Chiton Integument: Development of Sensory Organs in Juvenile *Mopalia muscosa*

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Abstract:
The girdle epidermis of adult *Mopalia muscosa* secretes several types of structures, including calcareous spicules and innervated hairs. Newly metamorphosed chitons superficially resemble adult animals, but they lack the adult girdle ornaments, shell sculpture, and coloration. The morphogenesis of the adult girdle structures has not been described previously for any species. Juvenile *Mopalia muscosa* secrete hairs at metamorphosis, but it was not known if these hairs were sensory or if they were retained as the animals grew. I discovered that the hairs of juveniles become the tips of adult hairs. When juvenile hairs are detectable by light microscopy the sensory components already exist, suggesting that they are functional receptor organs. The other girdle ornaments of young juveniles, the primary calcareous spicules, are lost as the animal grows. I also demonstrated that the hairs are not uniquely innervated; the same sensory structures are produced in conjunction with other girdle ornaments on the marginal and ventral faces.

Article:
After metamorphosis, juvenile chitons grossly resemble the adults. In most species, seven shell plates are secreted from the center of the mantle field, and a fringe of calcareous spicules arises from its outer edge (Kowalevsky, 1883; Heath, 1899; Hammarsten and Runnstrom, '26; Grave, '32; Okuda, '47; Matthews, '56; Thorpe, '62; Barnes, '72; Watanabe and Cox, '75; Haas et al., '78; Leise, '84). This type of spiculose girdle is not characteristic of any adult (Pilsbry, 1892, 1893). For only one species has the time of appearance of the adult girdle ornaments been documented: The tufts of long calcareous spines secreted by adult *Acanthochiton discrepans* first appear after 1.5 months (Hammarsten and Runnstrom, '26). The morphogenesis of these spines remains undescribed.

During metamorphosis, the girdles of juvenile *Mopalia muscosa* secrete small chitinous hairs, about 15 μm long, as well as the juvenile type of spicule (Leise, '84). In apparent contrast, adults produce stout chitinous hairs, up to 5 mm in length, that are innervated and probably mechanoreceptive (Leise and Cloney, '82). The relationship between the tiny hairs of juveniles and the large adult ones was unclear. The structure, function, and fate of the juvenile hairs was hitherto unknown. To resolve these problems, I raised *M. muscosa* from fertilized eggs for 1 year in the laboratory and examined the morphology of their integuments at various stages during that year. In this article I describe for the first time the morphogenetic events that occur as the girdle of a juvenile chiton attains its adult characteristics. I discovered that the hairs of juveniles are retained by the animals and that they become the tips of the adult hairs.

MATERIALS AND METHODS
Adult *Mopalia muscosa* (Gould, 1846) were collected from Cattle Point, San Juan Island, Washington, and maintained in sea tables at the Friday Harbor Laboratories, Washington. Naturally spawned eggs were fertilized with dissected or freely spawned sperm. Embryos were cultured in monolayers in pyrex custard dishes at temperatures between 9— 13°C. The water in the cultures was changed daily. Detailed methods were described previously (Leise, '84).

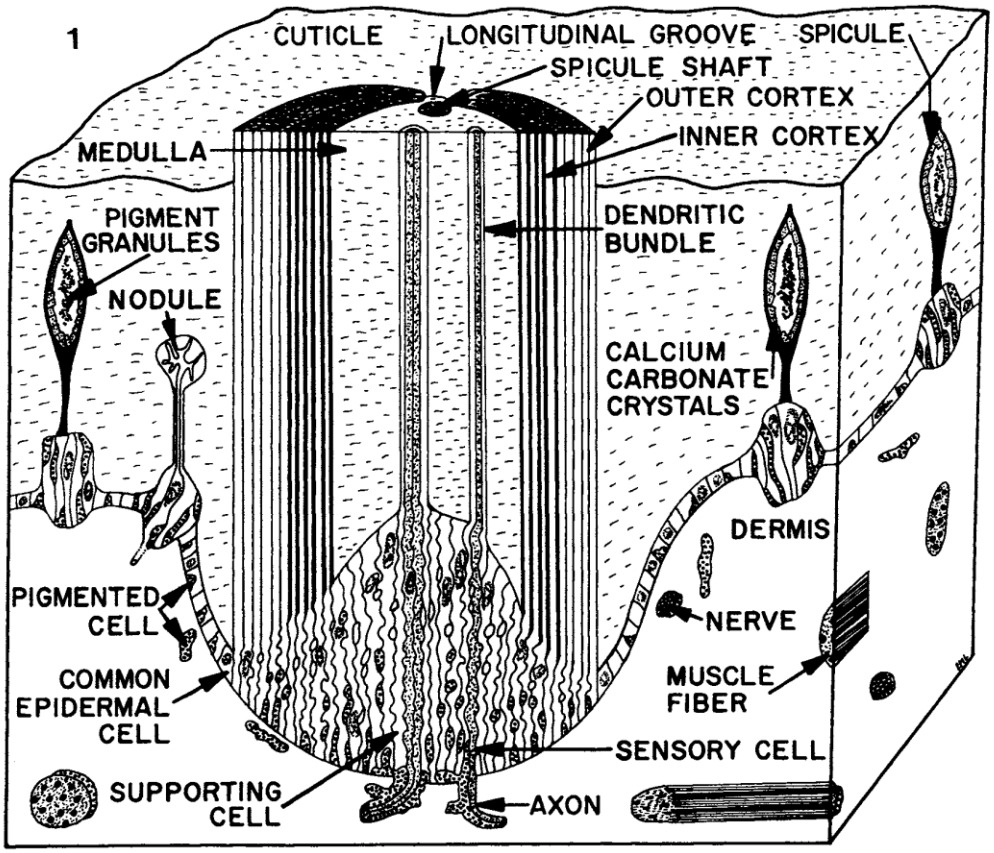


Fig. 1. Diagram of the adult dorsal epidermis. Calcareous spicules containing brown pigment granules are secreted by spiniferous papillae and lie within the chitinous cuticle. A layer of dense material, called a cup, surrounds each spicule. The thin basal extension or shaft of this cup extends to the apex of the papilla. Some spiniferous papillae produce sensory receptors, the stalked nodules. One hair, secreted by a trichogenous papilla, contains two similar nodules, although only their dendritic stalks are visible here. The hair is cut off just above the cuticle. The dendrites are diagrammed as straight tubes for simplicity. Two nerves emerge from the base of the papilla. Processes of pigment cells occur among the epidermal cells and in the dermis. Many of these processes, as diagrammed here, adjoin the papillary axons and the dermal nerves and muscle fibers.

When larvae were competent to settle (Leise, '84), small rocks covered with red or brown algal films were added to the culture dishes. Animals were raised from fertilization to over 1 year of age on these small stones in the same custard dishes.

Animals were fixed and embedded for light and electron microscopy following the protocol of Leise and Cloney ('82). Juveniles were decalcified after primary fixation by the addition of an equal volume of 10% disodium EDTA to the primary fixative solution. This 1:1 solution was replaced every 12 hours until decalcification was complete. After decalcification, specimens were postfixed and embedded as described in Leise and Cloney ('82). One-micrometer sections were stained with Richardson's solution (Richardson et al., '60). Thin sections (60-90 nm) were picked up on Parlodion- and carbon-coated copper grids and stained with uranyl acetate and lead citrate. Grids were examined on a Philips EM 300 electron microscope. Specimens for scanning electron microscopy were fixed as for transmission work, dehydrated in ethanol and acetone, and dried by the critical-point method. Specimens were mounted on stubs, coated with carbon and gold-palladium, and examined with a JEOL JSM 35 microscope.

RESULTS

The dorsal girdle epidermis of adult *Mopalia muscosa* has been described previously (Leise and Cloney, '82). Briefly, it is a simple epithelium, containing both columnar and cuboidal cells. The columnar cells occur in two types of papillae (Fig. 1). Trichogenous papillae secrete the large chitinous hairs (Fig. 2), and spiniferous papillae secrete calcareous, pigmented spicules and sensory nodules (Fig. 1). Common epidermal cells (CECs) occur ubiquitously between papillae. The girdle epidermis of newly metamorphosed juveniles is likewise a simple epithelium, but of low columnar cells, about 20 × 4 × 4 μm (Figs. 3, 4). The entire girdle is covered by a chitinous cuticle, about 5 μm high, into which the cells extend apical microvilli (Fig. 4). Some of these cells secrete calcareous spicules (Figs. 3a, 4c); others secrete small chitinous hairs (Figs. 3b, 5, 6). There are no

obvious common epidermal cells (Fig. 1) (Leise and Cloney, '82), but the fine structure of the juvenile cells that are neither trichogenous nor spiniferous resembles that of the CECs. Their junctional complexes are the same (Fig. 4b) (Leise and Cloney, '82), and small bundles of tonofilaments terminate in the proximal portions of the apical microvilli or on basal hemidesmosomes as they do in adult CECs (Leise and Cloney, '82).

The major morphogenetic changes occurring in the girdle are outlined in Table 1 and are described below in detail. The development of the hairs is treated in a separate section.

TABLE 1. The time from fertilization to the first appearance of cellular and extracellular structures in the girdles of larvae and juvenile Mopalia muscosa *at* 8–16°C

Time	Structure or event
Day 6	Spicule formation
Day 9	Metamorphosis begins
Day 10	Secretion of cuticle
	Shell deposition
	Tips of spicules protrude into cuticle
	Secretion of small girdle hairs
Day 13	Spicules completely extruded
1 month	Hairs with stalked nodules
2 months	Marginal spicules with stalked nodules
	Shell embedded in girdle tissue
6 months	Dorsal pigmented spicules
	Distinct epidermal papillae
	Ventral spicules
	Immature ventral stalked nodules
9 months	Mature ventral stalked nodules
	Trichogenous papillae secrete cortex
1 year	Dorsal stalked nodules

General development of the girdle

Juvenile hairs are rather small, being only 2 μm wide and 10-20 μm long 2 days after metamorphosis. These hairs are capped by very small calcareous spicules (Fig. 7) (Leise, '84) and initially are completely covered by the cuticle. The first or primary spicules produced by the spiniferous cells are also small and fusiform, about 8 × 2 μm, shortly after metamorphosis. These small spicules lack pigment granules and the dense chitinous cup of those in adults (Figs. 1, 3a, 4c).

The girdle of a young juvenile is a narrow rim, some 30-40 μm deep (measured dorso-ventrally) around the edge of the animal, ventrolateral to the shell plates (Fig. 7). Initially only the cuticle projects laterally beyond the shell plates. As the animal matures, the width and depth of the girdle increases by growth at first laterally and then ventrally. The edges of the shell plates also become embedded in the central muscular part of the girdle.

The girdle is about 40 μm deep after 1 month (Figs. 8, 9). The cuticle is fairly thick laterally (about 15 μm) but still relatively thin ventrally (Fig. 9b). It marks the extent of the girdle tissue and stains uniformly at this stage. The cuticle no longer completely covers the hairs; it erodes as the animal grows. The epidermal cells are still about 15 μm high and exist as an uneven layer of cells that are short in the dorsal part of the girdle, but tall in the ventral girdle (Fig. 9b). At this stage, the shell plates are angular caps that cover the dorsum and are not yet embedded in the girdle tissue (Fig. 9a).

After 2 months, the girdle has both lateral and ventral aspects (Figs. 10, 11). There are still no papillae, but the cells vary in height. Lines that separate secretory episodes occur in the cuticle's periphery. The outer layer stains more lightly than the newer (inner) cuticle (Figs. 10, 11), suggesting that there is an abrupt change in composition between layers. At this stage, immature stalked nodules are produced subjacently to some of the spicules (Figs. 10, 11). Immature nodules already contain some peripheral vacuoles (Figs. 10, 11) but are claviform epidermal bulges in both juveniles and adults (von Knorre, '25). These spicules also have short chitinous shafts (Fig. 10a), but they lack pigment granules and have nodules below them. They are more like adult ventral or marginal spicules than dorsal ones (Fig. 12) (Leise, '83). When ventral and marginal spicules can be distinguished, after about 6 months (Fig. 12), some marginal nodules are mature but the ventral ones are not. Thus, the spicules surmounting stalked nodules in the 2-month-old animals (Figs. 10, 11) belong to the first

row of marginal spicules. Two-month-old animals also secrete much larger spicules with long, basal, chitinous shafts (Fig. 11) that are part of incipient hairs and will be discussed in the following section. At 6 months, the cuticle no longer contains primary spicules, because they and the cuticular surface have been re-moved by erosion.

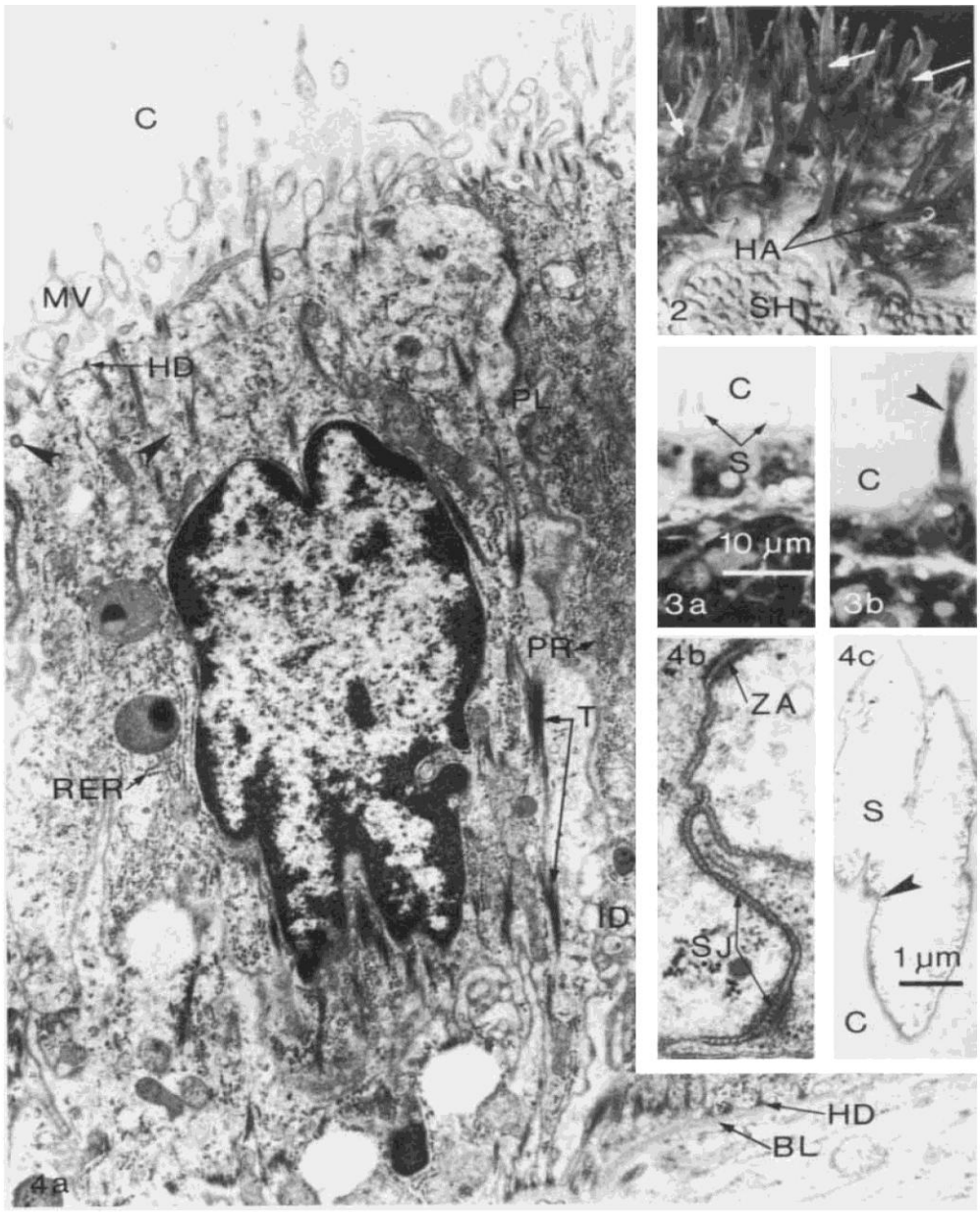


Fig. 2. Dorsal girdle integument of a live adult. Most of the spicules lying in the mesial groove (arrows) of each hair (HA) have been eroded. SH, shell plate. ×5.

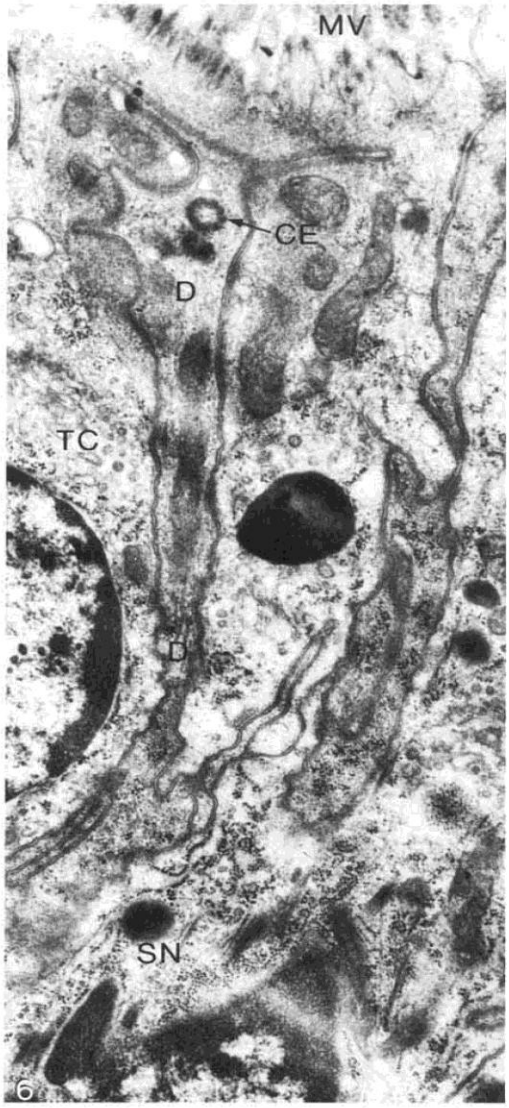
Fig. 3. One-micrometer sections through the girdle of a 13-day-old juvenile. a,b) Spicules (S) in a and hairs (arrowhead) in b lie within the cuticle (C). ×1,270.

Fig. 4. Electron micrographs of the girdle epidermis of a 13-day-old chiton. a) These cells produce neither spicules nor hairs. Interdigitations (ID) and plications (PL) occur at the cell margins. Small bundles of tonofilaments (T), many small vesicles (arrowheads), polyribosomes (PR), and rough endoplasmic reticulum (RER) occur throughout the cytoplasm. C, cuticle; BL, basal lamina; HD, hemidesmosome; MV, microvilli. ×11,400. b) The cells are joined by a distal zonula adherens (ZA) and proximal septate junctions (SJ). ×38,000. c) Spicules (S) have thin cups (arrowhead). The cuticle (C) is also electron lucent. ×7.900.

After 6 months, the girdles of juveniles closely resemble those of adults. The girdle has large lateral and ventral aspects, but a relatively small dorsal region. The lateral face of the girdle produces components that characterize both the dorsal and marginal surfaces of an adult girdle (Figs. 12, 13). As in adults, the shell plates are embedded in the girdle (F'ig. 13). The girdle integument on all three surfaces is papillate, but many of these papillae adjoin one another. Occasionally, common epidermal cells occur between the papillae (Fig. 13).

The girdles of 6-month-old juveniles produce four types of spicules (Figs. 12, 13): dorsal pigmented, marginal, ventral, and trichogenous spicules. Dorsal spicules, approximately 20 × 5 µm, generally are shorter than those in

large animals (Leise and Cloney, '82) and shorter than the marginal or ventral spicules (approximately 50 × 10 μm and 30 × 10 μm, respectively). The chitinous cups of the dorsal spicules are thinner than the ones in an adult. The marginal spicules form a band, two spicules across, that rings the outermost edge of the girdle (Figs. 12, 13). Each marginal spicule has a densely staining basal cup (Fig. 13), and the older ones surmount mature stalked nodules (Fig. 13). In younger marginal spicules, a narrow chitinous ring, or annulus, encircles the base of the cup and the tip of the subjacent nodule. Ventral spicules overlap, and their long axes point laterally. Younger ventral spicules surmount immature stalked nodules (F'ig. 12).



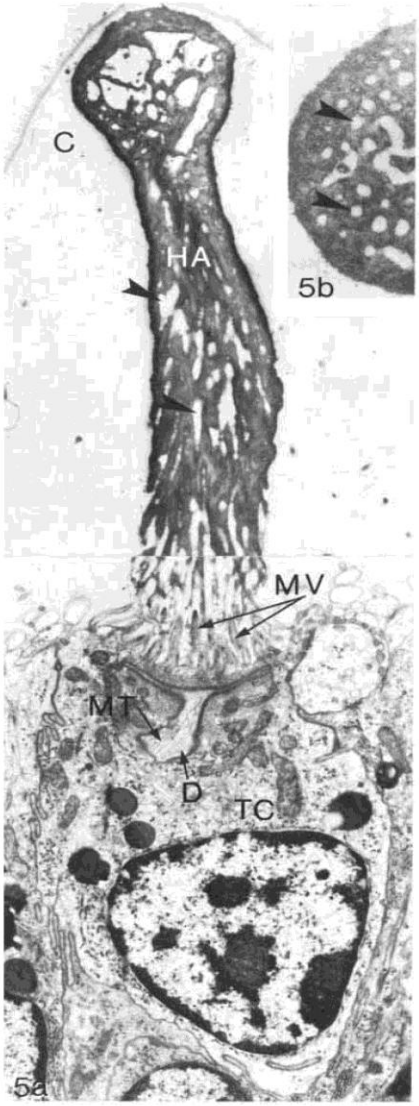


Fig. 5. a) Longitudinal section through a hair (HA) and its trichogenous cell (TC) in a 13-day-old juvenile. The dendrite (D), full of microtubules (MT), is from an adjacent neuron. The hair shaft, enclosed by the electron-lucent cuticle (C), has many lucent tracts (arrowheads), probably left by the apical trichogenous microvilli (MV). ×8,700. b) Transverse section from a hair of a 13-day-old juvenile showing the microvillar tracts (arrowheads). ×15,800.

Fig. 6. Electron micrograph of a trichogenous cell (TC) and part of the adjacent sensory neuron (SN) whose dendrite (D) protrudes into the trichogenous cell and ends stellately just below its apical surface. MV, apical microvilli; CE, centriole. ×20,800.

After 9 months, many ventral and dorsal papillae are discrete units, surrounded by common epidermal cells (Figs. 14, 15). Some papillae are still merged, especially on the ventral surface where both mature and immature stalked nodules occur (Fig. 14). Many dorsal spicules now have distinct basal shafts extending to the epidermal cells (Fig. 15).

When juveniles are 1 year old, the girdle has all the characteristics of an adult. The girdle is now cuneal in profile and has a rim of long, marginal spicules (Fig. 16). Papillae are discrete and separated by common epidermal cells. Dorsal spiniferous papillae produce both spicules and stalked nodules. The cuticle also stains more densely than it did in younger animals in similar 1-μm sections (cf. Fig. 3a,b, Fig. 16).

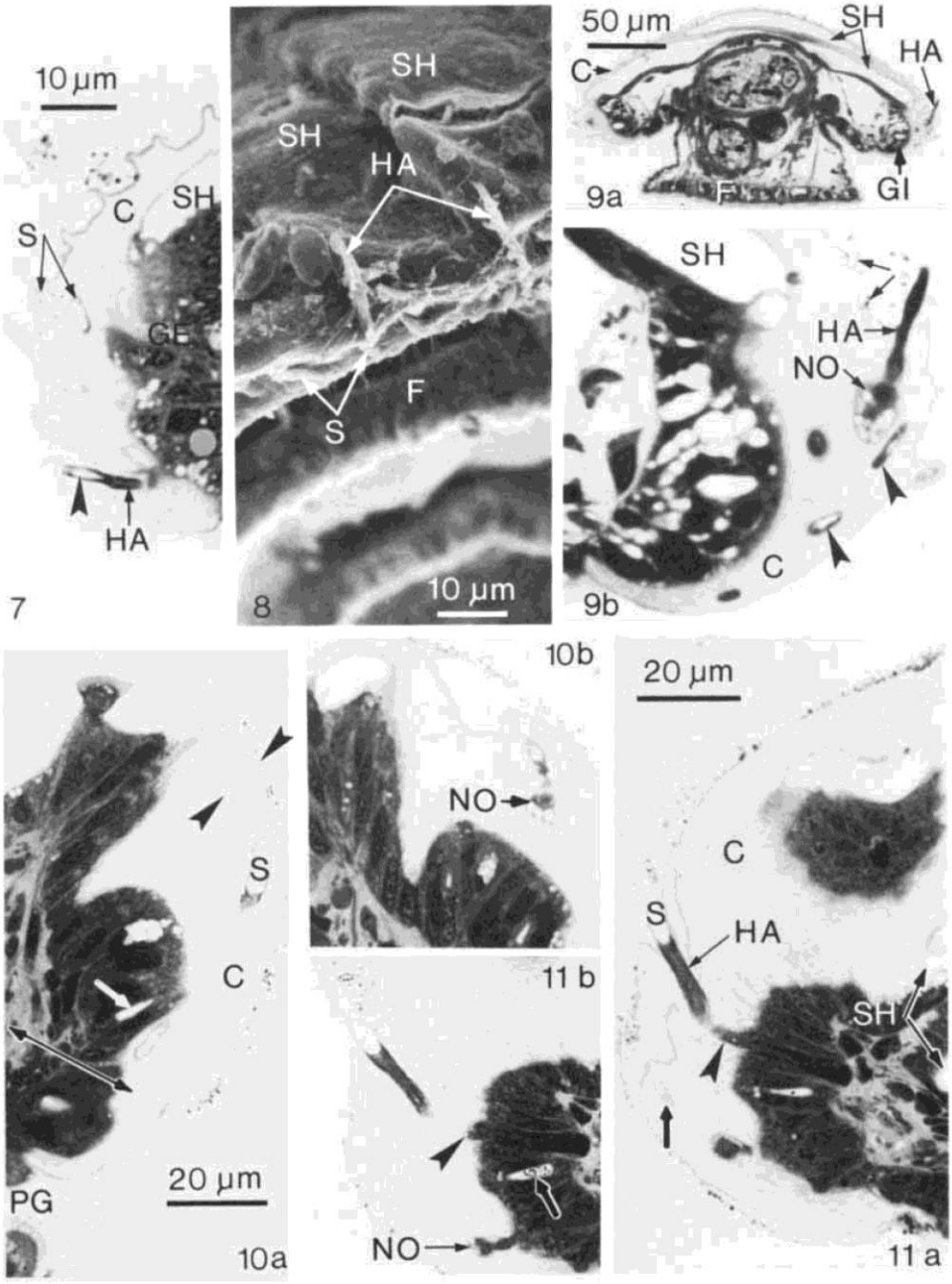


Fig. 7. *Transverse section through a 19-day-old chi-ton. The cuticle (C) covers the shell plates (SH) and girdle epidermis (GE) and contains spicules (S) and hairs (HA). The distal spicule atop the hair is still evident (arrow-head). × 1,060.*

Fig. 8. *Scanning electron micrograph of the edge of a 36-day-old juvenile. Two sutural hairs (HA), located at the junction of two shell plates (SH), protrude from the girdle and project dorsally. Spicules (S) bulge from the ventral side of the girdle. F, foot. × 960.*

Fig. 9. *a) Transverse section through a 36-day-old juvenile. The girdle (GI) rings the edge of the shell plates (SH), which cap the dorsum of the animal. × 230. b) Enlargement of the right side of the girdle in a. The cuticle (C) is narrow ventrally and is broad laterally, where it contains hairs (HA) and spicules surrounded by relatively thick chitinous cups (arrowheads). Older hairs surmount stalked nodules (NO). This nodule's stalk lies out of the plane of this section. Epiphytes (arrows) grow on the cuticle's surface. × 1,050.*

Fig. 10. *a,b) Serial, transverse sections through a 58- day-old juvenile. The double-headed arrow indicates the dorsoventral axis. The epidermal cells are of unequal heights, but no papillae are distinguishable. Nascent spicules (arrow) occur intracellularly; older ones lie within the cuticle (C). Dense lines within the cuticle separate episodes of cuticular secretion (one episode between arrowheads). One marginal spicule (S) in a has a dense chitinous cup and surmounts a stalked nodule (NO), which appears in b. The nodule's stalk is out of the plane of both sections. Section b is tilted with respect to section a. PG, pallial groove. × 700.*

Fig. 11. ***a,b) Serial transverse sections through a 58- day-old juvenile. The large spicule (S) and chitinous shaft are part of a young hair (HA) and protrude beyond the cuticle (C). The incipient nodule, only a slight epidermal bulge in b (arrowhead), meets the chitinous shaft in a. The stalked nodule (NO) in b is distinctly claviform and contains small vacuoles. The spicule (arrow) grazed in a surmounts this nodule. A few pieces of calcium carbon-ate in a nascent spicule (arrow) in b have refracted the light and appear to be, but are not, pigment. The shell (SH) is embedded in the girdle. × 760.***

Development of the hairs

During metamorphosis a fringe of small hairs is produced simultaneously around the perimeter of the girdle (Leise, '84). The hair shaft is produced by one trichogenous cell (Figs. 3b, 5a, 6) and has a small calcareous spicule about 2 µm wide and 4-8 µm long at its tip. The shaft of the young hair resembles a fibrous bundle of the adult hair cortex (Figs. 1, 5) (Leise and Cloney, '82). Both of these structures contain tracts, presumably left by the epidermal microvilli (Fig. 5). The trichogenous cell apex often bulges above the apices of the surrounding cells (Fig. 3b). In 13- day-old juveniles, each trichogenous cell has been penetrated by a dendrite from an adjacent sensory neuron (F'igs. 5a, 6, 17a). This anatomical arrangement was traced several times in serial thin sections and on rare occasions observed in a single fortuitous section (Fig. 6). This sensory neuron is the first of many that may occur within the future trichogenous papilla (Fig. 17) (Leise and Cloney, '82). The trichogenous cell will become one of the supporting cells of the nodule (Figs. 1, 17). The dendrites contain many microtubules, a few vesicles, and often a pair of centrioles (Fig. 6). The dendrites always terminate stellately below the apex of the trichogenous cell. The axon presumed to emerge from this cell has not been located, although the trichogenous papillae are known to be innervated (Leise and Cloney, '82).

Hairs of young juveniles tend to be directed laterally or dorsolaterally (Figs. 7, 9) and are usually eroded. Hairs with complete tips rarely occur in animals older than 3 weeks (Figs. 7, 9). The erosion continues as the animal grows; large hairs on adults are rarely whole.

At 1 month, each short hair shaft surmounts a stalked nodule (Fig. 9). New hairs are being produced continually, so after 2 months hairs are not uniform in size. Some hairs consist of large spicules atop thick chitinous shafts surmounting immature stalked nodules (Fig. 11). Others have lost their tips, are shorter, but surmount mature nodules (Fig. 18). As a hair grows, new spicules, each with its chitinous shaft and subjacent stalked nodule, are produced in succession by the trichogenous papilla (Fig. 17). However, even after 6 months, the hairs of juveniles do not fully resemble those of adults, mainly because they lack a cortex (Figs. 15, 19). The chitinous shafts of the trichogenous spicules become longer with each successive spicule, and the long, cellular stalks of the nodules embedded in the central cuticle form the medullary dendritic bundles of the mature hair (Figs. 1, 17d). Like marginal spicules, trichogenous spicules often have annulate shafts (Figs. 15, 16, 19).

After 9 months, trichogenous papillae be-gin to secrete a cortex (Fig. 20). Initially, the annulate shafts of the spicules are the only cortical material in a hair. The cortex is first secreted as a narrow border along one side of the hair (Fig. 20). After 12 months, the cortex of a hair is a crescentic structure lying along one side of the medulla. This cortex is at least three cells (and hence three fibrous bundles) wide (Figs. 16, 17d, 21). These fibrous bundles become part of the inner cortex of the mature hair (Fig. 1). The cortex does not yet contain an outer layer. A scanning electron micrograph (Fig. 22) of a hair at this stage shows that the spicules lie along the mesial surface, opposite to the cortical crescent, as they do in young hairs of adults (Fig. 23a-c). The hairs in 1-year-old individuals are about 0.3 mm in length (Fig. 24). These hairs rep-resent only the tips (that are usually eroded) of adult hairs that may be 5.0 mm long (Fig. 25).

DISCUSSION

The skin of most molluscs is highly sensitive to touch, and that of the chitons is not an exception. Stalked nodules occur on all three surfaces of the girdle of *Mopalia muscosa* and are incorporated into the large dorsal hairs. The epidermis is thus well endowed with sensory receptors. The basic neural components of the stalked nodules arise early in the animal's development, suggesting that the ability to respond to touch is important to the animal's existence. No other neuronal structures have been discovered in the girdle skin of this species

(reviewed in Leise, '83). The critical physiological tests of nodular functions remain to be accomplished, but the nodules are the best candidates for the role of epidermal mechanoreception.

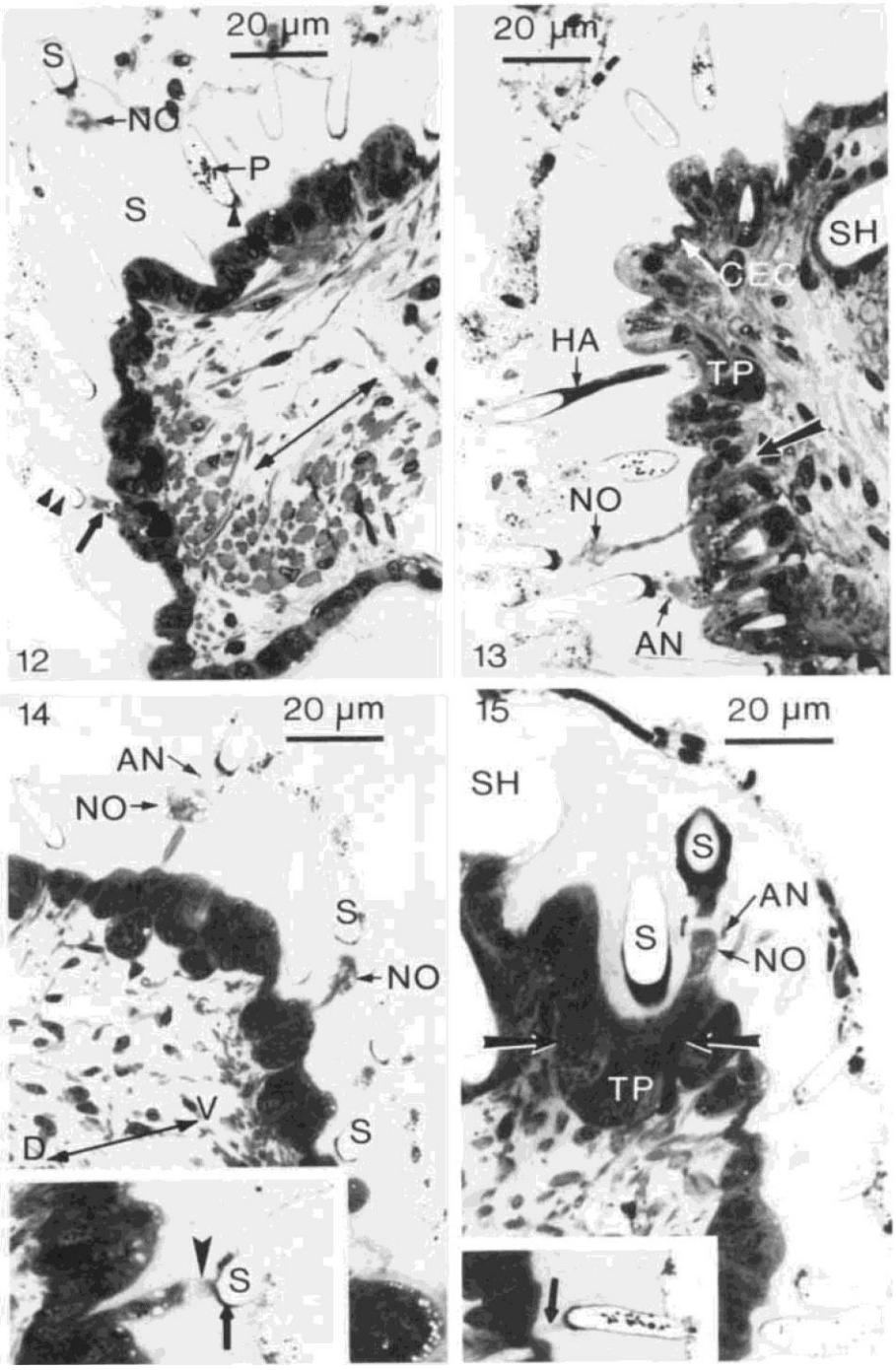


Fig. 12. ***Transverse section through the girdle of a 6- month-old (187-day) chiton. The double-headed arrow is the dorsoventral axis. The dorsal spicules have pigment granules (P) and dense basal chitinous cups (single arrowhead). The marginal spicules (S) are larger than the dorsal or ventral ones (double arrowheads) and surmount stalked nodules (NO). The ventral spicules have thinner chitinous cups than the dorsal spicules and surmount stalked nodules. The ventral nodules (arrow) are still immature. × 620.***

Fig. 13. ***Transverse section through a 6-month-old chiton. Dorsal is up. A trichogenous papilla (TP) has formed a hair (HA). Common epidermal cells (CEC) are evident between some papillae, although many papillae abut without intervening cells (arrow). Mature stalked nodules (NO) are below the older marginal spicules. Marginally, at the base of each of the younger chitinous cups, there is usually a thin annulus (AN). SH, shell. × 560.***

Fig. 14. ***Transverse section through a 9-month-old (266-day) chiton. The double-headed arrow is the dorso-ventral axis. A thin annulus (AN) is between each marginal spicule and its stalked nodule (NO). In adjacent sections a portion of the nodule is within the annulus. Some ventral spicules (S) now surmount mature nodules (NO). × 610. Inset:***

Ventral spicule (S) with a thin chitinous cup (arrow) and a short, thick shaft (arrowhead) surmounts an immature nodule. × 1,040.

Fig. 15. *Transverse section through a 9-month-old juvenile. One trichogenous papilla (TP, between arrows) has produced a hair that as yet has no cortex. The chitinous shafts of the two trichogenous spicules (S) are short. An annulus encloses (AN) the base of the spicule's cup and the tip of its nodule (NO). SH, shell. × 720. Inset: Dorsal spicule with an elongated, lightly staining chitinous shaft (arrow). × 930.*

Fig. 16. Transverse section through the girdle of a 1-year-old juvenile. A long dendritic stalk (arrow) to a marginal nodule occurs at the edge of the girdle. The hair produced by the trichogenous papilla (TP) has a rim of cortex (IC) on one side. The medulla, or central cuticular area, contains the nodules (NO), their stalks, and the bases of the mesial spicules (S). The distal spicule (*) has broken off from this hair. ×370.

Morphogenesis of the girdle ornaments

The hairs and spicules of newly metamorphosed juveniles appear to be morphologically distinct from their adult counterparts. In the case of the primary spicules, this distinction is real. Primary spicules have no central pigment granules, no thin chitinous cup, and no chitinous shaft. Primary spicules are lost, along with the enveloping cuticle, as the animal grows. Pigmented spicules are not produced until the animal is nearly 6 months old (Table 1). Even then, the spicules produced are smaller than those found in large animals and have a less dense chitinous cup. Successive generations of pigmented spicules gradually increase in size.

A comparison of the morphogenesis of the hairs on juveniles and small hairs of adults demonstrates the relationship between these seemingly disparate structures. Adults continually produce new hairs whose morphogenesis parallels that of juvenile hairs. Hairs first appear as spicules with heavy chitinous shafts. Sensory neurons can be identified concurrently. As the hair grows, more mesial spicules with their accompanying shafts and sensory nodules are formed. Thus, a hair contains one dendritic bundle for every mesial spicule. At first the hair has no cortex. When cortex is finally produced, the inner layer is produced first, as a horseshoe- or crescent-shaped mass along the outside of the medulla (Fig. 23). Gradually, more subcortical cells are created, and the crescent of the cortex enlarges until it surrounds the central cuticle or medulla, thus completing the hair.

The distance between successive spicules usually increases as a hair grows; the shaft of each spicule is often longer than preceding shafts. In large hairs, the shaft of a mesial spicule may be several millimeters long. In small hairs, or in tips of large hairs, two shafts from sequential spicules may occur simultaneously (Fig. 23).

Although each stalked nodule is mesial, the nodular stalks, or dendritic bundles, do not stay clustered along the mesial groove. Younger bundles tend to be closer to the longitudinal groove than older bundles.

Several factors may contribute to the continuous loss of the cuticle and the enclosed ornaments in juvenile chitons. The girdle faces ventrally for the first 6 months and is in contact with the substratum except at the rear anal elevation. Thus, the cuticle could be abraded as the juveniles crawl on the sub-stratum. New cuticle is

secreted below older cuticle, and, as a consequence of the interstitial growth of the epidermis, the older cuticle, which originally covered a smaller area of epidermis, cracks and is even more likely to be worn away as the animal moves. The adult hairs support extensive epiphytic and epifaunal communities (Phillips, '72), and even young juveniles are not free of affixed organisms (Fig. 9b). The attachment of these organisms may weaken or destroy the outer cuticular edge, thereby facilitating its removal.

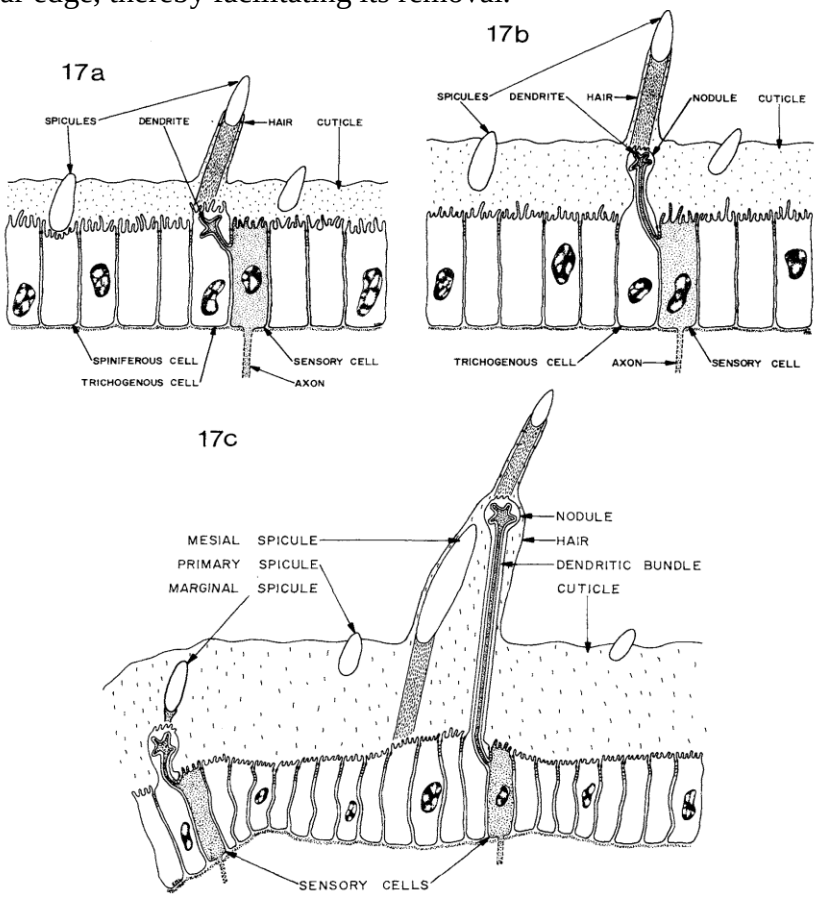


Fig. 17. A diagrammatic series illustrating the major stages in the development of the girdle hairs of juveniles. a) At 2 weeks, the cuticle contains primary spicules, surrounds the hairs, and overlays the entire girdle epidermis. Spicules and hairs protrude from the cuticle because the surface is eroded. The hair has a calcareous spicule at its tip and is produced by a single cell. A dendrite from an adjacent neuron penetrates the trichogenous cell and ends stellately below the hair. The axon from the putative sensory neuron is dotted, because it has not been located. b) After 1 month the secretion of more cuticle has carried the spicules away from the cells. The apical end of the trichogenous cell has elongated and become slightly claviform. The dendrite remains in the middle of this protuberance, and together these cells form the first nodule. Nodules may have up to 25 sensory neurons and several supporting cells (Leise and Cloney, '82), but the nodules here have been simplified and drawn with only two cells. c) After 3 months the tip of the hair is unchanged, but the first nodule has elongated; its stalk forms a dendritic bundle. As yet, the hair shaft has no cortex. A second spicule is being made along the mesial surface of the hair. This second spicule has a longer chitinous shaft than the previous one. A marginal spicule has been drawn for comparison. The morphogenesis of both stalked nodules is the same. d) After 1 year

Clarification of adult morphology from juvenile data

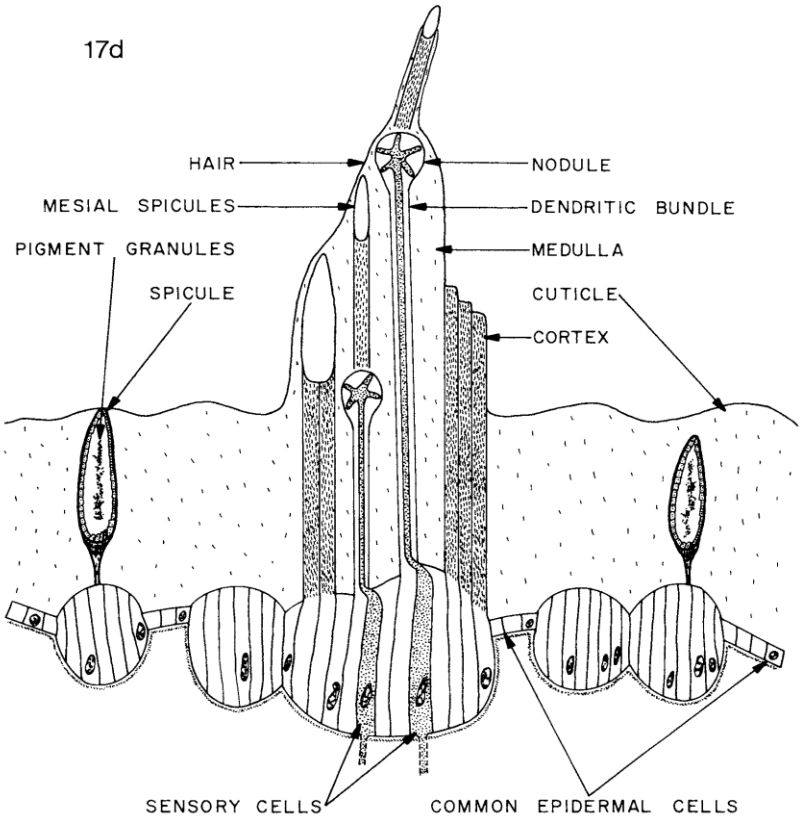
In an earlier article Leise and Cloney ('82) interpreted the trichogenous nodules in *M. muscosa* as being formed anywhere along the length of each dendritic bundle. Data from the present study suggested a reexamination of the adult material, whereupon it became clear that nodules only occur below the shaft of a mesial spicule. The general erosion of the hairs and their concomitant lack of spicules were among the misleading factors. Leloup's description ('42) of these hairs as being spiculose was the only such report in the literature (Pilsbry, 1892; review by Hyman, '67; Abbot, '74); hence, Leise and Cloney treated it skeptically. Careful observation of juvenile and adult material verified Leloup's findings.

Leloup found that the longest hairs tend to lose their calcareous spicules but retain the chitinous shafts. Leise and Cloney ('82) erroneously assumed old hairs to be the most complete, and incorrectly identified the residual shafts as cortical rods.

Girdle ornaments as assemblages of component parts

Girdle ornaments can be considered composite structures, consisting of a number of elementary components (Leise, '83). The basic components used by *Mopalia muscosa* are cups, spicules, pigment granules, chitinous

shafts, stalked nodules, annuli and cortical layers (e.g., the outer layers of large hairs), and cuticular or medullary material. Thus, any particular ornament is a unique structure, but shares some of its elementary units with others. Diversity, both within and between species, is generated in this fashion, although certain constraints limit the form of the various ornaments. One such constraint is that the serial order of certain components is always maintained. For example, spicules and shafts are produced in conjunction with stalked nodules in dorsal hairs, at the girdle's margin, and on the ventral surface. In all cases, the spicule is most distal, followed sequentially by the shaft and then the stalked nodule.



the cuticle contains pigmented spicules, but they are smaller than those in a larger adult. These spicules almost span the cuticle, which has been drawn as it appears in vivo; it contains no primary spicules because its outer edge has been eroded. A few cells on one side of the trichogenous papilla have secreted cortical material, and the medulla contains two stalked nodules. A third nodule would have developed below the shaft of the third spicule.

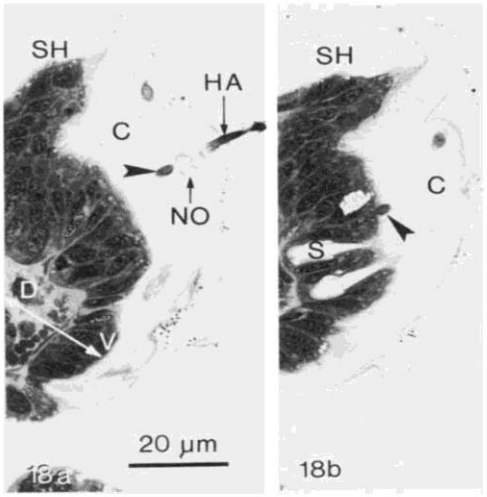


Fig. 18. Serial transverse sections from a 58-day-old juvenile. The double-headed arrow is the dorsoventral axis. a) The hair (HA) surmounts a nodule (NO) whose stalk (arrowhead) joins the epidermis in b (arrowhead). C, cuticle; S, spicule; SH, shell. ×760.

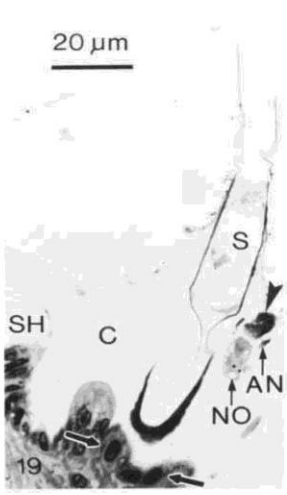
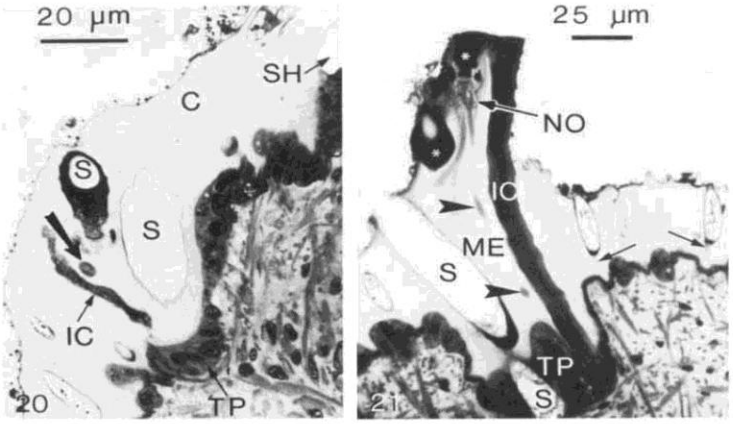
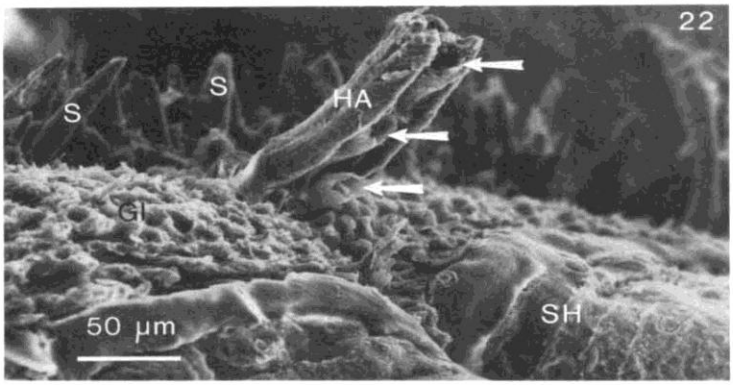


Fig. 19. Transverse section through a 6-month-old juvenile. The small trichogenous papilla (between arrows) has produced a hair that as yet has no cortex. One long trichogenous spicule (S) is unusually well preserved. A small nodule (NO) adjacent to it is below the annulus (AN) and base of the shaft (arrowhead) of a more distal spicule. C, cuticle; SH, shell. ×710.

Differences in the size or amount of the basic component also add to the diversity of girdle ornaments. For example, all spicules are calcareous, but the difference in size between primary and marginal spicules is

substantial. Consider also that the length of the dense chitinous material below a spicule determines whether it is merely a short extension of the cup, as occurs in the ventral spicules, or a true shaft, as in the trichogenous mesial spicules.





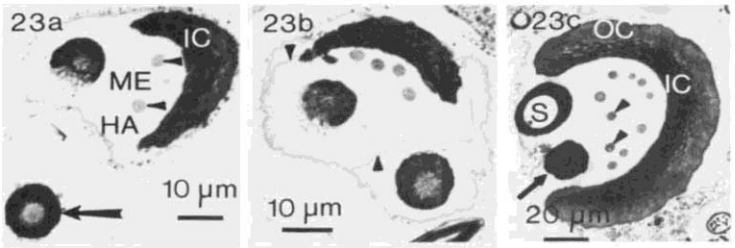


Fig. 20. Transverse section through a 9-month-old chiton. The trichogenous papilla (TP) has begun to secrete the inner cortical layer (IC). Portions of two mesial spicules (S) lie within the cuticle (C). The medulla contains a dendritic stalk (arrow) from a more distal nodule that is not in the plane of this section. SH, shell plate. ×660.

Fig. 21. Transverse section through a 1-year-old chiton. The dorsal spicules have lightly staining chitinous shafts (arrows). The medulla (ME) contains portions of one dendritic bundle (arrowheads) that emerges from a distinct papillary hillock and terminates distally in a nodule (NO). Two mesial spicules (S) and parts of the shafts (*) of two more distal spicules occur in this hair.

Fig. 22. ***Scanning electron micrograph of the dorsal surface of a 1-year-old chiton. Marginal spicules (S) rim the girdle (GI). The dorsal spicules crenulate the surface of the girdle. One sutural hair (HA) has at least three mesial spicules (arrows) that are eroded. SH, shell plates. x 330.***

Fig. 23. ***Three frontal sections through the girdle of an adult animal demonstrate small, immature hairs. a) The chitinous shaft (arrow) separated from the hair is the shaft of a mesial spicule. It joins the hair shaft (HA) below this level. The hair has two dendritic bundles (arrowheads) within the medulla (ME) and a lunate cortex (IC) opposite to the mesial spicules. Below the two chitinous shafts are nodules whose cellular stalks will form two additional dendritic bundles. x720. b) Layers in the medulla (arrowheads) indicate the successive addition of new elements. x730. c) The medulla of this larger hair is incompletely enclosed by the cortex. The two adult cortical layers (IC, inner cortex; OC, outer cortex; cf. Fig. 1) occur in this hair. The proximal end of one spicule (S) within its cup is adjacent to the shaft of a more distal spicule (arrow). The hair has 10 dendritic bundles (arrowheads) at this level, but 12 more proximally. x350.***

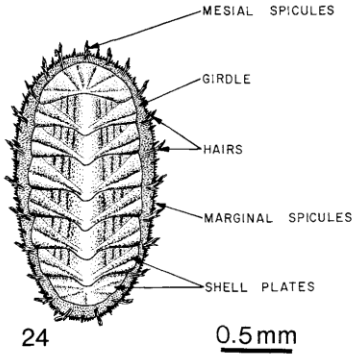
Hyman ('67) stated that hairs "may be understood as spicules in which the basal cup has elongated greatly. . . ." This description applies to species in other families, such as the Callochitonidae (Leise, 1983), but not to *M. muscosa.* As seen from their development, hairs contain girdle ornaments similar to those occurring elsewhere in the integument. In the hairs of *M muscosa* the basic replicated ornament consists of a spicule, its cup, shaft,

and stalked nodule. These ornaments are serially replicated along a cuticular out-growth, perpendicular to the surface of the animal.

Functions of the hairs and spicules

The function of the girdle hairs of *Mopalia muscosa* has been difficult to ascertain. Adult animals tighten their holds on the substratum or move off in the opposite direction if one or several hairs are bent or pinched (Leise, unpublished data). The possibility that the animal was responding to deformation of the skin was not eliminated. As the cuticle contains the same sensory organs, the stalked nodules, such a distinction may be unimportant. Hairs may serve several functions. They may be devices for increasing the distance from the body at which tactile input can be received. Their extensive epiphytic communities (Phillips, '72) may slow the animal's desiccation at low tides and hide it from potential predators. Predators such as the star-fish *Pisaster ochraceous* (Paine, '80) will touch and ignore overgrown chitons, but touch and prey on clean individuals. Such predators seem to find the girdle relatively unpalatable. They usually consume only the foot and viscera, leaving the shell and girdle intact (Leise, personal observation). Chitons that are ignored can move out of harm's way, but once detected as a food item, cannot outrun a hungry starfish (Leise, personal observation). The ability to detect and move away from potential predators may be of more importance to young juveniles that have not yet collected their epiphytic cloaks and who, because of their small size, may have a much wider range of potential predators.

The same mechanosensory structures that are in the hairs, the stalked nodules, occur above each of the overlapping ventral spicules. These nodules may enable chitons to detect features of the surface on which they move. Normally the animal holds the girdle in light contact with the substratum and only tightens its grip if disturbed. Feedback from some type of dermal or epidermal pressure receptor will be needed if the animal is to judge the strength of its hold. The ventral stalked nodules probably fill this role. Spicules may channel the forces involved directly to the stalked nodules.



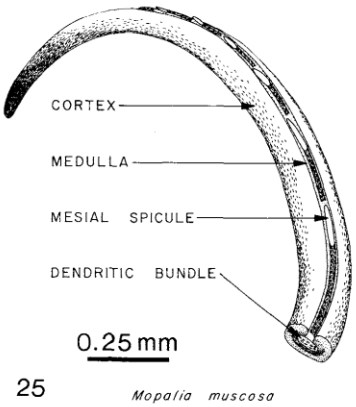


Fig. 24. Diagram of a juvenile about 1 year old. The aesthete canals have not been included. The animal superficially resembles an adult, although the girdle is relatively narrower than in an adult. The shell plates have some sculpturing, but lack most of the coloring and topography of adult shell plates. The first row of hairs nearest the shell plates formed at metamorphosis. A second row of hairs formed outside the first row. The marginal spicules that encircle the girdle are larger than the dorsal, ventral, or primary spicules.

Fig. 25. Diagram of the external anatomy of a hair from an adult. The base of the shaft of each mesial spicule is embedded in the medulla. Below each shaft is a stalked nodule that is also enclosed in the medulla. The stalked nodules are not drawn. This hair is drawn in its entirety and as if it were cut off just beyond the cuticle.

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