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<td>Mona, M. H.; Miyazaki, Katsumi</td>
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Kyoto University
Preliminary Observations on the Foot in the Homing Limpets, *Siphonaria acmaeoides* (Pilsbry) and *Patelloida saccharina lanx* (Reeve) with Light and Scanning Electron Microscopy

M.H. MONA

Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

and

KATSUMI MIYAZAKI

Seto Marine Biological Laboratory, Kyoto University, Shirahama, Wakayama 649–22, Japan

With Text-figures 1–16

Abstract The foot of the homing limpets, *Siphonaria acmaeoides* (Pilsbry) and *Patelloida saccharina lanx* (Reeve) has been preliminarily investigated by light and scanning electron microscopy. The constituents, of the foot, in both species are described in detail and their possible functions are discussed. Moreover, the differences between the foot of both species have been discussed in relation to their ecological behaviour.

Introduction

The foot is one of the most characteristic features in limpets with a capacity for remarkable modifications in form and function. It represents one of the main interfaces between the external and internal environments of limpets. It provides one of the main regions for contact with the outside world while at the same time it must protect the rest body from the rigors of its surroundings.

We often wonder why a certain limpet attaches firmly to the substratum and secretes more mucus than other. For any particular limitation, it is usually possible for a number of factors to operate simultaneously, and it is generally difficult to decide which has been the critical limiting factor.


limpets, *Siphonaria acmaeoides* (Pilsbry) and *Patelloida saccharina lanx* (Reeve), having variable degrees of adhesion and different ranges of locomotion. The most important point obtained from this description is giving evidences and deductions for the possible functions of various constituents of the foot in limpets. In addition, sometimes, we discuss the differences between the constituents of the foot in both investigated limpets in relation to their ecology and behaviour.

*Siphonaria acmaeoides* and *Patelloida saccharina lanx* are ones of the common limpets along most shores of Japan occurring attached to calcareous substratum. A visit of the senior author to Seto Marine Biological Laboratory as a visiting professor provided him an opportunity to collect the investigated specimens.

**Materials and Methods**

The specimens of *Siphonaria acmaeoides* and *Patelloida saccharina lanx* were collected in June 1994 from Bansho-Zaki Point near the Seto Marine Biological Laboratory of Kyoto University in the Kii Peninsula, middle Japan. The rocks with limpets were removed to the laboratory, where each limpet was allowed to move away from its home scar whilst immersed in sea water and then easily lifted from the rocks. After narcotized by 7% magnesium chloride solution in sea water for three hours, specimens were fixed overnight in 10% neutral formalin.

For light microscopy, the entire fixed foot was detached from the shell, then dehydrated in a series of ethanol, cleared with terpineol and embedded in paraffin. Sections of 5 μm thick were stained with Ehrlich's hematoxylin and eosin (H-E) for standard histology, Mallory's triple stain for connective tissue and muscles, periodic acid-Schiff (PAS) for neutral polysaccharides, and alcian blue for acid mucopolysaccharides.

For scanning electron microscopy (SEM) of some internal structures, paraffin-embedded specimens were made into SEM preparations followed by Miyazaki and Hirose (1993). The gold-coated specimens were observed with a JEOL JSM-T220 scanning electron microscope at 10kV.

**Results and Discussion**

The present investigation has drawn attention to the large volume of foot which nearly occupies 60% of the total volume in *Siphonaria acmaeoides* whereas less than 40% in *Patelloida saccharina lanx* as estimated by viewing sections. In both species, the surface of the foot is lined partly by mucous cells which are located principally in the lateral foot surface. There is no evidence of the presence of cilia in any cells lining the surface of the foot, in contrast to the previous description in other gastropods (Parker, 1911; Graham, 1957; Zylstra, 1972, Simkiss and Wilbur, 1977).

However, the foot in both investigated species has the similar plane, it is formed of pedal epithelium, compact fibrous layer, muscular portion.

The pedal epithelium is very thin and arisen into processes and furrows (Figs. 3, 4) to extend the surface area of the foot. The pedal epithelium is a single layer of epithelial cells resting on a basement membrane, and is supported by a mat of connective tissue through which run few muscle fibers. The pedal epithelium is formed essentially of two types of cells. The first type is compact pigmented columnar
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Fig. 1. Transverse section through the foot of *Siphonaria acmaeoides*. PAS. Scale: 100 μm.

Fig. 2. Transverse section through the foot of *Patelloïda saccharina lanx*. PAS. Scale: 100 μm.
cfl, compact fibrous layer; hv, hemocoelic vesicle; mp, muscular portion; n, nerve; pe, pedal epithelium.

cells lining the furrows of foot (Figs. 3, 4, 12). Each cell has a somewhat large nucleus occupying the basal portion. These characteristics suggest that the cells are chemoreceptor cells, and therefore, the furrows should play a significant role in the sensory functions of the foot (hence sensory furrows). The second type is evacuated or less compact columnar cells lining the remaining part of the foot (Fig. 3). Each cell has an unobvious small nucleus and may act in transporting of gases and other materials. A layer often covers the pedal epithelium (Fig. 12) forming what is sometimes confusingly referred to as a cuticle. Beneath the inner margins of pedal epithelium, there are a large number of vesicles and blood cells (Figs. 1, 6) particularly in lateral sides. The presence of these structures has been interpreted in other gastropods as indicating either an endocytotic or an exocytotic activity (Wondrak, 1968).

The sole of the contracted foot in *Patelloïda saccharina lanx* forms a deep furrow like ovate sucker (Fig. 13) surrounded by a pedal rim and its lateral wall. This
wall is thickened by tall epithelial glands producing secretion which may seal the entrance to the pallial cavity. These glands are classified into two types; the more common type with cells containing large spherules stained in red (proteinaceous) with Mallory, and the less one with cells stained in blue (the mucous cells).

Compact fibrous layer is formed of fibrous connective tissue and contains mucous glands, hemocoelic vesicles, pigments, blood cells.

The mucous glands in the foot of both investigated species are either diffused
Fig. 5. Lateral pedal lining epithelium of the foot of *Patelloida saccharina lanx*. Mallory. Scale: 25 μm.

Fig. 6. Lateral pedal lining epithelium of the foot of *Siphonaria acmaeoides*. H-E. Scale: 10 μm.

Fig. 7. Ventral pedal lining epithelium of the foot of *P. saccharina lanx* showing isolated mucous glands. H-E. Scale: 25 μm.

Fig. 8. Pedal rim of the foot of *S. acmaeoides*. H-E. Scale: 50 μm.

cmg, concentrated mucous gland; ecc, evacuated columnar cell; hv, hemocoelic vesicle; img, isolated mucous gland; pe, pedal epithelium; pi, pigment; pr, pedal rim.
or concentrated. Each of the isolated glands consist of the isolated unicellular gland cells, which are distributed over the whole surface of the foot and gathered in compact fibrous layer (Fig 14). In *Patelloidea saccharina lanx*, some unicellular gland cells were observed among the evacuated lining cells of the foot (Figs. 5–7), but such gland cells were not observed in *Siphonaria acmaeoides*. Each unicellular gland cell is enlarged flask-shaped whose neck forms a duct for discharging the fluid secreted by the bulbous portion.

The concentrated glands are two in number locating on both lateral sides of the foot each (Figs. 8–10). The gland cells are still flask-shaped, but their necks have become more elongated.

The two aggregated mucous glands would give a better sealing, particularly in *Patelloida saccharina lanx*, and the sucking force caused by the ventral ovate sucker would enhance the tenacity of the limpets.

There are significant differences in position and number of the mucous glands between the investigated species. For instance, in *Patelloidea saccharina lanx* numerous unicellular gland cells lie among the lining cells of the foot whereas such gland cells were not observed in *Siphonaria acmaeoides*. Similarly, the number of gland cells forming the compound glands in *S. acmaeoides* are smaller than that in *P. saccharina lanx*. These differences indicate that the mucous production of *S. acmaeoides* is less than that in *P. saccharina lanx*, which may be related to the fact that *S. acmaeoides* have a large distance of movement (K. Iwasaki and T. Yamamoto, personal communication) and are less adhesive than other limpets. However, Branch and Marsh (1978) reported the opposite result for several South African patellids, that limpets with high tenacity secrete less mucus than limpets with low tenacity. Further study is needed to make the relationship among the mobility, tenacity and amount of mucous secretion clear.

In spite of the differences between two investigated species, it is obvious that both have considerable power of secreting mucus, which is said in other limpets to be essential to excavation of the substratum to create home scars (Dwyer and Lindberg, 1980; Lindberg and Dwyer, 1983), prevention against sedimentation (Branch, 1981), reduction of drag forces (Denny, 1980) and locating mating-partners (for *Siphonaria acmaeoides*, Hirano, 1981). In addition, the two investigated limpets are homing species (for *Patelloida saccharina lanx*, Ohgushi, 1954; Hirano, 1981; Iwasaki, 1994, for *S. acmaeoides*, Takenouchi, unpublished data) and thus need to produce mucous trails that enable limpets to return to their homes by retracing (Funk, 1968; Cook et al., 1969) and to stimulate growth of their algal food (Connor and Quinn, 1984; Conner, 1986). Also the mucus would act as the enclosed fluid for creating a pressure-difference used for adhesive mechanism as suggested by Denny (1988). Under such a condition, the foot is admirably arranged to act as a piston.

In the foot, some small spherical hemocoelic vesicles, which often surround the mucous glands, were observed (Figs. 1, 6). Such an arrangement of vesicles and mucous glands suggests that the hemocoelic sinuses perform a significant role in the mechanisms for the control of the discharge of mucus; the increase of the inside
Fig. 9. Lateral wall of the foot of *Patelloida saccharina lanx* showing compound mucous gland. H-E. Scale: 100 μm.

Fig. 10. Enlarged part of compound mucous gland of the foot of *Siphonaria acmaeoides*. H-E. Scale: 10 μm.

Fig. 11. Muscular portion of the foot of *S. acmaeoides*. PAS. Scale: 100 μm.

cmg, concentrated mucous gland; mf, muscle fibers; tm, transverse muscles.
blood pressure may cause the release of mucus during normal locomotion. A similar suggestion was proposed by Machin (1964) in the terrestrial gastropod *Helix aspersa*.

There are numerous pigments, blood cells and specific nerves in the foot of both investigated limpets. The foot in both species is highly innervated by two large nerves situated in the muscular portion (Figs. 1, 2). In addition, some other nerve fibers were frequently observed among the muscle fibers. These nerve fibers sometimes appear in bundles with many fibers of various dimensions and in some other cases, they are in small group of several fibers.

The muscle fibers (Figs. 11, 15, 16) are arranged essentially in three directional planes within the foot; antero-posterior, transverse and dorso-ventral. As in other
Fig. 14. Compact fibrous layer of the foot of *Siphonaria acmaeoides*. SEM. Scale: 10 μm.

Fig. 15. Muscle fibers of the foot of *S. acmaeoides*. SEM. Scale: 10 μm.

Fig. 16. Transverse muscle of the foot of *Patelloida saccharina lanx*. SEM. Scale: 10 μm.

bc, blood cell; img, isolated mucous cell; mf, muscle fibers; tm, transverse muscles.
gastropods, those muscles in any plane antagonize the others by means of the fluid in the pedal hemocoel in the manner of a fluid skeleton (Chapman, 1958; Trueman and Brown, 1976). Maintenance of a steady tension in one set of muscles allows direct antagonism between those fibers against the two remaining planes and in this manner, changes may be effected in the shape of the foot the nature of which is considered in relation to locomotive stepping.

The muscle cells occur singly or are arranged in muscle blocks, which are tightly packed together with connective tissue. All the muscles are smooth irrespective of their orientation and position. The structure of the muscles of the foot in both investigated limpets is similar to that of other gastropods (Rogers, 1969; Plesch, 1977; Da Silva and Hodgson, 1987; Trueman and Hodgson, 1990). The distinctive features of the pedal muscles in these animals are a poorly developed sarcoplasmic reticulum and disordered large dense bodies (Trueman and Hodgson, 1990). These features suggest that the muscles contract and relax slowly but act powerfully.

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