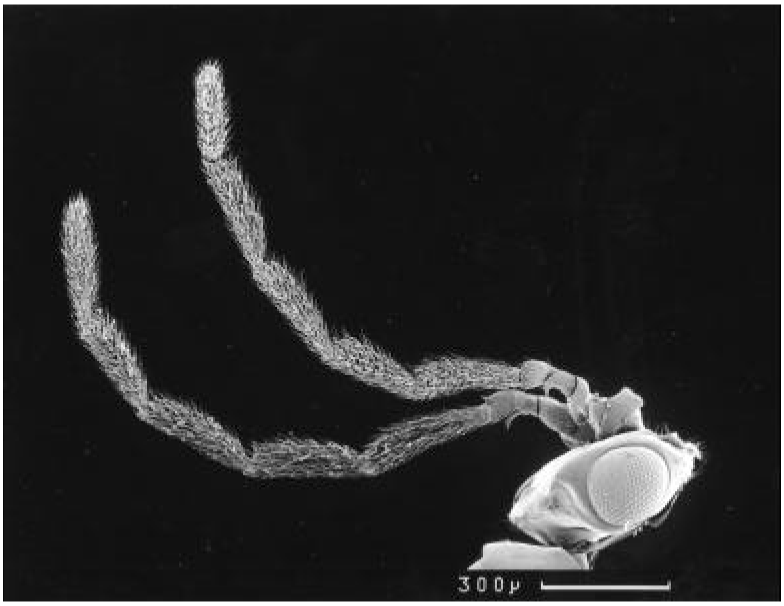
BLGY 3040 Project Report 2005

External Morphology of Antennal Sensilla in relation to the Host Searching Behaviour of *Liporrhopalum tentacularis* Grandi (Hymenoptera: Agaonidae)



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Contents

Abstract

External morphology of antennal sensilla on female *Liporrhopalum tentacularis* (Hymenoptera: Agaonidae) was examined using scanning electron microscopy. The antennae of live female wasps were manipulated and were found to break at a characteristic point in 87.5% of replicates. Antennae of female wasps removed from figs were examined and found to have broken at the same characteristic point. Video footage was taken of female wasps searching on the fig surface and entering figs. The female antenna displays 12 types of sensilla: basiconic capitate peg sensilla (1 type), basiconic sensilla (1 type), chaetica sensilla (6 types), placoid sensilla (2 types), trichoid sensilla (1 type) and a previously undescribed sensillum referred to as sensillum obscurum (1 type). The possible functions of these sensilla were discussed in relation to their position on the antenna and the behaviour observed in the video footage. The distributions and putative functions of sensilla were consistent with the life cycle of female *L. tentacularis*.

Introduction

Insect antennae are mobile, segmented, paired head-appendages and are found in nearly all insect groups (Gullan and Cranston, 1994; Schneider, 1964). Numerous sensory organs, or sensilla (singular: sensillum) occur on antennae in the form of hairs, pegs, pits or cones. Functions of antennal sensilla include chemoreceptivity (gustatory and olfactory), mechanoreceptivity, thermoreceptivity, hygroreceptivity and CO_2 receptivity (Keil, 1999). In this study we aim to investigate/describe the antennae of the fig wasp *Liporrhopalum tentacularis* Grandi, pollinator of the fig *Ficus montana* Blume, and relate this description to the function of the antennae in relation to the lifecycle of the species

The relationship between plants of the genus *Ficus* (Moraceae) and their pollinating wasps of the family Agaonidae (Hymenoptera: Chalcidoidea) constitutes a biologically important model system describing an obligate mutualism. This is reflected in the large number of publications describing their systematics, evolution, and ecology (Weiblen, 2002).

The genus *Ficus* is characterised by its inflorescence: an enclosed receptacle (syconium or fig) containing many unisexual flowers; staminate males and carpellate females of which mature at different times (Janzen, 1979; Weiblen, 2002; Cook and Rasplus, 2003). The relationship is generally highly host species-specific, although some departures from this specificity have been documented (Weibes, 1979; Michaloud *et al.*, 1996). Fig wasps show extreme sexual dimorphism, and both sexes have very different roles in the life cycle of the species.

As the female flowers within the fig mature it becomes receptive to female wasps and releases a species-specific volatile attractant to advertise this fact (van Noort *et al.*, 1989; Hossaert-Mckey *et al.*, 1994; Grison-Pige *et al.*, 2002). Winged female wasps enter the syconium through a bract-lined hole at the apex (ostiole) in order to oviposit in the ovaries of the enclosed female flowers. At least some species of pollinating fig wasps possess a hook on the pedicel of the antenna which is thought to assist it in entering a fig by clawing through the ostiolar bracts (S. G. Compton, pers. comm.). In entering the syconium a female wasp brings with it pollen, and depending on the species actively or passively pollinates these flowers. A female wasp lays her eggs in the fig by penetrating the styles of female flowers with her ovipositor and depositing eggs in the ovaries (generally a single egg in each ovary (Jousselin *et al*., 2001)). Individual larvae feed on the endosperm of the developing seed until hatching (Janzen, 1979; Wiebes, 1979; Weiblen, 2002).

The life cycle of the male wasp is restricted to the natal host fig. Male imagos hatch first and chew holes in 'galled' ovaries containing females (see Janzen, 1979 for a discussion of the term 'gall'). These males, apterous with vestigial eyes and antennae mate with females still inside their own 'galls' through these holes. The females then hatch, and pick up pollen from newly mature male flowers. Females escape the fig through holes excavated in the syconium wall by the male wasps, and take flight in order to colonise new figs (Weiblen, 2002). There is evidence to suggest that fig wasps may disperse long distances in order to find a receptive host fig. Compton *et al*. (2000) found evidence suggesting that fig wasps fly high above the rainforest canopy and use the wind to passively carry them long distances until a species-specific volatile attractant is detected, at which point the wasps may fly down into the canopy in order to locate figs. Harrison (2003) found evidence to support this theory, which suggests that fig wasps may disperse at least 30km in order to find an oviposition site. Ware and Compton (1994) found that female *Elisabethiella baijnathi* fig wasps can recognise figs of their host *Ficus burtt-davyi* after landing on the tree, this ability appearing to be via contact chemoreception. *E. baijnathi* could not distinguish between the figs and other parts of the tree when in flight. These studies suggest that fig wasps possess both long and short-range chemoreceptive ability, allowing them to locate fig plants and also specific fruits within a plant.

Much of the fig wasp literature states that upon entering a fig, a pollinator is at once trapped and remains here until death. The term 'tomb blossom' has been used to describe the fig at this stage, and seems to have been coined solely to describe this instance (e.g. Gibernau *et al*., 1996; Weiblen, 2002; Cook and Rasplus, 2003). Gibernau *et al*. (1996) present evidence to the contrary of this much quoted aspect of the mutualism, finding that some pollinator species can exit the fig and are able to oviposit in more than one fig. *L. tentacularis* is a species which is known to oviposit in more than one fig, and this behaviour has been studied in the laboratory (Moore *et al*., 2003a). As a female wasp enters a receptive fig through the ostiole, her wings and flagellar antennal segments are ‘easily detached’ (Cook and Rasplus, 2003) and generally lost as a result of the passage through the tight constrictions caused by the ostiolar bracts, as noted by Galil and Neeman (1977) in the wasp *Blastophaga psenes*. This divides oviposition behaviour in species able to enter more than one fig into a two-stage process: stage one in which a female is able to fly to a receptive fig and detect its volatile attractants with her antennae; and stage two in which a female is restricted to searching for receptive figs on one plant, with restricted or absent olfactory and gustatory senses.

L. tentacularis is a minute pollinating fig wasp which exists in an obligate mutualism with its host plant *F. Montana* (Moore *et al.*, 2003a). *F. montana* is a small pioneer gynodioecious shrub or scrambler found along rivers or in disturbed forest in the Eastern Malay Archipelago (Corner, 1952; cited from Moore *et al.*, 2003b).

This study attempts to link the morphology of female *L. tentacularis* antennae with this two-stage fig-searching behaviour by creating a map of antennal sensilla using scanning electron microscopy and observing antennal behaviour of wasps entering receptive figs. By manipulating the antennae of live wasps and examining specimens of post-entry wasps, I attempt to determine if the antennae have a characteristic break-point that could be consistent with the presence of a secondary stage in host-finding by recording the sensilla that such wasps still have available to them after entry into a fig.

Materials and Methods

Collection of wasps

Wasp specimens were collected from the University of Leeds glasshouse population of *L. tentacularis* and *F. montana*, which originated from the Center for International Forestry Research (CIFOR) plantation, West Java, Indonesia (06°34'S, 106°45'E) and from Rakata, Krakatau Islands, Indonesia (05°00'S 105°00'E) (Moore *et al.*, 2002; Moore *et al.*, 2003a; Moore *et al.*, 2003b).

Wasps were collected by picking and dissecting figs which were about to release wasps (D-phase figs) on functionally male plants. These figs are characterised by their relatively large size and spongy feel when squeezed, often coupled with a yellowing of the external fruit (S. Raja and N. Suleman, pers. comm.). Wasps were immediately placed in alcohol using a fine paintbrush and stored in a freezer.

Scanning Electron Microscopy (SEM)

Wasps were thawed to room temperature and critical point dried. Samples were mounted on aluminium stubs using double-sided sticky tape and sputter coated with gold. A Camscan Series III scanning electron microscope operated at 5-10kV was used to observe the specimens. Photomicrographs were taken using Ilford HP5 Plus 120 Roll Film. Wasps for photographic documentation of sensilla types were cleaned in alcohol for 30 seconds in an ultrasonic cleaner.

Mean numbers of antennal sensilla were obtained by observing 5 specimens on-screen. Estimates of total numbers of flagellar sensilla were obtained by counting sensilla in a 90µm section of each flagellar segment on 5 specimens. Individual flagellar segment lengths for each of these specimens were measured from photomicrographs in order to obtain full estimates for complete segments. Mean sensillum length was obtained by measuring photomicrographs of 3 to 7 sensilla and is given in µm ± standard errors. Terminologies of Snodgrass (1935), Schneider (1964), Zacharuk (1985), Amornsak *et al.* (1998) and Keil (1999) were used to describe the antennal sensilla (see discussion).

Manipulation Experiments

Live wasps were collected as outlined above and secured by holding the wings or thorax with forceps, under a dissecting microscope. The final flagellar segment of the antenna was gripped with forceps and slowly pulled until it gave way. The break point along the antenna was recorded. Fourteen antennae (from seven wasps) were manipulated in this way.

Figs which had been entered by female wasps were opened by making a longitudinal incision with a scalpel and bisecting the fig carefully with forceps. The wasps were removed and observed under a dissecting microscope. The natural break-points of 20 antennae (from 10 wasps) were recorded.

Observations of behaviour

Receptive functionally male figs (B-phase) were picked and immediately transferred to the lab. Receptive figs are characterised by their relatively small size, green colouration, and spongy feel often accompanied by a slight visible opening of the ostiole when squeezed (S. Raja and N. Suleman, pers. comm). Male figs were individually fixed under the dissecting microscope with sticky tape, and live wasps (collected as outlined above) were introduced onto the surface of the fig with a fine paintbrush. The behaviour of these wasps was filmed using a Teachcam PAL Rev. 6.0 microscope camera attachment connected to a dissecting microscope and recorded onto VHS tape using a SANYO VHR-H802E Video Cassette Recorder. The footage was later analysed and the time intervals between different behaviour classes measured using a digital stopwatch. Times are given in seconds ± standard error.

Results

Antennal map of female L. tentacularis

The antennae of female *L. tentacularis* is flagellar or annulated in form (see Schneider, 1964), and consists of a scape, pedicel and six-segmented flagellum. The scape is irregular in shape (Figs 3 and 4) and so a measurement of length was not possible. The pedicel consists of a rounded lozenge-shaped basal section (Figs 3 and 6) followed by a cylindrical segment with a large hook-shaped branch (Figs 3 and 7). The pedicel's irregular shape also prevented obtaining an accurate measurement of its length. The flagellum is differentiated into a short cylindrical section (flagellar segment 1 – see Figs 3, 7, 10, 15 and 16) followed by five elongate segments (Figs 3, 10, 11 and 13. Mean lengths of antennal flagellar segments are given in Table 1.

Table 1. Lengths of the antennal flagellar segments in female *L. tentacularis* (n = 5).

Antennal segments	Mean length ± SE (µm)
Flagellum 1	79.05 ± 2.65
Flagellum 2	279.04 ± 5.67
Flagellum 3	287.51 ± 3.57
Flagellum 4	294.00 ± 4.00
Flagellum 5	279.51 ± 5.17
Flagellum 6	272.29 ± 8.59
Total (segments 2-6)	1479.54 ± 29.26

A Kruskal-Wallis test showed no significant difference between the lengths of flagellar segments 2-6 measured in this study at the $P<0.05$ level ($P = 0.072$, $n = 5$).

Antennal sensilla types

Based on size, shape, distribution and cuticular attachment, 12 types of sensilla were recognised on the female antenna. The sensilla are: basiconic capitate peg sensilla (BCPS, 1 type), basiconic sensilla (BS, 1 type), chaetica sensilla (ChS, types 1-6), placoid sensilla (PS, types 1 and 2), trichoid sensilla (TS, 1 type) and a previously undescribed sensillum referred to as sensillum obscurum (SO, 1 type). The types, mean number and individual distribution of sensilla on the female antenna are given in Table 2. Mean lengths of sensilla are given in Table 3 (with the exception of BCPS – see below).

Table 2. Types, mean number ± SE, and distribution of sensilla on the antennae of female *L. tentacularis* (n = 5 antennae, from 5 wasps).

	Antennal Segments								
Sensilla Types	Scape	Pedicel	Flagellum 1	Flagellum 2	Flagellum 3	Flagellum 4	Flagellum 5	Flagellum 6	Total
BCPS 1	-	-	-	present	present	present	present	present	present
BS	-	-	-	-	-	-	-	7.6 ± 1.50	7.6 ± 1.50
ChS 1	17.0 ± 1.61	32.4 ± 1.12	-	-	-	-	-	-	54.6 ± 4.19
ChS 2	13.8 ± 0.80	-	-	-	-	-	-	-	13.8 ± 0.80
ChS 3	7.2 ± 1.28	-	-	-	-	-	-	-	7.2 ± 1.28
ChS 4	-	4.6 ± 0.24	9.0 ± 0.71	-	-	-	-	-	13.6 ± 0.81
ChS 5	-	1	-	-	-	-	-	-	1
ChS 6	-	-	-	266.8 ± 11.01	256.9 ± 10.26	293.4 ± 18.50	303.2 ± 22.54	312.3 ± 24.97	1432.5 ± 63.07
PS 1	-	-	16.6 ± 1.21	-	-	-	-	-	16.6 ± 1.21
PS 2	-	-	-	84.8 ± 8.86	81.6 ± 4.55	83.7 ± 5.35	87.0 ± 5.47	71.5 ± 3.13	408.6 ± 19.08
TS	-	1	-	-	-	-	-	-	1
SO	-	73.2 ± 0.80	-	-	-	-	-	-	73.2 ± 0.80

Table 3. Lengths of the measured sensillum types on the antennae of female *L. tentacularis*

Sensilla Types	Mean length ± SE (µm)	n
BS	18.97 ± 1.04	5
ChS 1	9.51 ± 0.85	5
ChS 2	3.54 ± 0.22	7
ChS 3	32.26 ± 2.83	5
ChS 4	44.87 ± 5.07	5
ChS 5	26.75 ± 2.68	3
ChS 6	46.29 ± 1.40	5
PS 1	33.87 ± 2.25	5
PS 2	55.58 ± 5.18	5
TS	6.24 ± 0.85	3
SO	15.90 ± 0.39	5

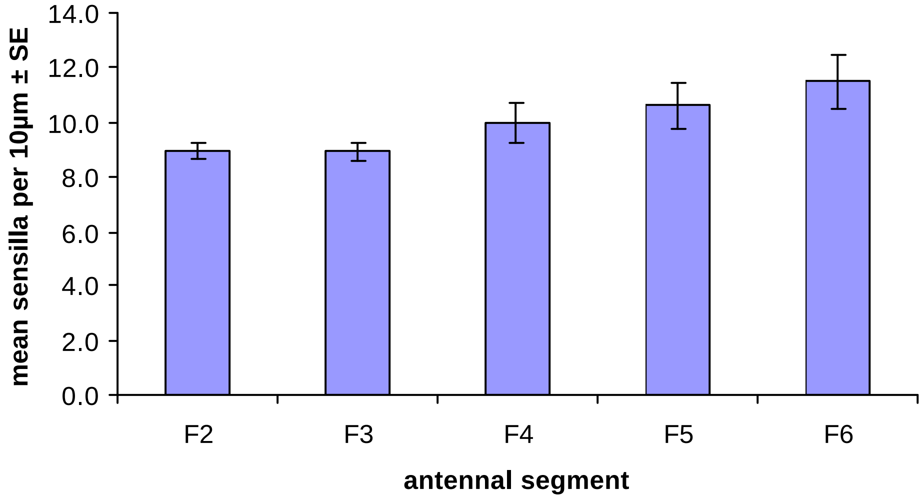

Fig. 1. Mean numbers of chaetica sensillum type 6 per 10µm on antennal segments 2-6 of female *L. tentacularis* (n = 5).

A Kruskal-Wallis test showed no significant difference between the densities of chaetica sensilla type 6 on flagellar segments 2-6 measured in this study at the $P<0.05$ level ($P = 0.083$, $n = 5$)

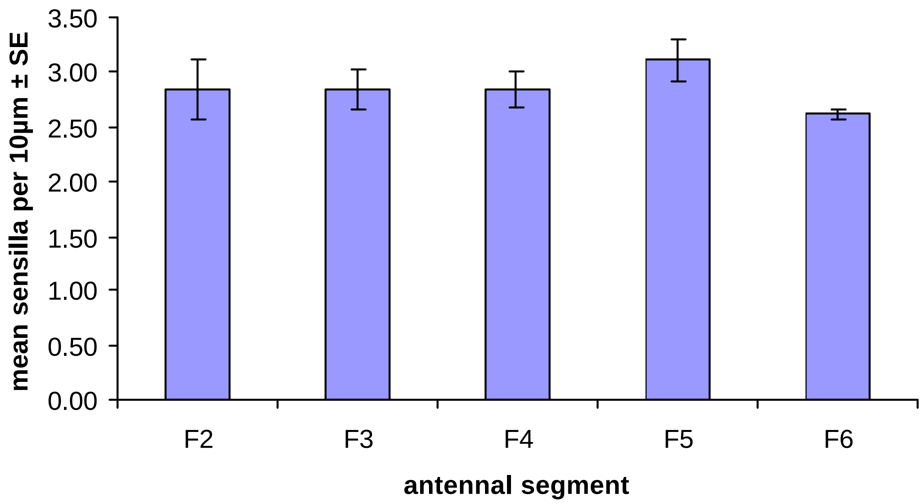

Fig. 2. Mean numbers of placoid sensillum type 2 per 10µm on antennal segments 2-6 of female *L. tentacularis* (n = 5).

A Kruskal-Wallis test showed no significant difference between the densities of placoid sensilla type 2 on flagellar segments 2-6 measured in this study (P = 0.325, n = 5)

Basiconic Capitate Peg Sensillum (BCPS)

See Figs 11 and 12. The BCPS in this study was classified according to its shape, cuticular attachment and distribution. BCPS are bulb-like structures, projecting from a depression in the centre of a circular raised area of cuticle. The BCPS in this study consist of a tapering stalk with a bulbous tip roughly the width of the base where the peg joins the cuticle. BCPS are distributed along the length of the second-sixth flagellar segments, arranged irregularly on the underside of the antenna i.e. the side which faces the ground if the wasp holds its antenna straight forward (hereafter: ventral). Due to the irregular distribution of these sensilla, numbers on each segment could not be estimated, so a presence or absence on each segment was recorded. BCPS are found in close proximity to chaetica sensilla type 6 and placoid sensilla type 2. They were thus obscured when viewed side-on and so an accurate measurement of sensillum length could not be made. Any other view would have incurred a parallax error.

Basiconic Sensillum (BS)

See Figs 13 and 14. The basiconic sensillum in this study was classified on the basis of its shape, distribution and cuticular attachment. Basiconic sensilla are relatively thick, weakly tapered, bristle-like sensilla set in a rounded projection of the cuticle, and possess a rounded tip. Basiconic sensilla are found in a small group at the tip of the antenna on flagellar segment 6. They are roughly parallel with this segment, and point away from it.

Chaetica Sensilla (ChS)

The chaetica sensilla in this study were classified on the basis of their size, shape, distribution and cuticular attachment. They are generally hair or bristle-like and are set into a basal socket.

Type 1 – See Figs 4 and 7 Small, socketed, strongly tapered, straight whiskers on the scape and pedicel, often near joints and other places where cuticle is likely to come into contact with other surfaces.

Type 2 – See Fig. 5. Relatively very small, strongly tapered protuberances set into sockets. Distributed across the rear face of the scape.

Type 3 – See Fig. 4. Hair-like, curved sensilla longer than ChS type 1, distributed on the front face and top ridge of the scape. Often arranged in a horizontal line halfway up the front face of the scape.

Type 4 – See Figs 7 and 10. Relatively long, weakly tapering, lightly grooved hair-like sensilla occurring on the pedicel, next to the join with the first flagellar segment and extending over this join onto the first flagellar segment. Also found on the first flagellar segment in a similar position, near the join of, and extending over the second flagellar segment.

Type 5 – See Figs 7 and 8. Relatively large and thick, sharply tapered sensillum existing as one example on the middle of the pedicel of each antenna.

Type 6 – See Figs 10, 11 and 13. Relatively very long, curved, strongly grooved, weakly tapered hair-like sensilla distributed in great numbers over the surface of the last five flagellar segments.

Placoid Sensilla (PS)

The placoid sensilla in this study were classified on the basis of their size, shape, cuticular attachment and distribution. They are elongate, plate-like sensilla with a relatively large surface area, arising from the cuticle or laying flat against it, differentiated by a surrounding groove.

Type 1 – See Figs 7 and 10. Elongate, plate-like structures surrounded by a groove in the cuticle, slightly tapering to a rounded point at their distal apex, where they may become separated from the cuticle. This projection occurs especially when distributed at the end of an antennal segment. Otherwise they lie flat against the cuticle. Arranged along the length of the first flagellar segment

Type 2 – See Figs 10, 11 and 13. Elongate raised ridge-like structures with height larger than their width, which become separated from the cuticle at their distal end, where they taper sharply to a rounded point. Distributed along the length of the second to the sixth flagellar segments.

Trichoid Sensillum (TS)

See Figs 7, 9 and 10. The trichoid sensillum in this study was classified according to its shape, cuticular attachment and distribution. This socketless pointed sensillum arises smoothly from the cuticle and exists as one example before the groove on the pedicel at the pedicel hook.

Sensillum Obscurum

See Fig. 6. This highly distinctive sensillum was classified according to its shape, cuticular attachment and distribution. Sensillum obscurum are flattened and leaf-like/teardrop-like in shape, strongly tapering to an elongate and thinned apex. They are attached to the cuticle at the deep edge of a depression which gradually slopes upwards to become level with the antennal surface. Occur in a patch on the inner face of the proximal segment of the pedicel, lying flat against the cuticle and pointing towards the head.

Manipulation Experiments

Of the 14 antennae manipulated with forceps, 12 (85.7%) broke at the join linking flagellar segments 1 and 2, i.e. all the elongate flagellar segments were removed. The remaining two antennae broke at the join linking the third and fourth flagellar segments and the join linking the fifth and final segments respectively.

All 20 antennae observed on wasps which had entered figs had broken at the join linking flagellar segments 1 and 2.

This characteristic break point between flagellar segments 1 and 2 was observed using SEM. The break point consists of a constriction in the cuticle at the tip of flagellar segment 1, creating a clean break with no damage to the remaining segment when flagellar segments 2-6 are removed (see Figs 15 and 16).

Observation of behaviour

Analysis of video footage showed a characteristic pattern of behaviour in females searching for an oviposition site. The behaviour of seven winged females was recorded.

Upon setting foot onto a fig, a female wasp starts walking across its surface with her antennae held parallel to it, waving them upwards and downwards from this position. During this first phase, on the downstroke of the waving motion, the wasp brushes the exterior of the fig with the ventral surface of the flagellar antennal segments, held flat against the fig surface. Whilst performing these actions, the wasp walks towards the ostiole at the apex of the fig. When the cylindrical projection at the top of the fig (which holds the ostiole) is reached (see Fig. 17), the wasp starts tapping the surface of the fig with the tip of the final flagellar antennal segment by curving her antennae downwards at the distal end. This tapping signifies the final stage of movement towards the ostiole, and is followed by the wasp chewing her way into the ostiolar bracts in order to make her entry into the fig lumen. This pattern of behaviour was present in all of the observed wasps.

Wasps started tapping with the antennal tip 2.91-15.52 seconds after being placed upon on the fig surface (mean = 7.22 ± 1.53 seconds, n = 7), and took a further 5.24-9.47 seconds to start entry into the fig (mean = 7.90 ± 0.61 seconds, n = 7). One wasp with flagellar antennal segments 2-6 removed was filmed on the surface of the fig and was seen to enter the fig after 23.05 seconds.

Scanning Electron Photomicrographs

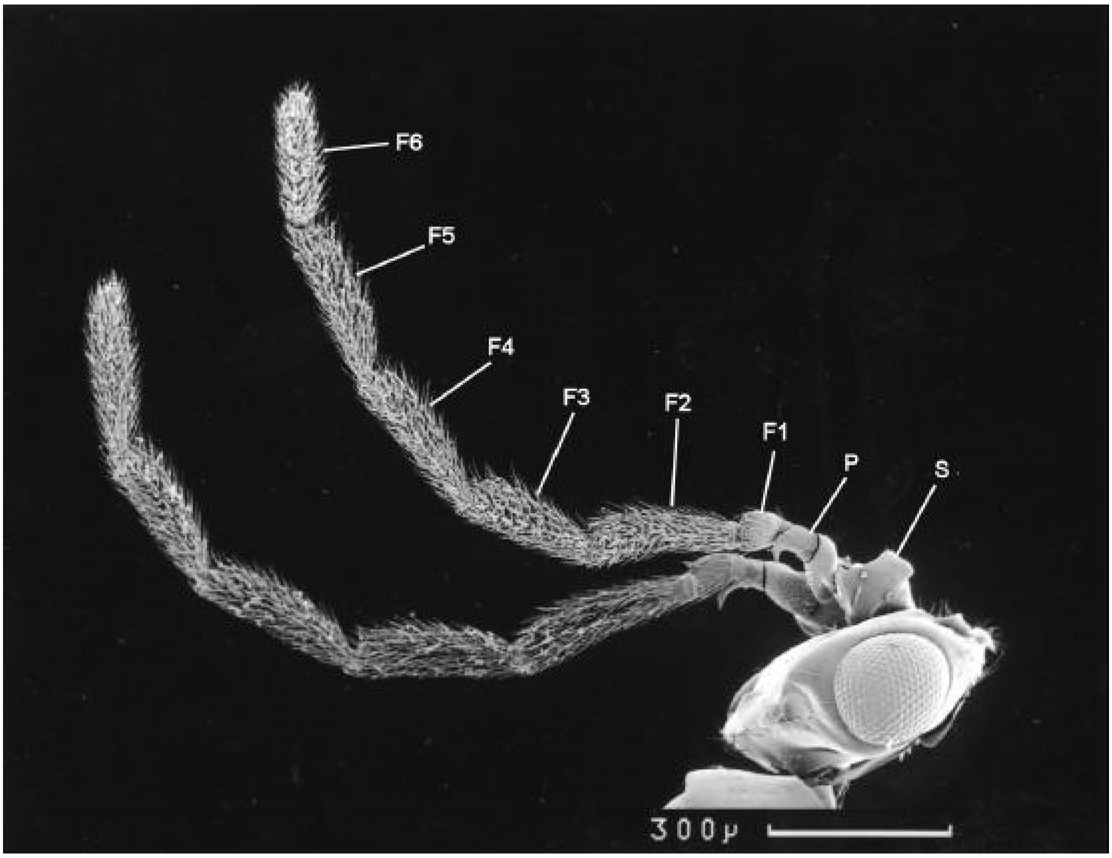

Fig. 3. SEM photomicrograph of female *L. tentacularis* head and antennae S = scape; P = pedicel; F1 = flagellum 1; F2 = flagellum 2; F3 = flagellum 3; F4 = flagellum 4; F5 = flagellu m 5; F6 = flagellum 6 of right antenna. Bar = 300µm.

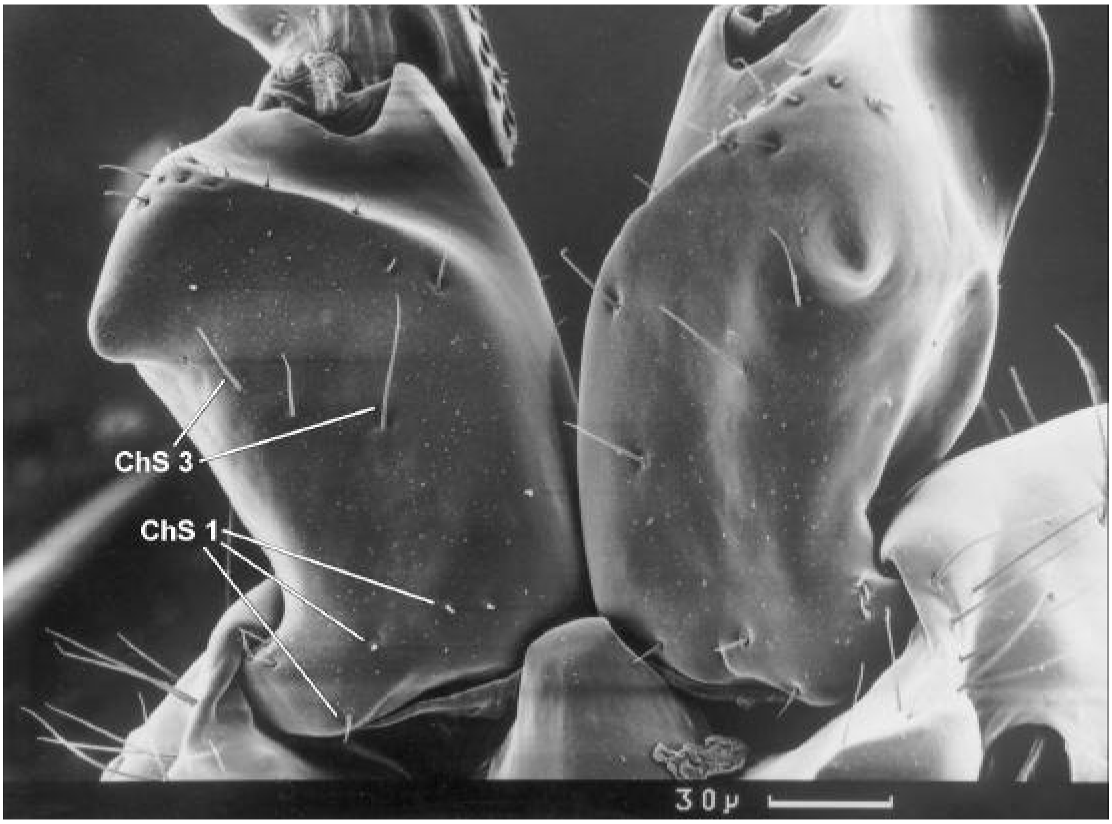

Fig. 4. SEM photomicrograph of female *L. tentacularis*. Front view of right and left scapes ChS 3 = two examples of chaetica sensillum type 3 on front face of right scape, in characteristic position along the mid-region; ChS 1 = three examples of chaetica sensillum type 1 on lower front face of scape, near join with head. Bar = 30µm.

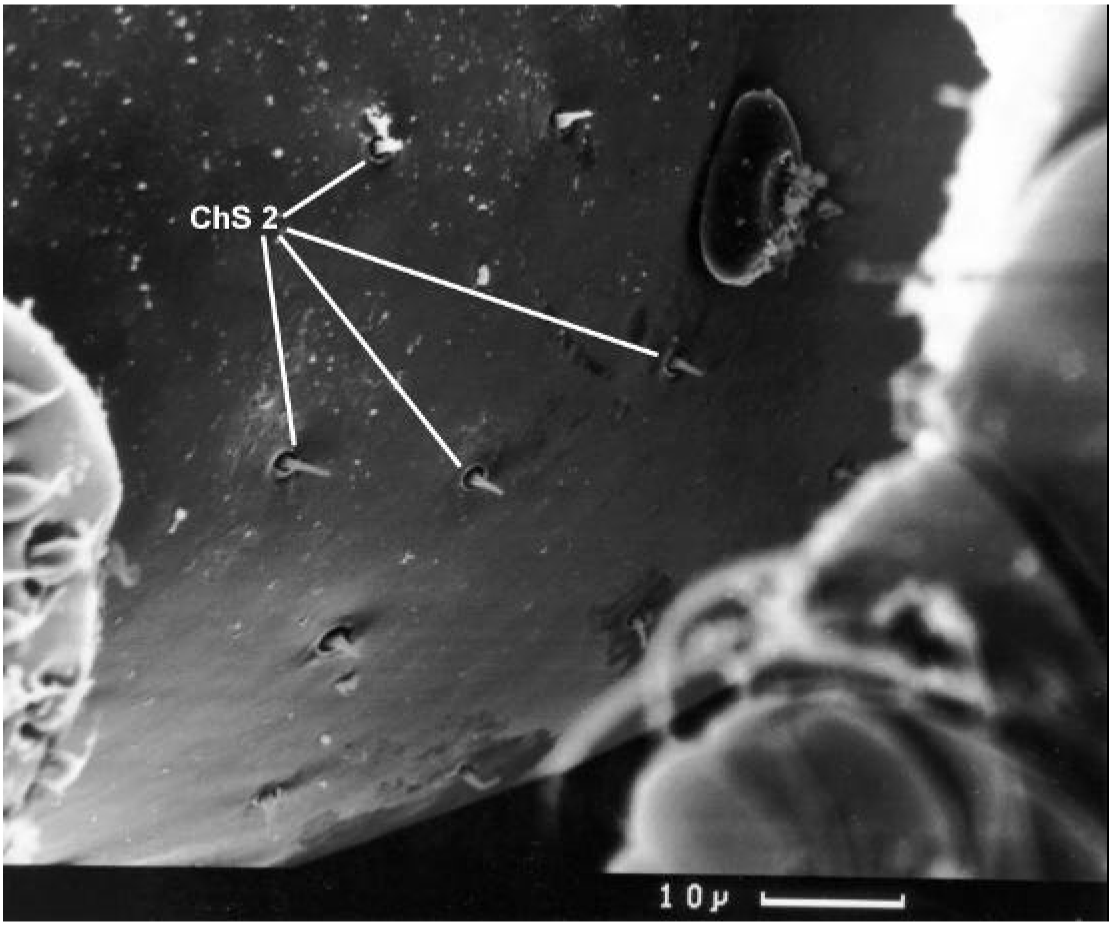

Fig. 5. SEM photomicrograph of female *L. tentacularis* antenna. Rear view of left scape. ChS 2 = four examples of chaetica sensillum type 2 on internal rear face of scape, where this sensillum is widely distributed. Bar = 10µm.

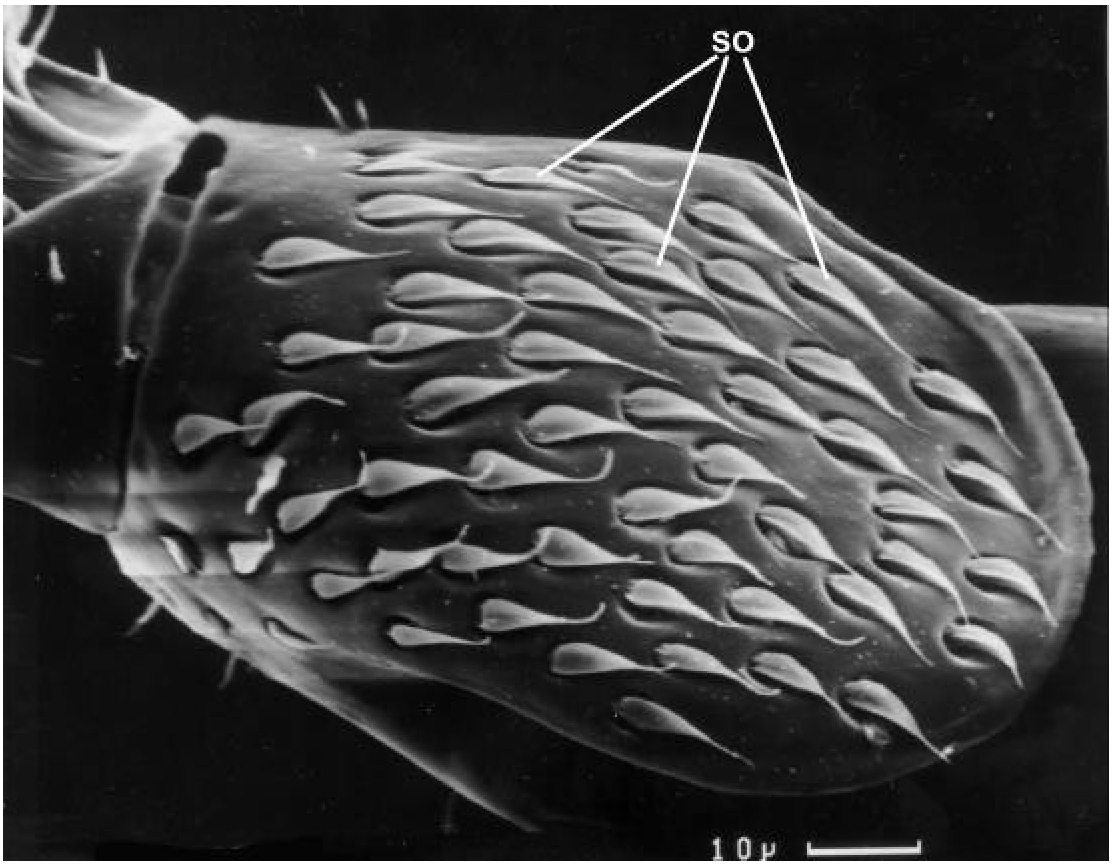

Fig. 6. SEM photomicrograph of female *L. tentacularis* antenna. Internal face of left pedicel. SO = three examples of sensillum obscurum on proximal section of pedicel, the only location of this sensillum on the antenna. Bar = 10µm.

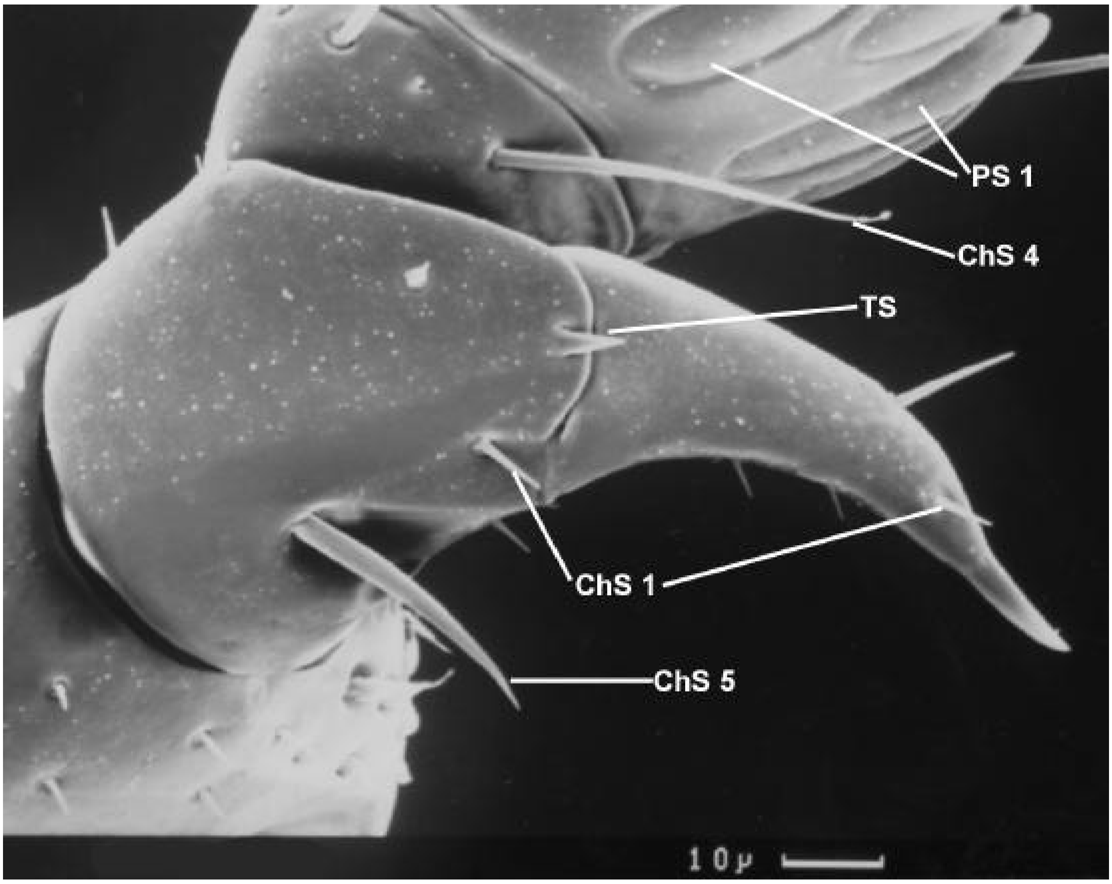

Fig. 7. SEM photomicrograph of female *L. tentacularis* antenna. External face of distal section of left pedicel, with first flagellar segment at top of view. ChS 1 = two examples of chaetica sensillum 1 on distal section of pedicel (left) and on pedicel hook (right); ChS 4 = example of chaetica sensillum 4 on pedicel reaching across over flagellar segment 1; ChS 5 = example of chaetica sensillum 5 in its characteristic position on the pedicel; PS 1 = two examples of placoid sensillum 1 on first flagellar segment; TS = example of trichoid sensillum in its characteristic position bridging the groove at the base of the pedicel hook. Bar = 10μm.

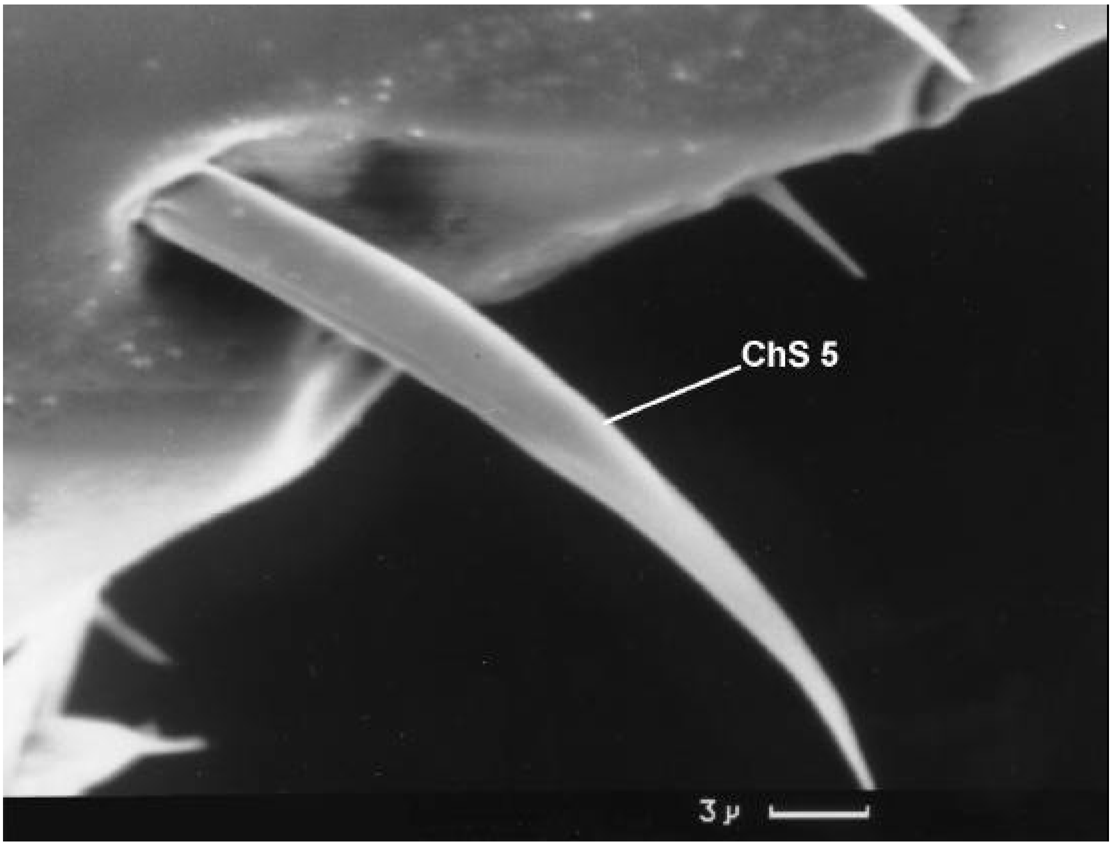

Fig. 8. SEM photomicrograph of female *L. tentacularis* antenna. External face of distal section of left pedicel. ChS 5 = close-up view of example of chaetica sensillum 5 showing socketed base set into depression in cuticle. Bar = 3µm.

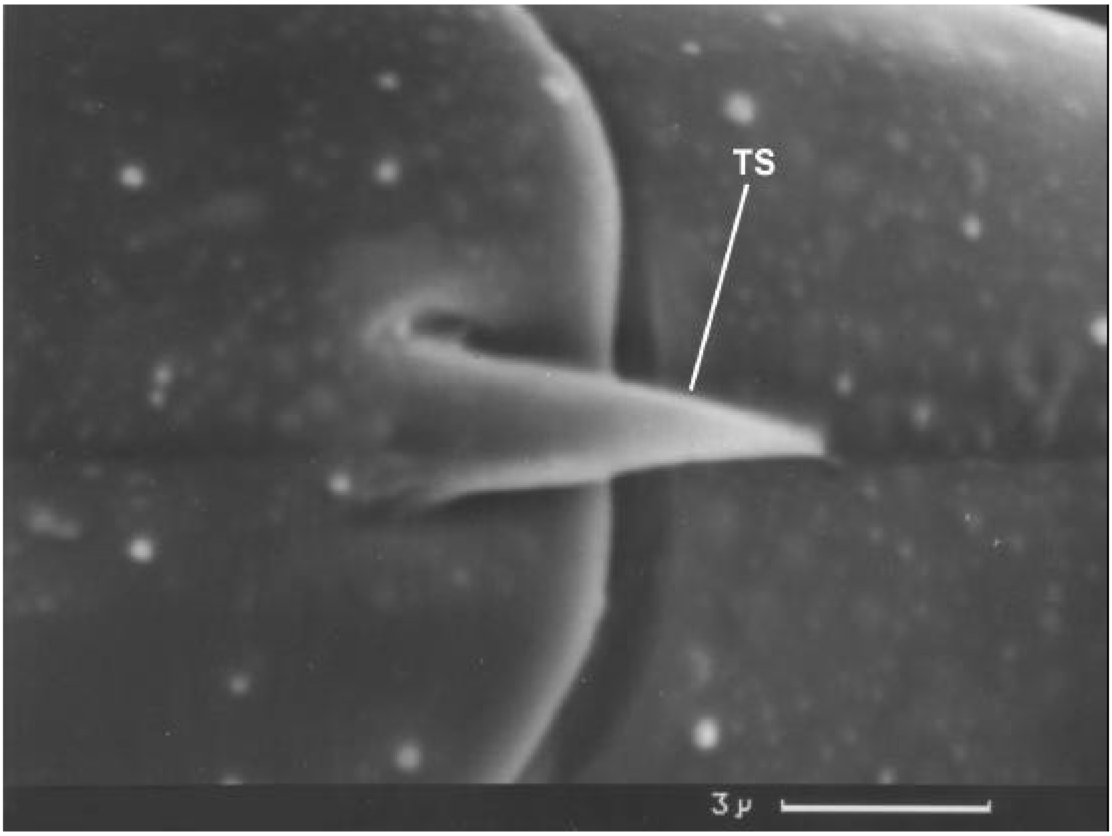

Fig. 9. SEM photomicrograph of female *L. tentacularis* antenna. External face of distal section of left pedicel. TS = close-up view of example of trichoid sensillum showing absence of socket at base where sensillum projects from cuticle. Bar = 3µm.

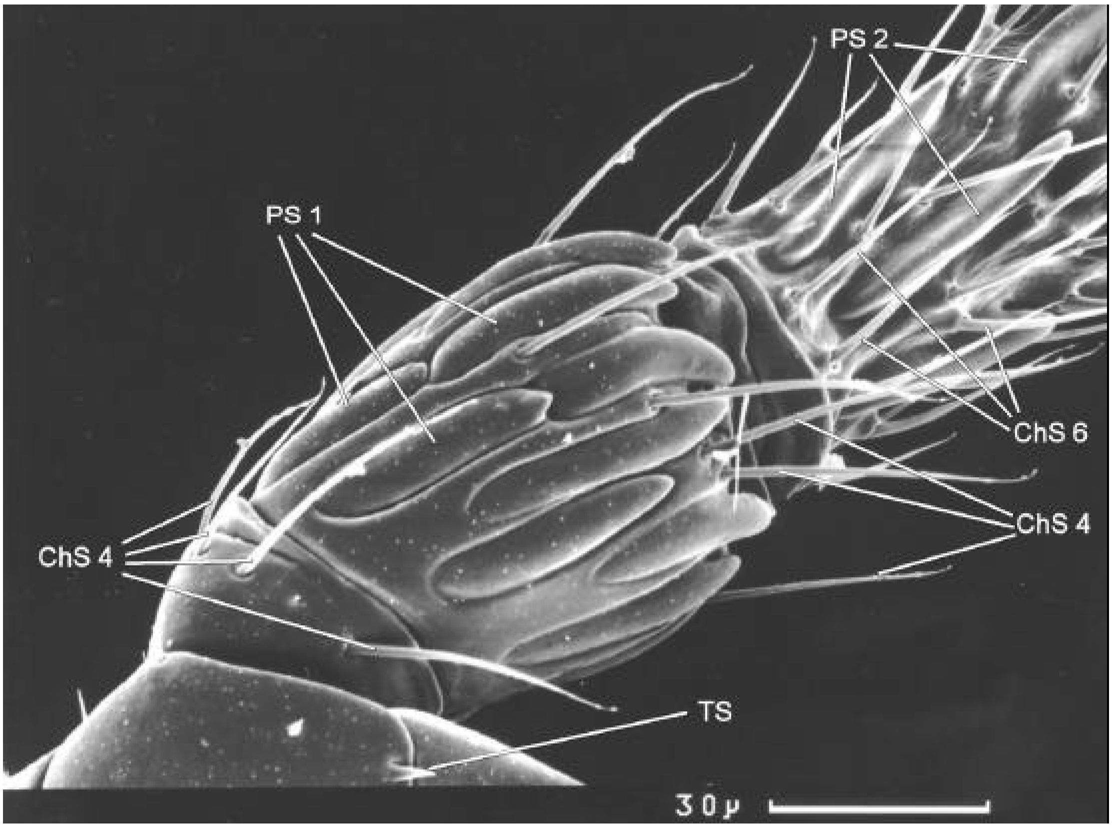

Fig. 10. SEM photomicrograph of female *L. tentacularis* antenna. First flagellar segment. External face of distal section of left pedicel at bottom of view, second flagellar segment at top of view. ChS 4 (left) = four examples of chaetica sensillum 4 on distal section of pedicel; ChS 4 (right) = three examples of chaetica sensillum 4 on flagellar segment reaching across towards and over flagellar segment 2; ChS 6 = three examples of chaetica sensillum 6 on the first flagellar segment; PS 1 = three examples of placoid sensillum 1 on first flagellar segment; PS 2 = three examples of placoid sensillum 2 on second flagellar segment; TS = example of trichoid sensillum in its characteristic position bridging the groove at the base of the pedicel hook. Bar = 30µm.

Fig. 11. SEM photomicrograph of female *L. tentacularis* antenna. Flagellar segment (after first). BCPS = two examples of basiconic capitate peg sensillum showing projecting peg surrounded by raised cuticular ring; ChS 6 = two examples of chaetica sensillum 6 – note lateral grooves on these and others in the photomicrograph, most apparent near the base; PS 2 = two examples of placoid sensillum 2. Bar = 10µm.

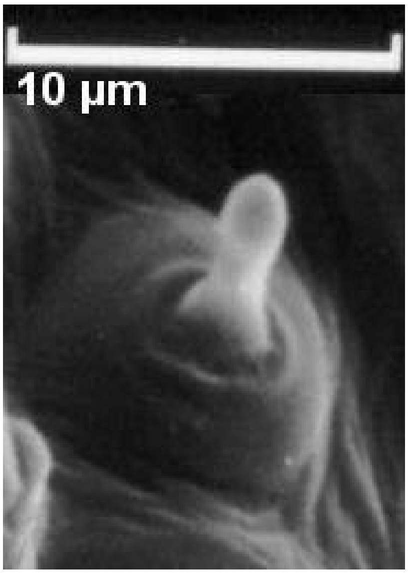

Fig. 12. SEM photomicrograph of female *L. tentacularis* antenna. Close-up view of basiconic capitate peg sensillum on a flagellar segment (after first). Note projecting peg set into depression in surrounding cuticular ring.

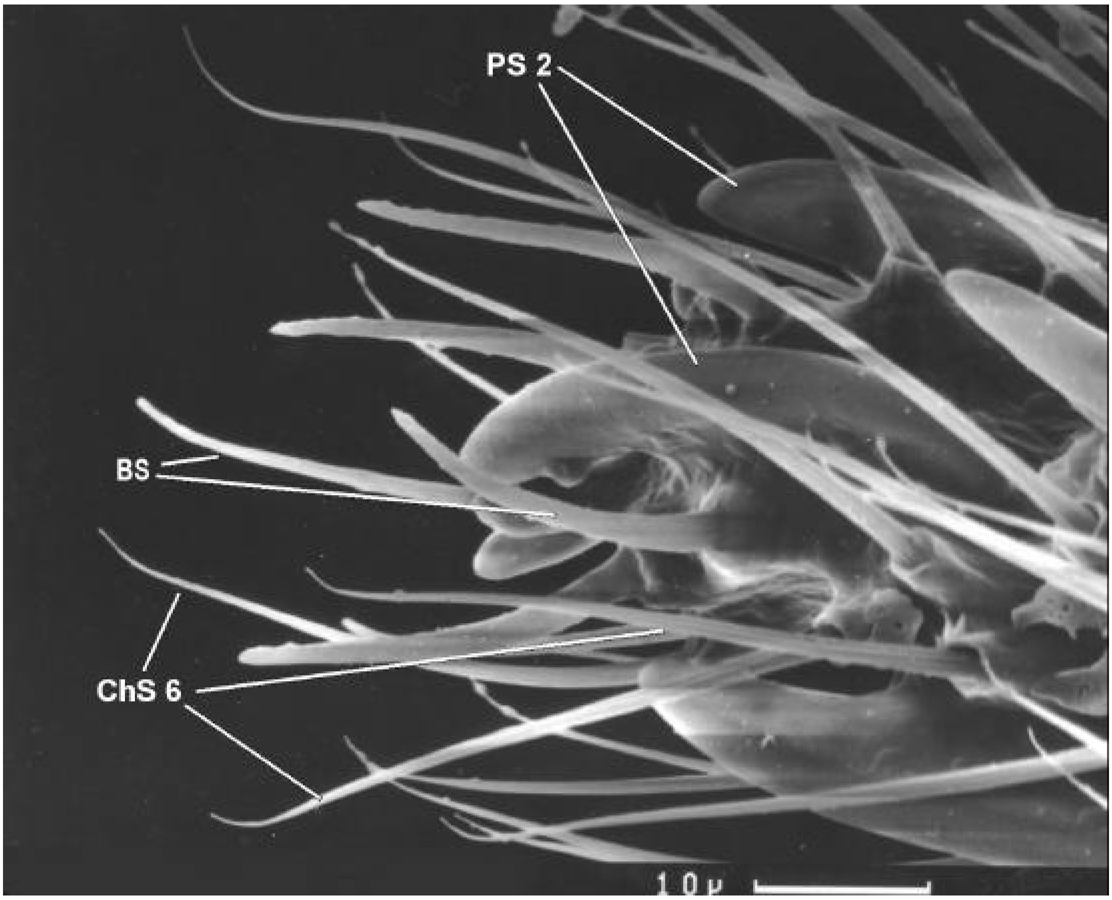

Fig. 13. SEM photomicrograph of female *L. tentacularis* antenna. Tip of final (sixth) flagellar segment. BS = two examples of basiconic sensillum showing rounded tip and shorter length compared to chaetica sensillum 6; ChS 6 = three examples of chaetica sensillum 6; PS 2 = two examples of plate sensillum 2. Bar = 10µm.

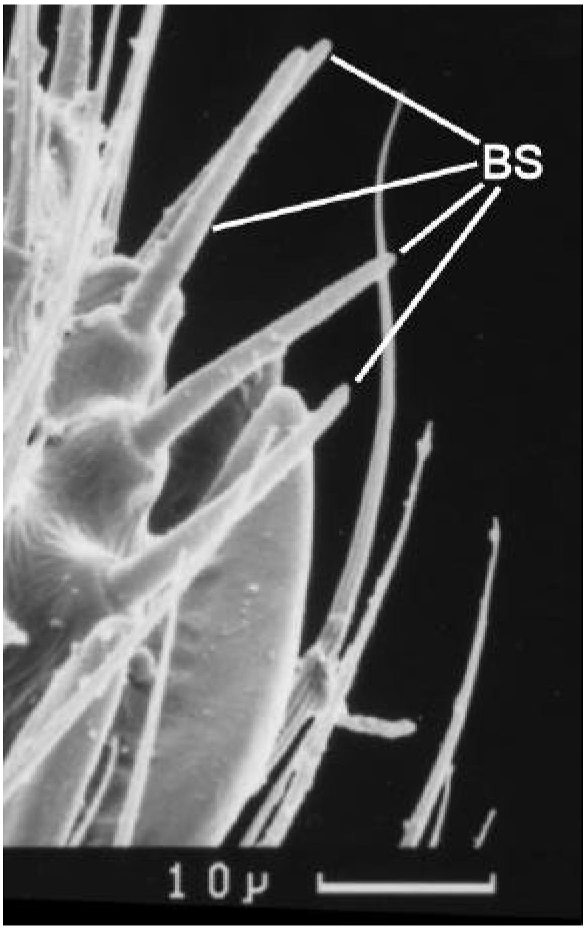

Fig. 14. SEM photomicrograph of female *L. tentacularis* antenna. Tip of final (sixth) flagellar segment. BS = four examples of basiconic sensillum showing general morphology and cuticular attachment. Bar = 10µm.

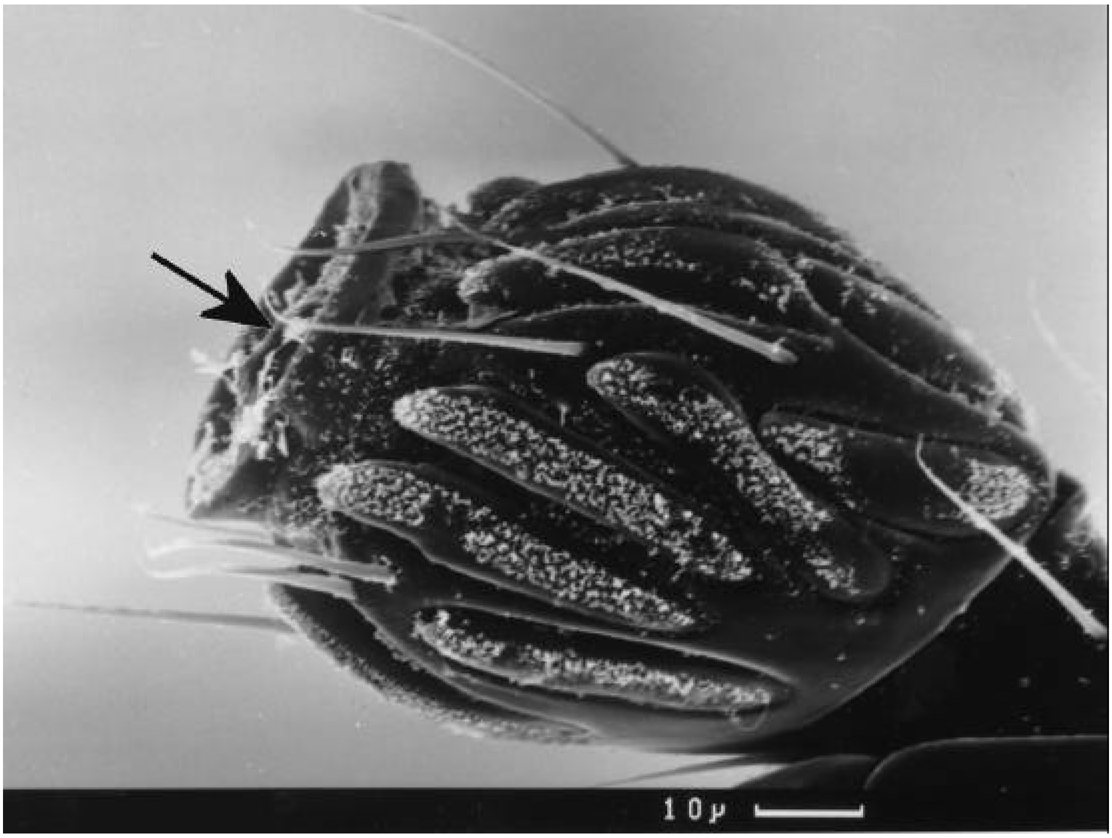

Fig. 15. SEM photomicrograph of female *L. tentacularis* antenna after loss of flagellar segments 2-6 by entering a fig. First flagellar segment. Arrow shows centre of constriction joining flagellar segments 1 and 2 where antenna has characteristically broken upon entering the fig. Bar = 10µm.

Fig. 16. SEM photomicrograph of female *L. tentacularis* head and antennae after removal of flagellar segments 2-6 through manipulation experiment (see method and results). Arrows show constrictions joining flagellar segments 1 and 2 where antennae have broken upon manipulation. Bar = 100µm.

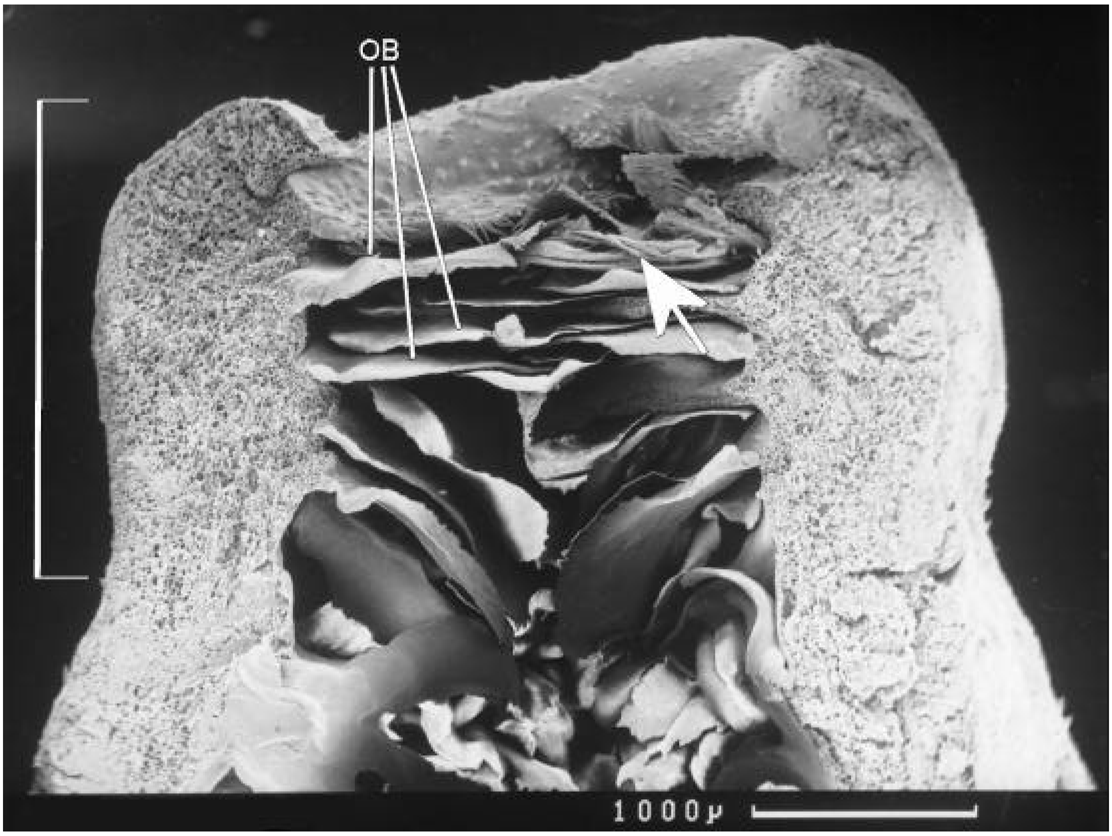

Fig. 17. SEM photomicrograph of functionally male fruit (fig) of *F. montana*. Apex of laterally bisected fig after wasps have entered through the ostiole. Bar on left shows the projection at the top of the fig which holds the ostiole and ostiolar bracts. OB = three examples of ostiolar bracts. Arrow shows a number of *L. tentacularis* flagellar antennal segments which have become caught in the bracts. Bar = 1000µm.

Discussion

Antennal map of female L. tentacularis

The Kruskal-Wallis analysis showed no significant differences between the lengths of the final 5 flagellar segments at the $P<0.05$ level, indicating that their lengths are equally distributed. As the sample size of 5 wasps is relatively small, and the P-value of 0.072 obtained is approaching statistical significance, further measurements of segment lengths would be helpful to clarify this issue, as the data and standard errors suggest that there could be some difference, especially between the second and final segments.

It is thought that the ostiole, with its occluding bracts, acts as a physical barrier contributing towards the maintenance of host-specificity by preventing access into the fig to all but the morphologically adapted specific pollinator species (Janzen, 1979; van Noort and Compton, 1996). Fig wasps show various adaptations to facilitate entry into the fig through the ostiole, e.g. using substantial and well-adapted mouthparts to chew their way through the ostiolar bracts (van Noort and Compton, 1996; Weiblen, 2002). Weiblen mentions a spine on the 'third segment' which serves as a hook for prying at the outer ostiolar bracts. It has been suggested that the pedicel hook functions in this way to aid the wasp in entering a fig through the ostiole (S. G. Compton, pers. comm.), although this hook is found on the second antennal segment, not the third. The magnification on the video footage taken in this study was insufficiently high to test this theory, and in most cases the pedicel was obscured by the head and outermost ostiolar bracts during entry. The theory is supported (but not confirmed) by the fact that the pedicel hook occurs on the 'sturdy' proximal section of the antenna which is not lost during entry to the fig, and so presumably is sufficiently robust to assist entry through the ostiole.

Sensillum types and observation of behaviour

The nomenclature of Amornsak *et al*. (1998) was followed when describing antennal sensilla in this study. One source was used as a guide rather than several because, as outlined by Amornsak *et al*., many different names have been assigned to sensilla types despite similarity in form and distribution. Amornsak *et al*. base their nomenclature upon the classic syntheses of Snodgrass (1935), Schneider (1964) and Zacharuk (1985), but define sensilla solely on their external morphology and employ some further distinctions to dismiss any ambiguity between these sources. The major distinction is that chaetica sensilla and trichoid sensilla are differentiated by the respective presence and absence of a cuticular socket at the point of insertion into the antennal surface (also in Schneider, 1964).

The following sections covering the specific sensillum types are influenced by the conclusions of Amornsak *et al*. (1998) in their review of antennal sensilla of parasitic chalcid wasps in the literature. Sensillum types not covered by Amornsak *et al*. were described according to the definitions of Snodgrass (1935), Schneider (1964), Zacharuk (1985) and Keil (1999); and are referenced accordingly. The presence of a flexible cuticular socket is used as a criterion to distinguish between some sensillum types; the presence of a joint membrane at the base of the sensillum was used as an indication of flexibility (see Keil, 1999).

Basiconic Capitate Peg Sensillum (BCPS)

BCPS have been suggested to be olfactory sensilla, this being supported by their punctate grooved appearance in many studies. The resolution at high magnification in this study was insufficiently high to determine whether BCPS displayed surface grooves or pores. Other suggestions include functions of BCPS as hygro-, thermo-, and mechanoreceptors (Amornsak *et al*., 1998). BCPS in this study were found to be distributed on the ventral surface of the final 5 flagellar segments; this seems consistent with the first stage of antennal searching behaviour observed in female *L. tentacularis* in which the ventral antennal surface is brushed along the fig surface. Amornsak *et al*. (1998) find general agreement that ventral flagellar sensilla in parasitoid Hymenoptera are likely to be olfactory, gustatory or mechanoreceptors. This suggests that BCPS could have a mechanoreceptive or gustatory function, allowing the wasp to orient itself on the fig surface and direct movement towards the ostiole, presumably by detection of a factor which varies throughout the longitude of the fig.

Basiconic Sensillum (BS)

The BS observed at the tip of the antennae were classified according to the definitions of Keil (1999); short with a rounded tip and non-flexible socket. The socket of BS in this study is signified by a shallow groove around the base of the sensillum where it attaches to the cuticular projection it is set into, and so was assumed to be non-flexible. Keil cites that BS are usually olfactory. The second stage of antennal searching behaviour in female *L. tentacularis* is characterised by a tapping of the fig surface with the tip of the antennae. As BS were found to occur only on the tip of the antenna, and no other sensillum is uniquely distributed in this area, we can assume that BS are associated with this tapping behaviour. This suggests a gustatory or mechanoreceptive function for similar reasons seen in BCPS. A transmission electron microscopy (TEM) or high-magnification SEM study of the porous or aporous nature of the surface of this sensillum would shed further light on the nature of its function.

Chaetica Sensilla (ChS)

Chaetica sensilla are classified mainly by their hair-like appearance and cuticular socket at the point of insertion into the antennal surface. This definition covers a large range of morphological sensillum forms, and presumably also encompasses sensilla with a number of functions. For example in this study the suggested functions of ChS types 1 and 6 are different despite their both being classified as chaetica sensilla.

Keil (1999) cites that trichoid sensilla with a flexible socket are either pure mechanoreceptors (with no surface pores) or gustatory sensilla (with pore(s) at the tip). Trichoid sensilla with a flexible socket as classified by Keil are classified as chaetica sensilla in this study. An examination using TEM or SEM at higher magnification would determine if ChS with flexible sockets have tip-pores or no pores, confirming their functions as gustatory sensilla or mechanoreceptors respectively. Sensilla found on the scape and pedicel are primarily mechanoreceptors (Amornsak *et al.*, 1998), so this function seems likely for ChS types 1-5, trichoid sensillum and sensillum obscurum in this study. **ChS 1** were found in low densities on the scape and pedicel, especially around intersegmental joints and other areas where they are likely to be stimulated by movement, suggesting a proprioceptive function. **ChS 2** on the front face of the scape possess flexible sockets and would be stimulated as the wasp pushes through the ostiolar bracts upon entry into a fig: this fact supports a mechanoreceptive and/or gustatory function. A proprioceptive function for **ChS 3** is supported by its morphology, and distribution on the rear face of the scape. TEM examination of this sensillum would be particularly useful in order to confirm its nature as a chaetica sensillum and not a coeloconic sensillum (a peg projecting from a pit in the cuticle). **ChS 4** were found on the pedicel and flagellar segment 1 and were seen to be set into a flexible socket, suggesting a mechanoreceptive function. ChS 4 were seen to be lightly grooved, suggesting that pores could be present and that ChS 4 possess a chemoreceptive function. However, grooves do not necessarily presuppose the presence of pores (see below). A mechanoreceptive/proprioceptive function is suggested for **ChS 5** due to its position on the pedicel hook. It is likely to possess a function related to fig entry, as the pedicel hook is also thought to be involved in this process (see above). **ChS 6** are strongly grooved along their longitude. Some studies suggest that grooves are indicative of surface pores (BCPS in Amornsak et al.,), but Ochieng et al. (2000) describe a sensillum morphologically similar to **ChS** type 6 (described as sensilla trichoidea) which does not possess surface pores. No olfactory function was assigned to this sensillum type; instead a mechanoreceptive function was suggested due to its flexible cuticular socket. Snodgrass (1925; cited from Ware and Compton, 1992) describes a division of multiporous plate sensilla as *sensilla chaetica*, considered to have an olfactory function. The distribution of these sensilla is restricted in part to the antennae of female fig wasps: these sensilla are likely to be the organs by which female fig wasps perceive their host figs (Ware and Compton, 1992). An examination of the porous or aporous nature of ChS 6 would be necessary to determine its function with more certainty; its similarity to sensilla trichoidea in Ochieng et al. (2000) suggests a mechanoreceptive function, but similarities with multiporous plate sensilla chaetica (Ware and Compton, 1992) suggest an olfactory function. If ChS 6 are in fact olfactory, their distribution in great numbers on the antennal segments would lead us to assume that the flagellar segments 2-6 constitute a highly sensitive olfactory organ.

Trichoid Sensillum (TS)

Keil (1999) cites that trichoid sensilla may be olfactory, but sensilla found on the pedicel are usually mainly mechanoreceptive (see above). The position of TS across the join of the pedicel hook suggests a proprioceptive function, but it does not possess standard proprioceptor morphology (it lacks a flexible socket). Schneider (1964) suggests that TS may be dye-permeable and so may possess chemoreceptivity. Further study by close examination of the surface structure of this sensillum is required to clarify its function.

Placoid Sensilla (PS)

Although no pores were observed on the surface of the plate-like sensilla in this study, their morphology conforms strongly to the descriptions of multiporous plate sensilla lineara (Ware and Compton, 1992) multiporous plate sensilla (Basibuyuk and Quicke, 1998), sensilla placodea, which are pierced by numerous pores (Keil, 1999), so are assumed to be porous and have a chemoreceptive function. These placoid sensilla are likely to be the olfactory receptors by which female fig wasps perceive the volatile attractants of host figs (Ware and Compton, 1992).

PS 1 and **PS 2** differ in that they respectively lay relatively flat against the cuticular surface and are raised ridge-like structures. PS 2 are also longer than PS 1. Ware and Compton (1992) explain how an increase in sensillum surface area through elongation and detachment from the antennal surface should result in an increased sensitivity. An increased number of sensilla, through lengthening, branching or thickening of antennal segments would also increase sensitivity. PS 2, through their elevated and lengthened structure, and apical detachment from the antennal surface, have a greater surface area than PS1, and appear on the antenna in far greater numbers on the elongate flagellar antennal segments. We can therefore assume that PS 2 are individually more sensitive to the detection of volatile attractants produced by figs, and also provide a more sensitive tool of detection than PS 1 when considered as a group of sensilla, due to their existing in greater numbers (on flagellar segments 2-6). An examination of the density of pores on the surface of PS types 1 and 2 could potentially support this assumption. This difference in olfactory sensitivity above and below the characteristic antennal break point can be linked to the life-cycle of female *L. tentacularis* (see below).

Sensillum Obscurum

To my knowledge, sensilla of this type do not appear elsewhere in the literature. Jefferson *et al*. (1970) describe *sensilla auricillica*; a rabbit's ear shaped sensillum found on the antennae of some noctuid moths, but this is likely to be distinct to SO due to morphological and taxonomic differences. Snodgrass (1935) and Schneider (1964) describe *sensilla squamiforma* as scale-like sensilla, but these are found on lepidopteran wings and so are also likely to be distinct from SO. Zacharuk (1985) describes s. squamiformia as trichoid hairs that resemble scales in external form, and assigns them with a mechano- or chemo receptive function. The gradation in form between hair-like and scale-like mentioned by Zacharuk could be seen towards the edges of the patches of SO seen in this study. None of the antennal behaviour observed in this study gave any clues as to the function of SO; other than suggesting that they could play a role in behaviour not observed here (e.g. detection of a factor inside the fig), we can only guess as to their function. An examination of the porous or aporous nature of SO would be necessary to determine its function with more certainty.

Manipulation experiments

The fact that 12 out of 14 manipulated antennae broke at the characteristic break point compared to all of the 20 naturally broken specimens suggests that there may be another factor which assists the antennae in breaking consistently after flagellar segment 1. The ridge at the rear of the wedge-shaped head of female *L. tentacularis* could be this factor, providing shear stress on the antenna along with the ostiolar bracts (Figs 3, 16 and 17).

Conclusions

One of the main functions of the female fig wasp antenna is to detect the volatile attractants produced by figs (Ware and Compton, 1992). The wasp must have some long range olfactory sensitivity in order to locate a fig tree with receptive fruits, and must also have short range olfactory or gustatory sensitivity in order to find receptive fruits on a tree and in order to find the ostiole on a fig. The putative functions of the sensilla types found on the antennae of female *L. tentacularis* suggest that flagellar segments 2-6 confer this long range olfactory sense through relatively sensitive and numerous PS 2; also possibly through ChS 6, should their function prove to be chemoreceptive. Short range chemosensitivity is likely to be conferred by PS 1 on flagellar segment 1; PS 2, BCPS and possibly ChS 6 on flagellar segments 2-6; and BS on flagellar segment 6.

All of the evidence suggests that the characteristic antennal break point is a morphological adaptation; presumably the wasp must lose its cumbersome antennae in order to move around inside the fig. The break point may be necessary for the wasp to successfully enter through the tight ostiole – without it a weak point the wasp could become trapped by its antennae and be restricted from entering the fig successfully. The antennae found in the outer ostiolar bracts of a fig which had been entered supports this hypothesis (Fig. 17). Retention of the scape, pedicel and first flagellar segment leaves the wasp with some olfactory and mechanoreceptive sensitivity in order to navigate within the fig and to another fig should it exit its first host. The wasp observed on the fig surface after flagellar segments 2-6 had been removed was seen to enter the fig, although it took longer than the mean time taken for wasps with intact antennae to enter. Although 'antennaless' wasps are able to navigate to an ostiole from another part of a plant, we should not assume that they are able to do this using chemoreception alone: vision, for example, could be an important aid at this stage.

The resolution of the SEM techniques at high magnification was insufficiently high to determine the surface structure of the sensilla observed in this study. The presence or absence of pores on the surface is important in determining the functions of sensilla, and in order to classify them according to the system suggested by Altner (1977; cited in Keil, 1999). With the application of specialised preparation techniques, it may be possible to obtain higher resolution images (Olson and Andow, 1993; cited in Basibuyuk and Quicke, 1998; A. Hick, pers. comm.). A TEM study of the sensilla of female *L. tentacularis* is also suggested in order to shed further light on the sensillar functions. Specimens of wasps with naturally removed flagellae were scarce in this study; comparative behavioural studies of wasps with and without flagellae would also shed further light on the functions of specific antennal segments.

This study showed that the antennal morphology of female *L. tentacularis* is consistent with the generic pollinating fig wasp life cycle, and the fact that this species may exit a fig after oviposition in search of another. Future work will tell if it is possible to predict the presence of re-entry behaviour in a species of fig wasp by examination of its antennal morphology.

Acknowledgements

I would like to thank Nazia Suleman and Shazia Raja for their help in collecting wasps and identifying receptive figs. Martin Lappage of the Experimental Gardens, Adrian Hick of the Electron Micsroscopy Suite and the staff of the university teaching laboratories were extremely helpful during practical work. Thanks finally go to Steve Compton for his enthusiastic suggestions and support throughout the study.

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