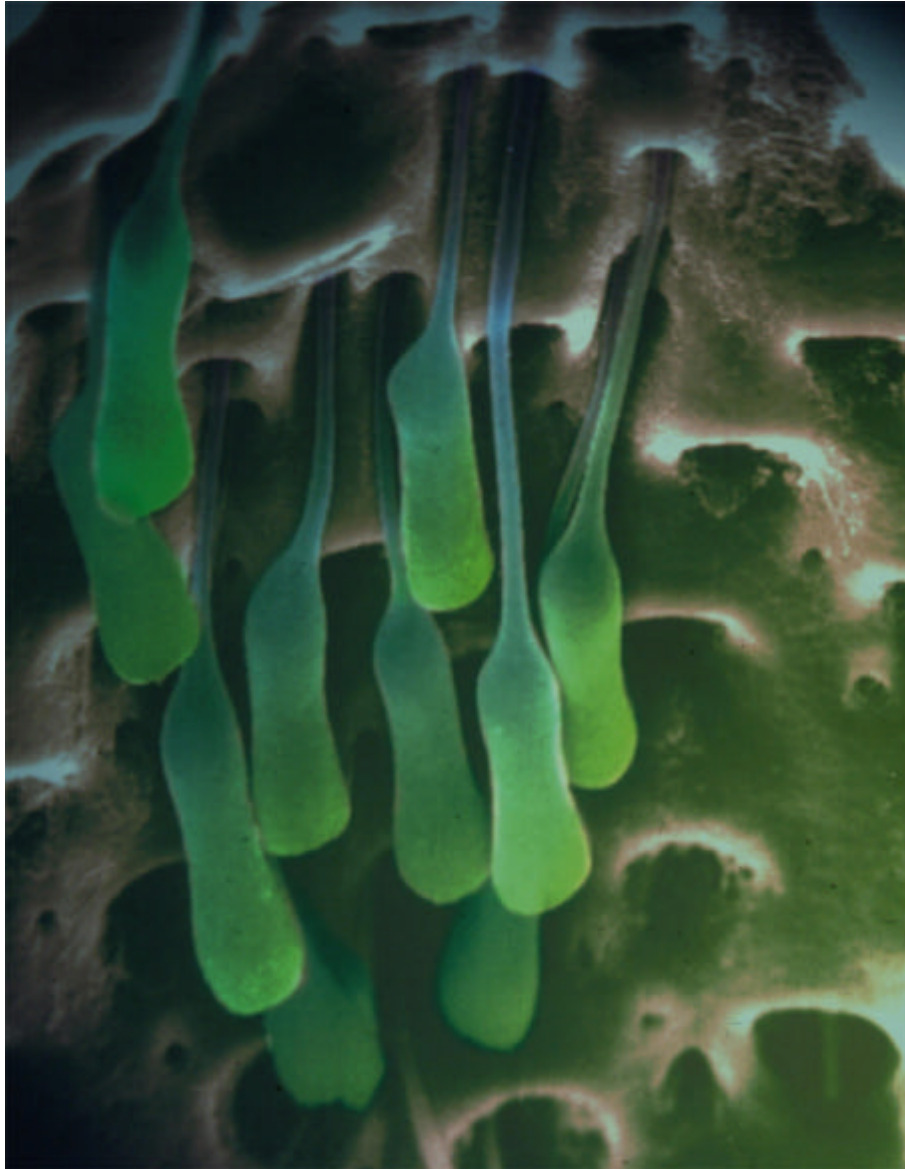


IV. MORPHOLOGY AND ULTRASTRUCTURE OF THE SENSILLUM RESPONSIBLE FOR CO₂ PERCEPTION



Scanning electron micrograph of the sensilla amullacea.
View of the inside of the antenna, tissue removed.

Introduction

In insects the sensory organs (sensilla) for smell, taste and mechanical stimuli are cuticular structures with associated neurons for stimulus perception. Chemosensory sensilla are found in a tremendous variety of types. Most sensilla are hair shaped, but pore plates and 'peg in pit' sensilla have also been described. The general bauplan of chemosensory sensilla is consistent for all insects. The sensilla are innervated by dendrites of bipolar primary receptor cells (sensory neurons). These dendrites can be simple in structure or lamellated, branched or invaginated. Different sheath cells envelope the soma regions of the neurons. The innermost sheath cell (thecogen cell) often forms a dendritic sheath around the outer dendritic segment and partially around the dendrites (Keil 1997). The outer sheath cells (trichogen and tormogen cells) are often characterized by a strongly folded apical membrane. Together with the cuticle of the hair shaft, the sheath cells enclose a separated cavity containing the sensillum lymph.

Tight contacts (septate junctions) between the sheath cells, the neurons and the base of the hair shaft cuticle form a barrier which is only penetrated by the dendrites. The outer sheath cells serve two functions: Firstly, they isolate the compartment for stimulus perception from the haemolymph. Secondly, they accumulate potassium in the sensillum lymph, generating a transepithelial potential which is supposed to support the excitability of the neurons (Thurm and Küppers 1980; De Kramer 1985).

Sensilla are classified by two typologies, mainly for historical reasons, and both are still used complementary to each other.

The first typology, introduced by Schenk almost 100 years ago is based on the external morphology of sensilla (Schenk 1903). Characters of cuticular structures which can be investigated by light microscopy, or nowadays by scanning electron microscopy (SEM) are used. Terms to distinguish the sensilla according e.g. the shape of the hair shaft are chaeticum, trichodeum, basiconicum etc. This typology has been used mainly for comparative morphology and phylogeny in several hymenopteran orders including the Formicidea (Prelinger 1940; Jaisson 1969; Walther 1979; Hashimoto 1990; Hashimoto 1992).

The second typology was proposed by Altner and is based on ultrastructural characters. Especially those structural characters of the sensilla are considered which can be expected to have functional relevance (Altner 1977). Unfortunately such characters can be investigated only using transmission electron microscopy (TEM). Discrimination is based on cuticular structures of the hair shaft such as the bauplan of the wall (single or double wall) and, if any, type of channels or pores (pore tubules or spoke channels). Equally important are cytological characters like dendritic features (branched, lamellated), expansion of the dendritic sheath, the lymph cavities and auxiliary cells.

It is tempting to think that the variety of sensilla types reflects differences in perception mechanisms. However, even though the number of ultrastructural investigations on sensilla has rapidly increased during the last 20 years and many different characters have been described, only few characters have been found to always occur in combination with a particular sensory modality. For instance, the wall of sensilla associated with olfactorial receptor cells is invariably multiporous (Zacharuk 1980; Steinbrecht 1997). Other characters occur frequently, but not exclusively with a certain modality e.g. a lamellated dendrite in temperature sensitive

neurons or a poreless wall and inflexible socket in sensilla for humidity perception (Altner and Loftus 1985; Tichy and Loftus 1996; Steinbrecht 1998). One sensillum often houses several sensory neurons responding to different stimuli. Thus, it is often hard to assign a certain character to a particular stimulus.

Perception of CO₂ is of particular interest for a better understanding of odor perception and adaptation of sensillar structures. Compared to most other odor molecules, CO₂ is a simple molecule which naturally occurs in high concentrations. This difference to larger and less abundant odor molecules is expected to be reflected in the morphology and ultrastructure of the CO₂ sensillum.

Numerous behavioral studies have revealed that perception of carbon dioxide is a widespread capacity in insects. Only in a few cases, however, have the sensilla responsible for CO₂ perception been identified.

They have been found on the maxillary palps of mosquitoes (Diptera) (Kellogg 1970; Sutcliffe 1994; Grant *et al.* 1995) and on the labial palps of different butterfly species (Lepidoptera) (Bogner *et al.* 1986; Bogner 1990; Stange 1992; Stange *et al.* 1995). In termites (Isoptera) a modulatory effect of CO₂ on pheromone receptor cells located on the antenna has been described (Wicklein *et al.* 1991; Kaib *et al.* 1993).

In Hymenoptera the neural activity of CO₂ receptor cells on the antenna was first described for the honey bee (*Apis mellifera*) by Lacher in 1964 (Lacher 1964). Unfortunately the kind of sensillum could not be identified but from the technique used (insertion of the electrode beside a small opening in the antenna) three different kinds of sensilla could be taken into consideration: sensillum ampullaceum, sensillum coeloconicum or sensillum coelocapitulum. These sensilla are located below the surface of the antenna and connect to the outside only with a small opening of about 1-2 µm. The sensillum coelocapitular was later identified to be responsible for humidity perception and no response to CO₂ was found (Yokohari *et al.* 1982). In ants the first extracellular recordings of CO₂ receptor cells were made by Dumpert who assumed that the cells he recorded from were located in the sensilla coeloconica (Dumpert 1972). Later, without further explanation he proposed the sensilla ampullacea for CO₂ perception (Dumpert 1978).

Based on these early studies, which are the only reports of CO₂ receptors in Hymenoptera, the sensilla coeloconica and ampullacea are the most likely candidates for CO₂ perception. Both sensilla types were already described by Forel in 1884 (Forel 1884). Regarding the function of one of them, the sensilla ampullacea he later mentioned:

‘Übrigens gestehe ich, dass man allerdings nur schwer versteht, wie Organe als Sinnesorgane funktionieren können, die in die Tiefe versenkt sind und mit der Aussenluft nur durch ein dünnes Luftfädchen in Verbindung stehen. Wenn ihre Funktion also eine sensorielle sein sollte, so ist doch sicher, dass sie weder in die Sphäre des Geruchs noch des Geschmacks fällt’ (Forel 1910).

The aim of the present study was to a) identify the sensillum responsible for CO₂ perception in leaf-cutting ants, b) investigate the morphology and ultrastructure of the sensillum in order to enable a comparison with other sensilla responsible for CO₂ perception and c) provide more information about sensillum characters and their functional relevance.

Methods

Workers were obtained from the same colony described in chapter three. For the experiments, workers were collected from the feeding site, thus it is assumed that only foragers were investigated.

Comparative morphology

Allometry between a sensillar character and body size was investigated using the size polymorphism of these workers. The mean size of the character (length of the duct of sensilla ampullacea) obtained from at least four sensilla in each individual (n=14) was used for correlation with body size. Body size was used as independent variable in the Pearson product moment correlation (Sokal and Rohlf 1995). For four of the 14 workers the duct length of 7-8 sensilla of each individual was measured and used in order to describe the variability of this character.

Workers were compared with males from a colony of *Atta sexdens* which has been collected by N. Weber in Guayana (1970). The males hatched in the laboratory in 1982 (E. Wilson's lab, No 470) and have been stored in 70% alcohol. In four males 4 to 7 sensilla could be investigated respectively and, like in workers, the individual means were used for the T-test.

External morphology

The external morphology of sensilla coeloconica and sensilla ampullacea was investigated with SEM and light microscopy.

For SEM the flagellum from CO₂ anaesthetized ants was excised and the last flagellar segment was sectioned oblique with a razor blade. These tip-fragments were cleaned with KOH-solution, sonicated in order to remove cell particles, dehydrated in a graded ethanol series and glued vertically on the dishes for the SEM in order to allow an investigation of the inner and outer side of the antenna. After critical point drying in a Bal-Tec CPD030 unit and gold coating in a Balzers Union MED010 sputter unit the preparations were examined with a Zeiss DSM962.

For light microscopy of semi-thin sections the last three segments of the flagellum of CO₂ anaesthetized ants were excised and immediately fixated with 5% glutaraldehyde in 0.1M posphat buffer for 1 hour at 4°C. After dehydration in a graded ethanol series the flagellar segments were embedded through propylene oxide in Durcupan ACM (Fluka, Buchs, Switzerland) and sectioned with a diamond knife on a microtom (RMC; MT-7000 Ultra).

Identification of the CO₂ sensilla

Using a fluorescent dye different sensilla were stained in intact animals. A glass electrode with a tip of about 0.5 µm was filled with 1 µl KCl (0.15M) containing the fluorescent dye, a labeled dextran (MW 3000 with texas red, Sigma). The remaining volume of the electrode was filled with 0.15 M KCl and mounted on a micromanipulator (Märzhäuser HS-6).

The electrode was superficially inserted under optic control with a microscope (equipped with

a Leitz objective, NPL-Fluotar L25/0.35) in the cuticle beside a visible opening of a sensillum coeloconicum or ampullaceum. The neural activity to a CO₂ stimulus was recorded (see next section) and the sensillum was simultaneously stained for 30 min. Only sensilla in the last flagellar segment were investigated. Afterwards, the last two segments were cut off with a razor blade and the same fixation described above was used. The semi-thin sections then were screened with a invert microscope (Zeiss, Axiovert 405M) for regions with the fluorescent dye.

Ultrastructure

For TEM investigations the flagellum of CO₂ anaesthetized ants was excised at the last segment, immediately immersed in Karnovsky's fixative with 2% Acrolein and left for 3 hours at 4°C. The flagellar segment was then washed overnight in cacodylate buffer, dehydrated in graded ethanol series, block stained with 1% uranyl acetate in 95% ethanol solution for 1 hour and finally embedded through propylene oxide in Durcupan ACM (Fluka, Buchs, Switzerland). Thin sections obtained by a ultramicrotome (L.K.B. Nova), sequentially stained with uranyl acetate and lead citrate were examined using a Philips TEM (EM 400T).

Results

Morphology of the sensillum coeloconicum and sensillum ampullaceum

Sensilla coeloconica and ampullacea belong to the 'peg in pit' sensilla and connect to the outside via a small opening. These openings (external pores) with a diameter of about 1-2 µm were found in a distinct area, on the ipsilateral side at the tip of the antenna (Fig.4.1a). SEM investigation revealed that the two types cannot be distinguished by characters at the surface of the antenna. Although there are variations, e.g. in rim structures around the external pores, transitions exist and no assignment to one sensillum type is possible (Fig.4.1b, c).

Differences in cuticular structures between both sensilla types can easily be found at the inside of the antenna. After removal of the tissue the sensilla ampullacea with their characteristic long and narrow duct, terminating in an ampulla at the base become visible (Fig.4.2b). The duct connects the ampulla with a rounded pit in the antennomere cuticle (Fig.4.3a). In contrast, the sensilla coeloconica do not have a duct and are embedded almost completely within the thick antennomere cuticle (Fig.4.2a).

The innervated pegs of the sensilla coeloconica are about 5 µm in length and have thick rim structures. In both sensilla types the pit has a diameter of about 5 µm. The pit, and in the sensilla ampullacea also the duct and the ampulla is filled with air. The sensilla ampullacea are embedded in the tissue below the antennomere cuticle (Fig.4.2c). From the pit in the antennomere cuticle a duct with about 1 µm in diameter expands into a 4 µm wide ampulla. In the sensilla ampullacea the peg with about 20 µm length is considerably longer than the peg of the sensilla coeloconica and almost as long as the whole ampulla (Fig.4.3b).

At the tip of the antenna the sensilla coeloconica are located distally from the group of sensilla ampullacea. About 10 sensilla coeloconica and a similar number of sensilla ampullacea are located in the last antennomere. In each of all the other flagellar segments only 0 to 3 sensilla of both types can be found.

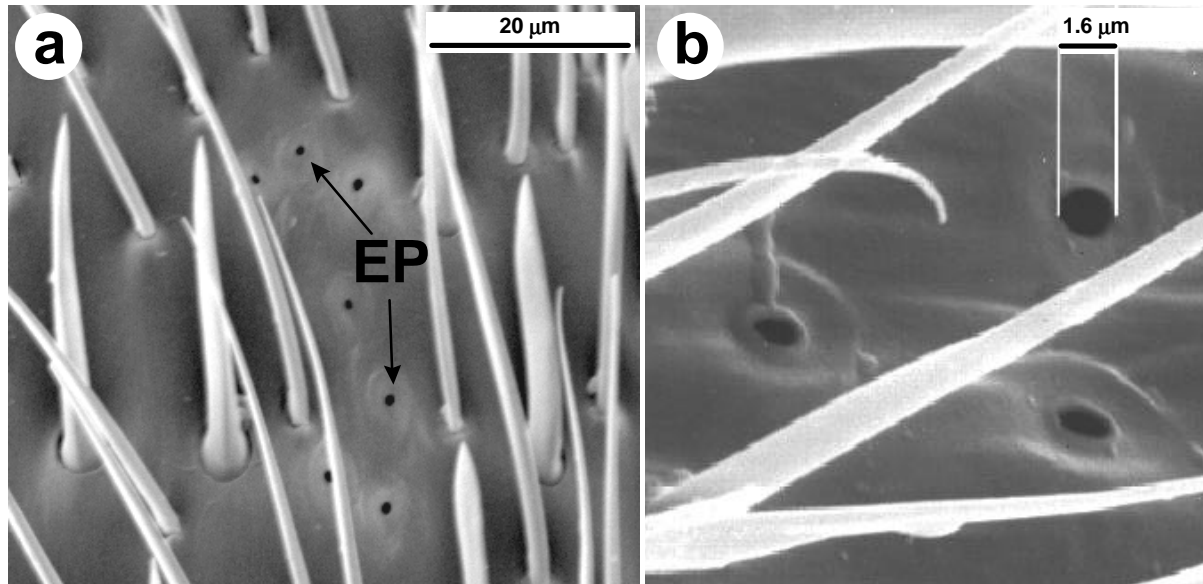


Fig.4.1 SEM photomicrographs of the apical antennomere with external pores (EP). Overview of the ipsilateral side of the antennomere a), and external pores with different rim structures b).

Ultrastructure of the sensillum ampullaceum

The surface of the peg has a constant number of about 20 finger-like ridges. These cuticular fingers taper from the base to the tip and are separated by furrows (Fig.4.4b-f). At the distal part of the peg the sensillar wall is reduced to less than $0.3\ \mu\text{m}$ and the profile of the fingers is mushroom-like. In the furrows separating the cuticular fingers, pores are present which enlarge into the cuticular wall (Fig.4.4b). At the base of the peg the walls of neighboring fingers are fused and intramural cavities are found. Here, the peg wall is thick ($\sim 1\ \mu\text{m}$) and encircles a sheath cell (Fig.4.4f).

Only a single sensory neuron innervates each peg and its soma is noticeably larger than that of other sensory neurons of surrounding sensilla (Fig.4.5e). The unbranched outer dendritic segment reaches the middle of the peg where first branches are visible (Fig.4.4d, e). At the tip the number of branches increases to more than 100 filling the shaft lumen completely (Fig.4.4c, 4.3c).

An undetermined number of sheath cells are wrapped around the soma of the sensory neuron (Fig.4.5d). The innermost sheath cell (thecogen cell) is lamellated and forms the dendritic sheath which is conspicuously thick at the base of the peg where it encircles the unbranched outer dendritic segment (Fig.4.5a-c). From thereon to the tip the dendritic sheath comes close to the cuticular fingers. Thus, in the distal part of the tip the furrows and the dendritic sheath are in close vicinity of each other (Fig.4.4c). The dendritic sheath encloses the

inner sensillum-lymph cavity with the naked dendritic branches.

The outer sensillum-lymph cavity between dendritic sheath and cuticular wall of the peg is reduced (Fig.4.4b). The inner dendritic segment measures about 3 μm in diameter and after the ciliary constriction forms a smaller outer dendritic segment (Fig.4.5d).

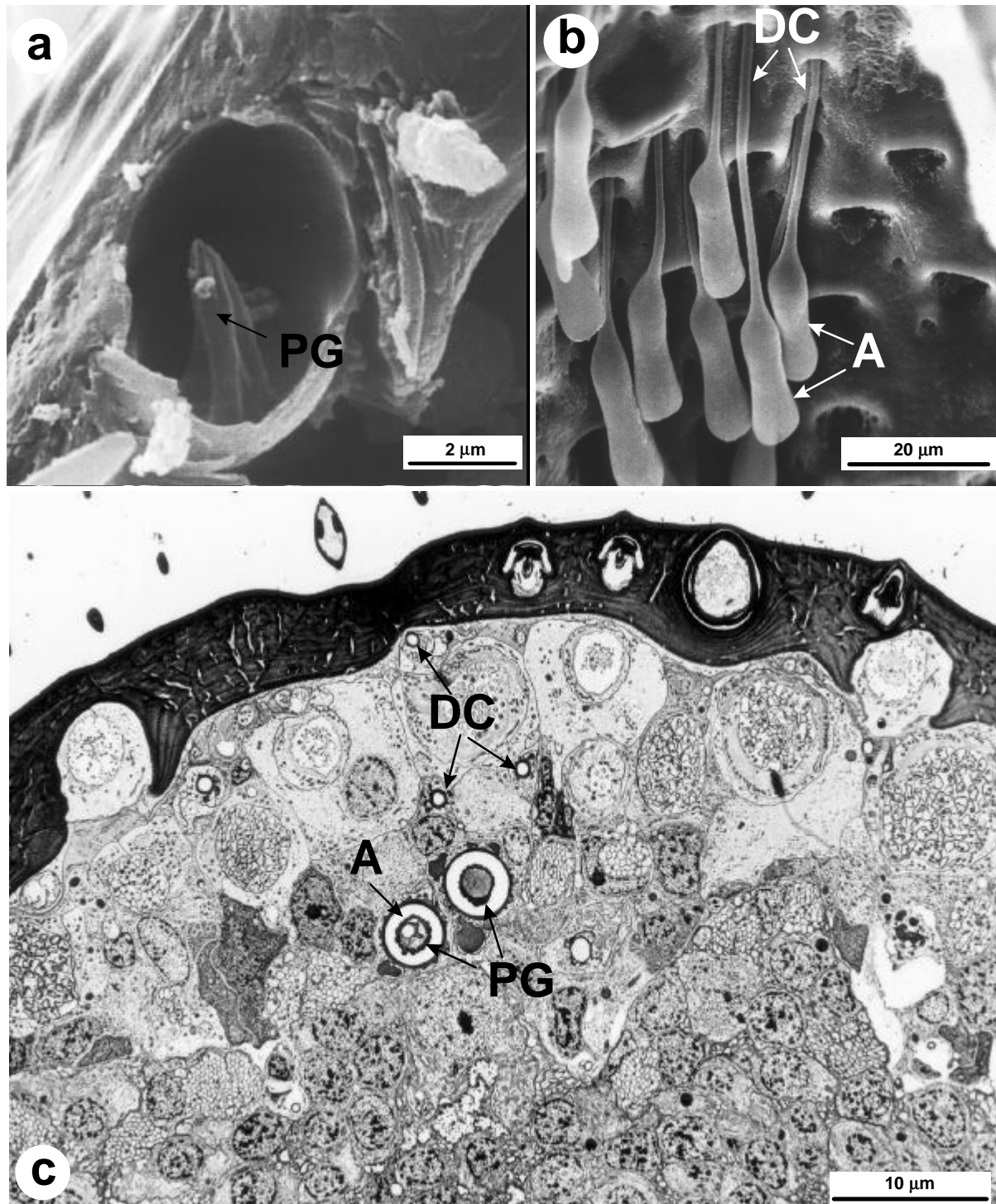


Fig.4.2 SEM photomicrographs showing a cross section of the antennomere cuticle with a sensillum coeloconicum a), and in b) the internal view of the same area with sensilla ampullacea. c) TEM micrograph showing a detail of a cross section of the apical antennomere with cuticular and cellular part of sensilla ampullacea. A, ampulla; DC, ducts; PG, peg.

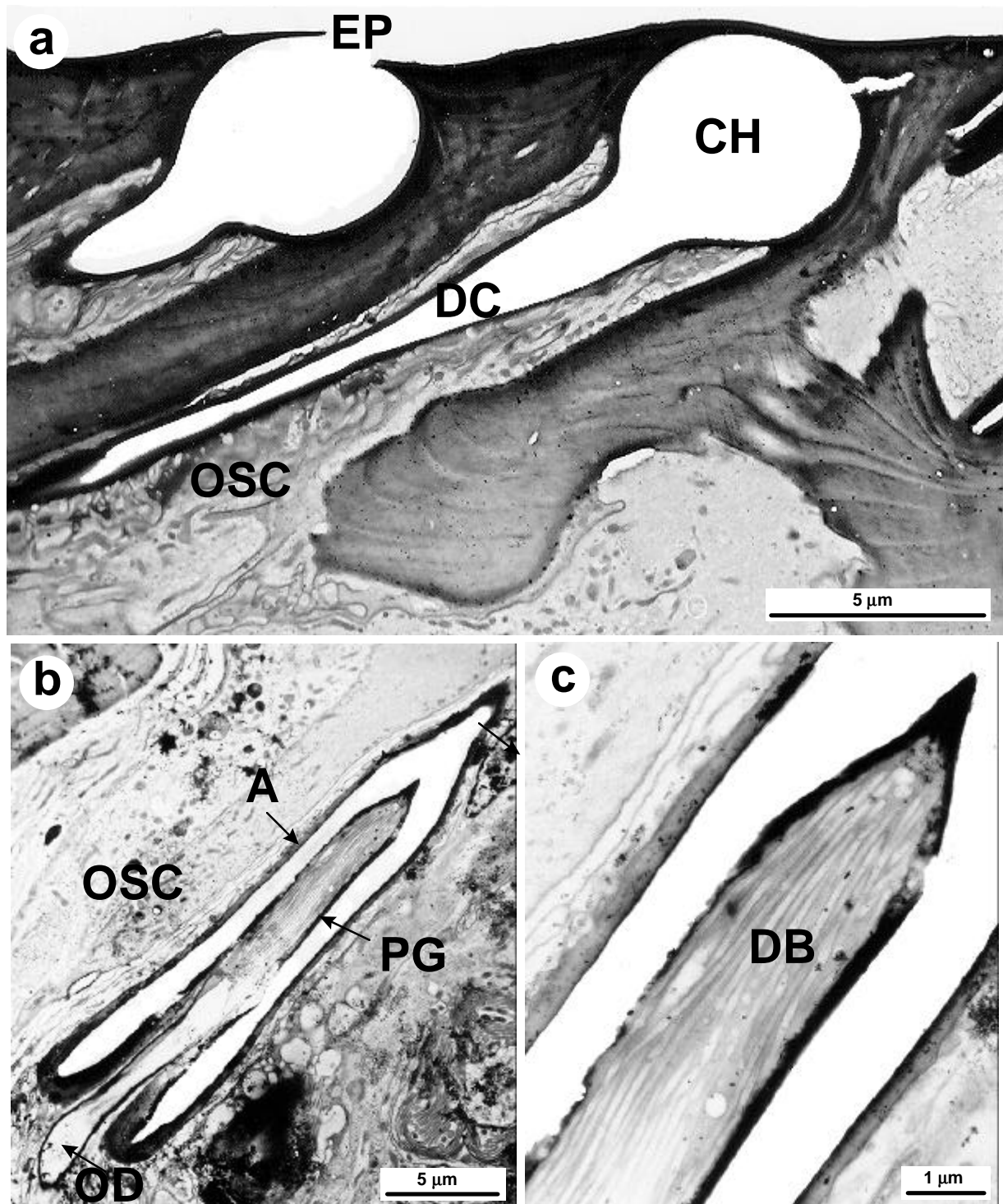


Fig.4.3 TEM photomicrographs of sensilla ampullacea. a) Longitudinal section of two sensilla ampullacea showing the external pore and the pit in the antennomere cuticular wall. b) longitudinal section of the ampulla showing the peg innervated by one dendrite which branches at about half length of the shaft. c) detail of the apical portion of the peg with the dendritic branches completely filling the lumen. A, ampulla; CH, pit; DB, dendritic branches; DC, duct; EP, external pore; OD, outer dendritic segment; OSC, outer sheath cell; PG, peg.

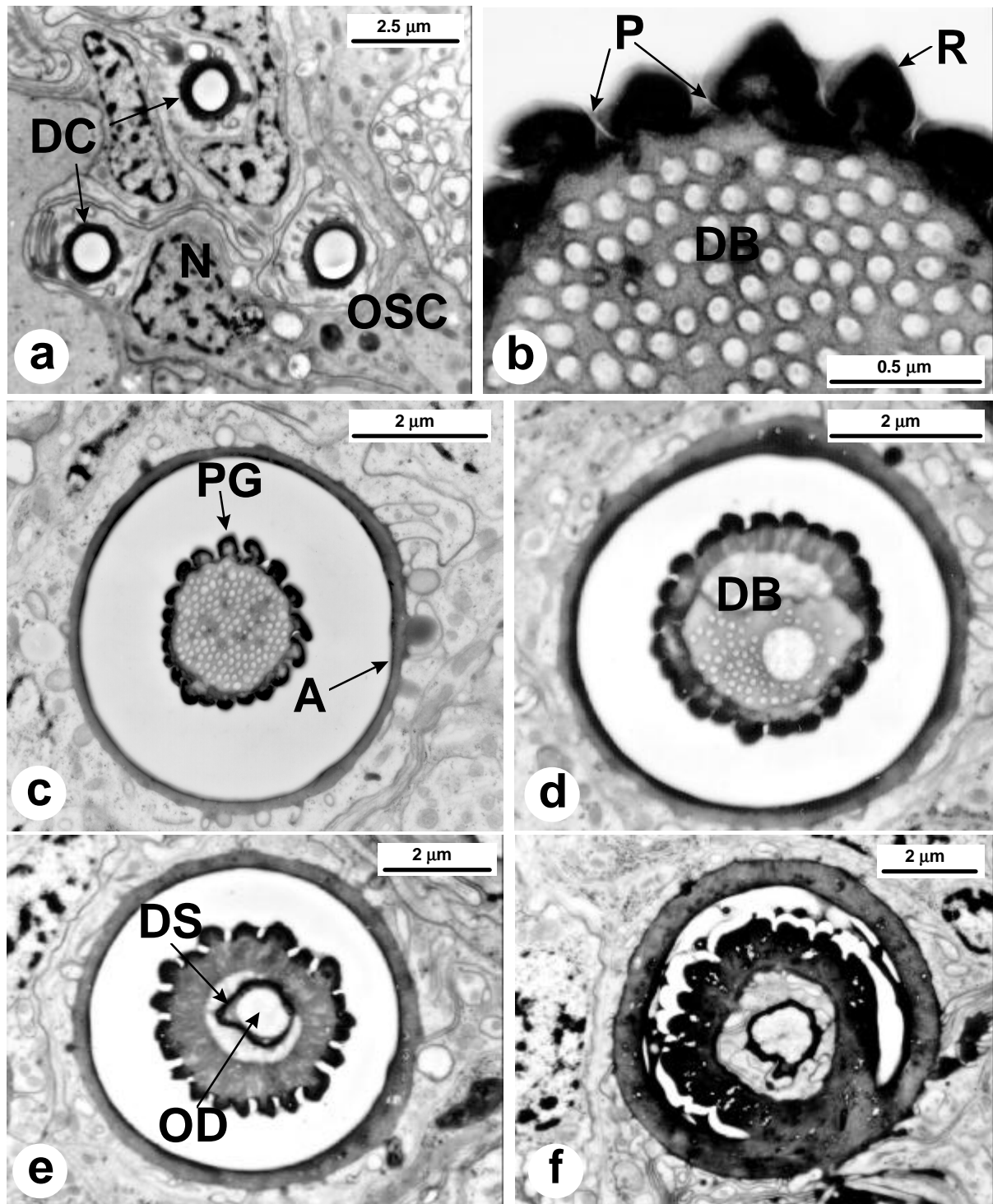


Fig.4.4 TEM photomicrographs of cuticular components of sensilla ampullacea: serial cross sections through the ducts (a), subapical (b and c), intermediate (d and e) and basal portion (f) of the peg inside the ampulla. A, ampulla; DB, dendritic branches; DC, ducts; DS, dendritic sheath; N, nucleus of a sheath cell; OD, outer dendritic segment; OSC, outer sheath cell; P, pores; PG, peg; R, ridge.

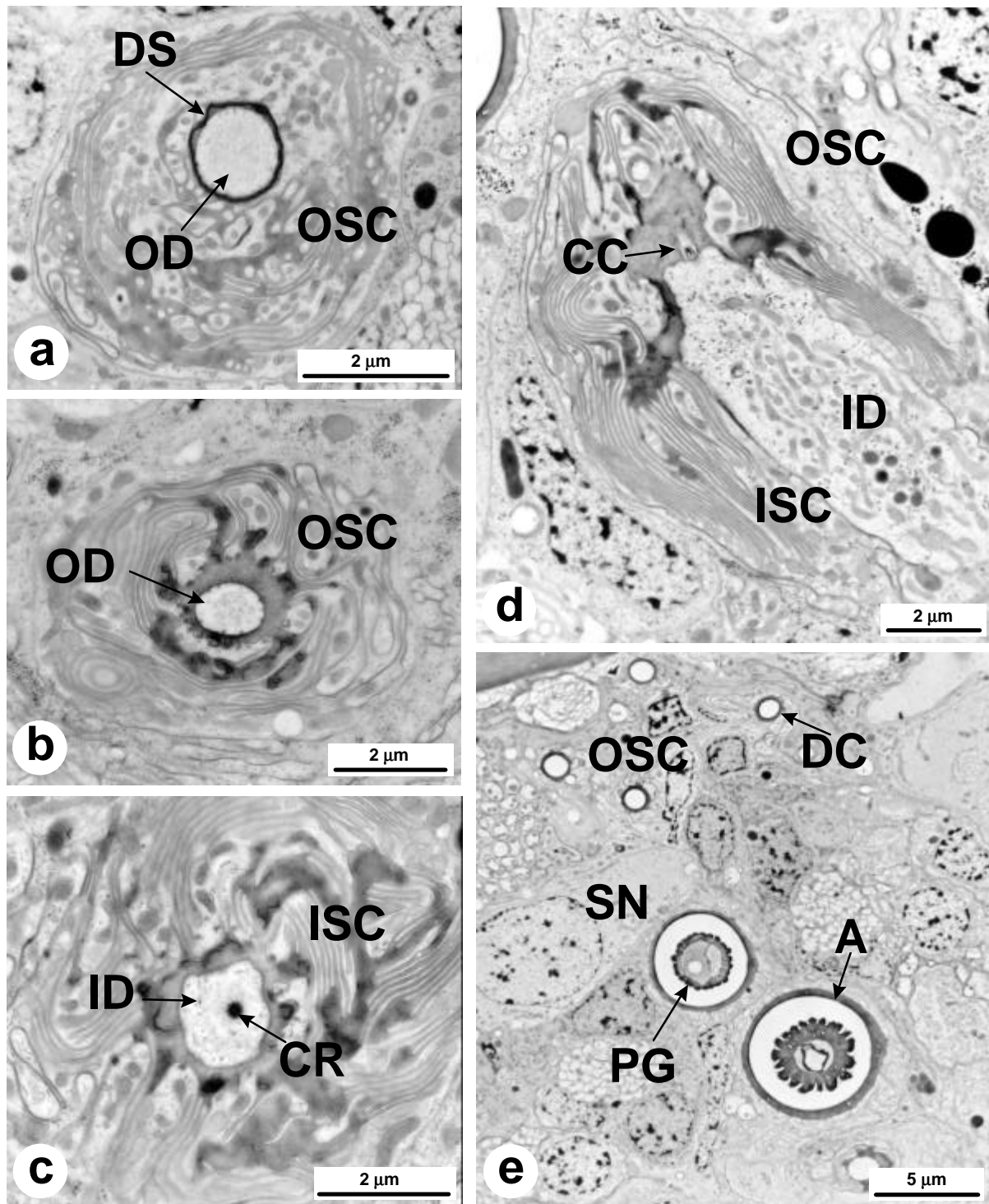


Fig.4.5 TEM photomicrographs of cellular components of sensilla ampullacea: cross sections through the outer dendritic segment encased in the dendritic sheath (a), the region near the ciliary constriction (b), and the ciliary rootlets (c). d) oblique section through the ciliary constriction and e) cross section overview showing the pericarian of the sensory cell noticeably larger than those of the other surrounding sensilla. A, ampulla; CC, ciliary constriction; CR, ciliary rootlets; DC, duct; DS, dendritic sheath; ID, inner dendritic segment; ISC, inner sheath cell; OD, outer dendritic segment; OSC, outer sheath cell; PG, peg; SN, sensory neuron.

Identification of the sensillum housing the CO₂ receptor cell

The sensilla ampullacea were identified as responsible for CO₂ perception in leaf-cutting ants. The used staining method revealed to be very specific. In repeated staining experiments only a single sensillum ampullaceum was marked with the fluorescent dye as shown in Fig.4.6. Neighboring sensilla ampullacea or coeloconica were not stained.

The fluorescent dye was found at the base of the peg and at a sheath cell (outer) near the antennomere cuticle. In all successful staining experiments where CO₂ was proven to be the specific stimulus for the receptor cell (with electrophysiological recording of neural activity) a sensillum ampullaceum was stained, never a sensillum coeloconicum. Thus, in ants the sensilla ampullacea are responsible for CO₂ perception.

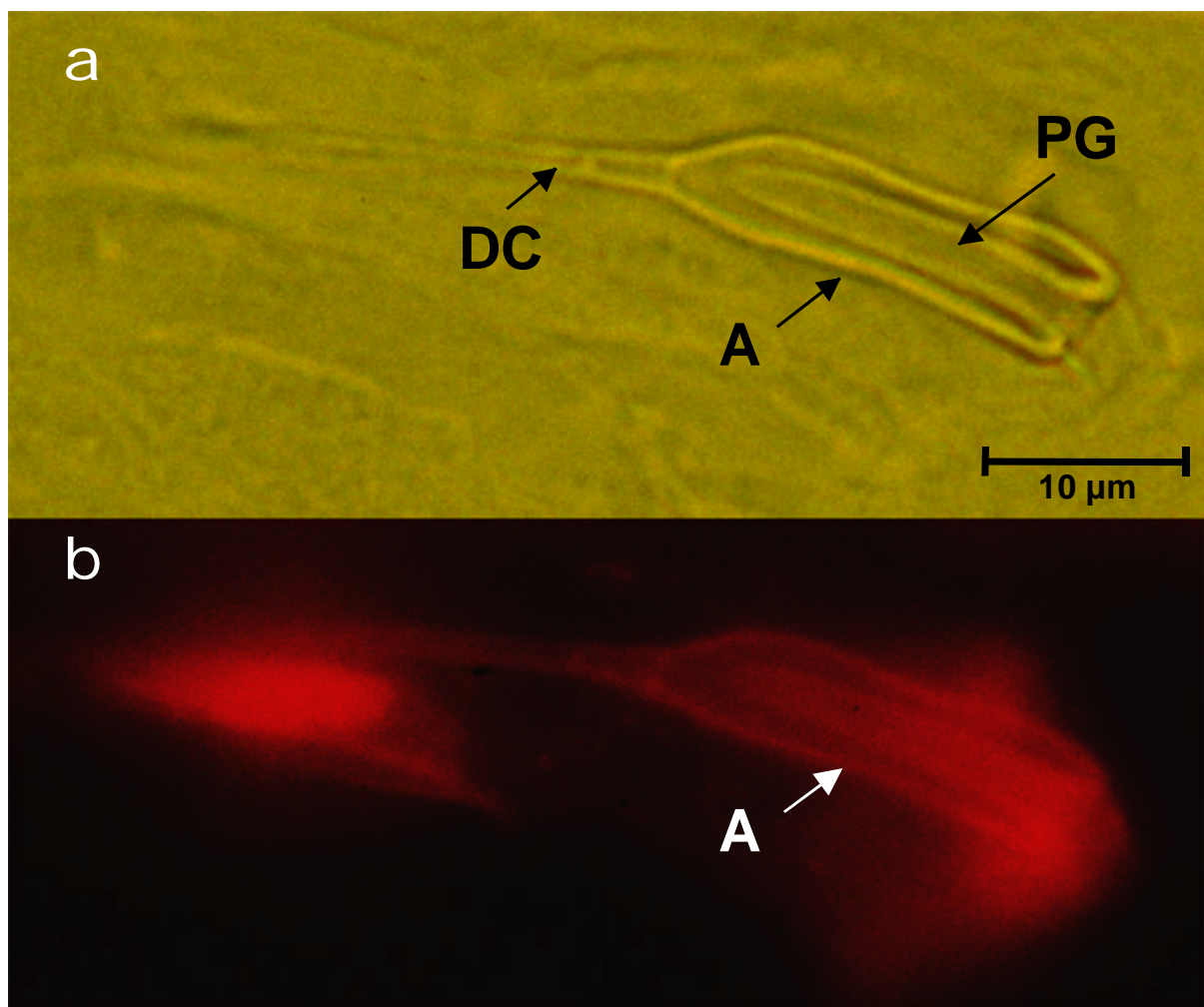


Fig.4.6 Photomicrograph showing a longitudinal section of a sensillum ampullaceum. a) light micrograph; b) fluorescent micrograph, stained with a Texas Red labeled dextran. A, ampulla; DC, ducts; PG, peg.

Comparative morphology of sensilla ampullacea

The duct length of different sensilla ampullacea is highly variable within single individuals. Fig.4.7 shows the mean duct length with standard deviation of four workers which differed notably in body size. The shortest duct length was 70%, 42%, 65% and 67% of the largest duct length.

Allometry between duct length and body size was tested with the mean duct length of a total of 14 workers ranging from 2.7 mm to 14.0 mm in head width. No allometry of this character was found. The mean duct length was 45.4 μm (SD = 4.0; n = 14) and the correlation coefficient between body size and duct length was $R = 0.24$ (Pearson R ; $p > 0.05$). Thus, there is no correlation between body size and duct length of the sensilla ampullacea (Fig.4.8).

In males the mean duct length was 25.6 μm (SD = 2.3; n = 4) and is significantly shorter than the duct length of workers (T-test; $p < 0.01$).

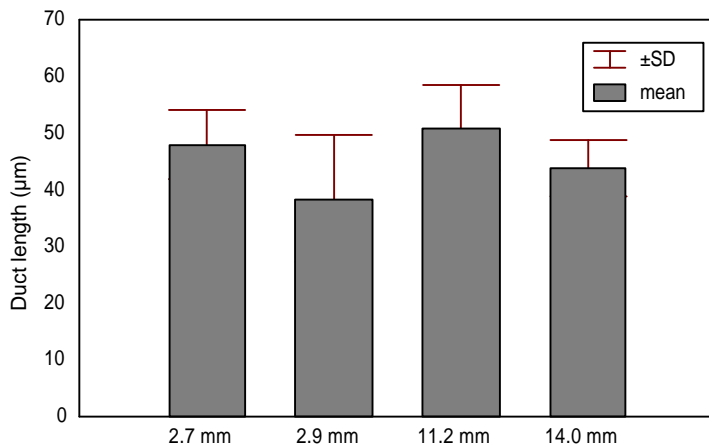


Fig.4.7 Comparative morphology of sensilla ampullacea of different workers. Size of four workers (head width) vs. duct length of the sensilla ampullacea. 7-8 sensilla were measured for each individual.

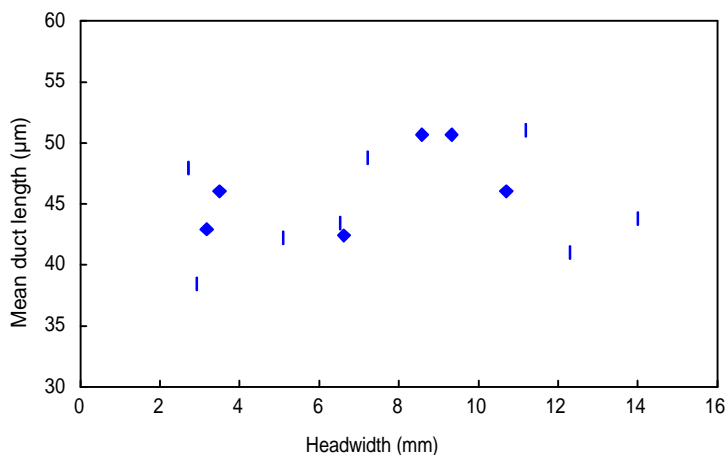


Fig.4.8 Comparative morphology of sensilla ampullacea of different workers. Size of workers (head width) vs. mean duct length of the sensilla ampullacea. For each individual (n = 14) the mean of at least four sensilla were calculated. No correlation ($R = 0.24$; $p > 0.05$), thus no allometry of the duct lengths were found.

Discussion

The location of CO₂ perception in Hymenoptera has long been an unsolved problem. Although there are several reports on the response characteristic of CO₂ receptor cells no successful attempt has been made so far to identify the sensillum containing the receptor cell. The present study fills this gap and is the first comprehensive ultrastructural description of the sensilla ampullacea in Hymenoptera. Using a specific staining technique during electro-physiological recordings the sensilla ampullacea were identified as CO₂ receptors in leaf-cutting ants.

In the literature two candidates have been proposed for CO₂ perception: sensilla coeloconica and sensilla ampullacea. Both share the character of a rounded pit embedded in the antennomere cuticle. Apically the pit has a sharp edged, circular opening (external pore).

A side-observation suggest a possible function of the pit with its sharp edged pore. While during preparation e.g. in ringer solution the duct remains air filled even if the flagellum is submerged, following a cut it is immediately filled with liquid by capillary forces. Thus, a possible explanation is protection against flooding with water as it was shown for the morphology of plant stomata (Schönherr and Bukovac 1972).

The most eminent character of the sensilla ampullacea are their ducts that distinguish them clearly from sensilla coeloconica. Unlike in all other olfactory sensilla stimulus perception in sensilla ampullacea takes place below the antennomere cuticle. CO₂ has to pass a long and narrow duct until perception can take place at the sensory peg inside the ampulla.

Besides in ants, sensilla ampullacea have been described for other Hymenoptera as well (Walther 1979; Martini 1984; Ågren and Hallberg 1996). In no case has their function been elucidated. The only other insect order where sensilla ampullacea have been described is Diptera (e.g. in mosquitoes), where their function for temperature and/or humidity perception has been discussed (McIver 1982; Sutcliffe 1994).

In *Atta sexdens*, the subject of this study, the sensilla ampullacea are accumulated at the tip of the antenna. This phenomenon has also been described for other ant species (Prelinger 1940; Dumpert 1972; Hashimoto 1991; Riedl 1995; Ehmer 1997). Since ants scan their surroundings with the antennae for orientation purposes the most distal part has a dominant role. Thus, the accumulation at the tip indicates that CO₂ is used as cue for orientation (see chapter three).

Prelinger was the first to discuss the long ducts of the sensilla ampullacea and to compare them between different ant species. He found shorter ducts in species which he suggested lead a 'hidden' life, like e.g. *Solenopsis* and *Leptothorax* and thus assumed a correlation between habitat and duct length (Prelinger 1940). Unfortunately, the only two categories he used, 'lively and big eyes' versus 'hidden life with small eyes' are somewhat superficial and can be assigned neither to a particular behavior nor to a particular habitat.

In order to investigate the functional relevance of the duct the following hypotheses are proposed:

- 1) The temporal resolution of the receptor cell is affected by the duct. The perception of changing CO₂ concentrations is delayed by diffusion through, and adsorption in the duct. Thus, the perception of short term fluctuations in CO₂ concentration is modulated.
- 2) The duct is an accessory structure increasing the specificity of perception. CO₂ is selectively transported to the ampulla and/or non-CO₂ substances which can excite the receptor cell are prevented from reaching the peg.
- 3) The sensilla are embedded below the antennomere cuticle to 'save space' at the surface. If the inventory of sensilla at the surface is at its maximum, embedded sensilla would increase the possible number of sensilla on the antenna.
- 4) The sensilla are embedded for protection (isolation) against environmental changes e.g. in temperature or humidity.

The first and second hypothesis will be discussed in the next chapter. The third and fourth hypothesis will be examined based on the data presented in this chapter.

Morphology of sensilla ampullacea

Save space?

The duct of the sensilla ampullacea originates at the bottom of a rounded pit in the antennomere cuticle. The diameter of this pit is 5 µm, comparable to e.g. the pit of sensilla coeloconica or the socket of sensilla basiconica. Thus, the long duct does not save any space at the antennomere cuticle. In addition, the 'save space' hypothesis can hardly explain duct lengths of more than 20 µm. These findings do not support the hypothesis that the duct presents a structure evolved to save space at the antennomere cuticle.

Isolation?

Isolation of the sensory peg against environmental temperature fluctuations should favor a central position in the lumen of the antenna. However, the ampullae are often located along the longitudinal axis of the antenna and are not centrally oriented. Thus, a function of the duct for isolation against temperature fluctuations is unlikely.

A second possibility for isolation would be protection against water loss. If the sensory peg has little protection against water loss long ducts might be favored in order to reduce evaporation.

The longest duct lengths have been described in the species *Lasius fuliginosus* which is of black color and often can be seen foraging in open space during sunshine (pers. observ.). In *Lasius fuliginosus* the ducts are often bent and sometimes even in spirals which is not known for any other species. In the desert ant *Cataglyphis bicolor* the sensilla ampullacea also have long ducts (Riedl 1995). If water loss is an important evolutionary parameter for duct length, it is not far fetched that these species need a better protection and thus have longer ducts.

The category 'hidden life' used by Prelinger (for which he found short duct lengths), often corresponds to a habitat with high relative humidity, thus long ducts might not be necessary for protection. The duct length of the sensilla ampullacea in workers of *Atta sexdens* are e.g. longer than in *Proceratium japonicum* (Ponerinae) and shorter than in *Lasius fuliginosus* (Formicinae) where the ducts are 80-100 μm (Dumpert 1972; Hashimoto 1990). The mean relative humidity in the habitat of *Atta sexdens* is high, but when foraging during daytime the workers might be exposed to low humidity.

The findings of this study and of the limited literature on comparative morphology support the hypothesis that the ducts act as protection against water loss. However, more detailed comparative studies are necessary in order to prove this hypothesis.

Sexual dimorphism is conspicuous in leaf-cutting ants, e.g. in males the antennae are much longer, the heads are smaller and the mandibles are reduced. Sexual dimorphism is also pronounced in the duct length of the sensilla ampullacea. Since the demands concerning the behavioral repertoire in males are completely different than in workers this finding suggests a functional relevance of the duct.

The intraindividual variability of duct length is in the same range as e.g. the variability in shaft length of sensilla chaetica. This indicates that there is no strong adaptive pressure on a specific duct length. Like in other types of sensilla no allometry was found between body size and size of sensilla ampullacea.

Another type of sensillum, the digitiform sensilla found in Coleoptera resembles the sensilla ampullacea. However, here the peg (or hair shaft) extends into a duct and morphological characters suggest a different morphogenesis. Digitiform sensilla have been discussed, but not electrophysiologically proven, as sensilla for CO_2 perception in *Tenebrio* (Coleoptera) (Honomichl and Guse 1981). The authors propose CO_2 perception at this sensillum due to loss of orientation behavior towards a CO_2 source after the last segment of the maxillary or labial palp is amputated (White *et al.* 1974). However, comparable experiments with a thermal source as stimulus gave similar results, thus thermoperception at the digitiform sensilla cannot be excluded and was also proposed by Honomichl (Gebhardt 1953). Moreover, since different other types of sensilla are located in the same area as the digitiform sensilla no assignment of CO_2 perception to a particular sensillum is possible.

Ultrastructure

Only a very limited number of both electrophysiological identification of the CO_2 receptor cell and ultrastructural description of the sensillum are available in other insect orders. CO_2 receptor cells have been found in the sensilla capitula on the maxillary palps of mosquitoes (Diptera) and there is some indication that their dendrites are lamellated (Kellogg 1970; Sutcliffe 1994; Grant *et al.* 1995). These sensilla are single-walled and multiporous (McIver 1982). On the labial palps in the pit organ of different butterfly species (Lepidoptera) CO_2 sensitive receptors have been found in sensilla basiconica (Bogner *et al.* 1986; Bogner 1990;

Stange 1992; Stange *et al.* 1995). These sensilla also have multiporous pegs with lamellicated dendrites and an extended dendritic sheath (Lee *et al.* 1985).

In termites (Isoptera) a modulatory effect of CO₂ on pheromone receptor cells located in sensilla basiconica has been found (Wicklein *et al.* 1991; Kaib *et al.* 1993). These sensilla are single-walled and multiporous but have branched dendrites.

Thus, in all insects investigated so far, the shared characters of all sensilla with associated CO₂ receptors are single-walled and multiporous pegs. In Lepidoptera, Diptera and Hymenoptera (this study) where the receptor cell specificity is tuned only to CO₂ the sensilla are located in a pit. The sensilla ampullacea with their long duct show the most extreme modification of this pit. The sensilla ampullacea investigated here have only a single receptor cell. Thus, the sensillum characters can be assumed to be adapted for CO₂ perception, whereas in the investigated Lepidoptera (at least a small number of sensilla bear more than one single neuron) and Diptera species the sensillum characters can also be shaped for neighboring cells with different stimulus specificity.

The peg of the sensilla ampullacea of the investigated leaf-cutting ants cannot be assigned easily to the group of single-walled or double-walled sensilla, since both were found. In order to distinguish unambiguously whether the peg is double- or single-walled the morphogenesis of the sensillum has to be investigated.

The finger-like ridges and particularly their constant number resembles the surface of double-walled sensilla. In double-walled sensilla these fingers are formed during morphogenesis by apical projections of the trichogen cell. Later, by cuticle secretion a characteristic eight shape of these fingers (in profile) is formed. After finishing cuticle deposition, the trichogen sprouts are degraded. The inner walls of the pegs are fused, especially at the tip. Inside the cuticular fingers of double wall sensilla electron dense material, remnants of the retracted trichogen cell is visible in cross sections (Ameismeier 1985; Kuhbandner 1985; Keil 1997). The sensilla ampullacea described here do not have these remnants of the trichogen cell. The cuticular wall is solid even at the base of the peg and punctured with intramural cavities. The intramural cavities are irregular and cannot be assigned to particular cuticular ridges.

Thus, the data presented in this study suggest that the morphogenesis of this sensillum is different than in double-walled sensilla. Single-walled sensilla are constructed by a single sprout of the trichogen cell, which later retracts after cuticle deposition and leaves a lumen into which the sensory dendrites grow (Keil and Steiner 1991). A similar wall structure as described has been found in sensilla of Lepidoptera and Coleoptera (Meinecke 1975; Lee *et al.* 1985). Judging from characters of the fully developed sensillum the peg in the ampulla has to be considered as belonging to the single-walled group.

A distinct stimulus specificity can be assigned neither to double-walled nor to single-walled sensilla, but a tendency has been found. Although there is an overlap in specificity, double-walled sensilla are more frequently sensitive to polar compounds (e.g. short chain fatty acids) than single-walled sensilla which receptor cells often respond to apolar long chain fatty alcohol's (Altner *et al.* 1977; Pophof 1997). CO₂ is a very small and apolar molecule and is perceived in single-walled sensilla ampullacea.

In single-walled sensilla pore-tubules normally extend from the pore-kettles into the sensillar lymph (Steinbrecht 1997). In the sensilla ampullacea pore-tubules were not distinguishable although the fixation protocol has previously proven useful for the identification of pore-tubules (Keil 1982).

The reason might be that the distance between the pores and the dendritic sheath is very low in the investigated sensilla ampullacea. According to the classification of Slifer, the peg belongs to the group of thin-walled sensilla (Slifer 1970). Based on ultrastructural characters no evidence could be obtained that the peg has low protection against water loss as discussed above. Thin-walled sensilla are abundant on the antennae of many species of insects and have been found, side by side on the antennal surface (Steinbrecht 1973). However, even if low protection of the peg against evaporation could be shown, this would not provide evidence that the ducts evolved for this function. The pegs of the sensilla ampullacea might have lost protection against evaporation after they were protected by long ducts that originally evolved for another function.

In conclusion, this study provides a detailed description of the sensillum ampullaceum. The findings show that the sensillum ampullaceum is innervated by a single neuron with highly branched dendrites. CO₂ perception of this neuron was shown with a specific staining technique which will be further examined in the next chapter. Thus, sensilla ampullacea are responsible for CO₂ perception in leaf-cutting ants. The extraordinary structure of the sensillum ampullaceum was discussed concerning its functional significance based on morphological and ultrastructural data. Of the proposed hypotheses for duct function, protection against water loss is the most likely, but remains to be examined with comparative morphology in further studies.