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**FINE MORPHOLOGY OF THE ANTENNAE OF *DIASPIS ECHINOCACTI*
(BOUCHÉ) 1833 (HEMIPTERA: DIASPIDIDAE).**

ABSTRACT

FINE MORPHOLOGY OF THE ANTENNAE OF *DIASPIS ECHINOCACTI* (BOUCHÉ) 1833 (HEMIPTERA: DIASPIDIDAE).

The antennae in the Diaspididae are reduced to minute appendages composed of one antennomere called the “antennal tubercle”. Few or no setae can be found on the antennal tubercle, although some small pegs in cuticular invaginations can be seen. In *Diaspis echinocacti*, the single antennal seta arises from two fused sensilla at its base. The chemoreceptive (olfactory) function of these multiporous sensilla was demonstrated using crystal violet and Transmission Electron Microscopy (TEM), while further information was obtained using light microscopy (LM), confocal laser scanning microscopy (CLSM) and Scanning Electron Microscopy (SEM). Some hypotheses on the possible function of these sensilla are discussed: (a) a feedback system to regulate the production of the female pheromone; (b) for detecting a male pheromone (postulated), or (c) for detecting a pheromone (postulated) produced by the crawlers. The possible evolutionary reduction in the number of antennal sensilla by fusion is evaluated. The lowest number of setae is accepted as the derived status for the character.

Key words: host-plant kairomones; mass-rearing; *Opuntia ficus-indica*, prickly pear, morphology.

INTRODUCTION

The antennae of adult female Diaspididae have received little attention by researchers working on this group. Berlese (1896) described the antennae of adult female Diaspididae as being “ridotte ad un semplice tubercolo, ai lati del quale sorge un lungo pelo semplice” but, on the other hand, he drew an antennal nerve from *Aonidiella aurantii* (Maskell). From an evolutionary point of view, Brown & McKenzie (1962) and Takagi (1969) considered that the number of setae may have gone on decreasing with each reduction in antennal size, eventually giving the highly reduced structure found today. This reduced structure has been variously described as: “reduced to an unsegmented tubercle, bearing one or more setae” (Kosztarab, 1963), a “simple tubercle with 1 or more setae” (Williams & Watson, 1988) and “a tubercle without joints but with setae at the apex” or “reduced to the size of a small tubercle” (Danzig, 1990).

In the Diaspididae, the number of antennal setae (in conjunction with other characters) is considered to be of taxonomic value at the tribal level

(Ben-Dov, 1990) and has also sometimes been used by taxonomists as a generic character, as, for instance, in separating the genera *Rugaspidiotinus* Balachowsky and *Poliaspoides* MacGillivray (now *Natalaspis* MacGillivray (Ben-Dov & Takagi, 1974)) (Takagi, 1969).

Apart from the morphological and taxonomic aspects, the function of the sensillae is uncertain. Ferris (1942) commented that “(the antennae) bear setae, which at times are somewhat ‘fleshy’ and are perhaps of a sensory nature”. This was also the view of McKenzie (1956), Koteja (1980), Rosciszewka (1989) and Bielenin *et al.* (1995), although only the latter provided evidence by reporting the presence of a nerve cell associated with the antenna of female *Quadraspidiotus ostreaeformis* (Curtis).

Due to this confusion and uncertainty, the aims of the present study were to clarify the sensillary nature of the antennal seta of *Diaspis echinocacti* (Bouché) and to discuss its possible function in connection with the general biology of the Diaspididae. In addition, the possible evolutionary trends in the morphology of these sensillae and their use as experimental systems are also discussed.

MATERIALS AND METHODS

These observations were made on more than 100 ovipositing adult females of *D. echinocacti* mass-reared on cladodes of prickly pear (*Opuntia ficus-indica* (L.) Mill.) maintained on turf under laboratory conditions (18-20°C and 65% RH). The culture was started from a few females collected in the field.

Light microscopy: the general morphology was studied on specimens mounted in Canada balsam using the methodology suggested by Wilkey (1990), although the inset picture in Fig. 1d was taken from a fresh, water-mounted, specimen. Observations and pictures were obtained using an Olympus BX 50 microscope equipped with bright field and phase-contrast at 400 and 1000 enlargement. Confocal laser scanning microscopy (CLSM) was performed using an Olympus BX 50 on the same slides, with the natural green fluorescence of the cuticle being collected by a CCD camera and elaborated on a Personal Computer.

The presence of pores in the cuticular wall of the sensilla was detected by using crystal violet, as suggested from Slifer (1960) and later modified by Porcelli (1995). A drop of the wetting agents Tween 80 or Triton X was added to 100cc of the staining solution to improve the results. More than 75 specimens have been observed over the past four years.

Scanning Electron Microscopy (SEM): specimens were observed “in vivo” or macerated in 10% potash in water, rinsed in 75% EtOH, repeatedly washed in chloroform (Mazzoni 1996), gold-palladium coated and observed at 5KV.

Transmission Electron Microscopy (TEM): specimens were fixed in Karnovsky (1965), post-fixed in Osmium tetroxide, dehydrated in EtOH, and embedded in araldite 502. Serial sections were obtained using a diamond knife and counter-stained with uranyl acetate (Robinson *et al.*, 1987) and lead citrate (Reynolds, 1963).

The terminology and interpretation are based on Berlese (1909), Snodgrass (1935), Schneider (1964), Zacharuk (1980, 1985), Altner & Prillinger (1980), Keil (1982) and Keil & Steinbrecht (1987).

The following abbreviations are used:

	In English	in Italian
Ant	antennomere	antennomero
AS	antennal seta/ae	setola antennale
asr	antennal seta roots	radici della setola antennale
cha	chamber of the seta/sensillum	vano all “interno della setola”
CS	ciliary sinus	seno ciliare
CU	cuticle	cuticola
DB	dendritic branches	terminazioni dendritiche
DS	dendritic sheath	guaina dendritica
Fa	fovea antennis	fovea antennis
Mf	foveal muscle	muscolo della fovea
MV	microvilli	microvilli
NC	nerve cell	cellula sensoriale
NU	nucleus	nucleo
oD	outer dendritic seg(s)	tratto (i) distale del dedrite
P	pore(s)	poro(i)
Pe	peg	sensillo basiconico
Pt	pore tubules	tubuli del poro
RL	receptor lymph	secreto linfatico
SC	sheath cell(s)	cellule della guaina
SJ	septate junctions	giunzioni settate
SS	sensillar sinus	seno sensillare
stu	arch at base of seta	varco della setola

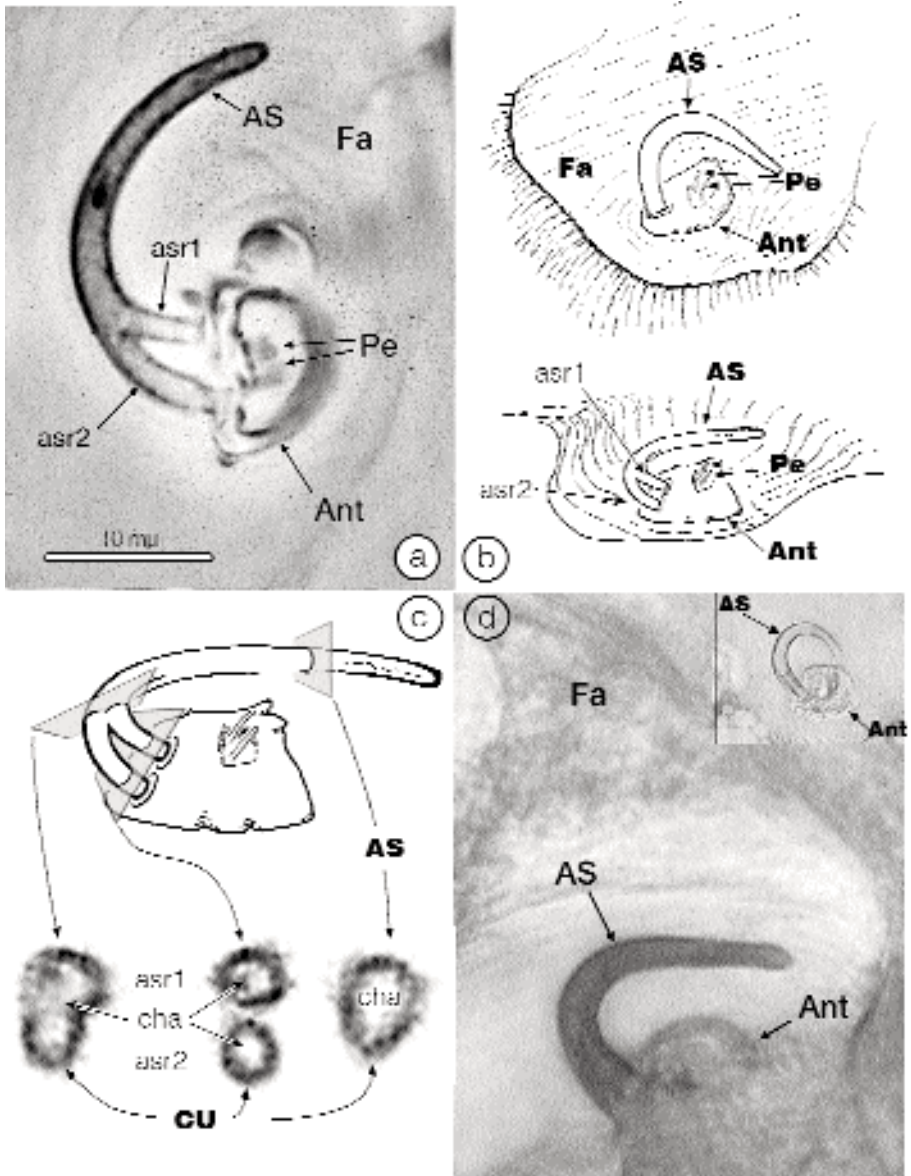


Fig. 1. *Diaspis echinocacti*: adult female antenna: a) light microscopy - phase contrast; b) schematic drawing of the antenna; c) Confocal Laser Scanning Microscope sections; d) crystal violet stained antennal seta (inset: natural coloured seta).

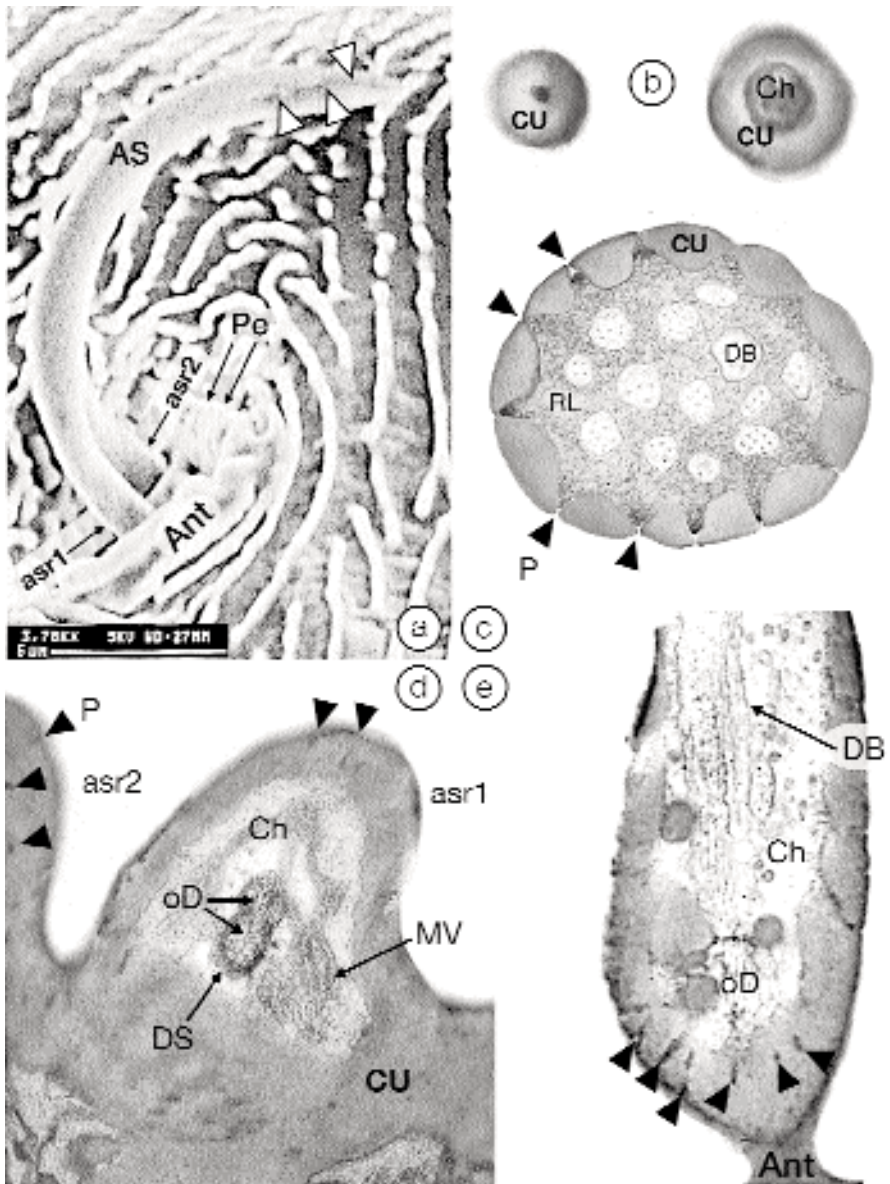


Fig. 2. *Diaspis echinocacti*: adult female antenna: a) SEM picture; b) transverse section of the pegs; c) transverse section of the antennal seta; d) tangential section of the roots; e) longitudinal section of one root.

RESULTS

Phase contrast light microscopy (Fig. 1a,b): the antennae (Ant) of *Diaspis echinocacti* are located on the “ventral” surface of the prosoma relatively far from the body margin and are encased in the antennal fovea (Fa).

The antennae are monomeric and each bears a stout, arch-shaped seta (AS) about 15µm long. The basal end of each antenna is composed of two distinctly separate roots (asr1 & asr2) which form an arch (stu), but which fuse distally to form the antenna. The antennomere is also provided with two small pegs (Pe) arising from a small invagination in the cuticle. The longitudinal (dorso-ventral) plane of the antennal seta is more or less parallel to the dorso-ventral plane of the insect. Some specimens have their antennal setae split almost to the apex or at least forming an arch-shaped seta.

These structures are best observed in very flat, clear specimens mounted in Canada balsam and observed at about 1000 enlargement under oil immersion.

Confocal Scanning Laser Microscope (Fig. 1c): optical sections showed two single and independent setal elements (roots = asr1 & asr2) arising from the antenna (Ant), which then joined together to form the antennal seta (AS).

All optical sections showed a dense peripheral cuticular wall surrounding a large chamber, with the arch (stu) between the two roots being delimited by the external surface of the cuticular wall. Sections through the seta at the point where the roots join show the confluence of the root chambers into the chamber of the antennal seta.

Crystal violet (Fig. 1d): in the natural position, only the side of one of the roots can be seen and the general morphology of the roots (asr) and of the setal arch can only be guessed by modifying the focus.

All crystal-violet treated antennae show a deep-blue stained seta (AS) in contrast to the clear seta of untreated specimens (Fig. 1d: inset). The antennomere and the pegs are unstained, as are all the other parts of the body. Rarely, in some specimens, some glandular ducts in the pygidium and part of the salivary ducts may become stained violet but this only occurs after several days in the staining solution.

Scanning Electron Microscope (Fig. 2a): the photograph shows the antennomere (Ant), the two pegs (Pe), the two roots (asr1 & 2) and the antennal seta (AS). At the distal end of the antennal seta, there is a scar (bordered arrows).

Transmission Electron Microscope: Pegs (Fig. 2b): the pegs are two similar, thick-walled, cuticular spurs. The cuticular wall (CU) is devoid of pores but a small, electron-dense area can be seen in sub-apical sections, while, in the

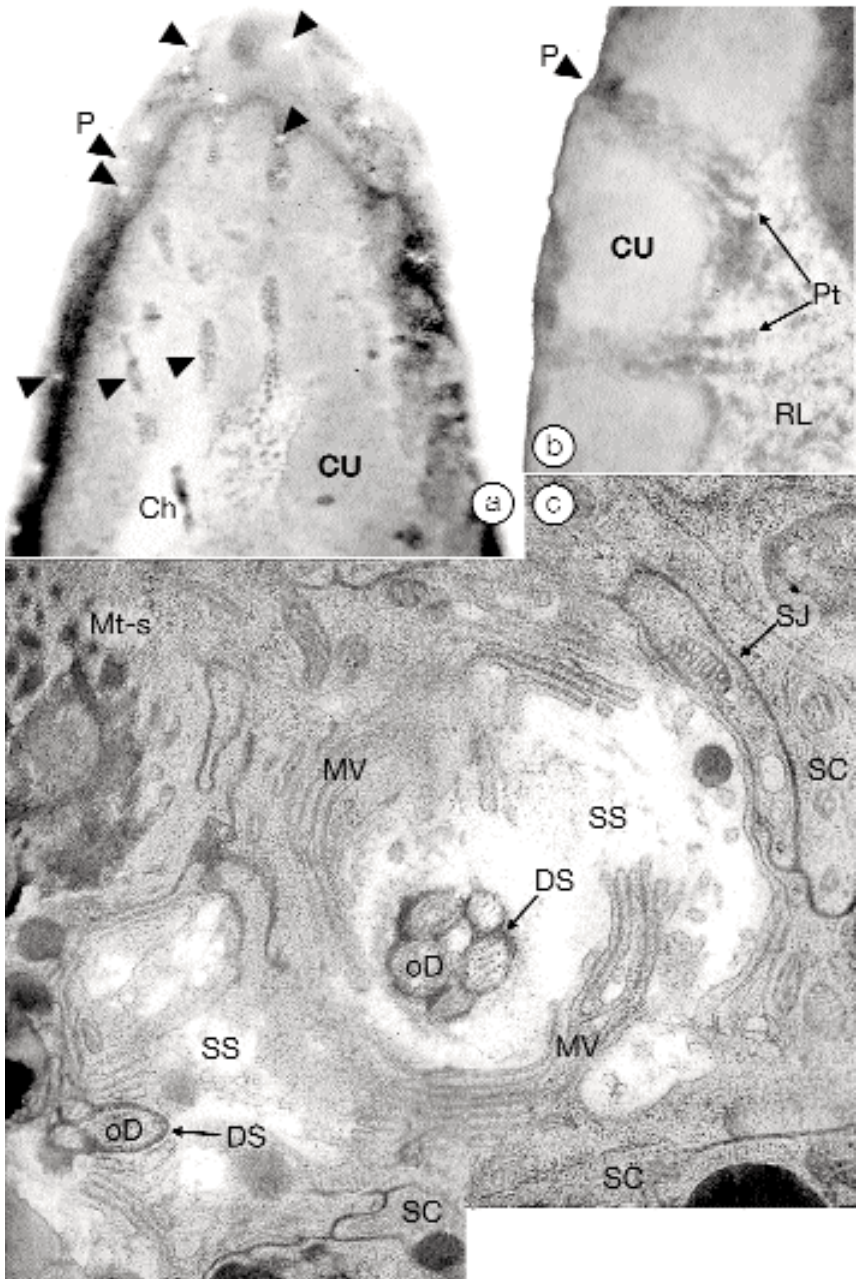


Fig. 3. *Diaspis echinocacti*: adult female sections: a) cuticular wall of the antennal seta; b) pores in the cuticular wall of the seta ▲; c) section at the level of sensillar sinus.

more proximal sections, a large chamber (cha) filled with moderately electron-dense matter with some membranes is visible. The two outer dendritic segments reach to the base of each peg. Neither a joint membrane nor any tubular bodies are found at the base of the pegs.

Setae (Fig. 2c,d,e; 3a,b): each seta has a single cuticular wall (CU) which is very rich in pores (P) with pore tubules. The large inner chamber of the seta is filled with receptor lymph (RL) which has several dendritic branches (DB) lying within it. The dendritic branches extend into the outer dendritic segments in the chamber of each root, where some microvilli (MV) are also found. Within the roots, an electron-dense dendritic sheath (DS) is found. Pores are present right to the base of each root.

Just beneath each antenna, within the body of the female, two distinct sensillary sinus (SS) are found (Fig. 3c). Each sinus is bordered with microvilli (MV) and contains up to five outer dendritic segments protected by a dendritic sheath (DS). Also present are sheath cells (SC) and related junctions (SJ), and a foveal muscle (Fig. 4b, Mf) connecting the fovea antennalis (Fig. 4b, Fa) with the dorsum of the insect. Each group of outer dendritic segments innervates one setal root. Neither a joint membrane nor any tubular bodies were present at the base of the roots.

DISCUSSION (FIG. 4)

Both light microscopy and confocal laser scanning microscopy showed that the antennal seta is composed of two joined elements and, on the basis of general morphology of setae among insects, it is proposed that the primitive status was two discrete elements and that the fused structure noted here is the derived state.

With regard to function, these antennal setae show all the features of chemoreceptors, i.e. the presence of pores in the cuticular wall, the large chamber filled with receptor lymph and provided with dendritic branches, and the connection of the sheltered dendritic branches with sensory neurons equipped with sensillary sinus and sheath cells. Some features are also of special interest with regard to the olfactory function of the antennal seta, i.e. the lack of a basal joint membrane and tubular bodies, and the position of the antenna itself in connection with a foveal muscle able to move the antenna (Fig. 4). The two pegs are possibly thermo-hygro-receptors, whose function is to tune the reaction of chemoreceptors.

Beyond the somewhat obvious presence of chemoreceptors and associated thermo-hygro-receptors, some aspects are of interest in connection with the

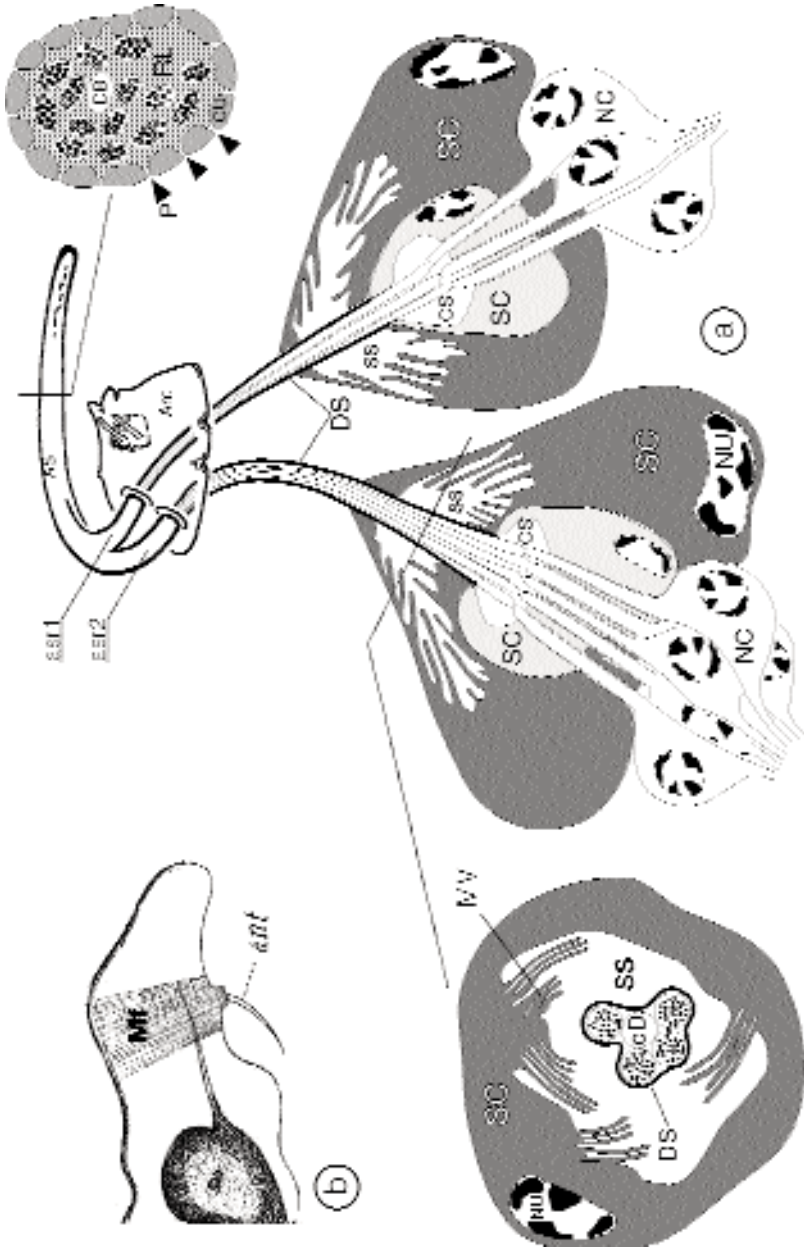


Fig. 4. *Diaspis echinocacti*: a) adult female antenna schematic drawing; b) schematic drawing of a section at the level of the foveal muscle (partly redrawn from Berlese, 1896).

special life-style of *D. echinocacti*. As armoured scales are fixed in position on their host plant, protected inside a self-produced protein-wax cover, one can hypothesise about what use the adult female can make with this chemosensory unit. Acceptable hypotheses regarding the antennal chemoreceptor are: (a) that it is part of a feedback system to regulate the secretion of the female sex pheromone that is absorbed by the scale cover from the anus; (b) that it is for the perception of a possible male pheromone (not yet discovered), or (c) it is for the perception of a pheromone (not yet identified) produced by the crawlers inside the scale cover, for instance, to avoid overcrowding.

Two further aspects of the antennal chemoreceptor of *D. echinocacti* are worthy of note. The first is the derived state of the joined setae as compared with the primitive state of two independent setae, and that the actual numerical reduction is by fusion. The second is to draw attention to the possible use of this experimental system to locate the site of selectivity or the redundancy of a sensory unit. In fact, when the semiochemicals involved in intraspecific relationships have been identified, it will be possible to test these substances using these different types of antennal setae.

CONCLUSION

The finding of chemoreceptors on the antennae of adult female diaspid scales will allow some interesting new perspectives on armoured scale biology to be studied. In particular: (a) the use of the special morphology of the antennal seta in experimental systems for testing possible pheromones, and also (b) phylogenetically, as the trend for reduction in antennal structures recalls the fusion of abdominal segments to form the pygidium. Other interesting aspects might be in relation to agricultural entomology if, for instance, either male or crawler pheromones were found or if it became possible to interfere with the secretion of the female pheromone.

The next step in this study will be to test the electrophysiological response of the antennal seta to several substances in order to identify the most promising classes of semiochemicals to search for in nature.

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