

Studies on the Female Reproductive System in the Aquatic Beetle *Cybister Tripunctatus* (Coleoptera; Dytiscidae)

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Abstract

The aquatic beetle *Cybister tripunctatus* occurs throughout India during all seasons of the year in ponds and tanks including the fish nurseries. It is a voracious feeder causing heavy loss of the fish crop every year. It is difficult to control this pest by using traditionally available mechanical, physical and chemical control measures. Only autocidal control based upon the principal of "control of a hazardous pest population by lowering down the birth rate rather than the death rate" may solve this problem in order to save the fish crop. In this regard, thorough study of the female reproductive system and mechanism of oocyte development and vitellogenesis is of prime importance. The present work has therefore undertaken to study the female reproductive system in *Cybister tripunctatus* with special reference to morphology of female reproductive system and cytological changes in developing oocytes, nurse cells and follicular epithelial cells. The histological and morphological details of the beetle are discussed in present paper.

KEYWORDS: *Cybister tripunctatus*, Morphology, Histology, female

INTRODUCTION

Coleoptera is considered as the largest order of the class insecta consisting about 330,000 species. All the members of this order are collectively called as beetles and their habits are extremely varied. There are about twelve families of aquatic beetles and Dytiscidae is one of them consisting of diving beetles. The Indian beetle, *Cybister tripunctatus* occurs commonly in the local region. The female reproductive system in Coleopteran shows wide variation in the anatomical organization, histological structure, process of egg development and mode of ovipositor among different species. Most of the work on the female reproductive system is carried out in Polyphagous beetles while very meager information is available such as, Datta Gupta, & Kumar, 1963.

The aquatic beetle *Cybister tripunctatus* occurs throughout India during all seasons of the year in ponds and tanks including the fish nurseries. It is the voracious feeder causing heavy loss of the fish crop every year. It is difficult to control this pest by using traditionally available mechanical, physical and chemical control measures. Only autocidal control based upon the principal of "Control of a hazardous pest population by lowering down the birth rate rather than the death rate" (Knippling, 1959), may solve this problem in order to save the fish crop. In this regard, thorough study of the female reproductive system and mechanism of oocyte development and vitellogenesis is of prime importance. The present work has therefore undertaken to study the female reproductive system in *Cybister tripunctatus* with special reference to the morphology of female reproductive system and cytological changes occurring in developing oocytes, nurse cells and follicular epithelial cells;

MATERIAL & METHODS

The present work is carried out in aquatic beetle, *Cybister tripunctatus* OL. (Richard and Davis 1977).

The aquatic carnivorous beetles were collected from the ponds located at- Pavani, Distt-Bhandara (M.S).The beetles were reared in laboratory throughout the year to carry out the present studies. In aquatic beetle *Cybister tripunctatus*, sexes are separate and sexual dimorphism is well marked as the forelegs of male beetles shows presence of adhesive pads, while such structures are absent in females. It possesses filiform antennae. Hind legs are notorial, functioning as swimming organs, flattened and fringed with hairs. Larvae are with long sickle shaped mandibles. Last two abdominal segments along with abdominal lobes are fringed with hairs. Elytra stores air beneath them. It is source of oxygen which is supplied to tracheal system by last two pairs of abdominal spiracles, during diving in deep water.

The female reproductive organs were dissected in insect Ringers solution under stereoscopic binocular microscope. The organs were fixed in Bouin's fluid for 18- 24 hrs.for histology and 6-12 hrs. in Cornoy's fixative for DNA, RNA, protein and carbohydrate histochemistry. The fixed tissue were, dehydrated and embedded in paraffin wax at 60-62^oc.The sections were cut at 4 and 10 um thickness on the microtome for histological and histochemical techniques respectively.

For histochemistry of lipids, the ovaries, colleterial gland and spermathecal gland were dissected gently, fixed in Bakers Calcium formal fixative and sections of 10-15um thickness were cut on the cryocut (Leica, U.S.A).

OBSERVATIONS:

Table1:

Developmental changes in weight of ovary, size of oocyte, nuclei of trophocyte and follicular cells in *Cybister tripunctatus*

Age of beetles(Days)	vit state	Weight of ovary(mg)	Histological changes		
			Oocyte length (µm)	Tc (µm)	Fc (µm)
0	NE	20.42±2.80	20±20	-	-
2	PV	20.42±2.80	62±4	96.10±0.48	8.10±0.5
4	EV	97.68±6.71	295.5±29	90.7±6	7±1
6	MV	227.24±7.11	400±23	97±11	11.00±0.69
8	LV	240±8.08	663.5±14.5	78±2.18	15.2±1.25
10	MS	273.25±7.95	657±27	-	12.4±1.80

Abb: PV-Previtellogenic stage
 MV-Mid-vitellogenic stage
 LV-Late-vitellogenic stage
 MS-Maturation stage
 TC- Trophocyte
 NE-Newly emerged±-standard err
 ±-standard error

RESULTS AND DISCUSSION

1. MORPHOLOGY

The female reproductive system is well developed in the adult beetle, *Cybister tripunctatus*(OL). It consists of-

1) A pair of ovaries. 2) A pair of lateral oviducts. 3) A common oviduct. 4) A vagina 5) A spermatheca with spermathecal gland. 6) A colleterial gland.

The female reproductive organs are located in the abdominal cavity occupying the region comprising 1st to 8th abdominal segments. The reproductive organs are intermingled with the fat body and trachea.

In the immature beetle, the ovaries are small laying ventral to the alimentary canal in the posterior 4th and 5th segments of the abdomen. In the matured beetles, the ovaries develop extensively and they occupy most of the region of abdominal cavity from 1st to 6th abdominal segments. Each ovary is large and oval in shape consisting of 20 to 25 ovarioles. Each ovariole is about 1.5 cm in length. An immature ovary measures about 8 to 12mg and the fully matured one about 46 to 54mg in weight. The ovaries are externally covered with thin peritoneal sheath. The ovaries are attached anteriorly to the inner surface of the wall of the 1st abdominal segment by a suspensory ligament. They open posteriorly into the lateral oviducts. The lateral oviducts are short and tubular structures. They run latero-medially from the 6th to 7th abdominal segments. Both the lateral oviducts unite together forming common oviduct. The common oviduct is a large tubular and convoluted structure. The posterior part of common oviduct is modified into the bulbus vagina

2. HISTOLOGY

The ovarioles

The ovarioles are long, tubular structures. They are antero-posteriorly differentiated into following four regions.

1. A terminal filament; 2. A germarium; 3. A vitellarium; 4. A pedicel

The terminal filament

Apical region of each ovariole contains stem line, the terminal filament. The terminal filament is anterior most thread like, fine structure of the ovariole. The terminal filament is composed of a mass of small spherical cells with oval nuclei. The cells are almost devoid of cytoplasm. Internally a large lumen is present. Externally, the terminal filament is covered with a fine layer of tunica propria. A transverse septum lies in between the terminal filament and germarium. The terminal filament of all ovarioles of an ovary unite together to form a thick cord, the suspensory ligament. (Giorgi, F.P. Lucchesi, J., Morelli, A., And Bownes, M., 1993)

The vitellarium

The posterior part of germarium is enclosed with the follicular epithelial cells. They migrate from germarium into the vitellarium in which further development of oocyte is taking place. It is the largest part of an ovariole enclosing the oocytes encircled with the follicular epithelium. The oocytes are arranged in a linear fashion. Each oocyte is provided with a group of 15 trophocytes or nurse cells. Each oocyte and group of nurse cells are separated from the preceding and succeeding follicles, by a single layer of cells, the inter follicular tissue. The ovaries, thus, represent polytrophic type.

The lateral oviduct

The wall of the lateral oviduct is composed of the columnar epithelial cells; the epithelial cells are characterized with small spherical nuclei and scanty cytoplasm. The lumen is large which forms a passage for the ovulating ova. It is externally covered with thin peritoneal membrane. Internally epithelium is devoid of cuticular intima. (Akopyan L.A. And Megakyan Y.U.A., 1984)

The common oviduct

The wall of common oviduct is composed of an outer thick muscle layer and inner thin epithelial layer. The muscle layer is composed of circular muscle while it is externally covered

with longitudinal muscles. The epithelium is composed of columnar cells. They possess distinct nuclei and granular cytoplasm. The epithelial layer is internally lined with cuticular intima. At some places epithelial folds are extended into the lumen.

The vagina

Vagina is greatly muscular. The wall of the vagina is composed of enormously outer thick muscle layer and inner thin epithelial layer. Dorsally it is differentiated into the bursa copulatrix by a cuticular plate. The bursa copulatrix receives spermatozoa from the spermatheca. The bursa copulatrix opens into the vagina through the fertilization pore which is present at the centre of cuticular plate. The lumen is internally lined with the cuticular intima.

The spermatheca

The wall of spermatheca is composed of outer muscle layer inner epithelial layer.

Seminal receptacle utriculus and lagena

The muscle layer of seminal receptacle is composed of longitudinal muscles while that of lagena is made up of inner circular and outer longitudinal muscles. The epithelial layer is composed of tall columnar cells possessing distinct spherical nuclei. They contain little cytoplasm in seminalis receptacle while abundant quantity of cytoplasm in lagena. The epithelium in both seminalis receptacle and lagena is lined with thick cuticular intima. The lumen is filled with a mass of spermatozoa.

Spermathecal Duct

The duct of spermatheca is composed of outer muscle layer and inner epithelial layer. The epithelial layer is almost degenerated and represented by spindle shaped nuclei. The cuticular intima is well developed. The lumen is filled with mass of spermatozoa.

Spermathecal gland

The spermathecal gland is composed of outer cuticular muscle layer and inner layer of tall columnar epithelial cells with prominent nuclei. The epithelial layer is folded enormously. The epithelial folds are extended into the lumen. The lumen is large and filled with secretion secreted by the epithelial cells. Thick cuticular intima is well evident all over inner surface of the epithelial layer.

Colleterial gland

The colleterial gland is about $65.60 \pm \mu\text{m}$ in diameter while the lumen is about $49.20 \pm \mu\text{m}$ in diameter. The wall of the gland composed of a layer of columnar epithelial cells with large spherical nuclei at the center and cytoplasmic inclusion in the cell bodies. The cell and nuclear diameter of the epithelial cells measures about $16.40 \pm \mu\text{m}$ and $12.30 \pm \mu\text{m}$ respectively. Externally they are covered with a thin peritoneal membrane. The epithelium is internally lined with a thin layer of cuticular intima. (Ghosh D., Chel., G. Amd Pal, S.G., 1998)

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