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Gustatory sensilla sensitive to protein kairomones trigger host acceptance by an endoparasitoid

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Proteins isolated from the host cocoon of *Acrolepiopsis assectella* (Lepidoptera: Yponomeutoidea) act as kairomones for host acceptance by the endoparasitoid wasp *Diadromus pulchellus* Wesmael (Hymenoptera: Ichneumonidae). In this study, morphological, ultrastructural and electrophysiological studies were carried out in order to identify the contact chemoreceptive sensilla on the parasitoid antennae that perceive the protein kairomones. Three types of sensillum on the antennae of the females were found to have a chemosensory function. The receptor cell(s) of one sensillar type were shown to give a positive electrophysiological response to protein kairomones. This sensillar type is apically multiporous and female specific. Consequently, this sensillum could be the one implicated in the perception of the protein kairomone that triggers the host-acceptance behaviour of *D. pulchellus* females.

Keywords: chemoreception; electrophysiology; electron microscopy; host acceptance; protein gustation

1. INTRODUCTION

Chemicals appear to play a major role at almost every step in the life of insects. They mediate interactions between organisms and are involved in many intra- and interspecific processes, such as host selection for feeding, egg laying or partner encounter (Ma & Shoonhoven 1973; Dethier 1976; Mitchell 1994; Städler *et al.* 1995, Van Loon 1996), and the avoidance or attraction of individuals in agonistic, mainly sexual, or antagonistic interactions (Städler 1984; Haynes & Birch 1985). The information they convey often elicits a series of responses, including behavioural responses.

There is extensive literature on the processes regulated by chemicals (Vinson 1985; Godfray 1994; Quicke 1997), their source (Guillot & Vinson 1972; Prokopy 1981; Harrisson *et al.* 1985; Dougherty *et al.* 1992; Höller *et al.* 1993; Godfray 1994; Quicke 1997) and the sensory organs involved in their perception (Kaissling 1971, 1987; Gullan & Cranston 1994; Quicke 1997). Even the fine details of the mechanisms involved in perception, including molecular recognition and chemo-electrical transduction, have been established (Payne *et al.* 1986; Kaissling 1986, 1996; Vogt 1987; Carlson 1996; Singh 1997; Krieger & Breer 1999; Clyne *et al.* 2000; Ishimoto *et al.* 2000). However, most of the data available concern the perception of volatiles (i.e. olfaction), particularly pheromones.

Studies related to the perception of non-volatile chemicals (i.e. gustation) usually refer to groups of molecules implicated in the gustatory system, by which the feeding or oviposition behaviour of insects is mediated through the perception of sugar or amino acids (Hansen-Deskelkamp 1972; Ma & Shoonhoven 1973; Dethier 1976; Städler *et al.* 1995; Du *et al.* 1995; Roessingh *et al.* 1997; Baur *et al.* 1998). However, there have been few attempts to investigate the influence of the gustatory system on other types of behaviour. The perception of non-volatile cuticular hydrocarbons through contact has been shown to contribute to intra- and intercolony recognition in many social insects (Nowbahari *et al.* 1990; Lorenzi *et al.* 1995; Vauchot *et al.* 1996). It has been reported that sex pheromones with low volatility control partner recognition in some cricket and fly species (Rence & Loher 1977; Huyton *et al.* 1980) and contact kairomones trigger host recognition in various parasitoid species (Vinson 1991; Bénédet 1999). The various studies that have been carried out on contact chemoreception in parasitoid species have never led to a clear identification of the receptors implicated in the host-acceptance step.

In the parasitoid *Diadromus pulchellus*, a specialist solitary endoparasitoid of the leek moth *Acrolepiopsis assectella*, host-acceptance behaviour is principally triggered following the perception of protein kairomones from the leek moth cocoon (Bénédet *et al.* 1999). Behavioural observations (Bénédet *et al.* 1999) and an experiment after removal of the antennae (Labeyrie 1960) performed on *D. pulchellus* females strongly suggest that chemoreceptors involved in host acceptance are present on the antennae.

The purpose of this study was to identify the antennal contact chemoreceptors of *D. pulchellus* that are sensitive to the host-acceptance protein kairomones. Sensillar perception of the protein kairomones was investigated by morphological, ultrastructural and electrophysiological studies.

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2. MATERIAL AND METHODS

(a) *Insects*

D. pulchellus and its host *A. assectella* were reared as previously described (Arnault 1982; Lecomte & Thibout 1984; Bénédet *et al.* 1999). *D. pulchellus* females used in this study were mated at emergence, and then isolated in cylindrical vials for 5–7 days, where they were fed on 10% (w/v) sucrose in water according to Bekkaoui & Thibout (1992).

(b) *Preparation of antennae for scanning electron microscopy*

To observe gustatory sensilla, the antennae of newly emerged *D. pulchellus* females were cut off and immediately dehydrated in a series of ethanols (50, 70, 90 and 100%) for 15 min and in an acetone bath for 3 min. They were dried, mounted on brass sample stubs and coated with gold–palladium. Samples were viewed through a DSM 982 Gemini scanning electron microscope.

Measurements (length and width) were obtained from photomicrographs of at least 10 sensilla of each type, and the mean value expressed in micrometres (± s.e.m.).

(c) *Preparation of antennae for transmission electron microscopy*

Antennae were removed from adult *D. pulchellus* females that were isolated from host pupae 2–3 days before emergence.

The flagella were fixed by immersing in 2.5% glutaraldehyde and 5% sucrose in 0.1 M sodium cacodylate buffer (pH 7.3) for 6 h at 4 °C. The flagella were then washed in 0.1 M sodium cacodylate buffer for 12 h at 4 °C, postfixed in 1% osmium tetroxide for 1 h at 4 °C, rinsed in the same buffer for 1–2 min, and then dehydrated in a series of graded ethanols (2 × (50% for 5 min, 70% for 10 min, 90% for 15 min and 100% for 20 min)) and in two propylene oxide baths for 30 min. Subsequently, they were embedded overnight using propylene oxide in Epon-Araldite at 4 °C. The flagella were correctly positioned within the resin, which was then allowed to set at 37 °C for 12 h followed by 48 h at 65 °C.

Thin sections (*ca.* 80 nm) were obtained using a diamond knife on an Ultracut microtome and were double-stained with uranyl acetate and lead citrate, and examined under a JEOL JEM 1010 electron microscope.

(d) *Isolation of the protein kairomones and behavioural experiments*

The isolation of the protein kairomones was obtained by washing 1000 cocoons with ultrapure water (Millipore; 19 MΩ; pH 5.5) as in previous studies (Bénédet 1999; Bénédet *et al.* 1999). This water cocoon extract was filtered through glass wool to eliminate traces of cocoon fibres, and then dialysed against five changes of 5 l of ultrapure water using dialysis tubes with a porosity of 12–14 kDa (Spectra/Por product) to ensure that all the small molecules (free carbohydrates, amino acids, inorganic or organic salts, etc.) were eliminated. The extract was concentrated by freeze drying and aliquots redissolved in a volume of ultrapure water or 50 mM of NaCl, allowing the acquisition of a final concentration corresponding to 20 cocoon washing equivalents.

To test the kairomonal activity of the protein extracts, behavioural experiments were performed by putting test parasitoid females in a two-choice situation between a control lure and an experimental lure. The control lure (absorbent cotton fibres) and experimental lure were fixed to the bottom of a 5 cm Petri dish at a distance of 1 cm from each other (Bénédet *et al.* 1999). Twenty microlitres of protein extract were deposited on the experimental lure, which was then allowed to dry. The control lures were moistened with 20 µl of ultrapure water or with 50 mM of NaCl. The females were placed individually in a Petri dish and observed for 5 min once contact had been made with a lure.

Two criteria were used to quantify host-acceptance behaviour: the total contact time and abdomen bending and probing (Bénédet *et al.* 1999). The values of the first criterion for a given experimental group of females were averaged, and then compared using the non-parametric Kruskal–Wallis test for independent samples. The second criterion, expressed as a percentage, was analysed using the χ^2-test. A 95% confidence interval was used for all statistical tests. The 11 most responsive female parasitoids were isolated in cylindrical vials for 1–2 days before being used for the electrophysiological experiments.

(e) *Electrophysiological experiments*

Spike activity from the chemosensory cells of antennal sensilla was recorded by a tip-recording technique (Hogson *et al.* 1955). The antennae were fixed on double-sided tape and a thin Ag–AgCl wire, serving as the ground electrode, was inserted into the thorax of intact live females restrained in plasticine. The recording electrode, a glass micropipette (diameter at the tip 4–8 µm) filled with the stimulating solution, was placed over the tip of the sensillum. An Ag–AgCl wire connected the stimulating/recording electrode to the input socket of the preamplifier (Biologic VF 180). Spike discharges were monitored on an oscilloscope (Tektronix 5100). The bioelectrical signals were recorded for the first 10 s, and analysed using the Autospike V4 software (Syntech), which counts the number of 'spikes' after eliminating the 'background noise'. Spike frequencies were calculated as the total number of spikes generated over 10 s.

Experiments were performed on three types of sensilla from the 11 previous females. A sensillum of each type was stimulated with the control solution (50 mM of NaCl) for 10 s, followed by stimulation for 10 s with a 50 mM NaCl protein extract contained in another electrode. NaCl (50 mM) was added to the solutions to ensure adequate electrical conductance.

3. RESULTS

(a) *Gustatory sensilla on the antennae*

Preliminary studies identified 16 morphologically distinct types of sensilla on the flagellum from the *D. pulchellus* antennae of both males and females (Lecomte *et al.* 1990). Three sensillar types could correspond to gustatory sensilla. In the present work, morphological studies of the distal antennomeres were carried out using a more refined electron microscope than previous works. The main morphological criterion used to identify gustatory sensilla is the presence of at least one apical pore.

The first sensillar type (S1), perpendicular to the antennal axis, is 13.3 ± 1.4 µm long and 1.2 ± 0.1 µm wide at the base. This type does not arise directly from the cuticle but emerges from a socket. There are a few longitudinal grooves on the surface of the sensilla (figure 1*a*). An average of four sensilla were counted per antennomere, i.e. 96 sensilla per antenna. They were located on the

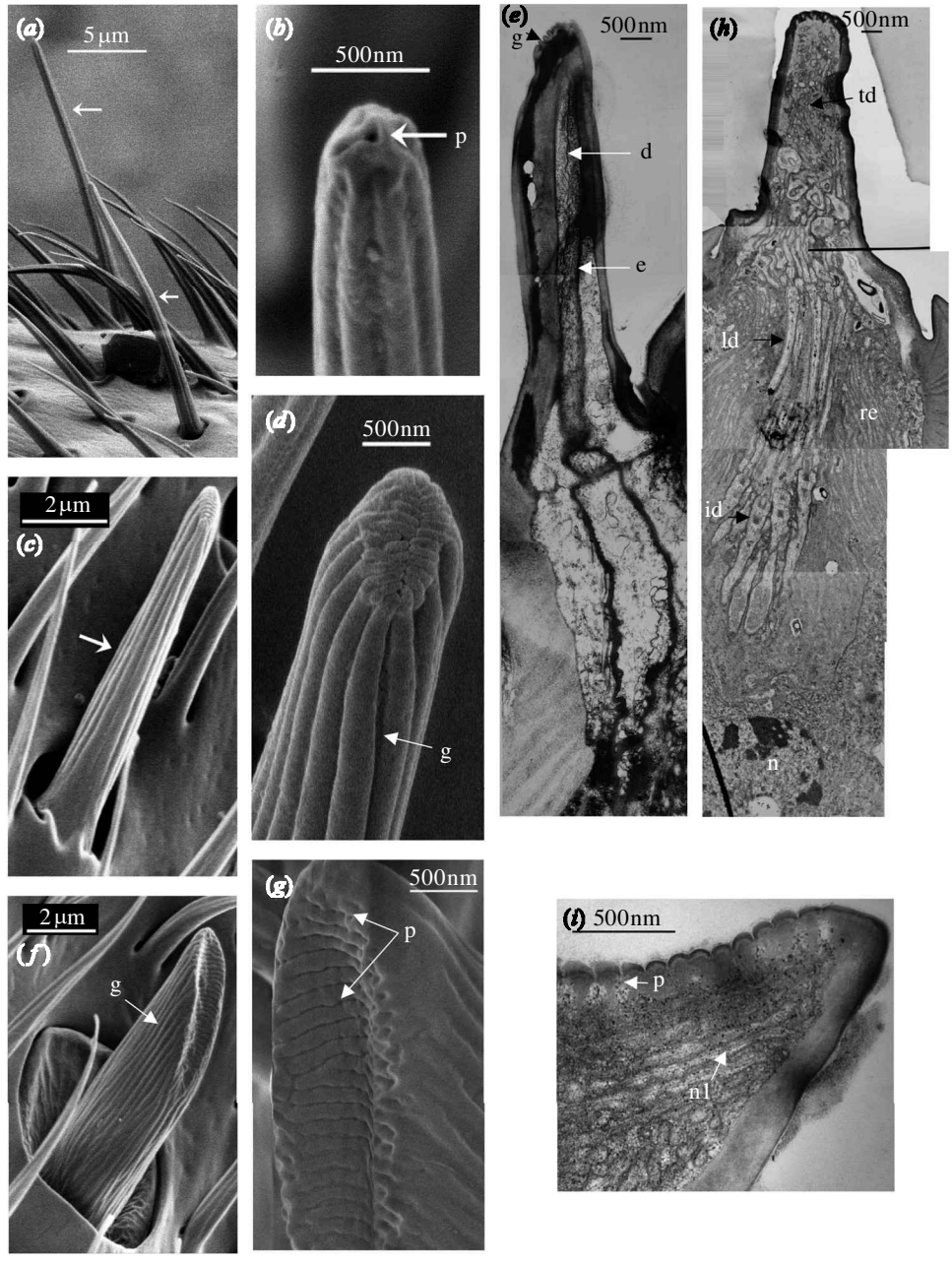

Figure 1. The three types of sensilla (S1, S2 and S3) observed under a SEM (*a–d*,*f*,*g*) and a TEM (*e*,*h*,*i*). (*a*,*b*) S1. (*c*,*d*) S2. (*f*,*g*) S3. (*e*) Longitudinal section through S2. (*h*,*i*) Longitudinal sections through S3. Abbreviations: d, dendrite; e, electron-dense sheath; g, groove; p, pore; ld, longitudinal section of dendrite; n, nucleus; nt, neurotubule; re, rough endoplasmic reticulum; td, transverse section of dendrite.

ventral and dorsal surfaces of the antenna (Lecomte *et al.* 1990). A single pore was observed at the tip of the sensillum (figure 1*b*). No ultrastructural thin sections of S1 could be examined because of their scarcity and their length, which makes it difficult to obtain an acceptable longitudinal section. Nevertheless, the presence of the apical pore strongly supports the gustatory nature of this S1 chemoreceptor.

The second type of selected sensilla (S2) is 9.1 ± 0.3 µm long and has an oval section measuring $1.8 \times 0.6 \pm 0.2$ µm. Half of these sensilla arise directly from the cuticle and the other half arise from a socket. The sensillum is oblique to the antennal axis and faces the apical part of the antenna (figure 1*c*). There were an estimated 680 sensilla per antenna, which were found only on the ventral surface of the 14 apical antennomeres (Lecomte *et al.* 1990). Some longitudinal grooves were present on the sensilla surface down to the tip (figure 1*c*). Even the flat tip had grooves that looked like interdigitations (figure 1*d*). The ultrastructural studies seem to indicate the presence of multiple pore-like structures between these interdigitations.

The S2 type contains at least one dendrite, consisting of numerous neurotubules, which is enclosed in an electron-dense sheath (figure 1*e*). This tubular dendritic sheath seems to be similar to the scolopoid sheath reported in the literature on contact chemoreceptors (Vinnikov 1974; Zacharuk 1980). The presence of a dendritic branch(es) within the projecting S2, and the apparently multiporous apical section, is consistent with a gustatory sensillum.

Table 1. Behavioural experiments with protein extracts.
(Mean contact time per female-min in seconds and percentage of females displaying abdominal probing on lures impregnated with protein extract or 50 mM of NaCl protein extract. The number of tested females is indicated in brackets. Data followed by the same superscript letters are not significantly different at $p > 0.05$ (non-parametric tests).)

	protein extract (20)		50 mM of NaCl protein extract (22)	
lures	experimental	control	experimental	control
contact time (s) (mean ± s.e.m.)	32.0 ± 5.2^{a}	0.7 ± 0.2^{b}	35.0 ± 4.2^{a}	0.8 ± 0.2^{b}
abdominal probing (%)	85^{x}	0^{y}	90.9^{x}	0^{y}

The third type of selected sensillum (S3) is 6.0 ± 0.8 μm long and has an oval section of $3.9 \pm 0.4 \times 1.0 \pm 0.2$ μm. Its base is connected to the cuticle via a joint membrane, which is set in an elliptical pit. The sensillum is oblique to the antennal axis and faces towards the apex of the antenna (figure 1*f*). The number of sensilla was estimated to be *ca.* 193 per antenna and they were located on the ventral surface only of the 13 apical antennomeres (Lecomte *et al.* 1990). The sensillum shaft had external grooves (figure 1*f*). The flat tip appeared to be multiporous (figure 1*g*).

The S3 contained numerous dendritic processes, which were characterized by having a bundle of neurotubules and no cuticular sheath. The only nucleus visible on the cross-section could have belonged to the neuron. It was surrounded by at least one auxiliary cell. This cell contained large quantities of the endoplasmic apparatus, as observed in the second sensillar type (not shown on the presented thin section) (figure 1*h*). The S3 tip was grooved and multiple pores could be seen (figure 1*i*). The morphological and ultrastructural data both showed that this S3 has a chemosensory function, probably gustatory.

(b) *Electrophysiological experiments*

The chemosensitivity of the three sensillar types was investigated through electrophysiology. As NaCl was used to improve the signal conductance in the electrophysiological tests, the influence of NaCl on *D. pulchellus* host-acceptance behaviour was investigated. The behaviour of females, therefore, was compared in the presence of lures impregnated with the NaCl protein extract and in the presence of a lure moistened with the aqueous protein extract.

The contact times and abdominal probing behaviour triggered by the aqueous and the NaCl protein extracts (table 1) were not significantly different (Kruskal–Wallis test, $p > 0.05$). The values for the control lures show very short mean contact time per female-min and no abdominal probing occurred (table 1). The presence of 50 mM of NaCl did not significantly modify the activity of the extract and did not disturb the host-acceptance behaviour of *D. pulchellus* females.

In the electrophysiological experiments, stimulation by the 50 mM NaCl control, which was used as the solvent for the stimulus, elicited very low responses (less than 10 spikes per 10 s) from the cell(s) of S1 and S2 (figure 2*a*,*c*). In contrast, no response was obtained with the S3 (figure 2*e*).

No response was obtained with S3 stimulated by the NaCl protein extract (figure 2*f*). None of our attempts to obtain an electrophysiological recording made from this sensillum, with either the NaCl control or the NaCl protein extract, yielded any response from any of the cells innervating it. We therefore concluded that S3 is definitely chemosensory, but that it is olfactory rather than gustatory.

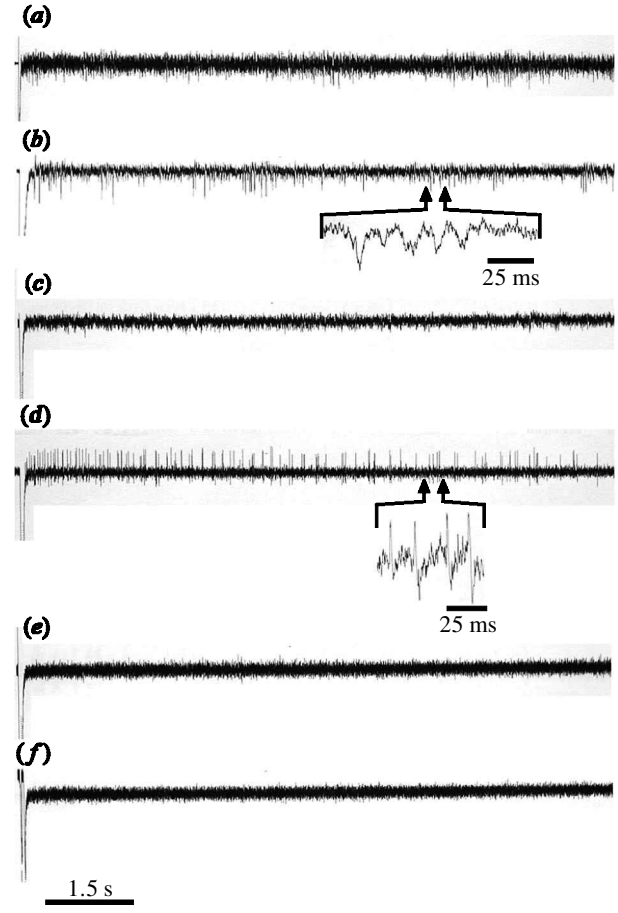

Figure 2. Samples of chemosensory recordings from the three sensillar types, S1 (*a*,*b*), S2 (*c*,*d*) and S3 (*e*,*f*), of mated *D. pulchellus* females to 50 mM of NaCl (control solution) (*a*,*c*,*e*) and to 50 mM of NaCl protein extract (*b*,*d*,*f*).

The NaCl protein extract elicited spikes from S1 and S2 (figure 2*b*,*d*). Interestingly, at least one receptor neuron in S2 responded much more strongly to this extract than the S1 at the same stimulus concentration. The total number of spikes generated over 10 s was significantly higher for S2 (139 ± 25 spikes) than for S1 (43 ± 11 spikes) (Mann–Whitney test, $p = 0.001$) (figure 3). Moreover, the greater sensitivity towards the stimulus of the S2 chemoreceptors

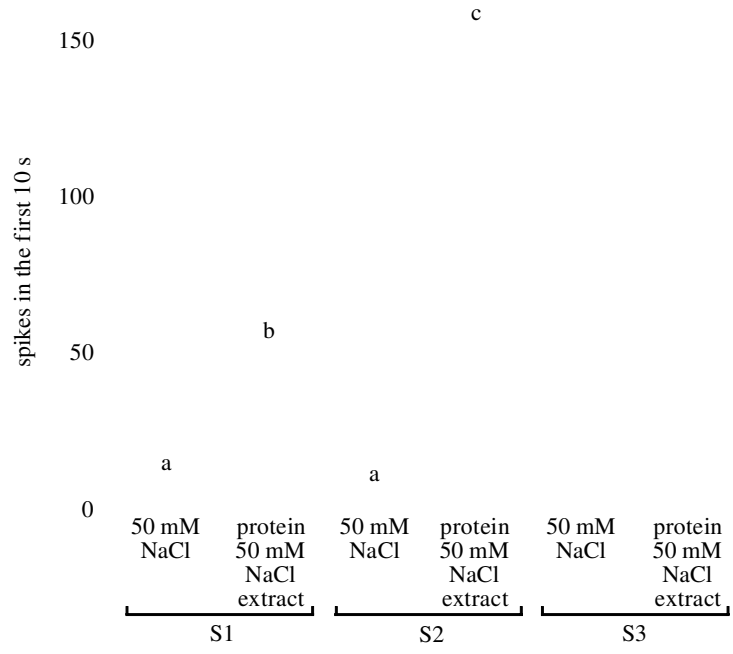

Figure 3. Comparison of spike activity from the three sensillar types (S1, S2 and S3) of mated *D. pulchellus* females to 50 mM of NaCl and to 50 mM of NaCl protein extract. Each datum is the mean value of spikes per recording from one sensillar type in 11 females (11 antennae) (± s.e.m., vertical bars). The values for S3 are equal to zero.

was observed within the first second of stimulation. The spike number in this first second was 30 ± 6 spikes for S2 versus 8 ± 2 spikes for S1, this difference was statistically significant (Mann–Whitney test, $p = 0.0001$).

It is significant that the shape of the electrical responses from the two responding sensilla was not the same. S1 elicited responses consisting only of negative impulses (figure 2*b*), which may not correspond to a real spike. The gustatory neuron(s) of the S2 provided unambiguous biphasic responses to the NaCl cocoon protein extract, in which a positive impulse was followed by a negative deflection (figure 2*d*).

All the data demonstrated that S1 and S2 were gustatory but that only S2 was very likely to contain a receptor neuron responding to the behaviourally active cocoon protein extract.

4. DISCUSSION

There are many behavioural steps in host selection and host acceptance, and while many infochemicals have been identified, the behaviour they elicit encompasses various steps. Moreover, it has seldom been possible to clarify which chemicals and which particular chemosensory receptors are involved in which behaviour. In the present study, the infochemicals and their source (Bénédet *et al.* 1999) and the specific sensillar receptors involved in the host-recognition behaviour exhibited by *D. pulchellus* females were clearly identified.

In the endoparasitic *D. pulchellus* species, protein kairomones have been shown to be essential for host-acceptance behaviour. These compounds come from the silk cocoon of their host, the *A. assectella* pupae (Bekkaoui & Thibout 1993; Bénédet *et al.* 1999). Given that protein kairomones are non-volatile compounds, they must be perceived by contact chemoreceptors. Behavioural observations (Bénédet *et al.* 1999) and previous ablation experiments performed by Labeyrie (1960) had already suggested that, in *D. pulchellus*, the chemoreceptors implicated in host recognition are located on the antennae. The antenna flagella of females are equipped with 13 types of sensillum (Lecomte *et al.* 1990; Bénédet 1999). The morphological, ultrastructural and electrophysiological studies showed that the females had three types of sensillum that were chemoreceptors, but that only one seemed to respond to the behaviourally active protein kairomone extract.

The presence of multiple pores on the apical tip and the dendrites in the projecting cuticular process supports the conclusion that S3 is a chemosensory sensillum. Despite differences in external shape, the ultrastructure of S3 is similar to some olfactory placoid sensilla already described in the antennae of *D. pulchellus* (Rojas-Rousse & Palevody 1983) and other species (Masson *et al.* 1972; Zacharuk 1980; Quicke 1997). Consequently, S3 seems to be olfactory rather than gustatory, and is probably not implicated in host acceptance. As they are present only on the antennae of females, the sensilla could well play a role in the selection of the plant. Indeed parasitoid females must perceive the characteristic sulphur-containing allelochemicals from *Allium* before they locate their potential host (Lecomte & Thibout 1986).

The presence of an apical pore on S1, which is reported for the first time, to our knowledge, in this paper suggests that it is indeed a chemosensory sensillum (Altner 1977; Zacharuk 1980, 1985). The spike discharges from this sensillar type do not correspond to the two-phase electrophysiological response normally expected with an active stimulus. This sensillum is not sex specific and is present in small numbers (four per antennomere; Lecomte *et al.* 1990). This suggests that S1 may not play any significant role in the perception of the cocoon protein kairomones or in host acceptance.

The morphology and ultrastructure of S2 reveal the presence of a multiporous apical part. Its structure and sensitivity to the behaviourally active extract was confirmed by the electrophysiological recordings, and lead to the conclusion that it has a gustatory function. Moreover, these sensilla are numerous (about 46 per antenna; Lecomte *et al.* 1990) and found on the ventral surface only of the distal antennomeres of females, as could be expected for sensilla implicated in host acceptance.

There is conclusive evidence that, in *D. pulchellus*, the multiporous S2 is the only one to respond to the cocoon protein kairomones that trigger host-acceptance behaviour. Multiporous gustatory sensilla are commonly found in parasitic Hymenoptera females and are located on the ventral surface of their antennae (Isidoro *et al.* 1996; Amornsak *et al.* 1998). They have been reported to play a significant role in host acceptance by contact in species such as *Cheiloneurus noxius* (Encyrtidae) (Weseloh 1972) and *Trissolcus basalis* (Scelionidae) (Bin *et al.* 1989; Isidoro *et al.* 1996). However, these receptors have rarely been shown to be implicated in the perception of a protein signal and, in clearly defined behaviour, this has seldom been demonstrated.

Large molecules, such as mucopolysaccharides and proteins, have already been reported to contribute to host recognition (Vinson 1991; Bin *et al.* 1993; Quicke 1997). For instance, it was suggested that infochemicals, probably proteins, from the *Lymantria dispar* silk cocoon are used by its parasitoid *Apantheles melanoscelus* in host acceptance but the evidence remains inconclusive (Weseloh 1977). It has been suggested that two large proteins present in the accessory gland of *Heliothis virescens* may serve as an egg-recognition kairomone for the parasitoid *Telenomus heliothidis* (Strand & Vinson 1983). However, this conclusion was based mainly on morphological and behavioural evidence (Vinson 1991; Quicke 1997) and there was usually no electrophysiological evidence.

Most of the electrophysiological tip recordings have been carried out using small molecules, such as sugars and amino acids (Städler 1984; Hollister & Mullin 1998; Bernays *et al.* 2000). It has been shown that sensilla perceive these molecules, which leads to specific behavioural responses, in particular feeding (Städler 1984). In *D. pulchellus*, the role of amino acids can be ruled out as cocoon proteins do not trigger host-acceptance behaviour after digestion by pronase (Bénédet *et al.* 1999), and in this study the dialysis of the protein extracts ensures that molecules smaller than 12–14 kDa were eliminated.

The literature reveals that kairomones constitute a large group with a widely varied biochemistry, but that they are mainly small molecules. Therefore, what could be the ecological significance of macromolecules in the *D. pulchellus*–host system interaction?

When its host is concealed, a parasitoid may use infochemicals from the container as well as from the host itself to recognize it (Chow & Mackauer 1999). Kairomones from the container and from the outside of the host are more easily recognizable by foraging females, since they can be located by means of antennal receptors and are immediately detectable. In contrast, internal cues, such as internal marks, or physiological changes within the host can be detected only after the host has been probed with the ovipositor. In *D. pulchellus*, kairomones in the host cocoon can always be detected since they are a permanent component of the cocoon.

The literature often describes the detectibility of kairomones as a limiting factor. They are believed to be biologically active for only a limited time because they are degraded or volatilized over time, leading to a decline in active concentration to below the threshold of perception at which infochemicals elicit the specific response from the parasitoid (Hoffmeister & Roitberg 1998). In *D. pulchellus*, 10-year-old cocoons conserved at room temperature are still able to trigger host-recognition behaviour by female wasps (Bénédet *et al.* 1999). This finding shows that these kairomones are very stable over time and suggests that they may be implicated in the recognition of host species rather than in the discrimination of host quality. Therefore, when pupae from other moth species, namely *Plodia interpunctella* and *Ephestia kuehniella*, were introduced into an *A. assectella* cocoon, *D. pulchellus* females still exhibited host-recognition behaviour and laid an egg, whereas these species were passed over for oviposition by the female wasps when concealed in their natural cocoon (Thibout 1988).

A comparative SDS–PAGE analysis of the main cocoon polypeptides previously carried out in other lepidopteran species, namely *Bombyx mori* and *E. kuehniella*, showed that the global protein pattern of the cocoon is species specific. The bands corresponding to the behaviourally active proteins were absent from the cocoons of the two other species (Bénédet 1999). The specificity of the cocoon had previously been suspected on the basis of behavioural experiments (Bekkaoui & Thibout 1993). These cocoon kairomones therefore seem to be specific and to provide very reliable cues for *D. pulchellus* females.

The ability to identify the concealed host from kairomones from the cocoon may make it possible to avoid wasting time and energy. These kairomones are easily accessible, stable over time, reliable and also easily detected as they are amongst the major components of the cocoon (Bénédet 1999). In such a pupal–parasitoid system, host recognition through the perception of protein kairomones may be highly advantageous for the parasitoid. As few examples of protein gustation are described in the literature, is the perception of proteins really an unusual feature in interactions between insects?

In the protein perception, an important question is how the molecules are adsorbed onto the sensillum surface and their transportation through the wall to the dendritic receptors in the sensillar lymph. Proteins are large molecules, and in the present study the kairomones were *ca.* 80 kDa (Bénédet *et al.* 1999). Is there any transducer, such as the odorant-binding proteins, involved in olfaction (Kaissling 1971, 1987, 1996; Krieger & Breer 1999)? A solubilization phase is probably required. In *D. pulchellus*, the protein kairomones are amongst the most hydrophylic polypeptides in the silk cocoon but this is probably not enough. They could be cut, and probably modified in other ways before they reach the cellular receptors. It has been suggested that some secretions from glands associated with gustatory sensilla may mediate the perception of kairomones (Bin *et al.* 1989; Isidoro *et al.* 1996). However, in *D. pulchellus*, no glandular structure has yet been observed in electron microscopy. Interestingly, *D. pulchellus* females are also able to recognize kairomonal

proteins even when they are presumably not in solution, i.e. a 10 year-old dry cocoon and a lure impregnated with an active extract then allowed to dry.

Our studies of this host–parasitoid system made it clear that the molecular mechanisms involved in the perception of large protein molecules and the role they play in specific behaviours remain to be elucidated.

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