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Author(s): Karen A. Mesce

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## MORPHOLOGICAL AND PHYSIOLOGICAL IDENTIFICATION OF CHELAR SENSORY STRUCTURES IN THE HERMIT CRAB *PAGURUS HIRSUTIUSCULUS* (DECAPODA)

*Karen A. Mesce*

### ABSTRACT

The hermit crab *Pagurus hirsutiusculus* uses its minor chela to examine gastropod shells that may be selected for habitation. Since tactile and chemical cues, especially calcium, are important for the recognition and selection of shells by this species, the minor chela was examined for the presence of mechano- and chemoreceptors. Chelar cuticular structures were examined with a scanning electron microscope, and electrophysiological methods were used to determine their sensory responses. Two structural types, simple setae and rows of teeth, function as mechanoreceptors. The simple setae also function as chemoreceptors, sensitive to calcium. A detailed description of the arrangement and external morphology of these bifunctional simple setae is provided and serves as background for further studies of their ultrastructural features.

Other structures found on the chelar surface were pits and nonsensory tubercles. The function of the pits remains unknown. Behavioral adaptations for calcium detection in marine environments are discussed, as is the possible occurrence of calcium detection in other crustaceans.

Hermit crabs are very selective about the types of gastropod shells they choose to inhabit, and frequently explore newly encountered shells in search of an optimal shell (Reese, 1962, 1963; Elwood and Neil, 1992, for review). Many species first locate potential shells from a distance using a variety of visual (Reese, 1963; Kinoshita and Okajima, 1968; Hazlett, 1982; Mesce, in press) and chemical cues (McLean, 1974; Rittschof, 1980; Kratt and Rittschof, 1991). Shell exploratory behavior is expressed once the crab comes in contact with a shell. This behavior involves a sequence of stereotyped motor patterns which in part are elicited by specific shell cues that ultimately direct the hermit crab into the shell. Behavioral studies of the hermit crab *Pagurus hirsutiusculus hirsutiusculus* (Dana) have demonstrated that contact with an object containing calcium is important for the initial recognition of the shell itself and the initiation of further exploratory behavior that leads to shell selection (Mesce, 1982, 1983, in press).

When a hermit crab explores a shell, it probes and scrapes the shell surface using the ventral side of the minor chela. The appendages of *P. hirsutiusculus*, especially the minor cheliped, are extremely setaceous, hence the crab's common name, hairy hermit crab. As in other crustaceans and insects, many of these setae are putative sensory structures (for reviews see Ache, 1982; Altner and Prillinger, 1980). Since both cal-

cium and tactile cues appear to contribute to shell selection, the aim of this study was to identify and characterize those chelar setae and other structures that function as calcium-sensitive chemoreceptors and mechanoreceptors.

A combined electrophysiological and anatomical approach was used to establish the sensory nature and specific functions of structures on the surface of the minor chela. In particular, the chelar simple setae were found to be bifunctional chemo- (calcium) and mechanosensitive receptors. Such information contributes to a preliminary understanding of the neurobiological bases of the decision-making processes involved in shell selection. In addition, this description of the arrangement and external morphology of these bimodal chemo- and mechanoreceptors serves as a complementary study to the subsequent examination of their ultrastructural features (Mesce, 1983; Mesce, in preparation). Together, these studies provide structural markers for the identification of modality-specific receptors in crustaceans, and contribute to a better understanding of how chemo- and mechanoreceptors function.

### MATERIALS AND METHODS

*Experimental Animals.*—Hermit crabs, *Pagurus hirsutiusculus hirsutiusculus* (Dana), and *Pagurus samuelis* (Stimpson) were caught at Heceta Head and Cape Arago, Oregon, respectively. They were kept in

round aquaria, 1 m in diameter, in 15°C natural sea water on a 12 h light : 12 h dark cycle. Hermit crabs were fed approximately twice weekly with fresh mussels (*Mytilus* sp.).

**Scanning Electron Microscopy.**—Minor chelae were fixed in 2.5% buffered glutaraldehyde and subsequently dehydrated in a graded alcohol series. The material was critical-point dried, placed on aluminum stubs with silver conductive paste, and coated with gold-palladium. Specimens were viewed with an AMR-100 scanning electron microscope (SEM) at 30 kV.

**Neurophysiology.**—Extracellular electrophysiological investigations were performed on minor chelae which had autotomized, and on intact chelae. The length of the chela and carpopodite together averaged approximately 1.5 cm. Extracellular electrophysiological recordings were made from fine nerve fibers within the chelar connective tissue or from teased leg nerves spanning the propodite-carpopodite joint. Sensory activity was recorded with either electrolytically sharpened tungsten bipolar hook electrodes or fine glass suction electrodes. Electrical signals were amplified and displayed using conventional electrophysiological equipment.

Minor chelipeds were mounted, ventral side up, in a small trough of wax lined with Vaseline. A Vaseline barrier was created to isolate the sensory axons from all stimulus solutions. For the experiments on chemosensation, Vaseline was used to cover all but a small patch of chelar surface containing the cluster of simple setae under investigation. Solutions were gently pipetted on to particular clusters of simple setae occupying regions of the ventral surface of the chelar tip, great care being used to isolate the solutions to the chelar setae. A fine burnished glass micropipette was used to apply mechanical stimulation to the chelar sensilla. A stimulus marker was manually pressed to provide a qualitative measure as to when chemical and mechanical stimuli were applied.

**Solutions.**—Various modifications of crustacean perfusates were used, including those described by Robertson (1953) and Cole (1941, in Lockwood, 1961), ranging in osmolarities between 960 and 1,000 mOSM/kg and pH 7.4–7.6. Stimulus solutions included Patin's (1946, in Lockwood, 1961), and MBL formulae (see Derby, 1982) artificial sea water (ASW) and Cole's saline, to which were added the appropriate amounts of calcium chloride (25 mM) or magnesium chloride (150 mM) salts. Stimulus solutions ranged in pH between 7.6–7.8, but were held constant in any given experiment. The osmolarities of control and stimulus solutions (980 mOSM/kg) were within 2.0% of each other. Additional chemical stimuli included an extract of *Mytilus* which was prepared by placing small pieces of soft tissue in 100 ml of ASW for several hours. This solution was filtered before use.

## RESULTS

### Anatomical Studies of Sensory Structures on the Minor Chela

**Minor Chela: Sensory Structures.**—A low magnification view of the minor chela and its use during exploration of a CaSO<sub>4</sub> (plaster)

block is illustrated in Fig. 1A, B. Note that the minor chela is opened (A) as the cheliped is used to scrape the plaster block's surface with back and forth movements (B). The cephalothorax length of the hermit crab (*P. hirsutiusculus*) measured 1.4 cm.

Four types of cuticular structures were identified on the minor chela (Fig. 2A–F) with the SEM. They are: (1) clusters or tufts of hairlike structures, termed simple setae (Fig. 2A, C), (2) a row of corneous teeth on the dactyl biting edge (Fig. 2C, D), (3) cuticular tubercles located primarily on the dorsal surface (Fig. 2B), and (4) an assortment of minute pitlike structures primarily located around the setal tufts (Fig. 2E, F). At the light microscope level, both the hairlike structures and the chelar teeth contain a lumen in which neuronal fibers exist in association with numerous supportlike cells (Mesce, 1983, personal observations). The cuticular tubercles show no evidence of neuronal innervation and a transmission electron microscope (TEM) study of the pitlike structures have yielded inconclusive results regarding their sensory nature (Mesce, 1983, personal observations).

**Morphology of Chelar Simple Setae.**—The hairlike structures found within each cluster can be classified as the simple or smooth type (Thomas, 1970; Farmer, 1974; Factor, 1978; Derby, 1982). These setae, which appear in discrete circular clusters or tufts over the minor chela, are most dense on the dactyl and index regions (Fig. 2A). They become more linearly arranged toward the propodite-carpopodite joint. The number of setae per cluster, on the ventral surface, can vary from as few as 3 to as many as 30. The average length is approximately 300 μm, although setae have been observed to reach 1.0 mm in length. The smallest of the simple setae are found at the periphery of each cluster. Tufts of setae at the dactyl and index tips are consistently shorter and thicker than setae closer to the propodite-carpopodite joint (Fig. 2A). This distinction was present in animals that had molted 1–2 days previously as well as in older animals, suggesting that these differences are not a consequence of use and abrasion (Hamilton and Case, 1983).

The majority of setal clusters are situated in an encompassing cuticular depression

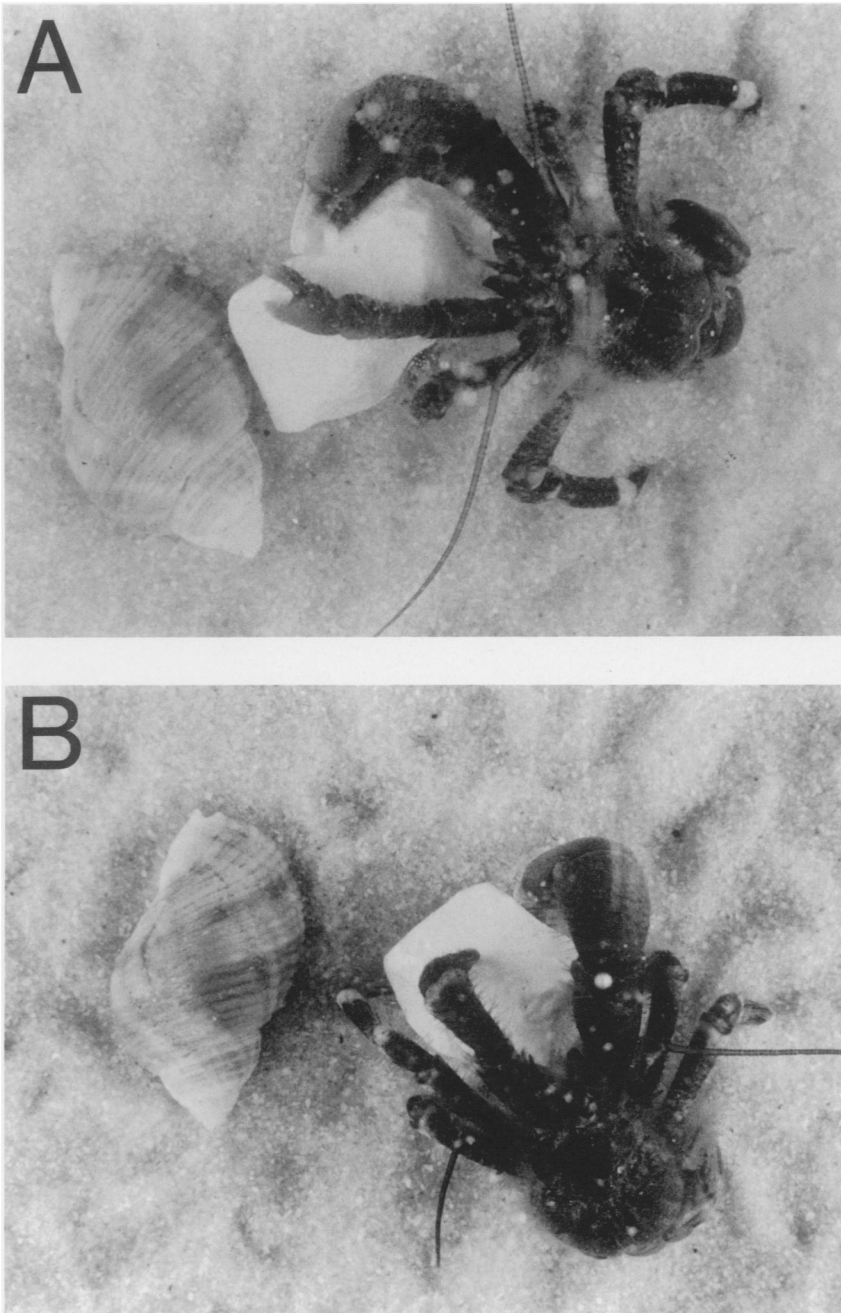


Fig. 1A, B. Photographs of the hermit crab *Pagurus hirsutiusculus* exploring the surfaces of a plaster ( $\text{CaSO}_4$ ) block. Note the use of the minor chela during exploration of the block. The silicone-coated shell of *Nucella canaliculata*, at left, which lacks the calcium cue is ignored. Length of shell is 2.5 cm.

(Fig. 3A). Within this depression each seta is set in a circular socket via a specialized articulation (Fig. 3B). Both the socket boundary and articulating membrane are asymmetrical around the setal shaft, with

one side having a wider rim (Fig. 3B). Individual setae are oblong, becoming acuminate at their more distal regions. When compared with the distal setal regions, the proximal (setal base) and intermediate

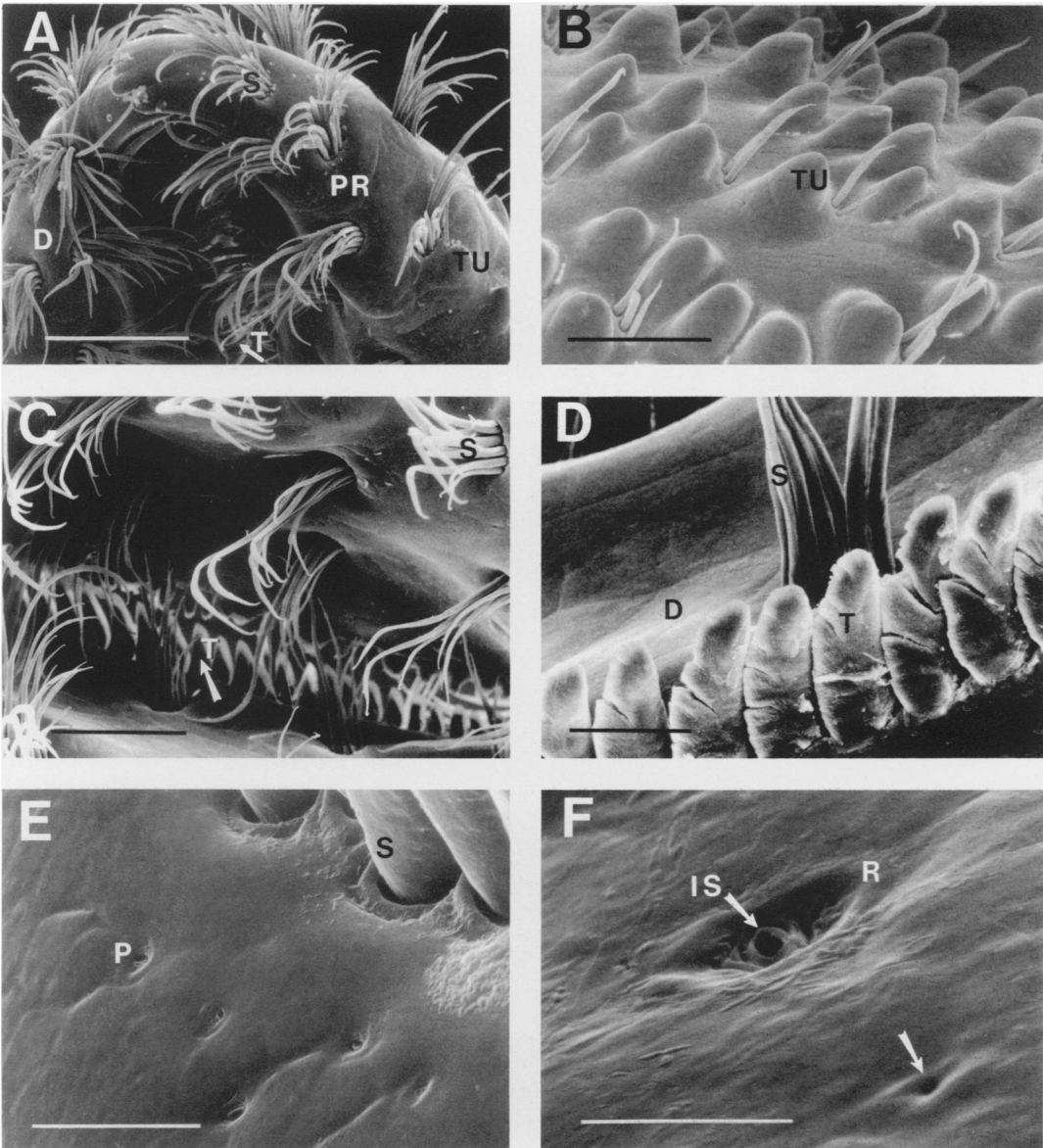


Fig. 2. SEM micrographs of chelar simple setae and other structures on the surface of the minor chela of *Pagurus hirsutiusculus*. A. Ventral surface of the minor chela including the circular clusters of simple setae, the teeth, tubercles, and minute pits (barely visible at lower magnifications). Note that the setae are slightly shorter and more stout at the chelar tip (bar = 300  $\mu\text{m}$ ). B. Cuticular tubercles on the dorsal surface of the minor chela. Here on the dorsal chelar surface, the arrangement of hairlike setae surrounding the tubercles differs from that on the ventral surface (bar = 200  $\mu\text{m}$ ). C, D. Cutting edges of the dactyl and propodite showing the chelar teeth at lower (C) and higher magnifications (D). A discernible socket apparatus was not observed at the bases of the teeth. Various surface indentations and small porelike structures were observed on the teeth, but none was consistently identified as the terminal or subterminal pore (C, bar = 300  $\mu\text{m}$ ; D, bar = 100  $\mu\text{m}$ ). E, F. Cuticular pits surrounding the simple setae at low (E) and high (F) magnifications. There are two distinct sizes of pits indicated by the two arrows in (F). Note the substructure in the larger pit and the inner sleeve or tubelike structure present (E, bar = 20  $\mu\text{m}$ ; F, bar = 5  $\mu\text{m}$ ). D = dactyl; IS = inner sleeve; P = pit; PR = propodite; R = rim of pit; S = simple setae; T = teeth; TU = tubercles.

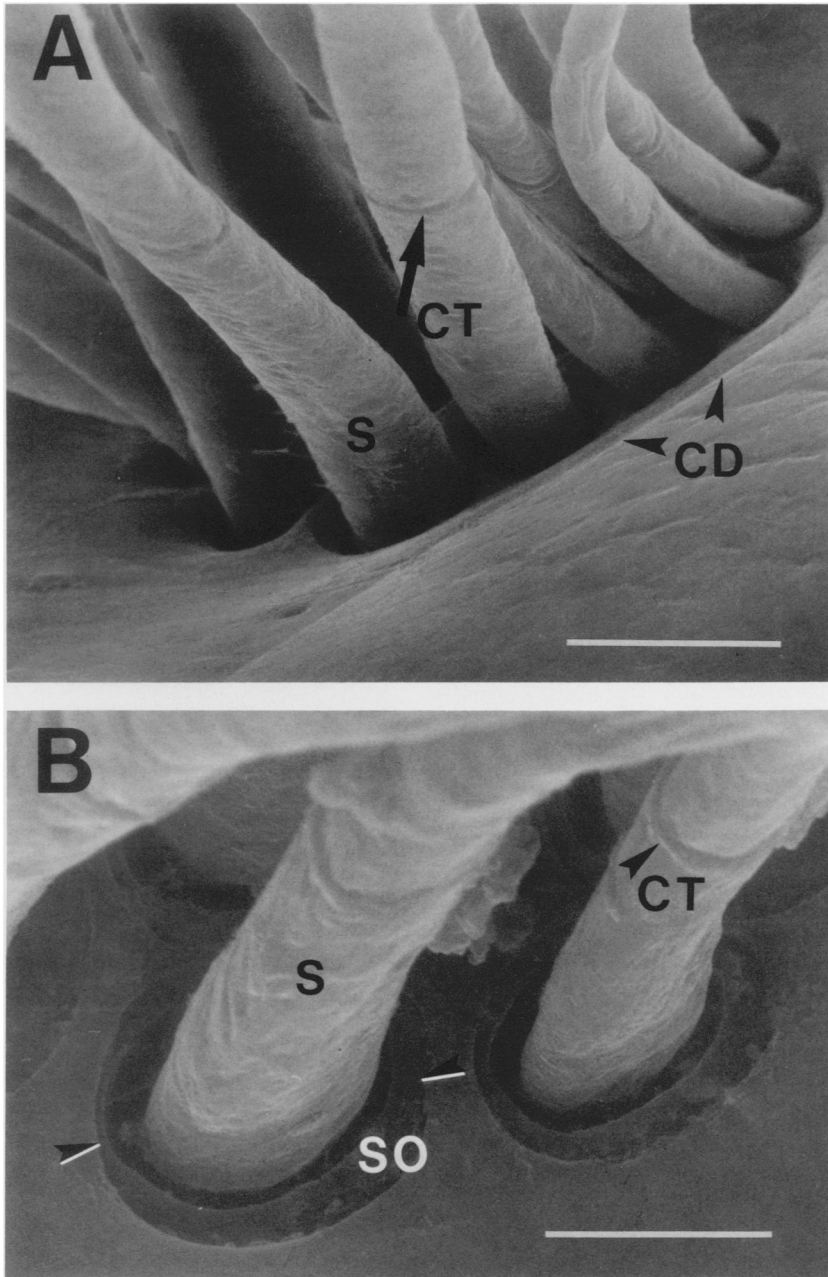


Fig. 3. SEM micrographs of a simple setal cluster on the ventral surface of the minor chela of *Pagurus hirsutiusc ulus*. A. The setal cluster is set down in a cuticular depression. Note the single annulus or constriction at the basal regions of each seta (bar = 30  $\mu\text{m}$ ). B. The socket apparatus of each seta is shown. Note that the setae are oblong and the socket boundaries are somewhat asymmetric as indicated by the arrowheads (bar = 10  $\mu\text{m}$ ). CT = constriction; CD = cuticular depression; S = simple seta; SO = socket.

regions are relatively smooth and homogeneous. Toward the setal base there is a single cuticular constriction or annulus (Fig. 3A, B). No porelike or other surface substructure was observed in these regions. Ap-

proximately 50  $\mu\text{m}$  from the setal tip, the surface begins to appear textured with several longitudinal fringes of cuticular prominences (Fig. 4C, D). These fringes range in form from ridges of stumplike structures

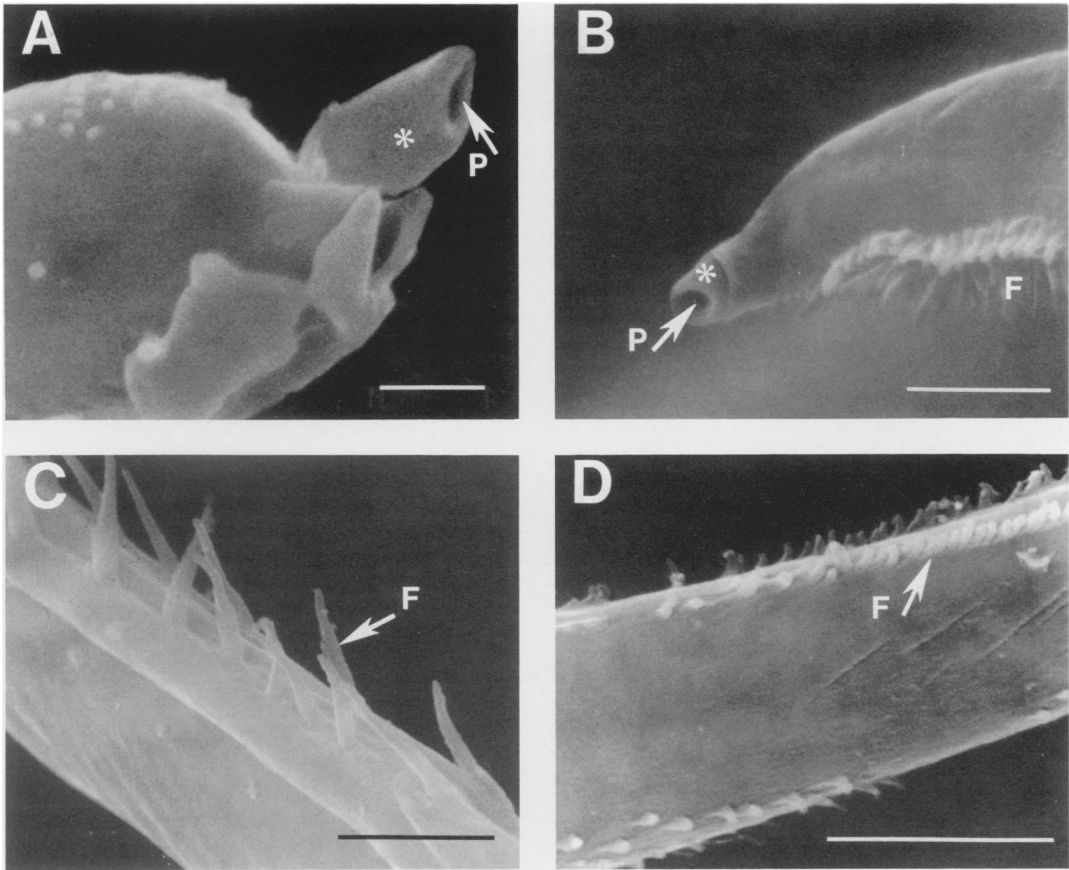


Fig. 4. SEM micrographs of chelar simple setae at and close to the terminal setal region. A, B. Terminal regions of two simple setae on the ventral surface of the minor chela of *Pagurus hirsutiusculus*. Both were situated in close proximity to one another. Of those setae which were observed to possess a terminal pore, the pore was usually found set in a tonguelike structure marked by the asterisks (A, bar = 1  $\mu\text{m}$ ; B, bar = 2  $\mu\text{m}$ ). C. Simple seta located closer to the carpopodite-propodite joint possessing fringes with spinelike protuberances most apparent near the terminal setal regions (bar = 3  $\mu\text{m}$ ). D. Stumplike structures located on the distal to medial regions of a simple seta (bar = 5  $\mu\text{m}$ ). F = fringe; P = pore.

(Fig. 4D) to rows of individually defined spinules (Fig. 4C). Chelar setae with well-defined spinules are most often found in clusters located toward the carpopodite-propodite joint.

In many of the intact setae, the setal tip bears a tonguelike structure whose tip contains a depression or possible pore (Fig. 4A, B). This terminal pore is most often found on setae belonging to animals that have recently molted. Chelae from hermit crabs in later molt stages had numerous broken setae with a high degree of surface abrasion and epizoid plant and animal growth (Hamilton, 1983b). Such setae did not bear any tonguelike structures and no porelike or fine surface structure was visible.

*Morphology of Chelar Teeth.*—Along the cutting edge of the chelar dactyl is a row of small corneous teeth (McLaughlin, 1974, fig. 2C, D). These teeth share general positional and morphological features with the stout hedgehog setae (lobster) and fringed setae (crayfish) studied by Shelton and Laverack (1970) and Derby (1982), and Altner *et al.* (1983), respectively. Altner *et al.* (1983) described the stout setae as having a subterminal pore. The stout setae studied here, also using SEM, possess various porelike depressions. However, no one particular porelike structure could be consistently identified in either the terminal or subterminal regions.

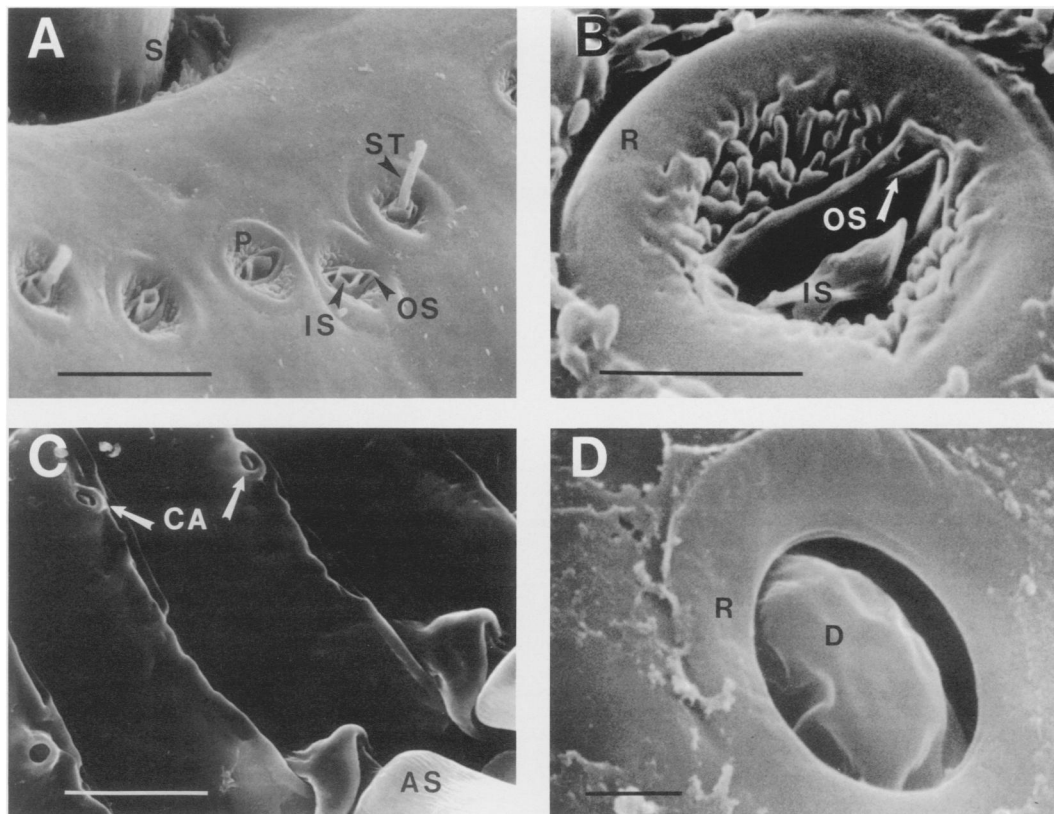


Fig. 5. SEM micrographs of cuticular pits in *Pagurus samuelis* (A, B) and campaniformlike structures on the antennular flagella of *Pagurus hirsutiusculus* (C, D). A. Cuticular pits surrounding the basal regions of the chelar simple setae. Note that some possess a stalklike structure which extends up from the center of the pit and is surrounded by an inner and an outer sleeve. In three of the pits shown, the stalk is absent and the inner sleeve has collapsed (bar = 10  $\mu$ m). B. Higher magnification SEM of a pit. Note the central tongue-like structure protruding from the central orifice which may represent a collapsed portion of the inner sleeve (bar = 3  $\mu$ m). C. Campaniformlike structures located on the antennular flagellum near the chemosensory aesthetascs (bar = 30  $\mu$ m). D. Higher magnification SEM of one of the campaniformlike structures which shows little topological resemblance to the chelar pits. Note the relatively smooth dome surrounded by a collarlike rim (bar = 1  $\mu$ m). AS = aesthetasc; D = dome; CA = campaniformlike structure; IS = inner sleeve; OS = outer sleeve; R = rim; S = simple seta; ST = stalklike structure.

**Morphology of Chelar Pits.**—Scattered on the surfaces of the minor chela, including the dactyl and index regions, are distinct pitlike structures. Such depressions are most numerous near cuticular areas surrounding the setal bases of the simple setae (Fig. 2E, F). The majority of depressions have a diameter of 5–7  $\mu$ m. In addition, several smaller depressions, which are approximately 1  $\mu$ m in diameter, were also observed (Fig. 2F). Figure 5A, B shows SEM micrographs of several cuticular pits on the minor chela of *Pagurus samuelis* (Stimpson), a closely related species. These pits are similar in size, distribution, and morphology to those found on *P. hirsutiusculus*. In-

terestingly, some of them possess a minute, central, stalklike projection situated within two concentric outer sleeves (Fig. 5A). These minute stalklike projections, however, were not commonly observed and were present on *P. samuelis* only. Because of the difficulties inherent in sectioning heavily calcified cuticle, only a few pits from *P. hirsutiusculus* have been examined. No obvious pattern of neuronal innervation was observed (Mesce, 1983; personal observation).

These pits may be the orifices of nonsensory dermal glands or the bases of broken microsetae. However, in other studies where similar pitlike structures have been observed, it has been mentioned that they



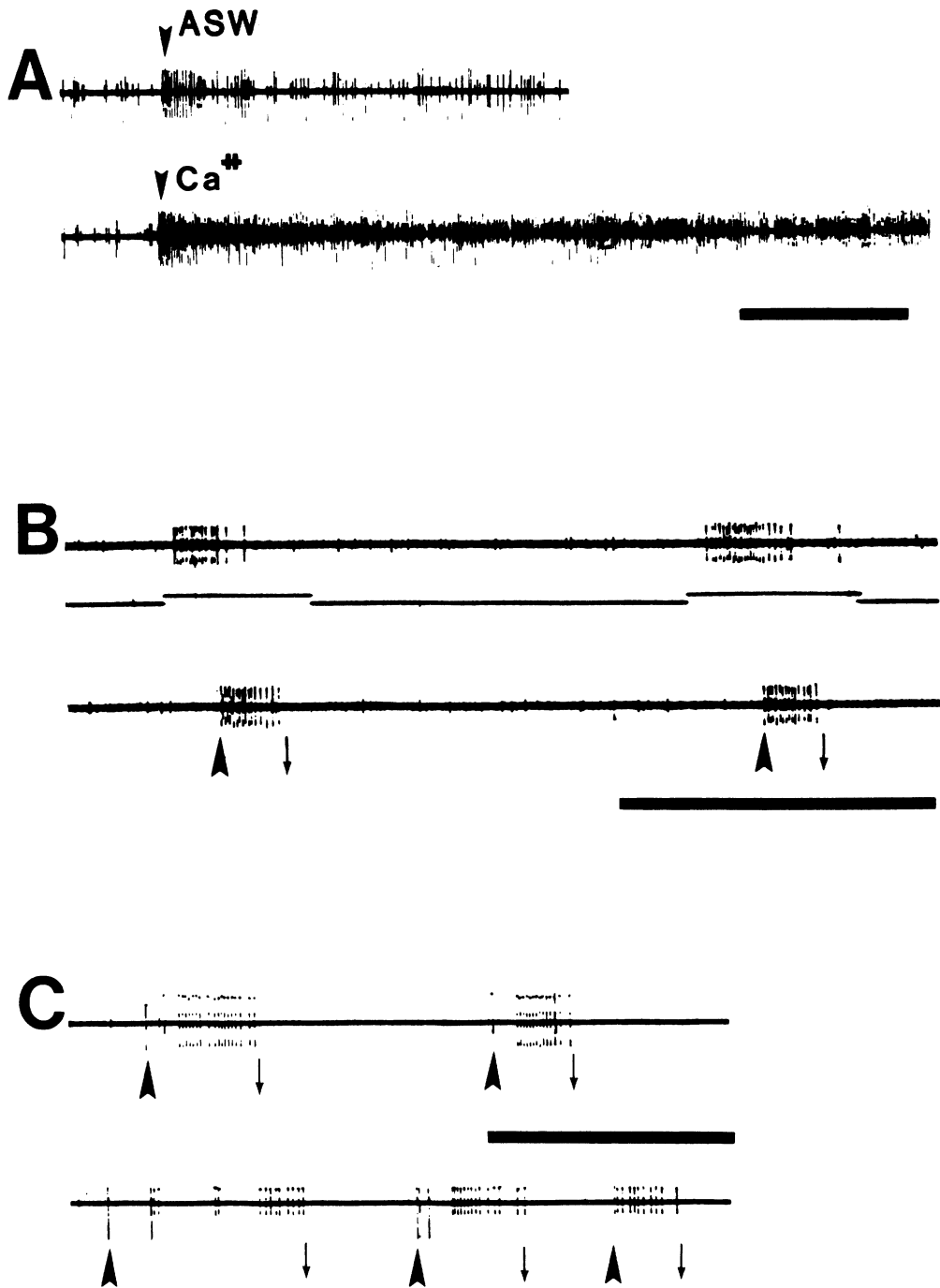


Fig. 6. Mechano- and chemosensory activity in *Pagurus hirsutiussculus* recorded extracellularly from the sensory fibers of the chelar simple setae (A, B) and mechanosensory activity recorded from the teeth (C). A. Upper trace: multi-unit mechanosensitive activity in response to a drop of ASW (control solution) directed over the simple setae. Lower trace: multi-unit mechano- and chemosensory activity in response to a 25 mM calcium solution. Note the increase in small amplitude units that are present for seconds after the initial stimulus application. B. Mechanosensory responses of one or possibly two units innervating a single simple sensillum. A manual stimulus marker was used as a qualitative indicator when a cluster of setae was gently brushed with a glass probe. The arrows more precisely mark the onset and offset of setal displacement. C. Extracellular recordings of mechanosensory neurons innervating the chelar teeth. Activity patterns of two mechanosensitive units in response to the pressure of a glass rod moved over the dactyl biting edge. Arrows mark the onset and offset of mechanical stimulation. Time scale = 1.0 s.

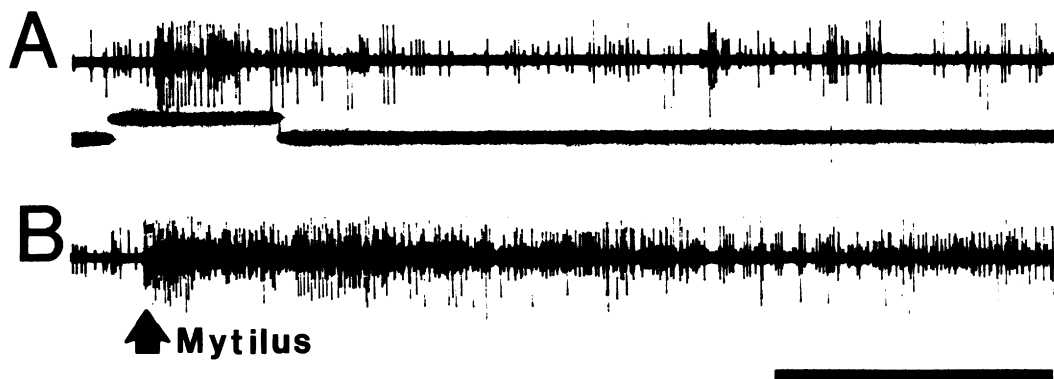


Fig. 7. Upper trace shows the response to a control ASW application. Chemosensory neurons from the simple setae of *Pagurus hirsutiusculus* are shown firing in response to two drops of extract of *Mytilus* (lower trace). Note the increase in the background level of small amplitude units in the lower trace. Time scale = 1.0 s.

might be campaniform sensilla (Shelton and Laverack, 1968; Laverack, 1976; Derby, 1982). Therefore, structures on the antennules of *Pagurus*, which greatly resemble identified insect campaniform sensilla, were compared to the pits of *Pagurus*. These campaniformlike structures, as judged by their distinctive morphology, were found only on the lateral aspects of antennular flagella of *Pagurus* (Fig. 5C, D), and resembled those found on other crustacean antennules (Snow, 1974; Laverack, 1976). These sensilla, which possess a smooth dome and collar (with no evidence of any slitlike structure on the dome), greatly differ in appearance from the cuticular pits, suggesting that the pits and campaniformlike structures may not share similar functions.

#### Electrophysiological Studies of Chelar Sensory Structures

**Mechanosensory Responses.**—Two types of cuticular structures were found to be mechanosensory: the chelar simple setae (because these structures are innervated, I can now refer to them as sensilla) and the chelar teeth. Multi-unit mechanosensory activity was observed when a small drop of ASW was placed on a cluster of simple sensilla (Fig. 6A). Single unit mechanosensory activity was also obtained from the simple sensilla. Several simple sensilla from a particular cluster, on the ventral surface of the fixed finger, were gently deflected by the movement of a fine glass rod. Figure 6B

shows the mechanosensory activity from a single cell innervating one simple sensillum. Note the short firing duration of the mechanosensitive units.

Figure 6C shows the activity of two mechanosensory cells belonging to either one or two chelar teeth in response to the pressure of a glass probe which was localized to a small number of teeth. These units are of relatively large amplitude, and fire only for the duration of stimulation.

**Chemosensory Responses.**—In response to chemical stimulation, multi-unit extracellular recordings were obtained from sensory fibers innervating the simple sensilla. Extract of *Mytilus* was used as a general excitatory stimulus, since it evokes a robust feeding response in the intact animal. Figure 7 shows a recording of multi-unit activity from simple sensilla in response to a mechanical stimulus (a single drop of ASW, top trace) and to the combined mechanical and chemical stimulation of a drop of extract of *Mytilus* (lower trace). Note that the addition of extract of *Mytilus* caused a significant increase in the firing of small amplitude units which persisted long after mechanical displacement had ceased. In addition to the filtered extract of *Mytilus*, sensory cells from the simple sensilla were responsive to 25-mM calcium solutions. Figures 6A and 8A show typical multi-unit responses to a 25-mM calcium solution (lower trace) and to drops of control solutions (ASW). Chemosensory responses to calcium solutions containing less than 25-

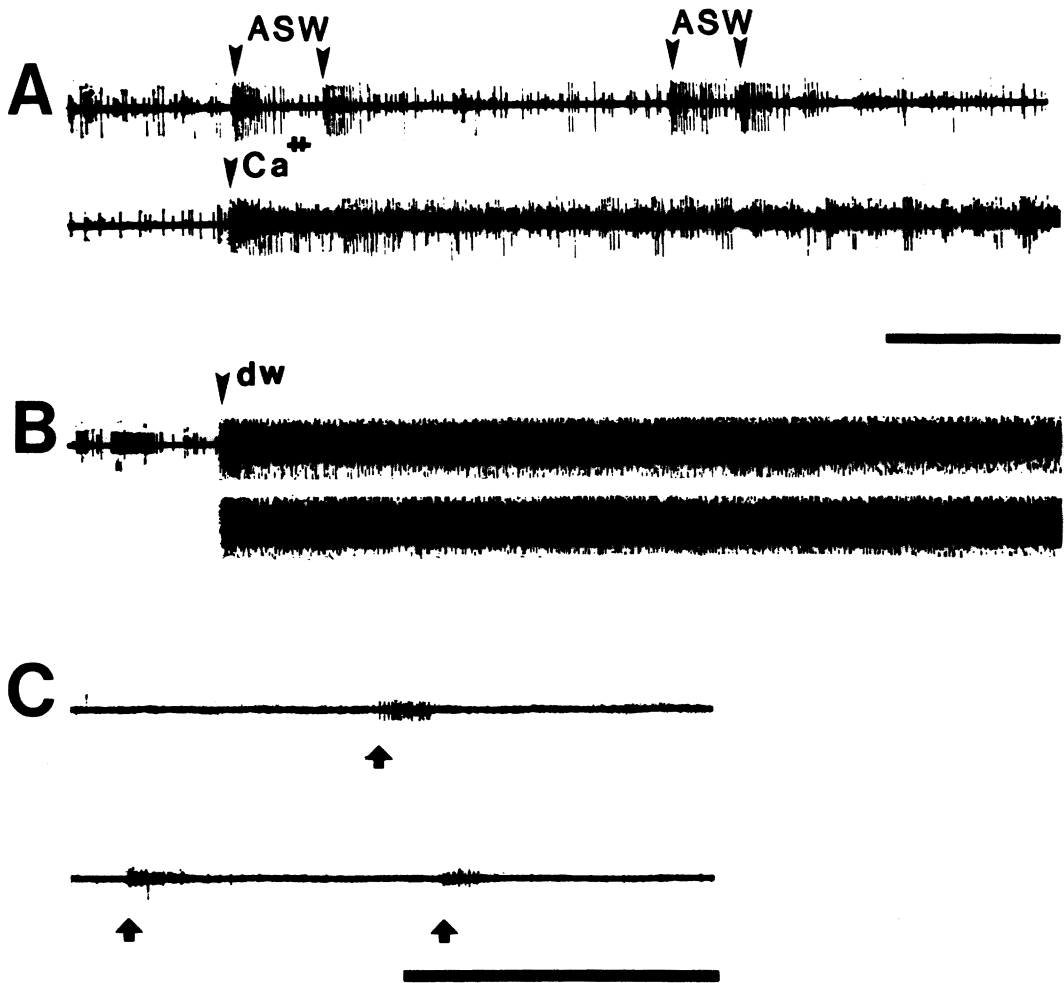


Fig. 8. Multi-unit sensory activity in response to mechanical, chemical, and osmotic stimulation of the simple setae in *Pagurus hirsutiusculus*. A. Upper trace: mechanosensitive units responding to multiple drops of a control ASW application. Lower trace: chemosensory responses to a 25 mM calcium solution. Note the large amount of background activity in both traces. B. The same preparation treated with 1 or 2 drops of distilled water. Resulting spike amplitudes were so great that recorded activity went off scale. Activity was still present 23 s after the initial application. C. Mechanosensory activity recorded from the simple setae, in a different preparation from above, after distilled water application. Only mechanosensory responses, not chemosensory ones, could be elicited after distilled water treatment. Time scale = 1 s.

mM calcium (2.5 times the calcium concentration found in ASW) could not be reliably elicited within and between preparations. The inability to record single-unit chemosensory activity from the simple sensilla made it difficult to ascertain whether higher calcium concentrations caused an increase in the number and frequency of particular calcium sensitive units. Chemosensory responses to presentations of ASW with 150-mM magnesium (a 3-fold increase above ASW) were not observed.

Since each bimodal sensillum is innervated by neurons that are mechanosensitive

in addition to those that are chemosensitive, it was not possible to record chemosensory activity without concomitant mechanosensory activity. In most preparations, however, purely mechanosensory activity could be obtained when chemosensory activity was eliminated with the application of distilled water to the simple setal clusters. Such a dramatic osmotic change of 980 mOSM/kg resulted in a barrage of long-lasting, high frequency, high amplitude injury discharges (Fig. 8B), similar to those observed by Laverack (1975), and Derby and Atema (1982) in *Homarus*. After

such treatment, negligible chemoreceptive activity could be evoked while mechanosensitive activity was elicited (Fig. 8C). The effect of distilled water on chemosensory activity was specific to the chelar simple sensilla and had no effect on neural activity from the chelar teeth.

#### DISCUSSION

The ability of hermit crabs to locate and select a particular gastropod shell for habitation is dependent on the detection and assessment of multiple shell features, involving a number of sensory mechanisms (for review see Elwood and Neil, 1992). In addition to visual, tactile, and proprioceptive stimuli contributing to shell-selection behavior, chemical cues convey important information about the location and type of shells possibly suitable for habitation.

Contact calcium reception enables hermit crabs to distinguish shells from pebbles and other objects (Mesce, 1982). This chemosensitivity also enables hermit crabs to detect partially buried shells (Mesce, in press). Distance chemoreception, as well, is important for shell detection. Peptide cues released from dead or dying gastropods attract hermit crabs to sources of newly available shells (McLean, 1974; Rittschof, 1980). Such chemical signals are highly specific, in that particular hermit crab species are attracted to the flesh of some gastropod species but not others (Rittschof, 1980; Gilchrist, 1984). Predacious gastropods often contribute to the mortality of particular prey gastropod species (Wilber and Herrnkind, 1984), and such predator-prey interactions may contribute to the specificity of peptide attractants released from gastropod muscle (Rittschof *et al.*, 1990; Kratt and Rittschof, 1991). Although hermit crabs possess a myriad of chemosensory organs, including antennal sensilla and antennular aesthetascs, the chelar chemosensory structures described in this study, sensitive to calcium, should also be considered as candidates mediating the detection of peptides involved in shell-acquisition behavior.

#### Morphological and Functional Characteristics of Chelar Sensory Structures

The minor chela of *Pagurus* is relatively free of the elaborate types of cuticular struc-

tures found on other crustacean appendages and in other species (Drach and Jacques, 1979; Derby, 1982; Solon and Cobb, 1980; Hamilton, 1983a; Laverack and Barrientos, 1985). Since the numerous tufts of setae on the chela consist of a homogeneous collection of just one setal type (simple setae), the electrophysiological characterization of their mechano- and chemosensory bimodal function was greatly facilitated.

One of the most striking features of the minor chela of *P. hirsutiusculus* is its densely setaceous nature. These clusters of simple sensilla, because of their sheer number and placement, are apparently the most important chelar structures for detecting the chemical and tactile nature of a gastropod shell. As the chela glides over the shell surface during the exploration of a shell for possible habitation, individual simple sensilla are in a position to monitor simultaneously the presence of a hard object and its chemical composition.

The overall pattern of setal investiture, including the setal tufts and chelar teeth, is very similar to that of the second pereopod chelae of the lobster *Homarus* (see Derby, 1982), and of the crayfish *Austropotamobius* (see Altner *et al.*, 1983). The external morphology of the chelar setae is basically of the simple or smooth type as described for setae in other crustaceans (Thomas, 1970; Farmer, 1974; Factor, 1978; Derby, 1982). Setae of *Pagurus*, however, are more elaborate and are marked by minute cuticular ridges and setules at their distal regions, structures that have not been previously observed (Factor, 1978). Derby (1982) reported that some of the chelar smooth setae in *Homarus* have "hair lines suggestive of the borders of scales closely pressed to the setal shaft." He suggested that this type of seta might be an immature squamous setal type. However, squamous or squamouslike setae were not observed on the chelae of *Pagurus*. It remains to be demonstrated whether smooth (simple) setae from other crustacean species also possess cuticular complexities apparent at higher levels of EM magnification, and what their functional significance is.

The single annulus around each seta reported here has also been observed on setae from a variety of decapod crustaceans (Thomas, 1970; Derby, 1982; Hamilton,

1983a). Most likely, the presence of this annulation is a consequence of setagenesis and marks the region where the newly formed seta becomes everted at each molt (Reaka, 1975; Drach and Tchernigovtzeff, 1967, as cited in Hamilton, 1983a). Unlike insect sensilla, the terminal pore found on the simple seta appears to be unnecessary for chemical transmission (Mesce, 1983). Instead, chemical stimuli may pass through a series of minute channels found to transect the relatively thick cuticle of the sensillum, and the terminal pore may be merely a molting pore (Mesce, 1983; Hamilton *et al.*, 1985).

Only a few studies of crustaceans have examined the electrophysiological responses of cuticular sensilla to both chemical and mechanical stimulation. As a result, the number of functionally identified bifunctional sensilla from various crustacean species is small. To date, bifunctional sensilla include the stout, squat setae in the lobster *Homarus* (see Shelton and Laverack, 1970; Derby and Atema, 1982), and the crayfish *Austropotamobius* (see Altner *et al.*, 1983); the antennal setae in the terrestrial isopod *Hemilepistus* (see Seelinger, 1977, 1983); the smooth and squamous (Derby and Atema, 1982), and serrate setae (Shelton and Laverack, 1970) in *Homarus*. The smooth setae on the claw of *Austropotamobius* were also found to be bifunctional (Hatt and Bauer, 1980). Typically, bifunctional sensilla contain neurons that are unimodal and are sensitive to either mechanical or chemical stimulation. In one study by Hatt (1986), however, individual sensory neurons (from the crayfish smooth setae) were demonstrated to respond to both chemical and mechanical stimulation.

The chelar simple setae of *P. hirsutiusculus*, as a class, were shown in this study to be bifunctional chemo- and mechanosensory sensilla. By visual inspection of which simple setae were mechanically displaced, mechanosensory activity was clearly associated with particular setal clusters. Subsequent chemical stimulation, directed specifically to these setal clusters, indicated that they were chemosensitive as well. Although the functions of individual simple sensilla were not determined, an ultrastructural examination of numerous simple sensilla (within and between setal clusters on the minor chela) revealed that they were

structurally homogeneous, containing between 20–23 unbranched dendrites (Mesce, 1983; Mesce, in preparation). Ultrastructural examinations of the simple (Mesce, 1983; Mesce, in preparation) and stout setae (Altner *et al.*, 1983), provide two studies whereby structural features have been examined in functionally identified, bifunctional sensilla. Such studies provide clues to the structural bases underlying mechano- and chemoreceptor function in crustaceans.

In this study, the mechanosensitive chelar teeth are the only other cuticular structures that can be assigned a definite sensory function. The morphology and placement of the teeth on the dactyl cutting edge are very similar to those of the bifunctional, stout setae found elsewhere (squat setae, Shelton and Laverack, 1970; hedgehog setae, Derby and Atema, 1982; and fringed setae, Altner *et al.*, 1983). Although chemosensory activity was not observed in the teeth, they may function as bimodal mechano- and chemoreceptors. Both the crayfish fringed setae, and the lobster hedgehog setae, require relatively strong mechanical stimulation for activation as do the teeth. It is thought that these structures provide only rough tactile information, probably concerning the presence of food materials when the food is pierced by the closing chelae (Derby, 1982; Derby and Atema, 1982; Altner *et al.*, 1983).

Mechanoreceptor activity obtained by Altner *et al.* (1983) from the fringed setae resembles the activity patterns reported here for the chelar teeth. In particular, two units, which Altner *et al.* (1983) suggested belong to the same sensillum, usually fire together. Their physiological data correlate well with their ultrastructural evidence which suggests that each fringed seta contains two mechanosensitive cells in addition to four chemosensitive cells, and two cells whose functions remain unclear. Whether an analogous situation exists for the teeth of *Pagurus* awaits further electrophysiological and ultrastructural analysis.

The cuticular tubercles are nonsensory structures whose functions remain unknown (Mesce, 1983). The function of the pits remains equivocal. The cuticular pits most likely are orifices for dermal (tegumentary) glands, which aid in the formation and maintenance of the cuticle. Both types

of pitlike structures, those 5–7  $\mu\text{m}$  and 1  $\mu\text{m}$  in diameter, are similar in size and form to the types of dermal gland orifices found in the insect *Tenebrio* (Delachambre, 1973). The larger depressions also resemble the dermal gland orifices located on the carapace of the crab *Carcinus* (see Bocquet *et al.*, 1976) and the shrimp *Atya* (see Felgenhauer and Abele, 1983).

The functional identity of the pits, however, still remains a curiosity, especially with the observation that the pits of *P. samuelis* contain a minute central rodlike projection, a structure that has not been previously observed in any gland orifices. Such projections, however, may be easily disrupted during fixation procedures and therefore have not been previously observed. If they are broken setae, they may represent structures similar to microsetae and the mechanosensitive peg sensilla (Drach and Jacques, 1979; Solon and Cobb, 1980; Derby, 1982). The diameter of the central rod or peg of the sensilla described previously, however, is an order of magnitude larger than the minute central stalk observed within each pit. In addition, peg sensilla do not possess the inner and outer sleeves observed within the pits.

Campaniformlike structures were found on the antennular regions of *Pagurus*. The campaniformlike structures in the lobster, similar in morphology to those studied here and also located on the antennular regions, have been found to be nonsensory dermal glands (Gnatzy, 1984). However, the lobster structures, unlike those in this study, contained a discernible slitlike opening on the surface dome. No such structure was observed on the campaniformlike structures studied here. Presently, no ultrastructural or electrophysiological studies are available to assess whether campaniform sensilla, similar to those in insects, exist in any crustacean species (Gnatzy, 1984; Gnatzy *et al.*, 1984; Schmidt and Gnatzy, 1984). Campaniformlike structures of *Pagurus*, however, appear to be likely candidates and deserve further examination.

#### Ion Sensitivity

Most investigations of ion sensitivity have focused on organisms living in fresh-water environments (Grant and Mackie, 1974; Hara, 1982). Most relevant to the work pre-

sented here are the behavioral and electrophysiological experiments conducted on the olfactory system of the sockeye salmon *Oncorhynchus nerka* (see Bodznick, 1978a–c). Salmon were sensitive to calcium concentrations in the range of 0.001–10 mM, and behavioral tests demonstrated that salmon can discriminate between two lake waters differing in calcium concentration by 0.33 mM. Since lake water can contain between 0.1 and 1.0 mM calcium, this study also demonstrates that aquatic organisms are sensitive to chemical signals (calcium ions) in especially noisy chemical environments (Derby and Atema, 1988).

Although not an aquatic crustacean, Seelinger (1977, 1983) found that the antennae of the terrestrial isopod *Hemilepistus reaumuri* were sensitive to calcium solutions in the range of 10–100 mM. For two sensory cells,  $\text{CaCl}_2$  solutions elicited 60–90 impulses/s that lasted up to 5 s. Calcium sensitive cells responded specifically to salts and best to calcium solutions.  $\text{KCl}$ ,  $\text{MgCl}_2$ , and  $\text{SrCl}_2$  were found to cause an order-of-magnitude less robust responses. Other compounds including amines, sugars, and amino acids were not effective stimuli. A similar type of single unit analysis will be required before a complete assessment of the calcium specificity of the chelar simple setae in *Pagurus* can be established. Understanding the actions of external calcium and other ions on the production of chemosensory responses may yield new insights into the mechanisms of chemosensory transduction (Hamilton and Case, 1983; Schmiedel-Jakob *et al.*, 1990).

#### Behavioral Adaptations for Chemosensitivity

The concentrations of calcium ions that the simple setae encounter at the surface of natural or plaster shells are not known. Detection of small amounts of shell surface calcium, in a sea-water background of 10 mM calcium, demonstrates the ability of the hermit crab's peripheral and/or central nervous system to detect a calcium signal above background chemical noise. It is possible that the concomitant activities of the chemo- and mechanosensitive neurons in each simple sensillum act on central neurons to enhance the detection of calcium ions at the surfaces of objects, especially if

they are at low concentrations. Interestingly, Hazlett (1971) has shown that hermit crab behaviors associated with feeding are elicited at lower stimulus concentrations with concomitant tactile stimulation.

Chemical signal amplification may also involve the movement of appendages across the shell surface. Such actions may act to splay apart the chemosensitive setae within dense tufts and expose them to chemical stimulation. In addition, when the hermit crab opens its minor chela, as it does when the shell surface is examined, a new set of setal clusters may become exposed to chemical cues. Derby and Atema (1982) pointed out that antennular flicking (Snow, 1973; Schmitt and Ache, 1979; Daniel and Derby, 1991) and possibly leg waving (Hazlett, 1971) are examples of behaviors whose functions may enable chemosensory setae in dense rows or tufts to obtain greater access to chemical stimulation. Stimulus thresholds in newly exposed setae may be lower (review in Ache, 1982), subsequently aiding in the earlier detection of distantly located food sources or the detection of important chemical constituents (such as shell chemical content) that are found in low concentrations.

One last consideration is whether other crustaceans have a similar sensitivity to calcium salts. Indirect evidence, based on observations that crustaceans often feed on their calcium-enriched exuviae, suggests that they might have such sensitivity. It has long been known that crabs and lobsters feed on their molted exoskeletons after ecdysis. It has been assumed that eating the exoskeleton supplies the newly ecdysed animal with calcium salts which are used to harden the new exoskeleton (Schmitt, 1985; Bliss, 1982). In addition, J. Atema and coworkers (personal communication) have observed that lobsters prior to ecdysis will specifically feed on calcium-containing objects, such as shells and pieces of cement. No previous reports, however, have addressed the question of whether crustaceans use their chemical senses to identify calcium-containing objects.

If calcium salts act as feeding stimuli, perhaps the ability of some hermit crabs to recognize shells as such, based on their calcium content, has evolved from a prior sensitivity to calcium as a feeding stimulus.

Future investigations of calcium sensitivity are needed to ascertain the kinds of animals and the varieties of yet unknown and adaptively significant behavioral responses that calcium salts evoke.

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Address: Department of Entomology and Graduate Program in Neuroscience, University of Minnesota, 219 Hodson Hall, St. Paul, Minnesota 55108, U.S.A.