SENSE ORGANS IN THE GIRDLE OF CHITON OLIVACEUS
(MOLLUSCA: POLYPLACOPHORA)

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ABSTRACT

A general scheme for the girdle sense organs in the Polyplacophora is put forward: a sensory papilla, inserted in the girdle epithelium, consists of a varying number of secretory cells, one ciliary cell and one spicule cell. The spicule cell is connected with an organic cup or shaft. In or on top of this structure there is a calcareous element (spicule, scale or small tip). The ciliary cell invaginates into the spicule cell. Around this invagination the cytoplasm of the spicule cell contains a dense network of microfilaments and a large number of mitochondria.

Modifications of this scheme are found in Chiton olivaceus. Behavioral experiments demonstrate that the girdle sense organs are mechanoreceptors.

The presence of sense organs (aesthetes and shell eyes) in the shell valves of chitons has been known since the work of Moseley (1884). The occurrence of sensory structures in the girdle that surrounds the valves has only recently been demonstrated (Haas and Kriesten, 1975; Fischer et al., 1980; Leise and Cloney, 1982; Leise, 1986). Previously, the hard structures of the girdle were considered as armament and ornamentation by most authors. However, Blumrich (1891) and Plate (1898, 1902) suggested that some girdle formations could be sensory. The ventral and dorsal surface differ in arrangement and form of these structures, differences which are species-specific. In addition, many species have different spines along the girdle margin. In several families, hair-like structures are also produced. A survey of the different forms is given in Hyman (1967). Hyman also suggests that hairs could be modified shafts of spicules.

The ultrastructure of the girdle is still poorly known. With the exception of Lepidochitona cinereum L. (Haas and Kriesten, 1975), the species that have been studied, Acanthochitona fascicularis L. (Fischer et al., 1980) and Mopalia muscosa Gould (Leise and Cloney, 1982; Leise, 1986), have a highly specialized girdle.

This study concerned Chiton olivaceus Spengler, the most common chiton in the Adriatic Sea; it is dominant in the tidal and low subtidal region (LeLoup and Volz, 1938). The girdle ornamentation has been described by Blumrich (1891) and no obvious specializations exist. We examined the ultrastructure of both scales and hairs in order to reveal the basic structure of the girdle sense organs.

MATERIAL AND METHODS

One to three year old individuals of Chiton olivaceus from the tidal zone of the coast of northern Yugoslavia were used in this study. For transmission electron microscopy, parts of the girdle were fixed in 5% glutaraldehyde in phosphate buffer (pH 7.4) for two hours. They were then decalcified in 3% EDTA in phosphate buffer overnight following postfixation in 2% osmium tetroxide for two hours. All this was done at 3°C. After dehydration (ethanol, propylene oxide) the specimens were embedded in Durcupan and ultrathin sections cut with a Reichert ultramicrotome. The sections were stained with uranyl acetate and lead citrate using the standard methods of Reynolds (1963) and studied in a Jeol electron microscope.

For scanning electron microscopy, some specimens, after complete dehydration in a graded series of ethanol, were critical point dried (Balzers CPD 020) from liquid carbon dioxide. Specimens were then coated with a 300 Å layer of gold and viewed in a Jeol 25S SEM.

In order to qualitatively test the reactions of the animals to touching of single spines or other formations of the girdle, a glass microelectrode filled with 3M KCl was connected via a preamplifier to an audiomonitor. Because the sound frequency changes due to a change in resistance of the...
kept in aquaria containing artificial sea water (20°C, natural day/night-cycle, simulation of high and low tides) for 6 months up to 3 years before the tests.

RESULTS

MORPHOLOGY OF THE GIRDLE

The girdle of *Chiton olivaceus* is covered with characteristic calcareous parts (Fig. 1). On the dorsal surface, large scales are arranged like tiles on a roof, with the open side directed towards the shell. The tallest scales (up to 180x130 μm) are found in the middle part of the girdle, whereas those near the girdle margin and near the shell valves are much smaller (50x20 μm). In most cases, the surface of the scales shows irregular elevations and ridges (Figs. 2, 3). The girdle margin is formed by one row of calcareous spines (50-90 μm long and 20-25 μm wide). They bear thin ridges running roughly parallel to their long axes (Fig. 2). Between the marginal spines and the dorsal scales, hair-like formations can be observed at regular distances. One hair is normally accompanied by one or two clapper-like structures. Each hair or clapper consists of a solid shaft of organic material and a calcareous tip, which can be lost in some hairs. The hair shaft is 70-110 μm long and between 5 μm (at the base) and 1.5 μm wide (distally). The calcareous tip structure can reach a length of 30 μm and a diameter up to 4 μm. The organic shaft of the clappers is 10 μm long and 2 μm wide; the calcareous tip is 10-15 μm long and about 7 μm in width.

Fig. 1. Schematic cross section through the girdle (a, articulamentum; ae, aesthetes in the tegmental shell layer; c, cuticle; cl, clapper; ct, connective tissue; ds, dorsal scales; h, hair; ms, marginal spines; t, tegmentum; vs, ventral scales) (after Maile, 1981).

Fig. 2. Scanning electron micrograph of the girdle margin (cl, clapper; ct, calcareous tip of a hair or clapper; c, cuticle; ds, dorsal scale; h, hair; ms, marginal spine). Fig. 3. Isolated dorsal scale, KOH-treated. A groove (arrow) shows the connection site with the spicule cell of the papilla. Fig. 4. Ventral scales (right side is lateral).
In living animals, the hairs are straight and oriented at an angle between 0° (parallel to the substratum) and 60°. Ventrally, the girdle is covered with rows of small scales (30-40 µm long, about 13 µm broad) (Fig. 4). The rows are oriented perpendicular to the girdle’s margin. All scales, at least at their base, are embedded in the cuticle.

**AVOIDANCE BEHAVIOR OF LIVING ANIMALS**

We studied qualitatively the avoidance behavior of 20 individuals of different ages (age can be estimated from the size of the animals). There was no difference in the reactions between these chitons.

Generally, there is a reaction to touch of any calcareous element but of a varying degree (Table 1). Girdle movements in the stimulated region can be observed upon touch of every structure. However, the weak reaction of the dorsal scales and the hairs could be accidental, as girdle movements can sometimes be registered without obvious external stimulation. Individual ventral scales are not moved. They are embedded in the cuticle except at their distal surface. The most effective stimulation is contact of a marginal spine. The touched spine is moved away immediately with a subsequent movement of neighboring spines. The girdle in the stimulated areas is withdrawn and, after repeated stimulation the animal often moves away. The reaction of the other structures to touch is much weaker; the weakest is that of the hairs.

Because the clappers are very small and inserted very close to the hairs, it was not possible to stimulate this structure without also possibly stimulating a hair. Therefore, the clappers were omitted in Table 1.

**PARENCHYME OF THE GIRDLE**

The parenchyme of the girdle consists of a network of connective tissue (Fig. 5). The nuclei of these cells are relatively small. The space between the cells is filled with irregular groups of collagen fibers and scattered muscle cells. The muscle cells insert at the basal lamina of the epidermis and not at the hard structures of the girdle. The movement of spines is obviously produced indirectly by contraction of the underlying muscle cells. Hemolymph filled lacunae of various sizes are bordered only partially by the cells of the connective tissue; they continue to a large extent into the intercellular substance.

**EPIDERMIS**

The girdle epidermis consists of 2.5-4.5 µm high cells that are intensively interdigitated. Distally, the epidermal cells are connected by zonulae adhaerens and septate junctions. Short microvilli (0.5-1 µm in length) protrude into the cuticle. The nucleus fills most of the cell's volume; its chromatin is highly condensed, a sign of relatively low metabolic activity. Many tonofibrils run from the basal lamina up to the tips of the microvilli (Fig. 6). Scattered ribosomes and only a few mitochondria are also found randomly in the epidermal cells. In areas where new papillae are formed, epidermal cells show ultrastructural features indicating higher metabolic activity. They have a larger cell volume, the chromatin is less condensed and the cytoplasm contains more mitochondria and some endoplasmic reticulum (ER). There is a regular transition to the secretory cell type of the papillae.

**GENERAL PATTERN OF THE GIRDLE PAPILLAE**

Papillae of various sizes (according to the position on the girdle) insert in the epithelial layer. Generally, a papilla contains a varying number of secretory cells, one ciliary cell and one spicule cell. The external appearance of the formations on the girdle looks very different (Fig. 2). However, the composition of the papillae (which are connected with these formations) is essentially the same. Therefore, a detailed description of the cell types found in a papilla is next described.

**SECRETORY CELLS**

Secretory cells, as well as all other cells of the papilla, are interconnected in the same way as the epidermal cells. Active secretory cells have a relatively large nucleus without much condensed chromatin which normally lies near the basal lamina. Granular ER, free ribosomes and numerous mitochondria are a regular feature of the cytoplasm. Golgi apparatus are rare, although the cell is filled to a large extent with membrane-bound secretory granules. Other granules, of varying electron density, and a few multivesicular bodies are also found. In older papillae, especially at the ventral side of the girdle, the secretory cells' activity decreases and the chromatin becomes more condensed (new papillae are formed mainly near the shell and the girdle margin, dorsally and ventrally the zone between these areas contains older papillae except in places where a scale had been lost.

**CILIARY CELL**

The ciliary cell also shows the ultrastructural features of high metabolic activity. The large nucleus is surrounded by granular ER; many mitochondria, a few granules and multi-

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**Table 1. Avoidance behavior of Chiton olivaceus upon touch of single elements in the girdle with a glass microelectrode**

<table>
<thead>
<tr>
<th>Movement of touched element</th>
<th>Movement of neighboring elements</th>
<th>Girdle movement</th>
<th>Animal moves away</th>
</tr>
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<tbody>
<tr>
<td>dorsal scale</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>marginal spine</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>hair</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ventral scale</td>
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vesicular bodies are also present. Distally, the cell is elongated and protrudes up to the cuticle. Relatively large (0.5 μm in diameter) microvilli protrude into the cuticle. One cilium (9+2 structure) runs to the base of the spine, scale, clapper or hair (in these sections we refer to all these formations as 'spicules') (Fig. 7). This cilium originates from a striated rootlet that consists of several parts (Fig. 8). Branches of the ciliary cell invaginate into other cells, especially into the distal area of the spicule cell (Figs. 9, 10). Two centrioles are present in this invagination. In longer papillae, the distal part of the ciliary cell contains many microtubules. This zone then resembles a dendrite.

**SPICULE CELL**

The spicule cell connects the spicule with the papilla. Again, the nucleus is large and does not contain much condensed chromatin. Especially in the distal half of the cell agranular ER, numerous microtubules and many mitochondria are present. This zone is also characterized by the invagination of the ciliary cell mentioned above. The membranes of both cells are parallel to each other, and the spicule cell forms a dense network of microfilaments around this part of the ciliary cell (Fig. 9). Distally, the spicule cell bears numerous microvilli that are connected with the organic cup or shaft of the spicule (Fig. 11). The calcareous element is placed in or on top of this organic structure. It does not contain any cellular elements. In all parts of the girdle, the ciliary of the ciliary cell at the base of the spicule is oriented towards the girdle margin, i.e. the papillae are polarized. Structures resembling small neurons (fibers containing numerous microtubules) can be found from the basal lamina far up into the papilla. However, no synapse or direct connection to a cell could be seen so far.

**VENTRAL PAPILLAE**

The ventral papillae are oriented at an angle of 10-20°
towards the girdle margin (Figs. 5, 12). A papilla consists of about seven cells (one spicule cell, one ciliary cell and about five secretory cells). The cup of the ventral scale consists of three zones. The proximal filaments are very thin (about 15 nm; in the median zone these filaments become thicker (150 nm). The area adjacent to the calcareous scale is homogeneous and surrounds the scale continuously in younger scales; in older ones the distal parts of the organic component has been eroded. Newly formed scales lie near the papillae, older ones have moved far into the cuticle. In these papillae the ciliary and spicule cells are elongated up to the scales' cup. Finally, the scale is dropped and a new one is formed (for a description of the formation of spicules see Haas and Kriesten, 19795).

**DORSAL PAPILLAE**

The size of the papillae on the dorsal side of the girdle varies considerably. They are small near the margin and the shell valves, where the scales are also small, and very large in the median area of the girdle. In this median area one cannot clearly distinguish between distinct papillae; a large scale can be surrounded by a ring of papillae-like cell complexes. Only on one side, towards the shell valve, are the spicule cell and the ciliary cell present (Fig. 11). Underneath the scale, normal epidermal cells are present (Fig. 13). All other cells are of the secretory type (Fig. 14).

The cup of a dorsal scale is composed of two parts (Fig. 15). In most cases the basal plate has a straight border towards the calcareous element; at the lower side, short branches run down between the microvilli of the epidermal cells (Fig. 6). At the lateral side, the scale is covered with an organic sheet that reaches the basal plate; there is often no direct connection between these two parts. The lateral part has a straight border towards the cuticle and many processes into the calcareous element. In new dorsal scales the basal plate is formed some time after the lateral part.

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**Fig. 8.** Longitudinal section through the base of a cilium of the ciliary cell (ci, cilium; mv, microvilli of the ciliary cell; r, striated rootlet of the cilium). **Fig. 9.** Distal part of a ventral papilla (c, cuticle; cc, ciliary cell; df, dense network of small fibers around the invagination of the ciliary cell; sc, spicule cell; scu, spicule cup; sec, secretory cell; arrow indicates basal body). **Fig. 10.** Tips of the ciliary cell and the spicule cell (cc, ciliary cell; iv, invagination of the ciliary cell into the spicule cell; mv, microvilli; r, striated rootlet of a cilium; sc, spicule cell; scu, spicule cup). **Fig. 11.** Section through the receptive part of a dorsal papilla (sc, spicule cell; scu, spicule cup; arrow indicates invagination of the ciliary cell into the spicule cell; double arrow indicates ciliary cell).
Fig. 12. Schematic drawing of a ventral papilla with its scale. Left side is lateral, upper side is ventral (c, cuticle; cc, ciliary cell; e, epidermal cell; n, neurite; sc, spicule cell; scu, spicule cup; vs, ventral scale).

Fig. 13. Schematic drawing of a dorsal papilla complex with its scale (c, cuticle; cc, ciliary cell; ds, dorsal scale; e, epidermal cells; n, neurite; p, papilla; sc, spicule cell; scu, spicule cup); arrow indicates the two parts of the cup of the dorsal scale are attached to each other (right side is lateral).

GIRDLE MARGIN
The greatest variety of structures is found in the girdle margin. The papillae of the marginal spines, of the hairs and the clappers are in many cases not distinct. All cells at the margin (except spicule and ciliary cells) resemble secretory cells; there are no typical epidermal cells.

At the margin, the papillae lie in three rows: ventrally, the papillae of the marginal spines; medially, the papillae of the clappers; dorsally, the papillae of the hairs (Fig. 16). The papilla of a marginal spine has numerous secretory granules concentrated in the upper side, whereas the papilla of a clapper has most of these granules in its lower side. The cup of the marginal spines consists of the same zones as in the ventral scales. In the clappers, the cup has been transformed into an elongated shaft, which is solid except near the tip of the spicule cell. Around the distal part of the papilla and the base of the shaft, a cortex of darkly stained granules is embedded in the cuticle. The middle zone of the papilla of a hair is quite narrow. All cells, spicule cells and ciliary cells as well as secretory cells, have a thin diameter in this zone. Numerous microtubules contribute to the dendritic appearance (Fig. 17). The distal part of the papilla is swollen (Fig. 18). Secretory cells are highly vacuolated around the spicule and ciliary cells. The hair shaft is solid except at the connection with the spicule cell. The distal (swollen) part and the base of the shaft, as in the clappers, are surrounded by a granulate cortex; in the hairs, it can protrude out of the cuticle for a short distance.

DISCUSSION
Despite the very different external appearance of the girdle formations, all papillae in Chiton olivaceus are of similar
construction. They are composed of a varying number of secretory cells that surround one spicule cell and one ciliary cell. The ciliary cell invaginates into the spicule cell which is highly specialized in this zone. The spicule cell is connected with the organic cup of a calcareous structure. Both these components vary considerably in size. The same pattern is also found in the primitive polyplacophoran *Lepidopleurus cajetanus* Poli (Fischer, unpublished) as well as in *Lepidochitonina cinereus* (Haas and Kirsten, 1975). In *Acanthochitona fascicularis* a similar appearance has been found (Fischer et al., 1980) with three major differences: the secretory cells are more prominent; photoreceptor cells are present in many papillae; a stalked nodule protrudes from many papillae into the cuticle. This nodule resembles the swelling of the hair papilla in *Chiton olivaceus*. It looks like a distal part of a papilla that has lost its spicule. In young *Mopalia muscosa* a pattern similar to *Chiton olivaceus* is found (Leise, 1986) (Fig. 6). It seems that the type described here is the basic structure of the girdle sense organs in the polyplacophora.

In *Acanthochitona fascicularis*, another type of spine has also been described, in addition to this general type. These spines are not connected with a papilla. Each is based on top of a large cup-like cell in the epidermal layer and grows basally as the animal gets larger. In contrast, the "normal" type of spine does not grow after it is produced. Behavioral observations and the fine structure of the cup-like cell suggest that this spine type in *A. fascicularis* is merely defensive (Fischer, 1979).

Adult mopalid chitons have elaborate sensory hairs in the girdle (Leise and Cloney, 1982; Leise, 1986). Leise (1986) has demonstrated that these hairs are formed by the growth of several spines (very similar to the hairs of *Chiton*) close to one another. As they grow, the whole bundle is surround-
ed by an organic cortex. Thus, the complex hair in *Mopalia* is an elaboration of the "normal" type.

In *Acanthochitona fascicularis* the spicule cell forms a neurite (Fischer et al., 1980). Nerves have also been demonstrated in the girdle sense organs of *Mopalia muscosa* (Leise and Cloney, 1982; Leise, 1986). In *Chiton olivaceus*, structures resembling neurons are present in the papillae of every type of girdle formation. However, the presence of such structures seen in the electron microscope is only an indication of a sensory function, for two reasons. Cells that are not sensory, such as the secretory cells in the aesthetes, can form fiber-like extensions that are very similar in structure to neurons. However, they certainly have another function, as they are not connected with the nervous system (Knorre, 1925). If the fibers observed in the papillae are nerves, they could have other functions such as stimulating the secretory cells. To establish a sensory function, appropriate neurophysiological or behavioral experiments must be carried out. Neurophysiology in chitons is very difficult, as single nerve fibers are thin and the amplitude of potential changes is quite low (Fischer et al., unpub. data).

The results of the stimulation experiments show that the girdle sense organs are mechanoreceptors. Due to the fact that the basic structure is the same in all species and in all areas in *Chiton*, we suggest that, apart from the function of the secretory cells, mechanoreception is the basic function of the girdle papillae. A possible function of the secretory cells could be to produce or impregnate the cuticle. The chemical composition of the secretory granules is unknown.

The presence of mechanoreceptors is certainly of great importance for a relatively small animal which lives in the tidal region and moves actively, but slowly, on exposed substrata for feeding. Individuals of *Chiton olivaceus* which have been detached from their stone have great difficulty settling again in turbulent water (pers. obs.). Under normal conditions, the girdle is pressed onto the substratum. There is no gap and the animals are not vulnerable to strong water movement. The ventral scales could provide feedback information about the pressure of the girdle on the substratum. The reactions to stimulation of the marginal spines show that these structures can detect an obstacle or movements of other animals.

Most chitons including *Chiton olivaceus* do not possess eyes. The photoreceptor cells in the aesthetes (Fischer, 1978) are involved in the photonegative behavior (Arey and Crozier, 1919; Boyle, 1972; Bergmann, 1984) and in the shadow
Fig. 16. Schematic drawing of the margin of the girdle [c, cuticle; cl, clapper; clp, papilla of a clapper; cls, clapper shaft; co, cortex-like structure; cti, calcareous tip of a hair or a clapper; e, epidermis; hs, hair shaft (a specialized spicule cup); h, hair; hp, papilla of a hair; ms, marginal spine; msp, papilla of a marginal spine; scu, spicule cup].

Response (Crozier and Arey, 1918). As mainly nocturnal animals, the light sense is not very specialized in chitons. C. olivaceus is frequently found on very irregular substratum. They hide in small holes (such as produced by the clam Lithophaga lithophaga L.) in stones. The great number of lateral mechanoreceptors obviously is involved in orientation.

Stimulation of the hairs does not evoke a strong reaction, touch apparently being not the appropriate stimulus. When the animal is feeding, the hairs are oriented towards the open water and are moved slightly by water motions (pers. obs.). A possible function could be to measure these movements.

The large dorsal scales certainly protect the animal against predators or strong water movement. Most predators usually only consume the foot and the viscera, not the valves and the girdle (Leise, 1986). When disturbed, Chiton olivaceus presses the girdle to the substratum very tightly. It is difficult to detach the animals. For nearly all possible predators, this species is unattractive because of the protection afforded by the valves and the dorsal scales. However, the dorsal papillae still retain the sensory elements, although they are relatively small. Further experiments must be carried out to define the exact function of the girdle sense organs of chitons.

Fig. 17. Dendrite-like appearance of different cells (arrows) in the base of the distal part of hair papilla. Fig. 18. Distal part of a hair papilla [cc, ciliary cell; sc, spicule cell; scu, spicule cup (here transformed into the shaft of the hair); vw, vacuolated secretory wall cells].
ACKNOWLEDGMENTS

We thank Birgit Seibel for the final drawings. Roland Gantner and Ulrike Kaltenhauser performed some preliminary studies. We are grateful to Renate Kammerer for reading the manuscript. Two anonymous reviewers provided useful comments. Last but not least, we would like to thank Prof. G. A. Manley for the revision of our English.

LITERATURE CITED


Date of manuscript acceptance: 16 October 1987