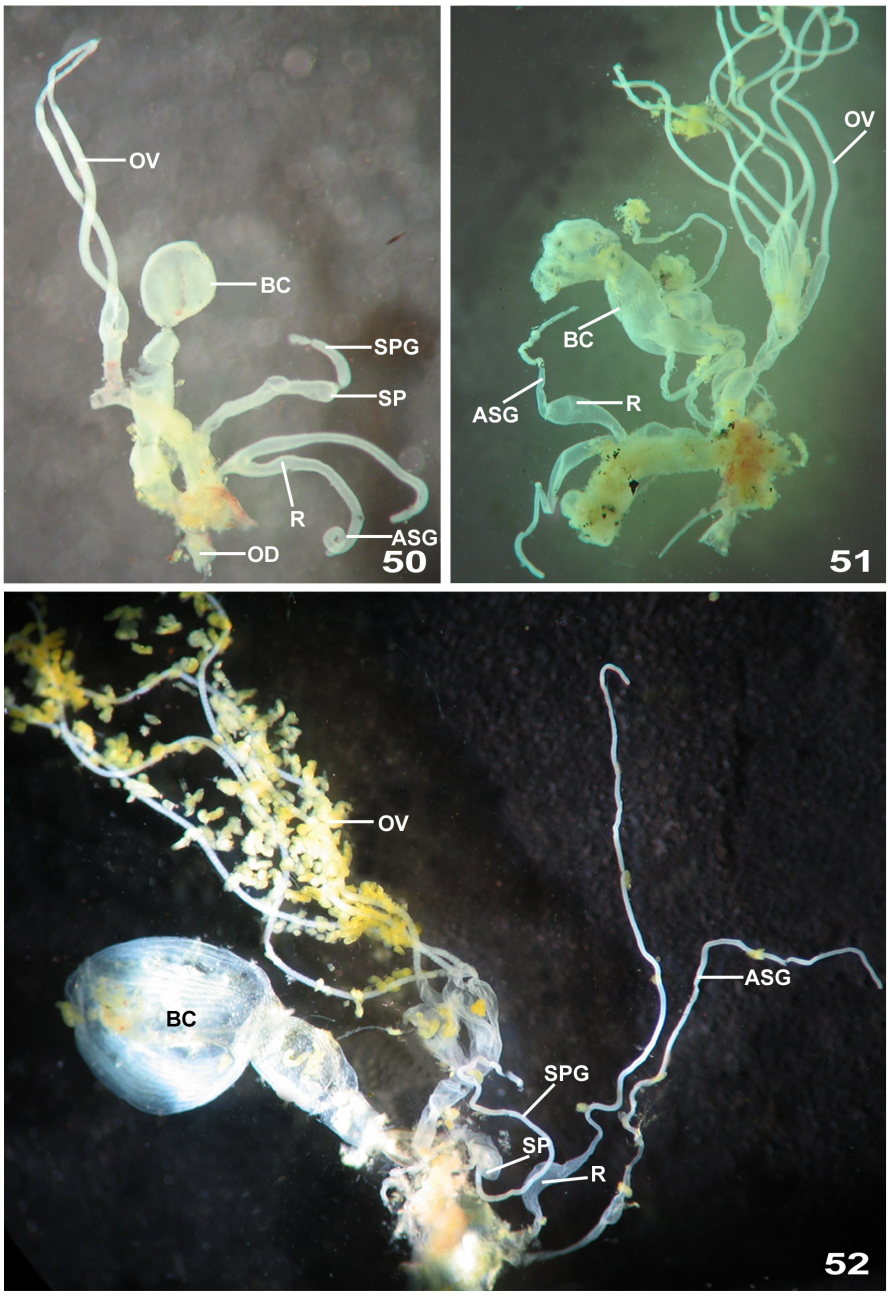
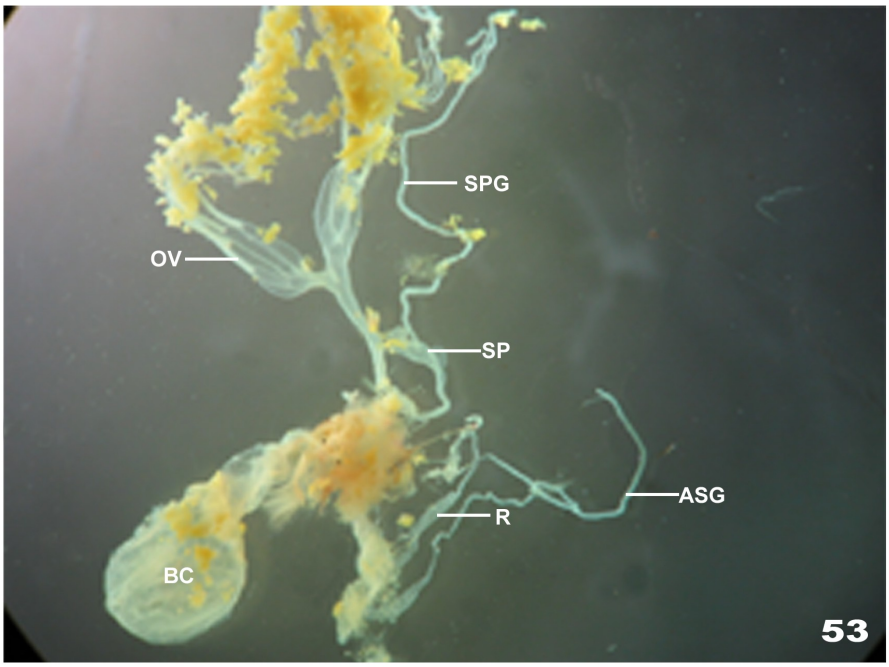


PLATE XIX



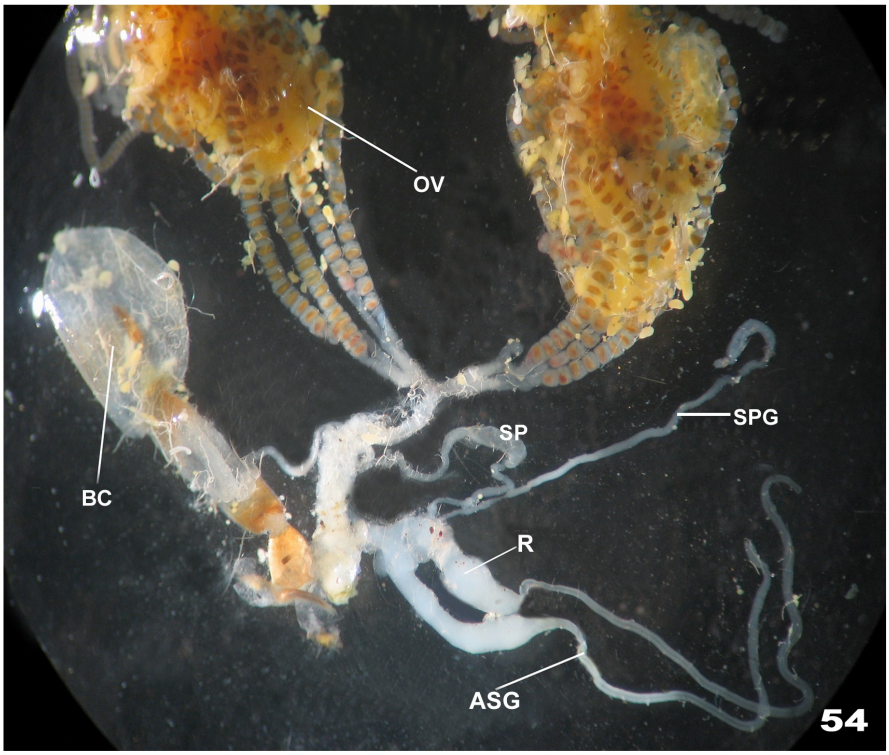
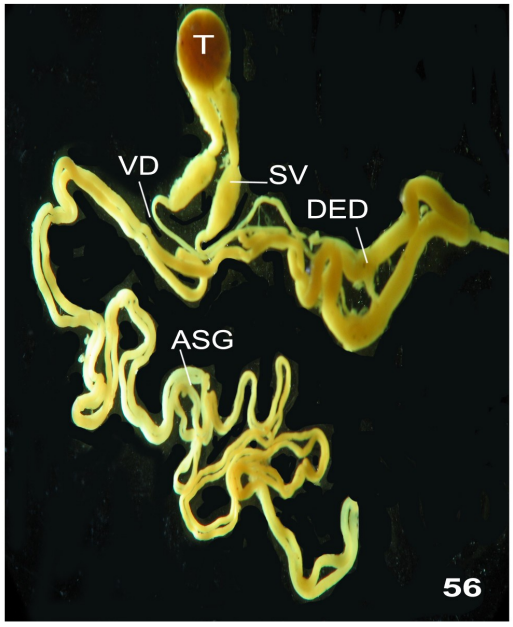
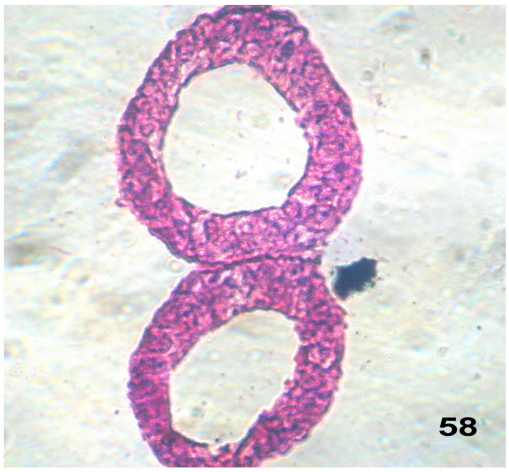


PLATE XX





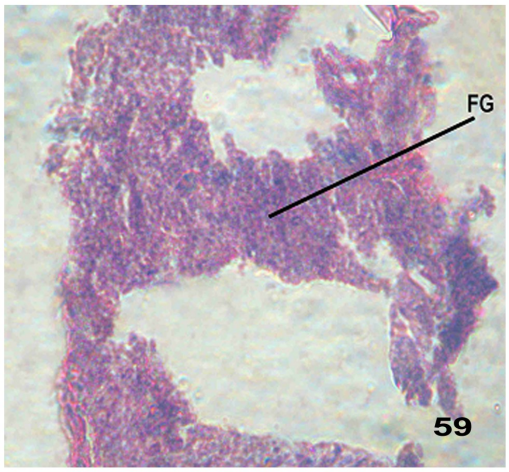
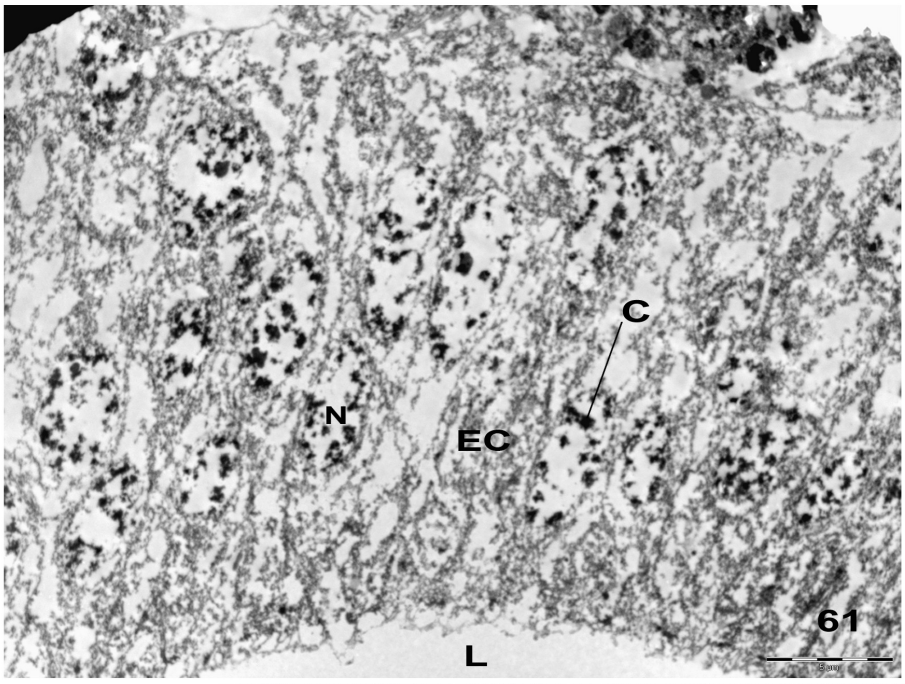


PLATE XXI



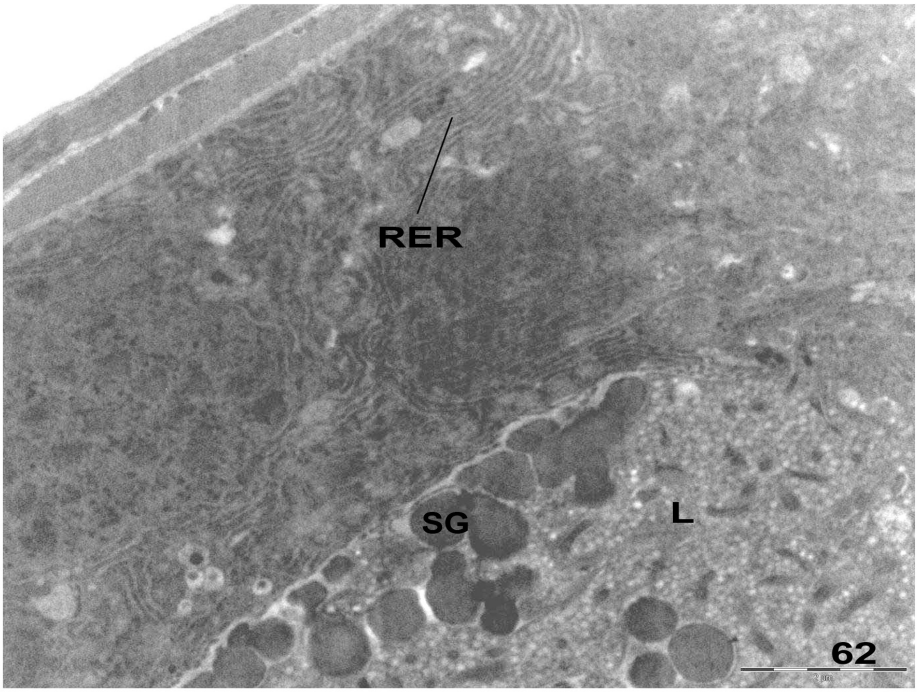
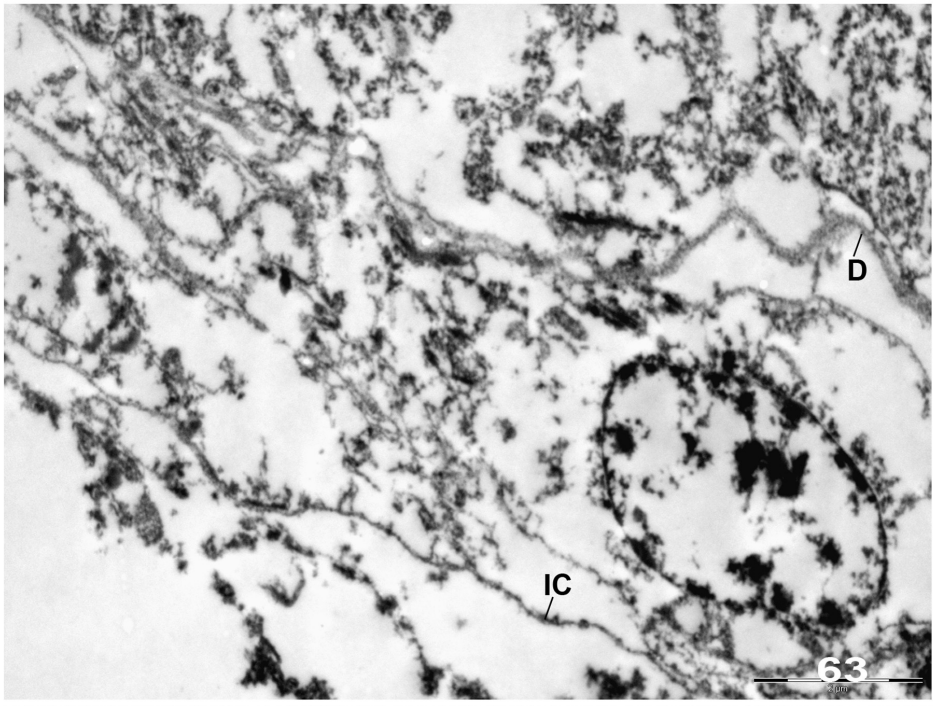


PLATE XXII



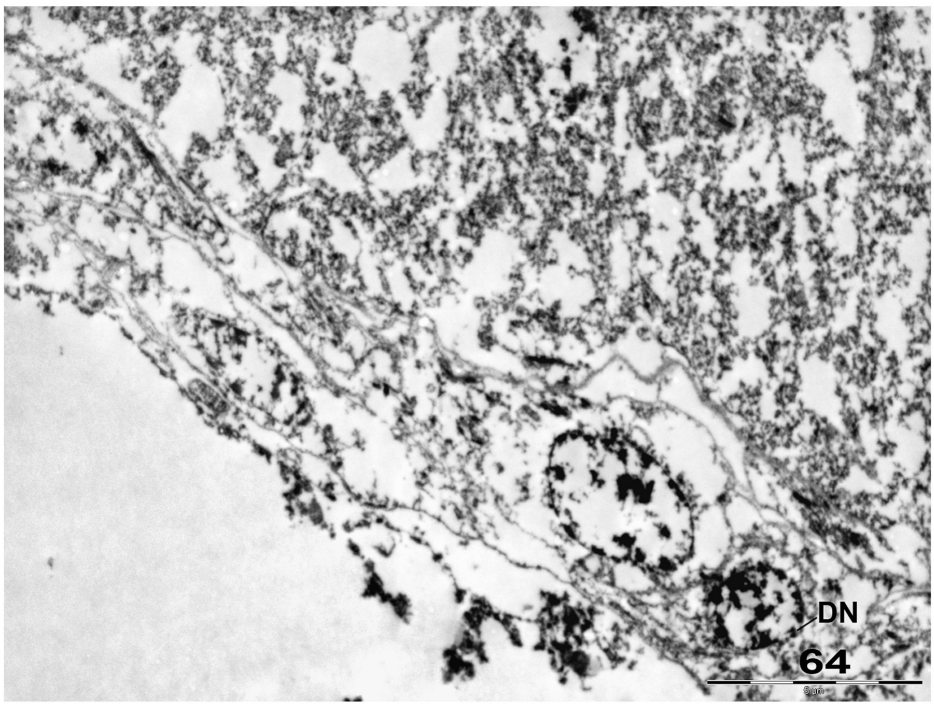
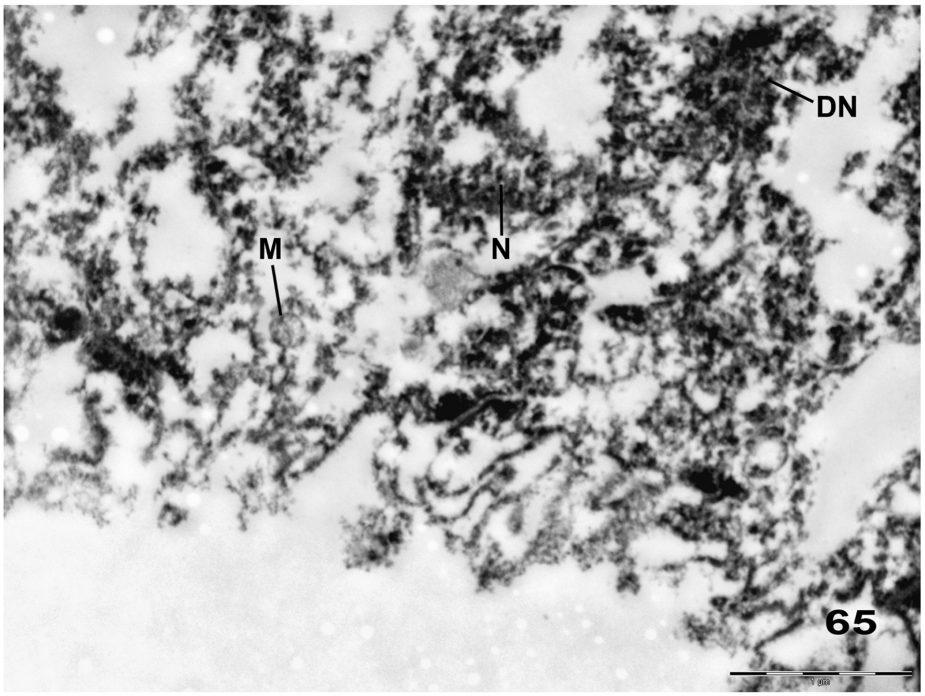


PLATE XXIII



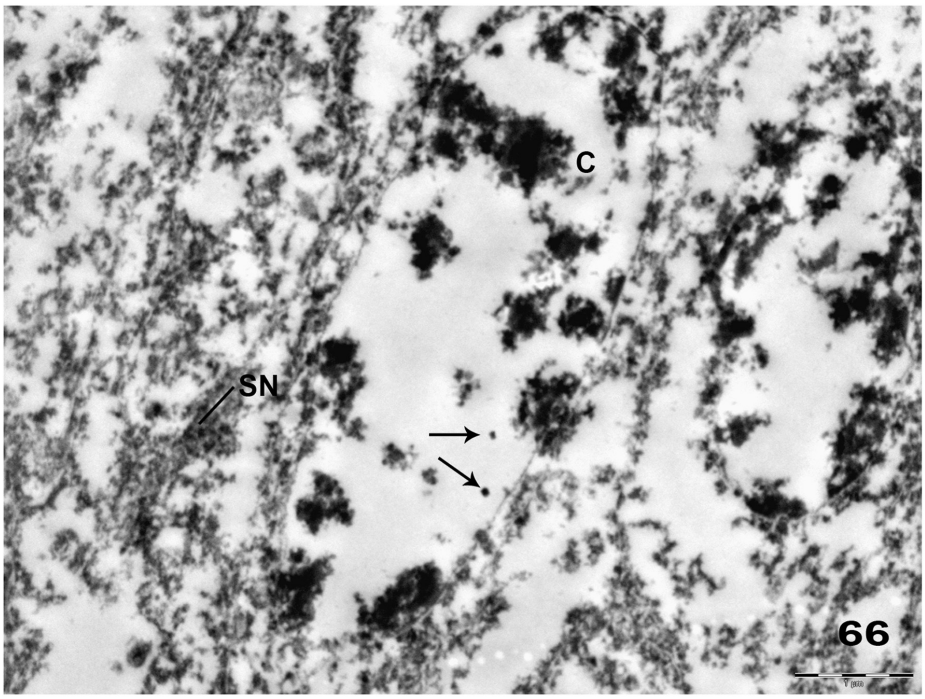


PLATE XXIV

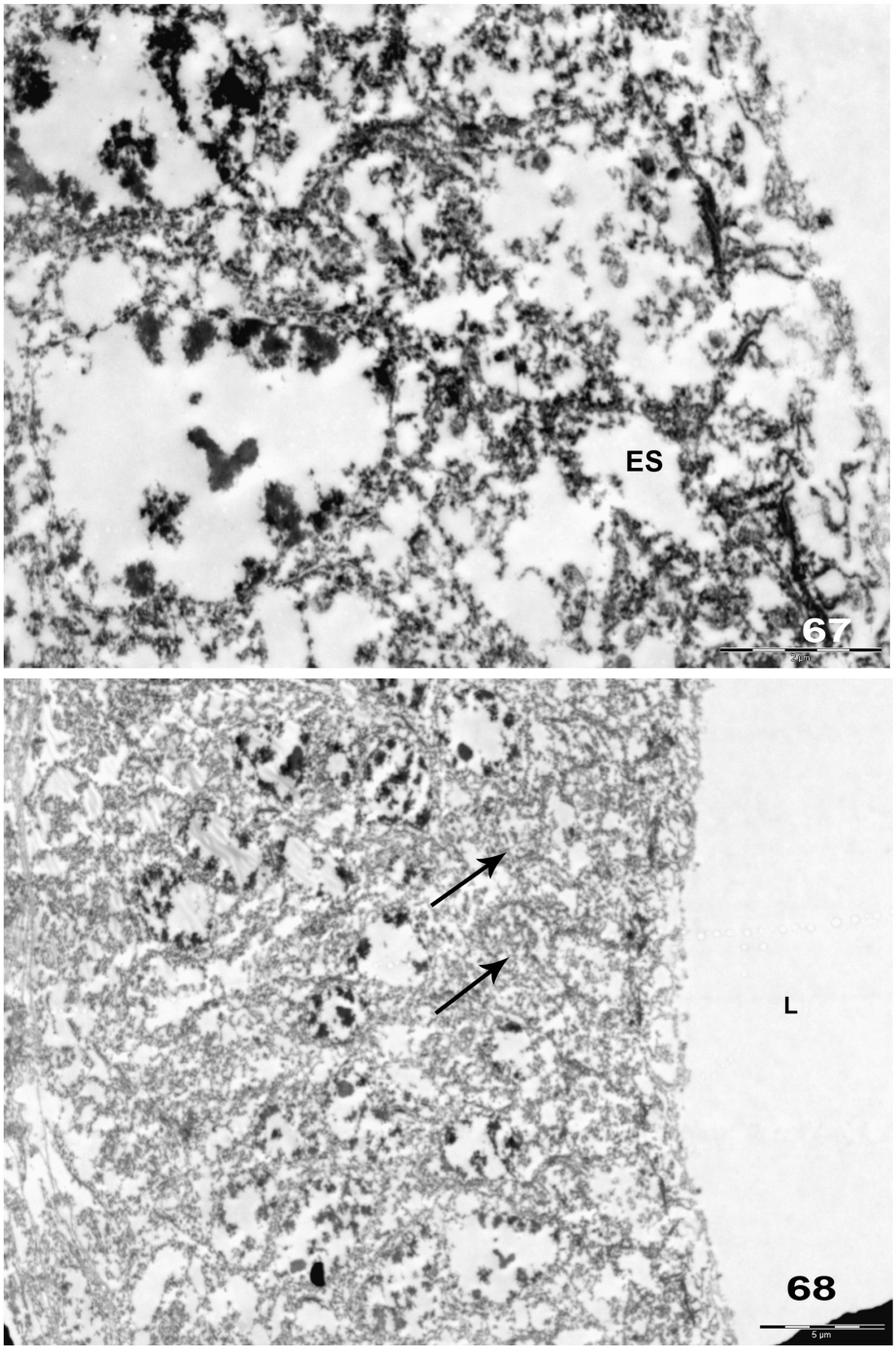
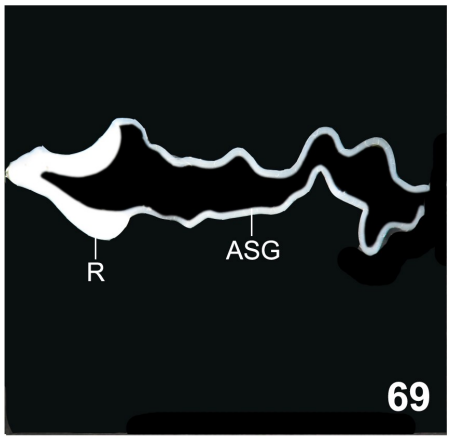
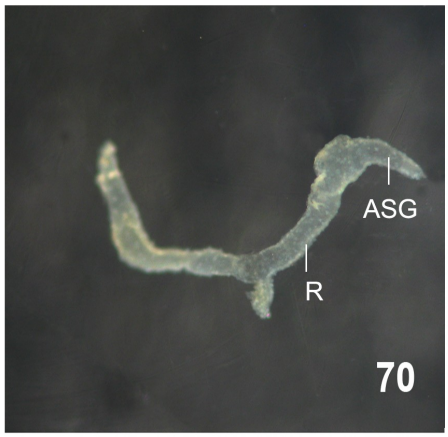


PLATE XXV





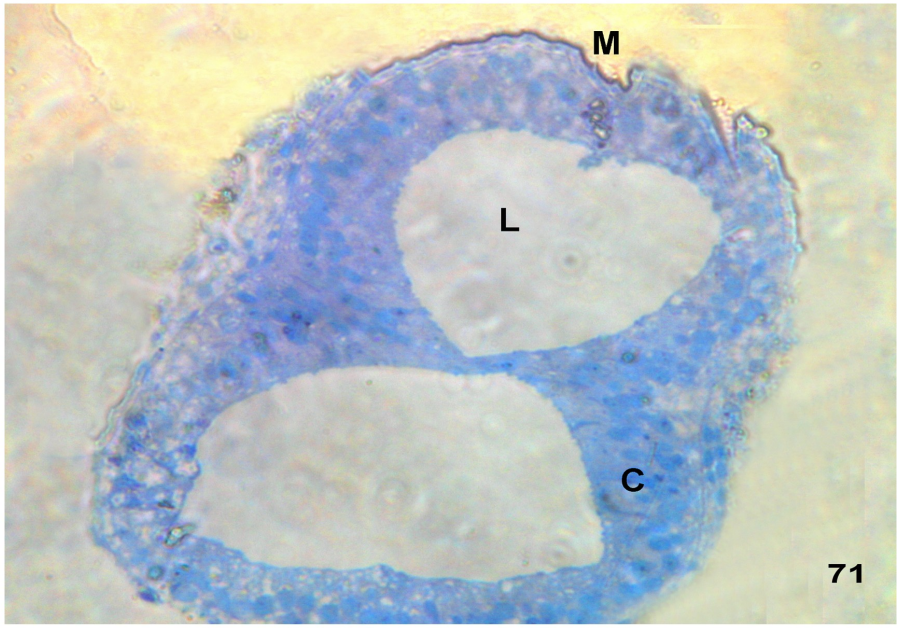
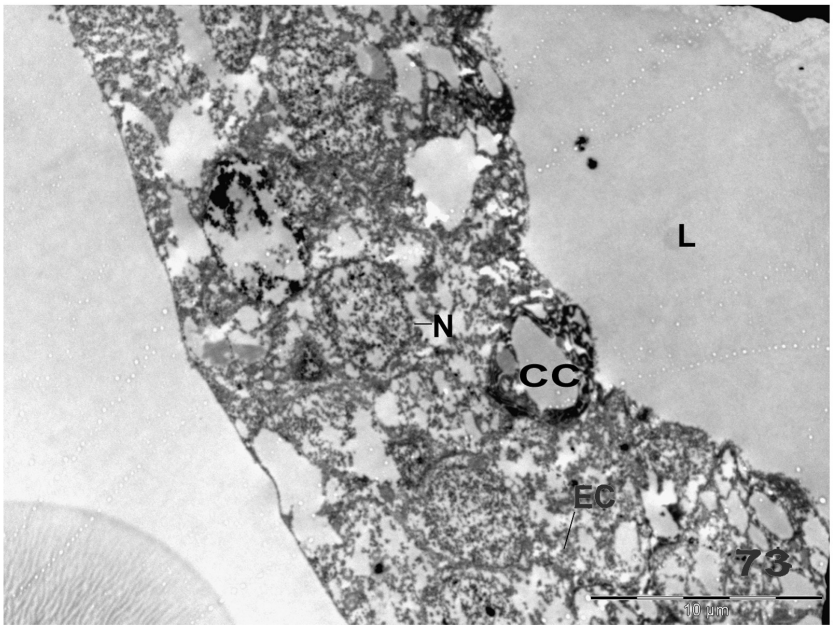


PLATE XXVI



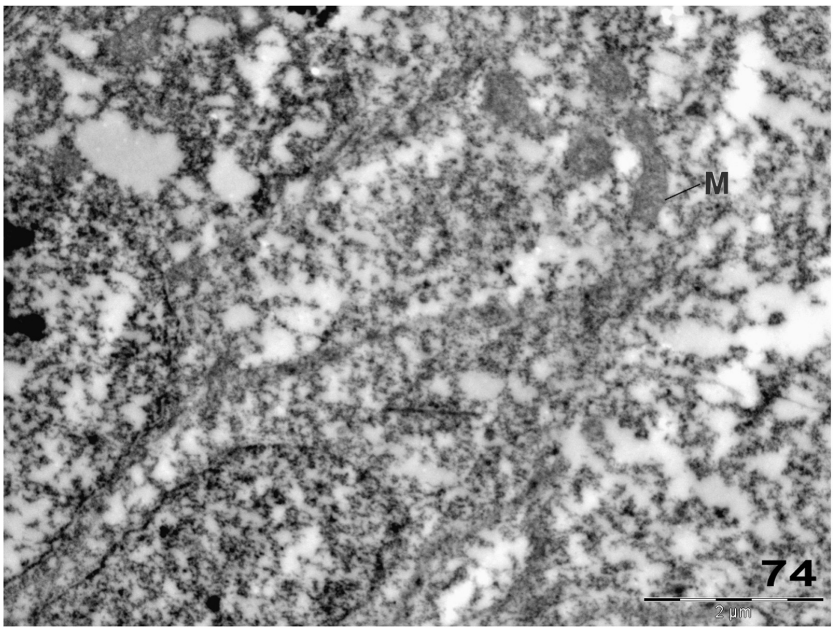


PLATE XXVII

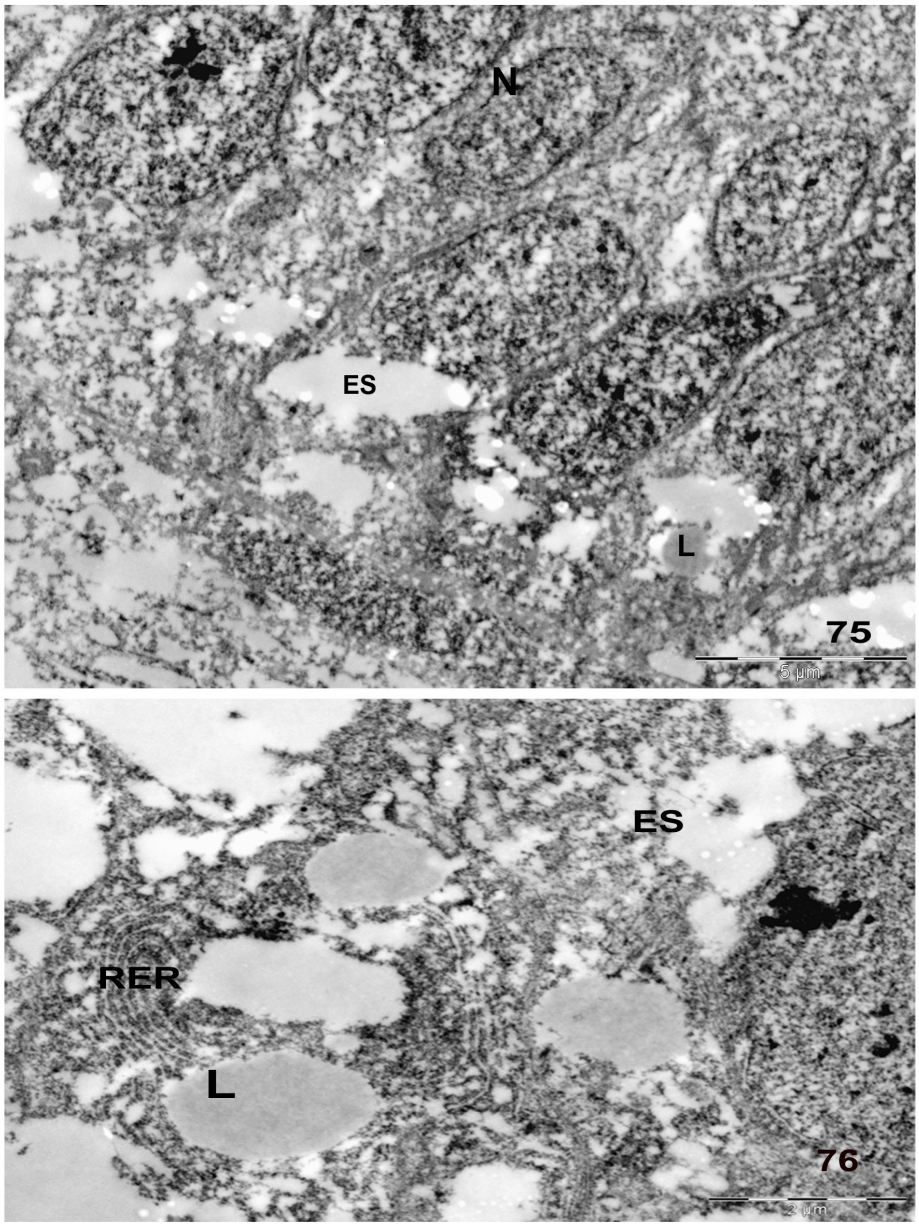


PLATE XXVIII

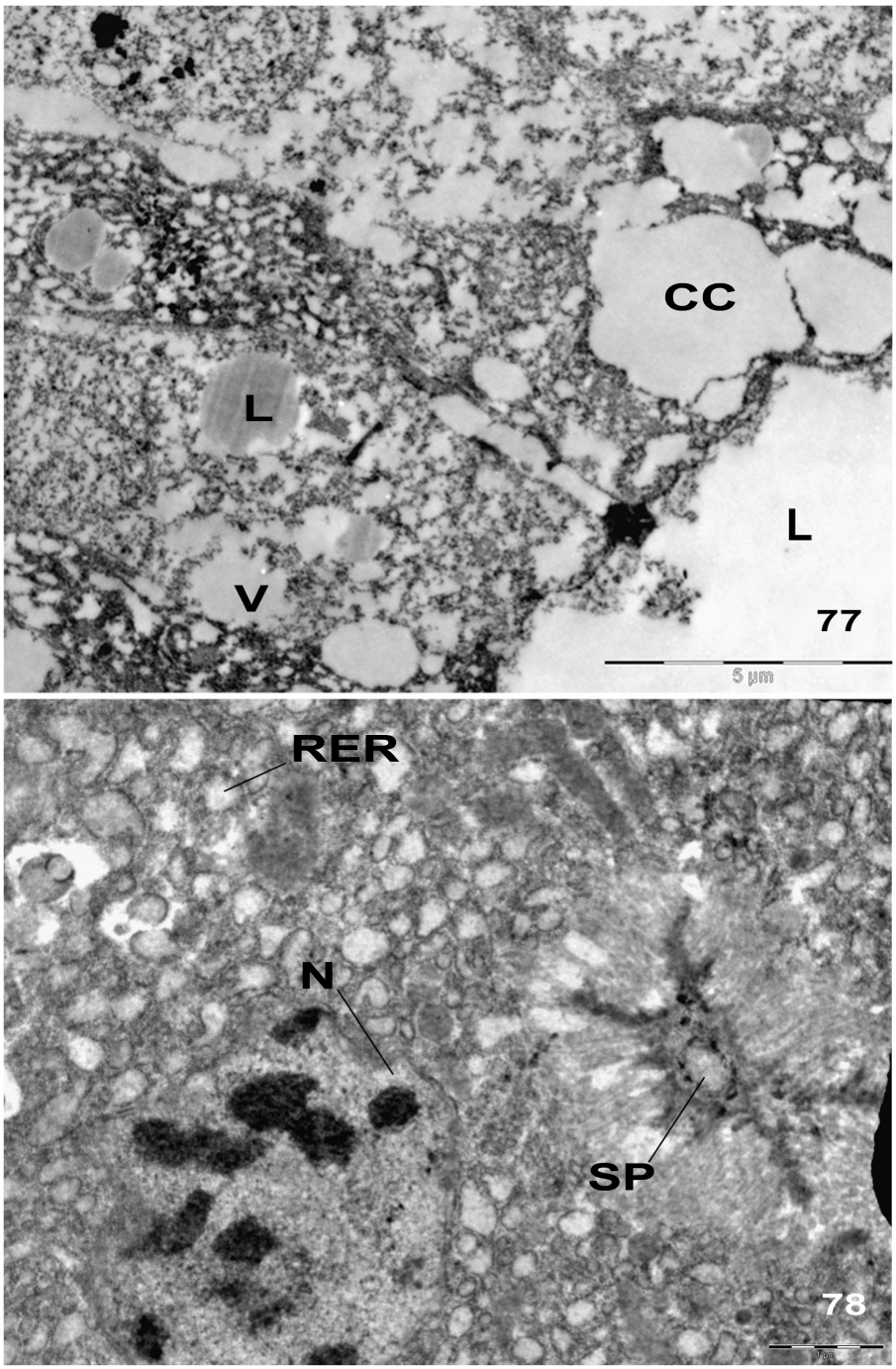


PLATE XXIX

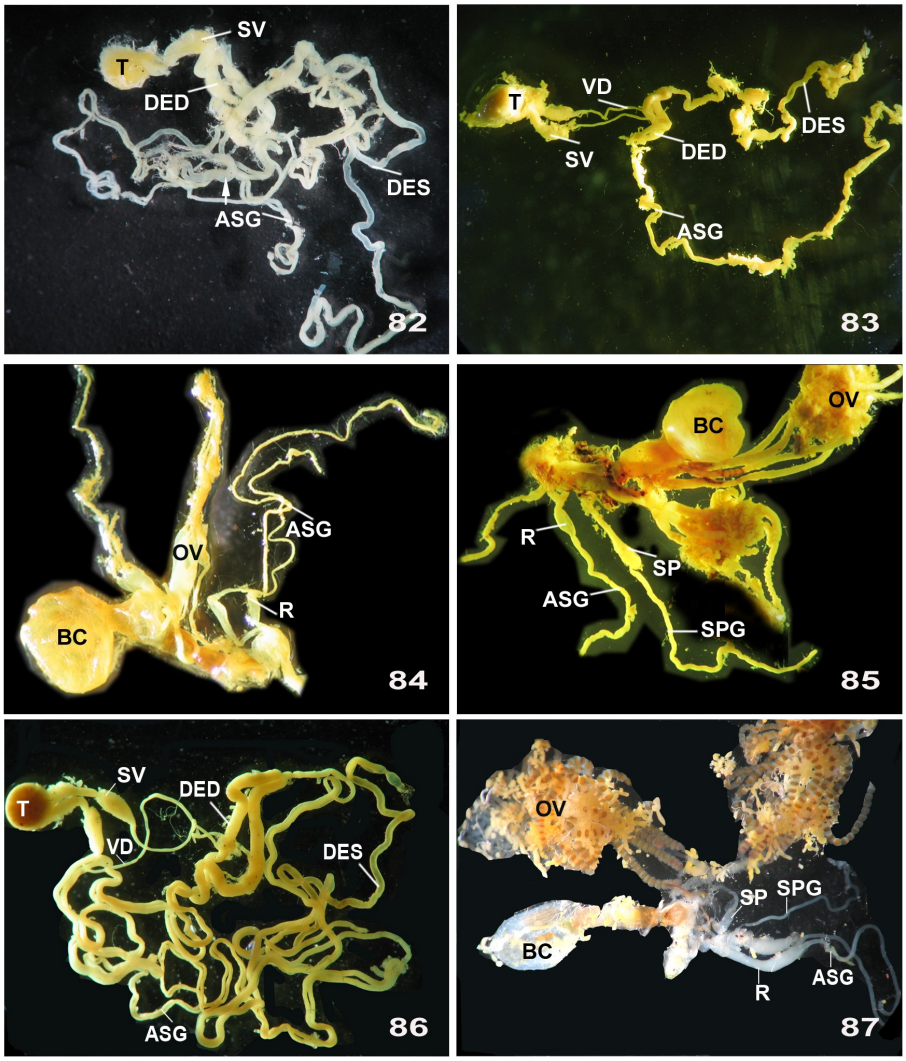

SV
T
DED
DES
ASG
82
T
VD
SV
DED
DES
ASG
83
OV
ASG
R
BC
84
BC
OV
R
SP
ASG
SPG
85
SV
DED
T
VD
DES
ASG
86
OV
SP
SPG
BC
R
ASG
87

PLATE I

Fig.1. Egg mass
Fig.2. Fifth instar larvae
a. Day 1 larva
b. Day 2 larva
Fig.3. Sixth instar larvae
a. Day 0 larva
b. Day 3 larva
Fig.4. Sixth instar larvae
a. Day 4 larva
b. Prepupa
Fig.5. Pupa
a. Ventral view
b. Dorsal view
Fig.6. Adults
a. Female
b. Male

PLATE II

Fig. 9. Section through the epithelial cells in the proximal region of the ASGs of adult male showing nucleus (N) and secretion inside the lumen (S).

Fig. 10. Section through the mid region of the ASGs of adult male showing secretion inside the lumen (S).

Fig. 11. Section through the ASGs of adult female showing epithelium (E), chitinogenous cells (C), chitinous intima (I) and secretion inside the lumen (S).

Fig. 12. Section through the reservoir of the ASGs of adult female showing columnar cells (Cc), lumen of the gland (L) and secretion inside the lumen (S).

PLATE III

Fig. 13. Section through the glandular epithelium of proximal region of the ASGs of adult male showing basement membrane (Bm), longitudinal muscles (Lm), Rough Endoplasmic reticulum (RER) and circular muscle (Cm) X 23000

Fig. 14. Section through the glandular epithelium of the proximal region of the ASGs of adult male showing nucleus (N), heterochromatin (Hc), tracheoles (T) lumen (L) of the gland and secretory globules (Sg) X 6000

PLATE IV

Figs. 15 and 16. Section through the glandular epithelium of proximal region of the ASGs of adult male showing the circular muscles (Cm), nucleus (N), nucleolus (Nu), nuclear envelope (Ne), heterochromatin (Hc), RER and secretory vesicles (Sv) near the lumen of the gland. Arrows represent coalescence of secretion inside the lumen. Fig. 15X 6800; Fig. 16 X 20500

PLATE VIII

Fig. 24. Section through the glandular epithelium of mid region of the ASGs of adult male showing secretory globule (Sg) inside lumen (L) of the gland, villi (V) X 20500

Fig. 25. Section through the distal region of the ASGs of adult male showing longitudinal muscles (Lm), circular muscles (Cm) and the basement membrane (Bm) of the epithelium X 49000

Fig. 26. Section through the glandular epithelium of distal region of the ASGs of adult male showing RER X 49000

PLATE X

Fig. 30. Section through the glandular epithelium of ASG of adult female showing RER, mitochondria (M) and infoldings (If) on the basement membrane X 23000

Fig. 31. Section through the glandular epithelium of ASG of adult female showing RER and connective tissue X 30000

PLATE XI

Fig. 32. Section through the glandular epithelium of ASG of adult female showing nucleus (N), nucleous (Nu) heterochromatin (Hc), mitochondria (M) and intercellular channels (Ic) X 23000

Fig. 33. Section through the glandular epithelium of ASG of adult female showing distended RER and mitochondria (M). Arrows represent granules inside RER X 30000

PLATE XII

Fig. 34. Section through the glandular epithelium of ASG of adult female showing nucleus (N), nucleolus (Nu) enlarged mitochondria (M) and RER X 13000

Fig. 35. Section through the glandular epithelium of ASG of adult female showing specialized secretory apparatus (Sp) with radiating arms (Ra) and felt work like structure (F), RER and mitochondria (M) X 30000

PLATE XIX

Fig. 53. Reproductive system of day 6 female pupa showing Ovariole (OV), Bursa copulatrix (BC), Spermatheca (SP), Spermathecal gland (SPG), Accessory sex glands (ASG), Reservoir (R).

Fig. 54. Reproductive system of adult female showing Ovariole (OV), Bursa copulatrix (BC), Spermatheca (SP), Spermathecal gland (SPG), Accessory sex glands (ASG), Reservoir (R)

PLATE XVI

Fig. 42. Reproductive system of day 1 male pupa showing Testis (T), Seminal vesicle (SV), Ductus ejaculatorius duplex (DED), Accessory sex glands (ASG)

Fig. 43. Reproductive system of day 2 male pupa showing Testis (T), Seminal vesicle (SV), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

Fig. 44. Reproductive system of day 3 male pupa showing Testis (T), Seminal vesicle (SV), Vas deferens (VD) Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

Fig. 45. Reproductive system of day 4 male pupa showing Testis (T), Seminal vesicle (SV), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

PLATE XVII

Fig. 46. Reproductive system of day 5 male pupa showing Testis (T), Seminal vesicle (SV), Vas deferens (VD), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

Fig. 47. Reproductive system of day 7 male pupa showing Testis (T), Seminal vesicle (SV), Vas deferens (VD), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

Fig. 48. Reproductive system of adult male showing Testis (T), Seminal vesicle (SV), Vas deferens (VD), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG), Proximal region (P), Mid region (M), Distal region (D), Aedeagus (A)

PLATE XVIII

Fig. 50. Reproductive system of day 1 female pupa showing Ovariole (OV), Oviduct (OD), Bursa copulatrix (BC), Spermatheca (SP), Spermathecal gland (SPG), Accessory sex glands (ASG), Reservoir (R)

Fig. 51. Reproductive system of day 2 female pupa showing Ovariole (OV), Bursa copulatrix (BC), Accessory sex glands (ASG), Reservoir (R)

Fig. 52. Reproductive system of day 5 female pupa showing Ovariole (OV), Bursa copulatrix (BC), Spermatheca (SP), Spermathecal gland (SPG), Accessory sex glands (ASG), Reservoir (R)

.

PLATE XX

Fig. 56. Reproductive system of male kept as control showing Testis (T), Vas deferens (VD), Seminal vesicles (SV), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

Fig. 57. Male Reproductive system of male adultoid showing Accessory sex glands (ASG)

Fig. 58. Section through the ASG of the day 4 male pupa kept as control showing the multiple layer of epithelial cells and empty lumen

Fig. 59. Section through the ASG of the male adultoid showing the fused glands (FG).

PLATE XXI

Fig. 61. Section through the glandular epithelium of ASG of the male adultoid showing the epithelial cells (EC), Nucleus (N), Chromatin (C) and empty Lumen (L) X 2900

Fig. 62. Section through the glandular epithelium of ASG of male kept as control showing RER and secretory globules (SG) in the lumen (L) X 1900

PLATE XXII

Fig. 63. Section through the glandular epithelium of ASG of the male adultoid showing the desmosomes (D) and crumpled intercellular membrane (IC) X 9300

Fig. 64. Section through the necrotic area of glandular epithelium of ASG of male adultoids showing disintegrating nucleus (DN) X 4800

PLATE XXIII

Fig. 65. Section through the glandular epithelium of ASG of the male adultoid showing the mitochondria (M), pycnotic nucleus (N) and disintegrating nucleus (DN) X 18500

Fig. 66. Section through ASG of male adultoid showing chromatin (C) and shrinking nucleus (SN). Arrows represent dense granules inside the nucleus X 13000

PLATE XXIV

Fig. 67. Section through the glandular epithelium of ASG of the male adultoid showing empty spaces (ES) X 9300

Fig. 68. Section through the glandular epithelium of ASG of male adultoids showing lumen (L). Arrows represent nuclei devoid of condensed heterochromatin X 2900

PLATE XXIX

Fig. 82. Reproductive system of 3 µg methoxyfenozide treated male showing Testis (T), Seminal vesicles (SV), Ductus ejaculatorius duplex (DED), Accessory sex glands (ASG) and Ductus ejaculatorius simplex (DES). Arrow represents the voluminous region of ASG.

Fig. 83. Reproductive system of 30 µg methoxyfenozide treated male showing Testis (T), Seminal vesicles (SV), Vas deferens (VD), Ductus ejaculatorius duplex (DED), Accessory sex glands (ASG) and Ductus ejaculatorius simplex (DES)

Fig. 84. Reproductive system of 3µg methoxyfenozide treated female showing Ovarioles (O), Bursa copulatrix (BC), Accessory sex glands (ASG) and Reservoir (R)

Fig. 85. Reproductive system of 30 µg methoxyfenozide treated female showing Ovarioles (O), Bursa copulatrix (BC), Spermatheca (SP), Spermathecal gland (SPG), Accessory sex glands (ASG) and Reservoir (R)

Fig. 86. Reproductive system of male kept as control showing Testis (T), Seminal vesicles (SV), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)

Fig. 87. Reproductive system of female kept as control showing Ovarioles (OV), Bursa copulatrix (BC), Spermatheca (SP), Spermathecal gland (SPG), Accessory sex glands (ASG) and Reservoir (R)

PLATE XXV

Fig. 69. Accessory sex glands (ASG) of female kept as control showing Reservoir (R)

Fig. 70. Accessory sex glands (ASG) of female adultoids showing Reservoir (R)

Fig. 71. Semithin section through the ASG of female adultoids showing empty lumen (L), small columnar cells (C) and degenerated muscle layer (M)

PLATE XXVI

Fig. 73. Section through the glandular epithelium of ASG of the female adultoid showing the epithelial cells (EC), nucleus (N) and ruptured chitinogenous cells (CC) and empty lumen X 2900

Fig. 74. Section through the glandular epithelium of ASG of female adultoids showing abnormal mitochondria (M) X 11000

PLATE XXVII

Fig. 75. Section through the glandular epithelium of ASG of the female adultoid showing the empty spaces (ES), nucleus (N) and lysosomes (L) X 4800

Fig. 76. Section through the glandular epithelium of glandular epithelium of ASG of female adultoids showing rough endoplasmic reticulum (RER), empty spaces (ES) and lysosomes (L) X 11000

PLATE IX

Fig. 27. Section through the glandular epithelium of distal region of the ASGs of adult male showing RER, nucleus (N) and heterochromatin (Hc) X 23000

Fig. 28. Section through the glandular epithelium of distal region of the ASGs of adult male showing Golgi bodies (G), mitochondria (M) and lumen containing secretory globules (Sg) X 13000

Fig. 29. Section through the distal region of the ASGs of adult male showing the lumen (L) containing secretory globules (Sg) and cytoplasmic debris (Cd) X 13000

PLATE V

Fig. 17. Section through the glandular epithelium of proximal region of the ASGs of adult male showing parallel arrays of RER near nucleus (N) X 11000

Fig. 18. Section through the glandular epithelium of the proximal region of the ASGs of adult male showing secretory globules (Sg) and multivesicular bodies (Mvb). Arrows represent electron dense patches on secretory globules X 30000

PLATE VI

Fig. 19. Section through the mid region of the ASGs of adult male showing multiple layer of epithelium (E) and nucleus (N) X 4200

Fig. 20. Section through the glandular epithelium of the ASGs of adult male showing longitudinal muscles (Lm), circular muscles (Cm), plasma membrane (P) and vesiculated rough endoplasmic reticulum (RER) X 20500

Fig. 21. Section through the glandular epithelium of the mid region of the ASGs of adult male showing nucleus (N), nuclear envelope (Ne), mitochondria (M) and RER. Arrows represent granules inside RER X 23000

PLATE VII

Fig. 22. Section through the glandular epithelium of the mid region of the ASGs of adult male showing abundant mitochondria (M) and nucleus (N) X 4200

Fig. 23. Section through the glandular epithelium of the mid region of the ASGs of adult male showing nucleus (N) and mitochondria (M) X 13000

PLATE XIII

Fig. 36. Section through the glandular epithelium of ASG of adult female showing the secretory apparatus. Arrows represent electron dense materials near the arms X 13000

Fig. 37. Section through the glandular epithelium of ASG of adult female showing collection of mitochondria (M) near the specialized secretory apparatus X 30000

PLATE XV

Fig. 40. Section through the ASG of adult female showing chitinous intima (I) and the lumen of the gland (L) X 30000

Fig. 41. Section through the chitinogenous cells of ASG of adult female showing septate desmosomes (Sd), looped plasma membrane (Lp) on the margin of the chitinogenous cells and microtubules (Mt) X 23000

PLATE XIV

Fig. 38. Section through the glandular epithelium of ASG of adult female showing chitinogenous cells (C), nucleus (N) and lumen of the gland (L) X 23000

Fig. 39. Section through the chitinogenous cells of ASG of adult female showing septate desmosomes (Sd), looped plasma membrane (Lp) on the margin of the chitinogenous cells and chitinous intima (I) X 48000

PLATE XXVIII

Fig.77. Section through the glandular epithelium of ASG of female adultoids showing lysosomes (L) and ruptured chitinogenous cells (CC) X 6800

Fig.78. Section through the glandular epithelium of ASG of female kept as Controls showing RER, nucleus (N), Specialized secretory apparatus (SP)

Fig. 7 Male Reproductive system

T	Testis
SV	Seminal vesicles
VD	Vas deferens
DED	Ductus ejaculatorius duplex
DES	Ductus ejaculatorius simplex
ASG	Accessory sex glands
P	Proximal region
M	Mid region
D	Distal region
A	Aedeagus

Fig. 81. SDS-PAGE profile of ASG proteins in PPN treated and control male insects

Lane 1. ASG protein profile of day 1adult controls
Lane 2. ASG protein profile of day 1 adults treated with 20 µg PPN immediately after eclosion on day 0
Lane 3. ASG protein profile of adultoids
Lane 4. ASG protein profile of day 0 adult controls
Lane 5. Molecular weight markers

Fig. 8 Female Reproductive system

OV	Ovarioles
LO	Lateral oviduct
MD	Median oviduct
BC	Bursa copulatrix
SD	Seminal duct
SP	Spermatheca
SPG	Spermathecal gland
ASG	Accessory sex glands
R	Reservoir