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Investigation of Poison Gland of Sphex flavipennis Fabrius, 1793 (Hymenoptera: Sphecidae): Morphology and Ultrastructure

Menderes Suiçmez[1], Mustafa Duran[2] and Kasım Özmen[3]
[1] Hitit University, Department of Biology, Çorum, TURKEY
[2]Pamukkale University, Department of Biology, Denizli, TURKEY
[3] State Hospital of Dr. Cevdet AYKAN, Tokat, TURKEY

ABSTRACT

The structure of poison gland of Sphex flavipennis was investigated by using, scannig electron microscopy and transmission electron microscopy. Poison apparatus consists of poison gland, poison sac, Dufour's gland and poison needle. Poison is produced in a gland consisting of two ramified glandular tubules terminating in a common sac. Each tubule is 6-8 mm in length and approximately 90 µm in diameter. These tubules are lined with the secretory cells and duct cells. The secretory cells have a well-developed secretory unit which is open to the lumen of tubules. In addition, there are free ribosomes, large secretory vesicle and a few mitochondria in the cells. Apical surface of cells are lined by irregular microvilli. Glandular tubules go into pear-like sac. Apical surface of the cells in the poison sac are lined cuticle. Outer surface of poison sac is surrounded muscle fibril and connective tissue. Lumen side of glandular tubules and poison sac are surrounded with monolayer epithelial cells.

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***Correspondence to**: Menderes Suiçmez, Hitit University, Department of Biology, Çorum, TURKEY*
Tel: +90 (364) 223-8000-3376
Fax: +90 (364) 223-8004
E-Mail: menderessuicmez@hitit.edu.tr, menderessuicmez@gmail.com

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INTRODUCTION

Poison glands are ectodermic-originated and their organization has the same model in all Hymenoptera studied [1]. A portion of the poison apparatus of *Pimpla turionellae* was placed in the abdomen, whereas another part is on the 8th, 9th and 10th ventral segments [2]. The other one of the part is placed at the end of the abdomen as a unique part and extended position. The complex-structured poison glands of *Pimple turionellae* is composed of a sac, a toxin channel and a poison tube [3]. The needle of *Liris niger* lies down in between rectum and nervous system at the rear end of the abdomen [4]. All species investigated (*Amblyopo reclinata, Mystrium camillae, Prionopelta kraepelini, Onchomyrmex hedleyi*) have poison glands with two secretory tubes characterized by non-folded gland and combined with a poison sac [5]. The needle apparatus of *Pepsis pallidolimbata* consists of three anatomical structures: needle, poison gland and Dufour's gland [6]. The poison glands in *Polistes versicolor* consists of two independent tubes connected separately to poison sac [7].

Parasitic wasps lay eggs onto or inside of the hosts that make up a large part of the Hymenoptera species and the significant regulators of insect populations [8]. Poisonous bees contribute significantly to the protection of ecological balance due to the fact that they use some other insects both to breed and to be fed. Female ones among sharp-needle wasps living alone (including fossorial wasps) produce paralyzing poisons for victims (insects or spiders) they needle once or several times. The victim then taken to a prepared nest and the egg is laid down over or inside the victim. The suitable means to inject the poisons into the circulatory or neural system is an injection needle device with a modified ovipositor. Once they needle the victim, they inject poison into the haemolymph or muscle-nerve (neuromuscular) connections regions. Paralyzed victim acts as a live food source for the larvae of wasps [4]. Some pompilids sting their victims and cause temporary paralysis, then the stung spider is caught actively, consumed and normally survive until the emerging (developing) wasps will savage it [9]. The poison apparatus of Hymenoptera is directly grown from the ovipositor and makes function

as an effective defensive weapons for the colony in Aculeata [10].

Morphology and the ultrastructure of poison apparatus of many Hymenoptera have been studied [4,6,8,9,11,12,13], but there are no studies on *Sphex flavipennis*. In this study, the poison glands and poison sac were investigated using scanning and transmission electron microscopes.

MATERIALS AND METHODS

Adults of *Sphex flavipennis* Fabricus, 1793 (Hymenoptera: Sphecidae) were collected from Tokat, Pınarlı village, Turkey. The samples were killed by freezing and their poison gland was rapidly dissected from posterior of the abdomen under a binocular microscope.

Scanning Electron Microscopy (SEM)

For the scanning electron microscopic studies, the alimentary canal was fixed in 3% glutaraldehyde in phosphate buffer, pH 7.2, for 4 h at 4°C. Then samples were dehydrated in a graded acetone series (70%, 80%, 90%, 100%), critical point dried, coated with gold, and analysed under a Jeol 5400 scanning electron microscope [7].

Transmission Electron Microscopy (TEM)

For the transmission electron microscope examination, gut tissues of *S. flavipennis* were fixed in 3% glutaraldehyde in sodium phosphate buffer, pH 7.2, for 4 h at 4°C. After fixation, samples were washed with the same buffer and post-fixed in 1% osmium tetroxide and in sodium phosphate buffer, pH 7.2, for 1 h at 4°C. Tissue samples were washed with the same buffer for 2 h at 4°C, dehydrated in a graded acetone series and embedded in Araldite resin. Thin sections were stained with 2% uranyl acetate and lead citrate. The sections were viewed and photographed under a Zeiss Libra 120 transmission electron microscope [13].

RESULTS

Anatomically, the poison apparatus of *Sphex flavipennis* consists of poison glands, poison sac, Dufour's gland and poison needle. Poison glands consists of two tubular portions connected separately to poison sac. The great numbers of trachea recess along the convoluted tubes branched at the cylindrical distal ends. Each tube has a length of 6-8 mm and approximately 90 µm in diameter. (Figure1 A). Poison sac (reservoir) are surrounded by pear-shaped externally network-like muscle fibres (Figure1 B).

Ultrastructural observation of poison glands make one thought that the secretory and duct cells are positioned around the lumen of collecting duct. Secretory cells constitute the secretory unit with the duct cells. Secretory

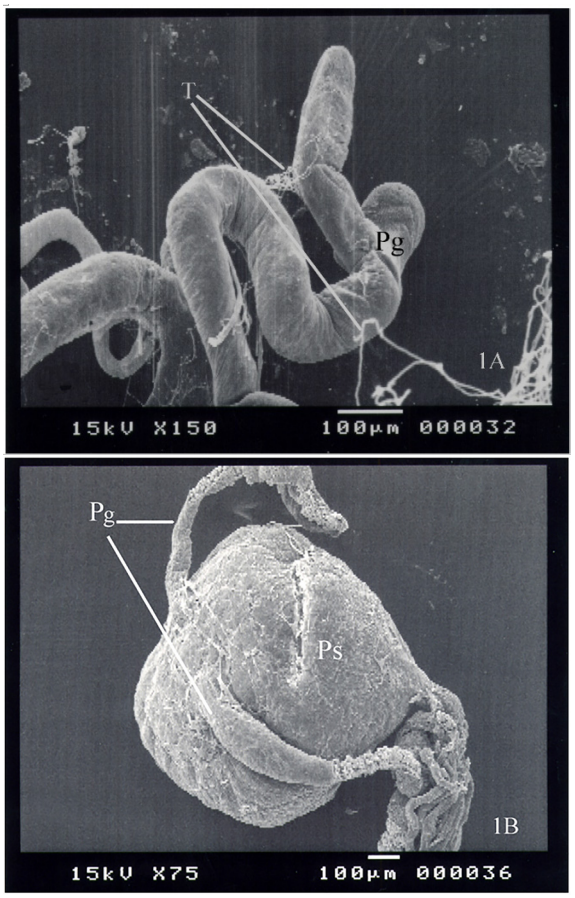

Figure 1 A. Poison Gland. Pg, Poison gland; T, Trachea. (SEM) X 150.1 B. Poison gland and Poison sac. Pg, Poison gland; Ps, Poison sac.(SEM) X 75.

unit is connected with tubulin by means of a canaliculi that is lined by a thin cuticle (Figure 2).

Each secretory unit has a large extracellular space including a great number of microvilli and each of secretory cell contains a porous outer epicuticular layer and a inner fibrous layer. A great number of microvillus is directed into the apical surface of cuticular lining of secretion apparatus. Irregularly shaped nuclei of secretory cells are relatively large and almost in central location. These cells have

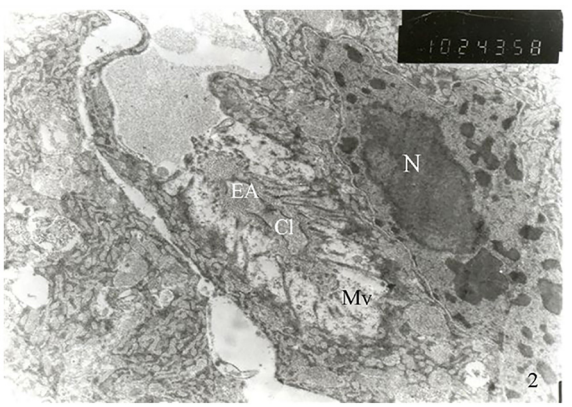

Figure 2. Secretory unit. **N,** Nucleus; **Mv,** Microvilli; **EA**, End apparatus; **Cl,** Cuticular lining. (TEM) X 10 000.

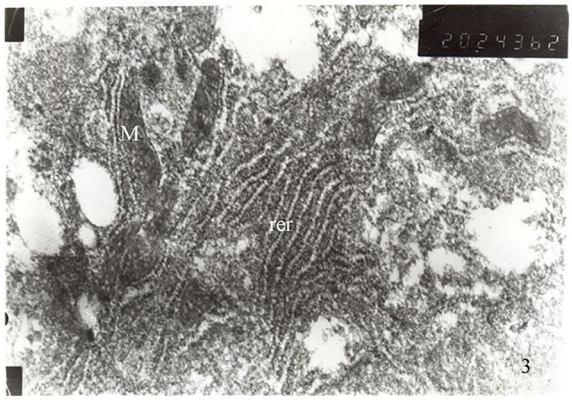

Figure 3. Secretory cell. **M**, Mitochondria; **rer**, Rough endoplasmic reticulum. (TEM) X 20 000.

numerous free ribosomes, rough endoplasmic reticulum, mitochondria, and secretory vesicles. A very dense granular endoplasmic reticulum is in the form of cisternae (Figure 3).

Secretory vesicles are observed abundantly in the peripheral cytoplasm of the cell. Some of these vesicles have light-electron density while some do intense electron-density (Figure4 A, 4 B).

Duct cells have little amount of cytoplasm, granular endoplasmic reticulum, a few mitochondria and free ribosome (Figure 2). Poison sac is coated with by a single layer of epithelium surrounded by a thick intima layer inside. Epithelial cells are surrounded externally by the basal membrane and the muscle layer. These cells contain a relatively extended nucleus containing many mitochondria and heterogeneously dispersed chromatin (Figure 5).

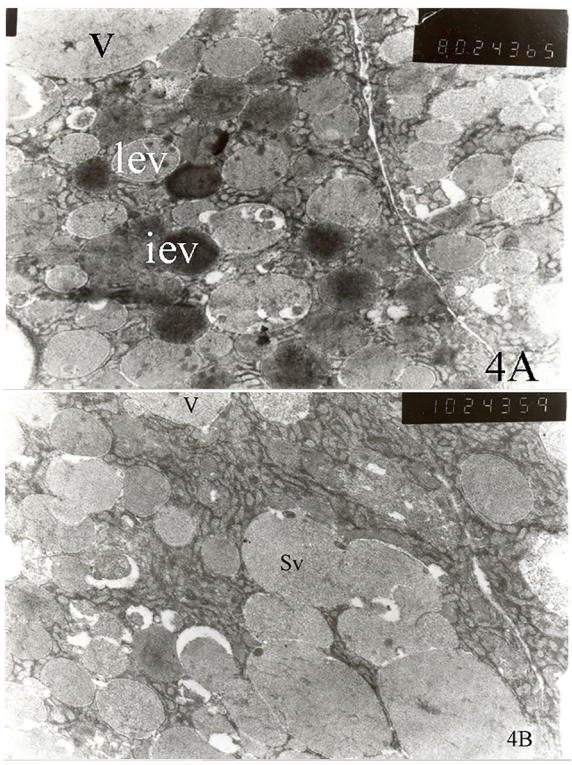

Figure 4. Peripheral cytoplasm of Secretory cell. **Sv,** Secretory vesicles; **iev,** intense electron-density vesicle; **lev,** light electron-density vesicle; **V**, Vacuole. (TEM) **A** X 8 000, **B** X 10 000.

DISCUSSION

Morphology

Some pompilids cause their victims have temporary paralysis, then needle-spider is caught actively, and it continues to function normally until growing wasps will consumed it [9]. The *Sphex flavipennis* that we have studied use some Orthoptera species for larval development. As defined in many studies for different species of Hymenoptera [6,8,11,12], the poison apparatus of *S. flavipennis* consists of the poison glands, poison sac, Dufour gland and poison needle.

The poison apparatus of female *Rhynchium cyanopterum* consists of two parts as secretory part and a poison sac. The secretion part of the gland consists of two cylindrical convoluted glands, proximal end of which opens by entering separately to the poison sac while the distal end extends freely within the haemolymph [13]. The poison glands of *Sphex flavipennis* consists of two convoluted tubular parts connected separately to the poison sacs. Cylindrical tubules extends to the haemolymph freely and ramifies in distal ends.

The poison glands consist of two tree-like branched tubular glands connected to a joint bladder-like reservoir. Each of tubular glands has a length of 5-8 mm (Φ about 150 μm). Near the reservoir entry point, branches of each tubular glands are united to form a common generating channel (Φ about 80 μm). Poison sac is spherical when the sac is filled (Φ about 0.8 mm; volume approximately 270 nl) and is surrounded by bundles of thin network of muscle fibers [4]. Muscle fibers arranged in the form of transverse and oblique forms the sac essentially muscled in the form of four different lobes. The tubes are cylindrical and there

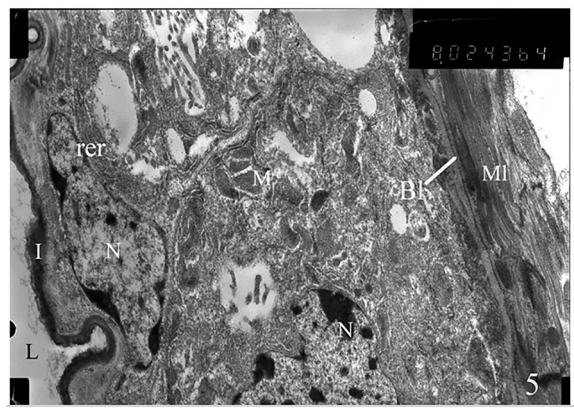

Figure 5. The cell of poison sac. N, Nucleus; M, Mitochondria; I, intima; Bl, Basal lamina; Ml, Muscular layer; L, Lumen; **rer**, Rough endoplasmic reticulum (TEM) X 8 000.

are some slight protrusions formed on the surface due to the press of the epithelial cells [7]. The wall of poison sac in all individuals under investigation is surrounded by fairly well-developed muscle sheath [6]. The pear-shaped poison sac of *Sphex flavipennis* is surrounded outside by muscle fibres. The convoluted poison gland connected separately to poison sac have the length of 6-8 mm long and a diameter of 90 μm. The morphology and ultrastructure of poison apparatus have been studied in most of the Hymenoptera [4,5,6,8,9,11,13], but there are no studies found on this issue about *Sphex flavipennis*.

Ultrastructure

Secretion apparatus (secretory unit) tip is the terminal part containing numerous amount of microvilli and opening to secretion ducts in each of the secretory cells with large extracellular surface and epicuticular layer fibrillated inside and porous outside. In addition to microvillus, end of each apparatus is provided with secretion duct making the secretion go to the lumen [12,14]. There are some open electron density vacuoles (Φ ranges from 0.54 to 1.57 μm) available in the cytoplasm of *Pteromalus puparum* secretory cells. Secretory vesicles are often observed as scattered in peripheral cytoplasm in the cell. There are also secretory granules, a nucleus, rough endoplasmic reticulum and vacuoles available in these cells [12]. In *Sphex flavipennis,* there is a large surface with numerous amount of microvillus including epicutucular layer fibrillated inside and porous outside in secretory unit. The numerous amount of microvillus are directed towards cuticular lining on the apical surface of the secretion apparatus as defined for *Pteromalus puparum* [12] and in *Pachycondyl striata* [14]. Irregularly shaped nuclei of secretory cells are relatively large and centrally located. As noted by the researchers, these cells have granulated endoplasmic reticulum, the nucleus, secretory vesicles and secretory granules. In addition, there are a lot of rough endoplasmic reticulum and mitochondria for expression of secretory proteins in these cell types and to meet the energy required in the process of secretion and absorption, respectively. The cytoplasm of the secretory cell is characterized with abundant free ribosomes distributed to all around the cytoplasm and with a large number of spherical ergastoplasmic sac [13].

The poison sac of *Polistes versicolor* exhibits a dense muscular structure combined with thick cuticle on the reservoir wall. In reservoir, there is a well-developed epithelium continue with the outer tubular part anatomically. However, there is no secretory cell observed on the reservoir wall [7,11]. Similarly, it is observed that the sac of *Sphex flavipennis* is surrounded by dense muscle fibres, but not secretory cells on the reservoir wall. On the contrary, Abreu implies that there are secretory cell on the reservoir wall of *Apis mellifera* [15]. Although it is noted that there are no specific organelles in the cytoplasm of reservoir epithelium cells of *Pteromalus puparum* [12], there are numerous amount of mitochondria observed in the epithelial cells of the sac of *Sphex flavipennis.*

CONCLUSIONS

In this study, we investigated morphological and ultrastructural features of poison gland of *S. flavipennis*. The features of poison gland and poison sac were found to have differences from both morphological and ultrastructural structures which help us to clarify and better understand each portion in the poison gland of this species.

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