2011

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Medical and Veterinary Entomology

Medical and Veterinary Entomology (2011) **25**, 327–336 doi: 10.1111/j.1365-2915.2011.00946.x

Characterization of olfactory sensilla of *Stomoxys calcitrans* and electrophysiological responses to odorant compounds associated with hosts and oviposition media

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Abstract. Stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae), are economically important biting flies that have caused billions of dollars in losses in the livestock industry. Field monitoring studies have indicated that olfaction plays an important role in host location. To further our understanding of stable fly olfaction, we examined the antennal morphology of adults using scanning electron microscopy techniques. Four major types of sensillum were found and classified as: (a) basiconic sensilla; (b) trichoid sensilla with three subtypes; (c) clavate sensilla, and (d) coeloconic sensilla. No significant differences between male and female flies in abundances (total numbers) of these sensillum types were observed, except for medium-sized trichoid sensilla. The distinctive pore structures found on the surface of basiconic and clavate sensilla suggest their olfactory functions. No wall pores were found in trichoid and coeloconic sensilla, which suggests that these two types of sensillum may function as mechano-receptors. Details of the distributions of different sensillum types located on the funicle of the fly antenna were also recorded. Electroantennogram results indicated significant antennal responses to host-associated compounds. The importance of stable fly olfaction relative to host and host environment seeking is discussed. This research provides valuable new information that will enhance future developments in integrated stable fly management.

Key words. *Stomoxys calcitrans*, antennal morphology, electroantennogram (EAG), odorants, scanning electron microscopy (SEM), stable fly.

Introduction

Stable flies, *Stomoxys calcitrans* L., are obligate, blood-feeding insects. They are considered significant economic pests of livestock and other warm-blooded animals in many parts of the world (Zumpt, 1973; Mullens *et al*., 1988; Masmeatathip *et al*., 2006). Females lay eggs in decaying vegetable matter (including straw and hay) mixed with or

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without excrement from horses, cattle and sheep (Bishopp, 1913; Pinkus, 1913; Broce *et al*., 2005). Stable flies are known to use semiochemical facilities for host location and to select oviposition sites (Birkett *et al*., 2004; Jeanbourquin & Guerin, 2007). To detect their hosts or oviposition sites, stable flies presumably use specific cues including visual and/or olfactory stimuli associated with the host and acceptable larval environments. Jeanbourquin & Guerin (2007) demonstrated that stable flies are able to locate either horse or cow dung by relying on odour-based cues, without contact with the substrate. Laboratory wind tunnel studies have shown that 1-octen-3-ol, 6-methyl-5-hepten-2-one and 3-octanol increase stable fly upwind flight, whereas naphthalene, propyl butanoate and linalool reduce upwind flight of the face fly, *Musca autumnalis* D.G. (Diptera: Muscidae), horn fly, *Haematobia irritans* L. (Diptera: Muscidae), screwworm fly, *Wohlfahrtia magnifica* S. (Diptera: Sarcophagidae), sheep headfly, *Hydrotaea irritans* L. (Diptera: Muscidae), and stable fly (Birkett *et al*., 2004). These findings indicate a critical role for olfaction as used by flies to search for appropriate hosts and to avoid inadequate environments.

Sensory organs on the antennae of insects are known to be used in locating mates, hosts, habitats and oviposition sites (Weseloh, 1972; Vinson *et al*., 1986; Bin *et al*., 1989; Isidoro *et al*., 1996). With a few exceptions, studies of the antennal sensilla in dipteran species have revealed an abundance of basiconic, coeloconic and trichoid sensilla (Sutcliffe *et al*., 1990; Pfeil *et al*., 1994; Shanbhag *et al*., 1999; Fernandes *et al*., 2004; Sukontason *et al*., 2004, 2005; Castrejon-Gomez Victor & RoJas Julio, 2009). In muscoid flies, most sensory organs used for the perception of chemical odorants are located on the funicles of antennae (Lewis, 1970; Bay & Pitts, 1976; White & Bay, 1980). These sensory organs have been reported to respond to various stimuli such as warmth, humidity, skin odours, ammonia and carbon dioxide (Krijgsman, 1930; Hopkins, 1964; Zdarek & Pospisil, 1965; Gatehouse, 1969). Lewis (1971), using transmission electron microscopic (TEM) images, described the internal structures of seven types of sensillum and provided limited descriptions of their external morphology. The present study characterizes the morphology, abundance and distribution of presumptive olfactory sensilla on the funicles of stable flies and their electrophysiological responses to selected host- and oviposition site-associated odorants.

Materials and methods

Scanning electron microscopy

Stable flies were obtained from the U.S. Department of Agriculture (USDA) Agroecosystem Management Research Unit (AMRU) laboratory in Lincoln, Nebraska, U.S.A. and maintained at 23 ± 2 °C under variable humidity [30–50% relative humidity (RH)] and an LD 12 : 12 h photoperiod. Adults were fed citrated bovine blood (3.7 g sodium citrate/litter) soaked into a feminine hygiene napkin (Stayfree®; McNeil-Ppc Inc., Skillman, NJ, U.S.A.) placed on top of a screen cage.

The antennae of male and female stable flies (at least 10 from each sex) were observed using scanning electron microscope (SEM). Stable fly heads were removed under a dissecting microscope (Olympus SZ-6; Olympus America Inc., Center Valley, PA, U.S.A.) using microsurgical scissors. The antennae were excised and fixed in 2.5% glutaraldehyde mixed with 0.1 M Sorenson's phosphate buffer solution at pH 7.4 at 4 °C for 24 h. Antennae were then rinsed twice in 0.1 M Sorenson's phosphate buffer and dehydrated with ethanol. The dehydration process was sequentially subjected to increasing ethanol concentrations of 30%, 50%, 70%, 80% and 90%. Antennae were held in each concentration of ethanol for 1 h during the dehydration process. Following dehydration, antennae were twice placed in absolute ethanol for a 12-h period for further dehydration and then subjected to critical point drying. Dehydrated antennae were mounted vertically on aluminium stubs (allowing angle imaging) and were coated with gold in a sputter coating apparatus. Samples were observed using a variable pressure scanning electron microscope and a field emission scanning electron microscope (Hitachi 3000N and 4700; Hitachi Corp., Tokyo, Japan) at the Microscopy Core Facility, Biological Technology Center, Beadle Center, University of Nebraska (Lincoln, NE, U.S.A.).

Micrographs of the dorsal, ventral, outer and inner sides and tips of antennae in both male and female stable flies were taken at ×500 original magnification (OM). The distribution and density of various types of sensillum were determined using the grid technique described by Kelling-Johannes (2001). The micrographs were divided into approximately 187 sections and the number of sensilla in each area of 1000 μm^2 was counted twice.

Higher magnifications (up to ×35 000) were used to further investigate the fine structure of individual sensilla. The following procedures were used to obtain accurate sensilla maps: (a) comprehensive, large images (OM ×3500) of the funicle were constructed by assembling a series of micro photographs (ranging in size from 30 to 60 sections) into whole pictures of each antenna; (b) the antennal area was estimated from the number of sections in the assembled images, and the mean number of sensilla per funicle was calculated. The terminologies and nomenclatures used to describe antennal morphology and to classify sensillum types were adapted from Lewis (1971), Steinbrecht (1997) and Keil (1999).

Electroantennogram

Electroantennograms (EAG) were recorded by connecting an electrogel-filled (Spectro 360; Park Laboratories, Inc., Fairfield, NJ, U.S.A.) glass electrode to the excised head of a stable fly (as a ground contact). A recording electrode filled with the same electrode gel was connected to the tip of the funicle. Antennae were exposed to a charcoal-filtered, humidified airstream of 0.5 m/s, and EAGs were recorded at room temperature (25 ± 1 °C). The EAG system consisted of a high-impedance DC amplifier with automatic baseline drift compensation (SYNTECH Equipment & Research, Kirchzarten,

Germany). An EAG program (SYNTECH EAG-Pro 4.6) was used to record and analyse the amplified EAG signals. A quantity of 500 μg of each selected stable fly host-associated odorant compound was dissolved in 500 μL of redistilled high-performance liquid chromatography (HPLC)-grade hexane and 10 μL of the prepared solution were applied to strips of filter paper (0.5 × 2.5 cm, Whatman No. 1; Whatman International Ltd, Maidstone, U.K.). The filter paper strips were air-dried and inserted into Pasteur pipettes (15 cm in length). A 5-mL puff of odorant compound was blown through the pipette and directed across the antennae to elicit an EAG response. Control puffs of air were applied after each puff of a test stimulus. The absolute EAG response of each stimulus was recorded as the mean of at least six replicated measurements. The sequence of exposure of each stimulus to each antenna was randomly defined.

Test odorant compounds

The test odorant compounds (indole, dimethyl trisulphide, 1-octenol-3-ol, phenol, *p*-cresol, 2-heptanone, acetic acid, butyric acid, isovaleric acid and hexanoic acid) thought to be associated with stable fly host and oviposition sites were purchased from Sigma-Aldrich Corp. (St Louis, MO, U.S.A.). Labelled purities for these odorants ranged from 98.0% to 99.5%.

Results

Stable fly sensilla

As in most Muscidae, the antenna of the stable fly consists of three segments: a proximal scape; a medial pedicel, and the funicle, the distal third antennal segment. Most sensilla were found on the funicle. Based on SEM observations, we recorded four major types of sensillum: basiconic; trichoid; clavate, and coeloconic (Fig. 1). Trichoid sensilla types were grouped into three subtypes based on hair length (Fig. 1F).

Trichoid sensilla

Trichoid sensilla were the most abundant type of sensillum on the funicle in both sexes. Based on the length, shape and surface morphology, three subtypes of trichoid sensilla were characterized as short (<9 μm long), medium (12–15 μm long) and long (>20 μm long) (Fig. 1F). The long trichoid sensillum had a smooth cuticular surface, but the short and medium sensilla had grooved surfaces (Fig. 1G). The short, curved trichoid sensillum was the most common of the three subtypes. Most were on the inner and outer regions of the funicle, with fewer (35%) distributed over the remainder of the funicle. Significantly more medium trichoid sensilla were found on males than females ($t = 9.3$, $P < 0.05$). No sexual dimorphisms were found in total numbers of sensilla for the other two subtypes (long trichoids, $t = 4.73$, $P < 0.05$; short trichoids, $t = 1.60$, $P < 0.05$). No pore structures were identified on any of the trichoid sensillum types (Fig. 1G).

Basiconic sensilla

Mean numbers of basiconic sensilla on the funicle in female and male stable flies were 1190 and 1149, respectively (Table 1). This type of sensillum had a basal diameter of 1.5–2.5 μm and a length of 4.7–5.0 μm with a blunt tip (Fig. 1C, H). The surface of the sensilla wall was perforated by numerous pores of about 0.01 μm in diameter. Basiconic sensilla were mostly distributed on the ventral and outer and inner sides of the funicle (Fig. 2A).

Clavate sensilla

The length, shape and size of clavate sensilla were similar to those of basiconic sensilla except that they were distally enlarged (Fig. 1D) and the average diameter of the tip of the basiconic sensillum was 1.0 μm, whereas that of the clavate sensillum measured 1.5 μm ($t = 2.92$, $P < 0.05$). There were significantly fewer clavate sensilla relative to basiconic and trichoid sensilla ($F = 16.9$, $P < 0.05$). There were a mean of 218 and 169 clavate sensilla on the funicles of female and male antennae, respectively. Like basiconic sensilla, clavate sensilla were distributed mostly in the outer and inner regions, with relatively few located on other parts of the funicle. There were no significant differences in total numbers of clavate sensilla between male and female stable flies ($t = 12.7$, $P > 0.05$).

Coeloconic sensilla

Coeloconic sensilla were the shortest (0.2–0.25 μm) and least numerous of the various types of sensillum on the funicle. They arose from a wide base cone and featured nine to 12 cuticular fingers that met at the distal tip. The shaft of a coeloconic sensillum was longitudinally grooved (Fig. 1E). They were most abundant on the outer region of the funicle and were sparse elsewhere (Table 1). Female and male stable flies had an average of 57 and 51 coeloconic sensilla, respectively.

Sensilla distribution

No differences in the distributions and abundances of sensillum types were detected between male and female stable flies, except for the medium trichoid sensillum (Table 1). Trichoid and basiconic sensilla were the two most abundant sensillum types and were distributed on all surfaces of the funicle. Basiconic sensilla were embedded among trichoid sensilla. Over 70% of clavate sensilla were present on the inner and outer sides of the funicle, with a few observed on the tip of the funicle. Half of the coeloconic sensilla appeared on the outer side and the rest were located on other regions of the funicle. Figure 2 shows the distributions of each sensillum type on the stable fly funicle.

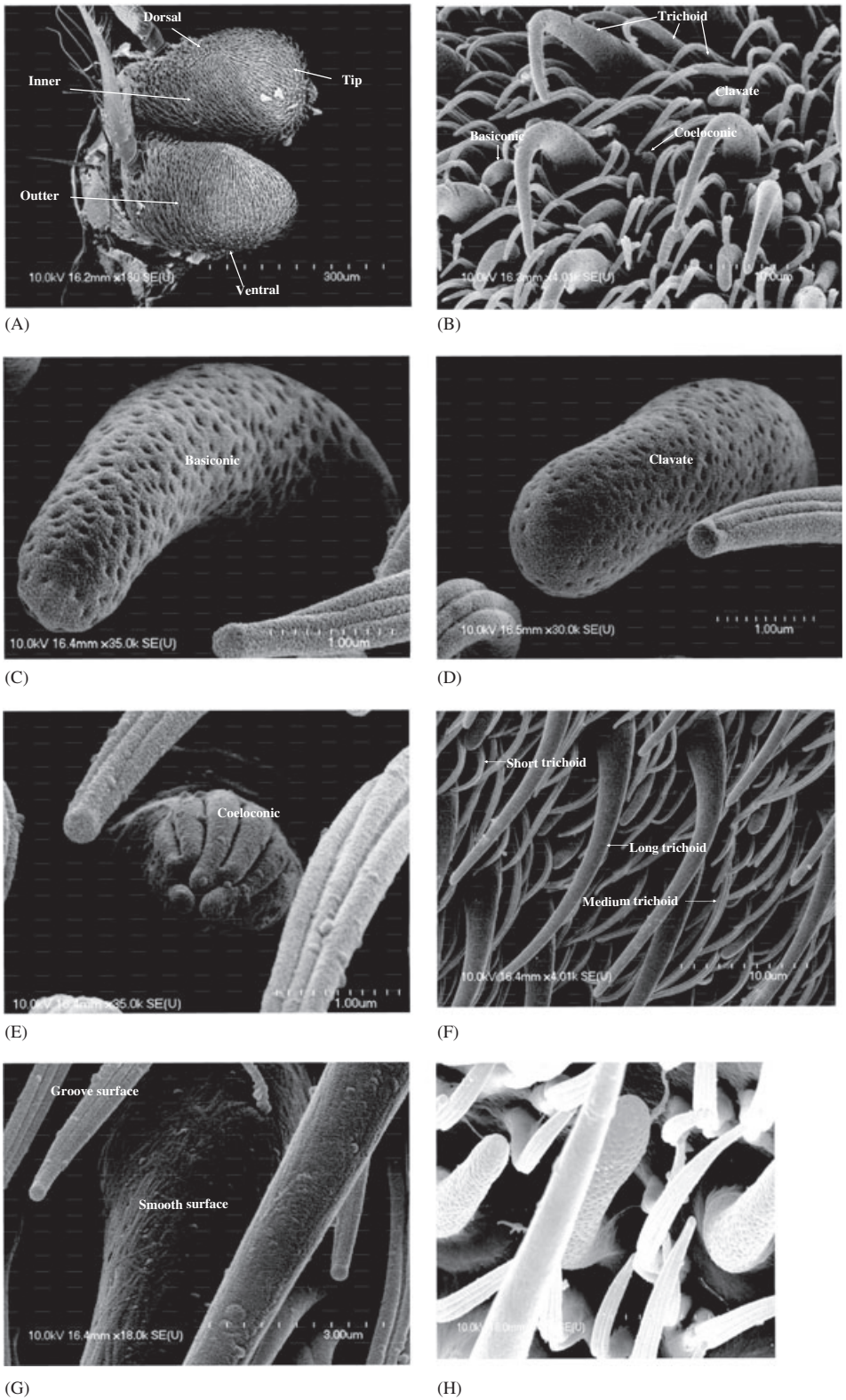

Fig. 1. Scanning electron microscopy (SEM) of the antennae of the stable fly, *Stomoxys calcitrans*. (A) Regions divided by sensilla type. (B) Dorsal view of the funicle showing the distribution of all types of sensillum. (C, D) Shape differences between basiconic and clavate sensilla, with pore structures on the wall surface. (E) High-resolution SEM of a coeloconic sensillum. (F) Three types of trichoid sensilla. (G) Close-up view showing the smooth surface wall of the trichoid sensillum. (H) Basal structures of the basiconica and clavate sensilla.

Table 1. Abundance and distribution of sensillum types on the funicle of the stable fly antenna (mean ± standard error); n = 10 antennae in each sex.

Areas of funicle		Basiconic	Clavate	Coeloconic	Trichoid, long	Trichoid, medium	Trichoid, short
Tip	Male	100 ± 34	6 ± 1	5 ± 1	611 ± 2	302 ± 32	587 ± 86
	Female	159 ± 36	26 ± 6	9 ± 4	542 ± 204	379 ± 79	1165 ± 306
Dorsal	Male	120 ± 40	10 ± 6	1 ± 1	371 ± 78	549 ± 76	949 ± 269
	Female	158 ± 18	31 ± 27	1 ± 1	626 ± 142	852 ± 161	1339 ± 400
Inner side	Male	263 ± 82	70 ± 29	9 ± 3	767 ± 439	2157 ± 1059	2542 ± 535
	Female	238 ± 39	41 ± 5	10 ± 4	907 ± 590	1452 ± 404	2316 ± 1389
Outer side	Male	400 ± 64	54 ± 4	26 ± 3	616 ± 64	2566 ± 273	3196 ± 470
	Female	325 ± 10	73 ± 2	26 ± 1	670 ± 7	1028 ± 4	3258 ± 400
Ventral	Male	266 ± 37	29 ± 3	11 ± 4	450 ± 117	1651 ± 267	1102 ± 375
	Female	311 ± 9	47 ± 2	11 ± 1	338 ± 7	1002 ± 4	996 ± 4
Total	**Male**	**1149 ± 110**	**169 ± 30**	**52 ± 3**	**2815 ± 462**	**7225 ± 475***	**8376 ± 1025**
	Female	**1190 ± 35**	**218 ± 40**	**57 ± 11**	**3082 ± 382**	**4713 ± 97**	**9072 ± 1641**

*Student's t-test, $P < 0.05$.

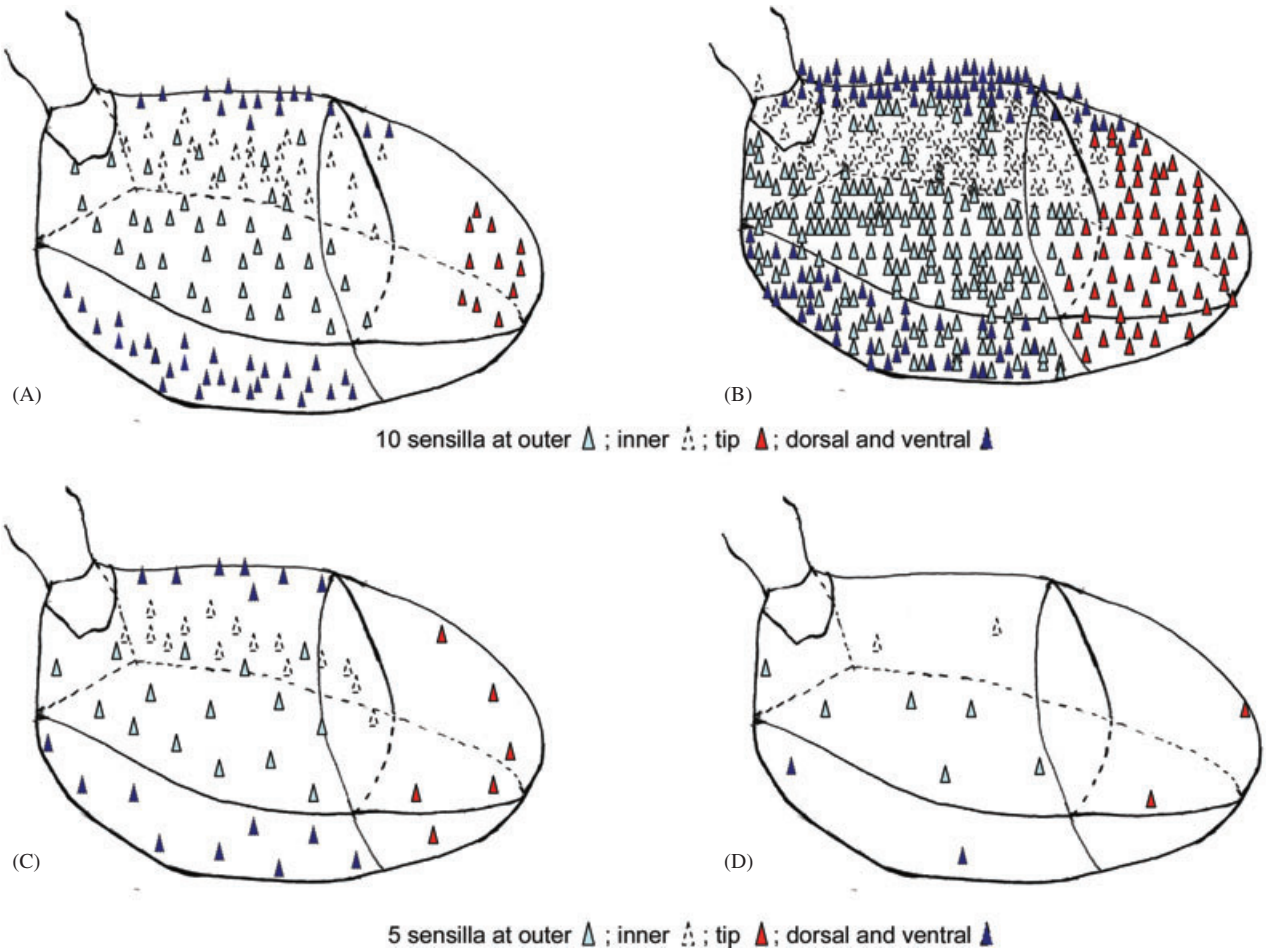

Fig. 2. Three-dimensional diagrams constructed to show the distributions of the four principal types of sensillum on the funicle of *Stomoxys calcitrans.* (A) Basiconic sensilla. (B) Trichoid sensilla. (C) Clavate sensilla. (D) Coeloconic sensilla.

Electroantenogram results

Absolute EAG responses to 10 selected odorant compounds associated with stable fly hosts and oviposition sites are shown in Fig. 3. Significant EAG responses were elicited from stable fly antennae responding to all tested compounds, compared with the control ($t = 2.96 - 22.6$, $P < 0.05$). Average EAG responses to the control from antennae of female and male

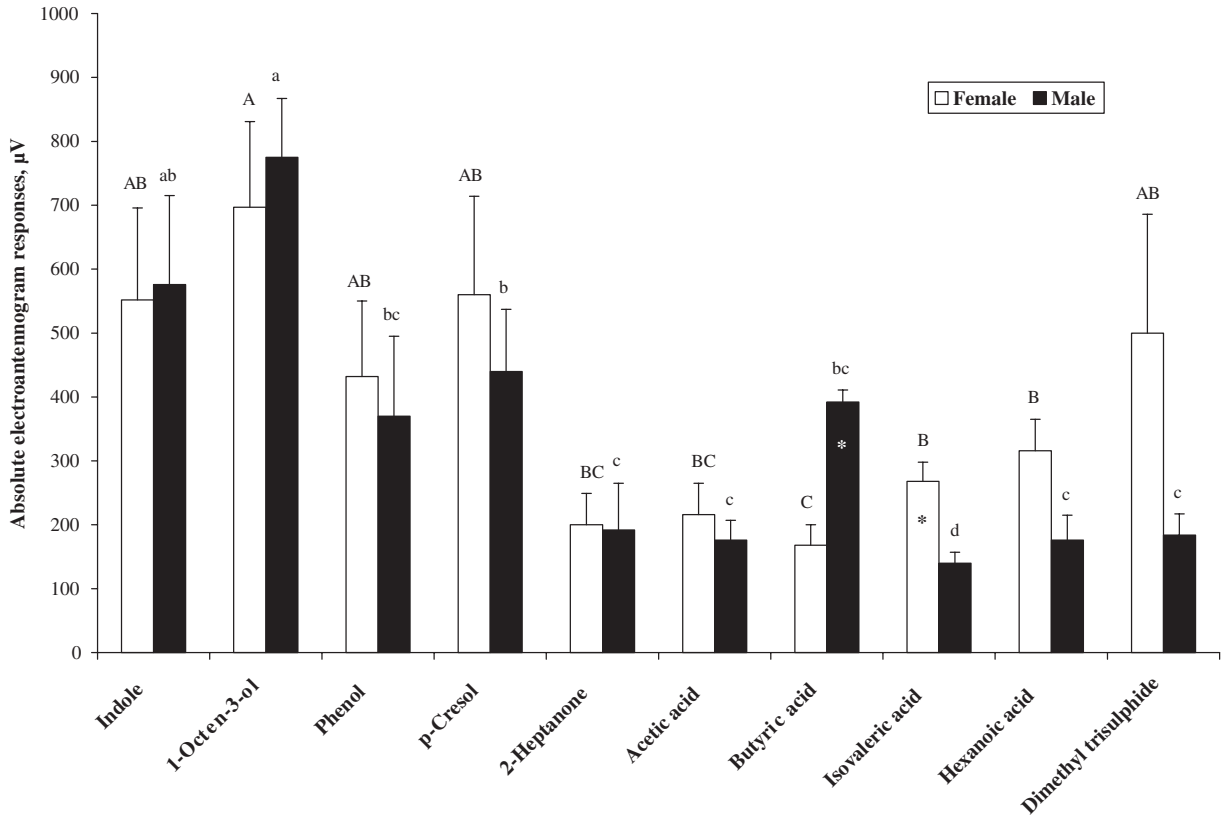

Fig. 3. Relative electroantennograms showing responses of male and female *Stomoxys calcitrans* to host-associated odorant compounds. Means with different letters above the bars are significantly different at $P < 0.05$ (SAS Version 9.1, performed on the least-square means). * indicates significant differences in responses between males and females.

stable flies were 0.15 ± 0.01 mV and 0.11 ± 0.01 mV, respectively. The highest EAG responses to 1-octen-3-ol, indole, phenol and *p*-cresol were observed in both female and male antennae ($F = 2.77$, d.f. = 9,42, $P < 0.05$ for females; $F = 6.55$, d.f. = 9,46, $P < 0.001$ for males). No differences in EAG responses to the test compounds were found between the sexes except that male antennae responded more strongly to butyric acid and isovaleric acid than did female antennae (for butyric acid, $t = 2.77$, $P < 0.05$; for isovaleric acid, $t = 2.79$, $P < 0.05$). Although a mean EAG of 500 µV was elicited from female stable fly antennae in response to dimethyl trisulphide, compared with a male response of 184 µV, no statistical difference was found ($t = 1.55$, $P = 0.09$).

Discussion

In the current study, three potential olfactory sensilla, the basiconic, coeloconic and clavate types, were all found on the funicles of the antennae. However, the numbers and distribution patterns of these sensilla differed from those reported by Lewis (1971). Whereas Lewis (1971) found an average of 3300 basiconic sensilla on the funicle, our specimens had an average of 1200 of this type of sensilla. In the Lewis (1971) study, most basiconic sensilla were located in the proximal lateral and ventral regions of the funicle, whereas in our study basiconic and clavate sensilla were concentrated on the inner side of the funicle. Five times as many basiconic sensilla as clavate sensilla were found on the funicle, compared with the 30 : 1 ratio reported by Lewis (1971). Based on our SEM observations and the TEM images recorded by Lewis (1971), there are approximately 700–1000 pores on each basiconic and clavate sensillum, suggesting an olfactory function. In most insects, olfactory sensilla are characterized by numerous pores in the sensillum cuticle that allow for the entry of odorant compounds (Steinbrecht, 1996). The least common sensillum was the coeloconic type, which were mostly located on the outer side of the funicle. Coeloconic sensilla were the shortest in length and had a finger-like structure. Discrepancies between the findings of our study and those of Lewis (1971) may reflect differences in local fly populations (European vs. American populations) and the use of scanning vs. transmission electron microscopy.

Trichoid sensilla were the most abundant sensilla observed on the funicle in both stable fly sexes. The three subtypes of these thin, conical and sharply pointed sensilla had either smooth or grooved surfaces and differed in length. Similar morphologies have been reported in other dipteran species (Ross & Anderson, 1987; Rahal *et al*., 1996; Fernandes *et al*., 2004; Chen & Fadamiro, 2008). Lewis (1971) estimated

there were approximately 700 relatively thick-walled trichoid sensilla and a variety of thin-walled sensilla on the funicle. However, in the current study, over 16 000 trichoids were counted on the funicle in both sexes. As no pores were found on trichoid sensilla, a mechano-receptor function is suggested for stable fly trichoid sensilla. Similar sensilla without surface pores are also found on the primary screwworm, *Cochliomyia hominivorax* C. (Diptera: Calliphoridae) (Fernandes *et al*., 2004), and the papaya fruit fly, *Toxotrypana curvicauda* G. (Diptera: Tephritidae) (Arzuffi *et al*., 2008). Trichoid sensilla with a mechanical function have been reported in the parasitoid, *Microplitis croceipes* C. (Hymenoptera: Braconidae) (Ochieng *et al*., 2000), the human bot fly, *Dermatobia hominis* L. (Diptera: Oestridae) (Fernandes *et al*., 2002), and the fire ant, *Solenopsis invicta* B. (Hymenoptera: Formicidae) (Renthal *et al*., 2003). However, Lewis (1971), using TEM, observed about 500 pores per thick-walled trichoid sensillum, which is generally similar to numbers on the long trichoids observed in this study. Trichoid sensilla with pores have also been reported in other fly species (Stocker, 1994; Riesgo-Escovar *et al*., 1997; Shanbhag *et al*., 1999). Several studies have attributed an olfactory function for trichoid sensilla in *Drosophila* spp. (Diptera: Drosophilidae) (Clyne *et al*., 1997; Riesgo-Escovar *et al*., 1997; Shanbhag *et al*., 1999) and Clyne *et al*. (1999) used electrophysiological studies to confirm pheromone sensitivity. It is surprising that significant differences in numbers of medium-sized trichoids were found between male and female antennae, which may indicate differences in antennal responses to different odours, as shown in EAG tests. However, our SEM study has shown that trichoids are not olfactory sensory. Further investigation is needed to reveal the function of medium-sized trichoid sensilla.

Basiconic sensilla were the second most abundant type of sensillum found on stable fly antennae. Basiconic sensilla have been reported as the most common sensillum type on male and female antennae in *Lucilia cuprina* W., *Chrysomya megacephala* F., *Chrysomya rufifacies* and *Chrysomya nigripes* (all, Diptera: Calliphoridae), *Musca domestica* L. and *Synthesiomyia nudiseta* W. (both, Diptera: Muscidae), *Megaselia scalaris* L. (Diptera: Phoridae) and *Trichopoda pennipes* F. (Diptera: Tachinidae) (Giangiuliani *et al*., 1994; Sukontason *et al*., 2004; Ngern-Klun *et al*., 2007). Three subtypes of basiconic sensilla were identified in *Pseudoperichaeta nigrolineata* W. (Diptera: Tachinidae), *Drosophila melanogaster* M. and *C. hominivorax* (Rahal *et al*., 1996; Shanbhag *et al*., 1999; Fernandes *et al*., 2004), but we did not identify any subtypes of basiconic sensilla on stable fly antennae. In our study, clavate sensilla looked similar in morphology and pore structure to basiconic sensilla, except that they had enlarged tips. The pore density on the surface wall of both basiconic and clavate sensilla is approximately 35–40 pores per μm^2. These findings are in agreement with earlier studies on *S. calcitrans* (Lewis, 1971) and *D. melanogaster* (Shanbhag *et al*., 1999). The presence of the pore structure on the surface wall of basiconic and clavate sensilla suggests their olfactory function, which will be further confirmed using single sensillum recording techniques. In *D. melanogaster* and *Phoracantha semipunctata* F. (Coleoptera: Cerambycidae) similar sensilla were demonstrated to respond to specific odorant compounds (Siddiqi, 1983, 1987; Lopes *et al*., 2002).

Coeloconic sensilla were the most distinctive (finger-like) and least abundant sensillum type (<0.3% of total sensilla) on the stable fly funicle. They were mainly distributed along the outer side of the funicle. Coeloconic sensilla have nine to 12 closely apposed cuticular fingers. In *D. hominis* L., coeloconic sensilla have pegs and are located in pits surrounded by microtrichia. However, in stable flies they arise directly from the surface of the funicle, which is similar to those found in several *Drosophila* species (Riesgo-Escovar *et al*., 1997; Shanbhag *et al*., 1999; Fernandes *et al*., 2002). Similar types of coeloconic sensilla in many insect orders are not considered to have a chemosensory function (Steinbrecht, 1997; Yao *et al*., 2005). In the current study, the absence of cuticular pores on coeloconic sensilla suggests they are unlikely to function as chemoreceptors. However, Schneider & Steinbrecht (1968) described coeloconic sensilla as olfactory receptors. Those sensilla lacked pores on the surface wall, but had terminal tubule structures. Using a single sensillum recording technique, Schneider & Steinbrecht (1968) recorded responses from this type of sensillum to CO_2, temperature and humidity. Furthermore, pore channels, reported in grooves on similar sensilla on female antenna in *Aedes aegypti* (Diptera: Culicidae) were reported to respond to lactic acid (Cribb & Jones, 1995). The grooves between the fingers at the distal half of the coeloconic sensillum in the stable fly may have the same chemosensory capacity. Further studies including TEM with negative staining and single sensillum recording are underway to demonstrate whether coeloconic sensilla function as chemosensory receptors, or not.

Stable flies use a wide variety of visual, olfactory, gustatory and physical stimuli for host location (Zhu *et al*., 2008). Among these stimuli, volatile semiochemicals emitted from the host play a major role in mediating host location and oviposition site selection. Several cow urine, manure and rumen-associated odorants that are attractive to stable flies have been identified (Logan & Birkett, 2007; Jeanbourquin & Guerin, 2007). Gravid stable fly females are capable of selecting an oviposition site based on microbe-derived stimuli that indicate the suitability of the substrate for larval development (Romero *et al*., 2006; Zhu *et al*., unpublished data, 2011).

Three major types of stable fly attractant, derivatives of fatty acid and amino acids, and isoprenoids, have been identified so far. 1-Octen-3-ol elicited the strongest EAG responses in both female and male stable fly antennae. This volatile, associated with rumen digesta compounds, has also been identified in cattle urine (Birkett *et al*., 2004; Jeanbourquin & Guerin, 2007). Traps baited with 1-octen-3-ol have been reported to significantly increase stable fly catches (Holloway & Phelps, 1991; Mihok *et al*., 1996). A second group of odorants associated with cattle manure and urine compounds, including phenol, *p*-cresol, dimethyl trisulphide and indole, also elicit strong EAG responses. These compounds, when produced by anaerobic bacteria isolated from aged horse manure, were attractive to gravid stable flies (Mohammed *et al*., 2003; Romero *et al*., 2006; Zhu *et al*., unpublished data, 2011). Significant behavioural responses (activation and

attraction) of stable flies were elicited by lures containing 10 μg of dimethyl trisulphide, 1-octen-3-ol and *p*-cresol (alone and in combinations) in wind tunnel bioassays (Jeanbourquin & Guerin, 2007). Although EAG responses were detected in stable fly antennae tested with a range of straight and branched carboxylic acid compounds, the response levels were significantly lower than for the compounds associated with cattle manure. Of these, butyric acid and isovaleric acid caused the lowest EAG responses, similarly to findings in Jeanbourquin & Guerin (2007). No differences between the two sexes of stable fly were observed in EAG responses to most of the compounds tested ($t = 0.42$, $P > 0.05$), except that significantly higher EAG responses were recorded in male antennae in response to butyric acid ($t = 2.31$, $P < 0.001$) and in female antennae in response to isovaleric acid ($t = 1.86$, $P < 0.01$). However, why the antennal responses of stable flies to these two particular compounds (25% of odorants identified from rumen digesta in Jeanbourquin & Guerin, 2007) differ remains a mystery and further behavioural studies are underway with the aim of increasing understanding of how these cues are used differently (for host searching and oviposition site selection).

In conclusion, the current study characterized the morphology and distribution of four different types of sensillum on the funicle of the stable fly. Three of these (basiconic, clavate and coeloconic sensilla) may have potential olfactory chemoreceptor function. Trichoid sensilla are likely to be involved in mechano-reception only. Stable fly antennal sensilla are similar to those described in other muscoid flies. In general, there are no morphological differences in two sexes of stable fly antennae in terms of sensilla types. Significant EAG responses detected in stable fly antennae to some selected host-associated volatile compounds indicate the use of olfactory cues for host and oviposition site searching. The characterization of morphological details and data on olfactory sensilla mapping lay a foundation for future trials using single sensillum recording that will aim to advance our understanding of the mechanisms underlying stable fly chemical ecology, sensory physiology and neuroethology. The knowledge gained from these studies will ultimately benefit the development of stable fly management strategies.

Acknowledgements

We express our appreciation to D. Berkebile and T. Weinhold for their technical support in this study. This work was conducted in cooperation with the Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, and supported in part by Regional Project 1030. We also thank J. Kalisch for his help in constructing the three-dimensional drawings of sensilla distributions.

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Accepted 19 November 2010
First published online 20 February 2011