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Characterization of Antennal Trichoid Sensilla from Female Southern House Mosquito, *Culex quinquefasciatus* Say

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Abstract

Culex quinquefasciatus, the southern house mosquito, is highly dependent on its olfactory system for vector-related activities such as host seeking and oviposition. The antennae are the primary olfactory organs in mosquitoes. We describe 5 morphological types of sensilla on the antenna of *C. quinquefasciatus*: 1) a pair of sensilla coeloconica located at the distal tip, 2) long and short sensilla chaetica present on all 13 antennal flagella, 3) sensilla ampullacea found on the 2 proximal-most flagella, 4) 2 morphological types of grooved pegs dispersed throughout the flagella, and 5) 5 morphological subtypes of sensilla trichodea distributed among all flagella. Antennal trichoid and grooved peg sensilla of mosquitoes have been demonstrated to house the olfactory receptor neurons (ORNs) that detect many of the odors involved in eliciting vector-related behaviors. In order to initiate the functional characterization of the peripheral olfactory system in female *C. quinquefasciatus*, we mapped the physiological responses of all 5 morphological subtypes of sensilla trichodea to an odor panel of 44 behaviorally relevant odor compounds. We identified 17 functional classes of sensilla trichodea: 3 short sharp-tipped, 9 short blunt-tipped type I, and 5 short blunt-tipped type II sensilla. One morphological subtype remains unclassified as the long sharp-tipped sensilla did not respond to any of the volatiles tested. The functional classes of the ORNs were analyzed with respect to stimulus response profiles, stimuli sensitivity, and temporal coding patterns. Comparisons with other functionally classified mosquito antennal sensilla trichodea are discussed.

Key words: chemoreception, electrophysiology, host cues, olfactory receptor neurons, oviposition cues, plant volatiles, semiochemicals and southern house mosquito

Introduction

The close association of the southern house mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), with humans makes them exceptional vectors of disease. *Culex quinquefasciatus* is a vector of lymphatic filariasis (*Wuchereria bancrofti*) affecting 120 million people worldwide with an estimated impact of 5 644 000 disability-adjusted life years (World Health Organization, fact sheets). In addition, *C. quinquefasciatus* is a vector of the West Nile virus, a flavivirus, currently spreading throughout North America with a 1–4% mortality rate among confirmed cases (Centers of Disease Control, USA; Health Canada, Canada). The endophilic and nocturnal behavior of these mosquitoes increase their disease vector efficacy (Deane and Damasceno 1952; Rachou 1956). Their preference for moderately aerated, polluted water rich in decaying organic matter for oviposition sites also increase the ability of *C. quinquefasciatus* to vector disease because these sites are commonly prevalent near human habitation (Blackwell et al. 1993; Chavasse et al. 1995).

The primary sensory modality used by mosquitoes during long-range host/oviposition site seeking is olfaction (McIver 1982; Bentley and Day 1989; Davis and Bowen 1994). Most studies on *C. quinquefasciatus* have been behavioral investigations focused on oviposition attractants: identifying a good blend of attractants for monitoring population size and host preference. An infusion of Bermuda grass (*Cynodon dactylon*), a common lawn and pasture grass found in warm temperate and tropical regions, was determined to be a strong attractant for gravid female *C. quinquefasciatus* using both oviposition assays and gas chromatography coupled to electroantennodetection (GC-EAD) analysis (Millar et al. 1992;

Blackwell et al. 1993; Isoe et al. 1995; Du and Millar 1999; Burkett-Cadena and Mullen 2007). Also, infusions of cow manure and dairy effluent have been shown to be strong oviposition attractants for *C. quinquefasciatus* (Allan et al. 2005). Compounds identified from the grass infusions and dairy effluents that are used in this study include 3-methylindole, indole, phenol, 4-ethylphenol, 4-methylphenol, 2-undecanone, 2-tridecanone, eugenol, nonanal, naphthalene, dimethyl trisulfide, α-pinene, and limonene.

More recent studies have begun to focus on the response of *C. quinquefasciatus* to blood host–related volatiles (Mboera et al. 1998; Allan et al. 2006a, 2006b; Puri et al. 2006) due to the recent introduction of the West Nile virus to North America and the identification of *C. quinquefasciatus* as the primary vector of this disease in the southern United States (Zinser et al. 2004; Molaei et al. 2007). The life cycle of the West Nile virus includes both birds and mammals as hosts and as such research has focused on the volatiles emitted from these 2 sources. Allan et al. (2006a, 2006b) assessed the attractiveness of volatiles collected from chicken feathers as well as those volatiles emitted from bovine blood to *C. quinquefasciatus* in a dual-choice olfactometer. These behaviorally active volatiles identified from bovine blood and chicken feathers were included in our odor panel: lactic acid, acetic acid, propionic acid, butanoic acid, heptanoic acid, hexadecanoic acid, stearic acid, and methyl propyl disulfide. Puri et al. (2006) chose to focus their attention on human skin emanations and to assess the effects through both behavioral and physiological assays. Saturated carboxylic acids of various chain lengths (C_3, C_6–C_{18}), 6 alcohols/heterocyclics, and 4 aldehydes were tested. All these volatiles except C_{14} and C_{18} were found to elicit electroantennogram responses in 4–5 days postemergence host-seeking females, and thus, representatives of each of these chemical groups were included in the current study. Recently, a GC-EAD study compared the odor profiles of 8 people, 4 males and 4 females (Curran et al. 2005). Not only were differences in the ratios of volatile emanations reported but also variations in the presence of the volatiles themselves. Among the volatiles present in all samples were nonanal and phenol; those with few representations in the samples were benzyl alcohol, naphthalene, and α-pinene (Curran et al. 2005). By including these compounds in our odor panel, we attempted to ensure that those human volatiles common to everyone and which may confer differences in the attractiveness of individuals to *C. quinquefasciatus* are represented as it may be within the variation in human volatile emanations that we may find novel mosquito attractants/repellents (Logan et al. 2008).

Mosquitoes sense host emanations, and in fact all olfactory cues, through the activation of sensory neurons housed in hair-like structures called sensilla. These olfactory sensilla located on the antennae, the maxillary palps, and the proboscis constitute the mosquito's peripheral olfactory system. The majority of these sensilla are sensilla trichodea located on the antennae and, as such, are thus considered to be the principal "drivers" of various behaviors. The sensilla trichodea morphological subtypes of culicine mosquitoes are conserved: 2 sharp or pointed tip subtypes, long and short; and 2 short blunt-tipped subtypes, I and II (McIver and Charlton 1970). This apparent morphological conservation may indicate an evolutionary constraint on the peripheral olfactory system in culicine mosquitoes which may, in turn, translate into a conservation of function as well. In short, this conserved morphology may reflect conservation in function. In culicine mosquitoes, both pointed-tipped sensilla subtypes are evenly tapered along their lengths ending in an obvious point (McIver 1982). In all previously studied *Culex* spp. the long sharp-tipped (LST) sensilla trichodea LST are distributed evenly among all but the most proximal and distal antennal flagella with either no increase or a slight increase in number (McIver 1982). The LSTs in another culicine species, the yellow fever mosquito, *Aedes aegypti*, however, are concentrated solely on the distal flagella (Ismail 1964). Short pointed-tipped sensilla trichodea, SSTs, were identified as a new subtype, separate from the blunt-tipped sensilla trichodea, SBTs, by McIver (1971). Because the majority of antennal sensilla descriptions were made prior to 1971, separate estimates of SST and SBT subtype numbers are not available for many species. In *Culex* spp., there is a marked reduction in the number of short sensilla from proximal to distal flagella (McIver and Charlton 1970) (Table 2). There are 2 subtypes of blunt-tipped sensilla trichodea in the culicines: short blunt-tipped type I (SBT I) are thin walled sensilla trichodea with moderate tapering of the sensillum shaft terminating in a blunt tip (McIver and Charlton 1970) and short blunt-tipped type II (SBT II) sensilla trichodea have extremely thin walls with foreshortened, blunt tips and no tapering of the diameter (Ismail 1964; McIver 1969). According to Ismail (1964) both culicine and anopheline mosquitoes have this sensillum type. Since then, the sensilla types for anopheline mosquitoes have been renamed: The SBT II culicine class appears to correspond to the anopheline trichoid type D, TD class, as both of these sensilla are similar in length with a relatively consistent diameter from base to tip and a shaft that parallels the flagella surface (McIver 1982).

McIver (1970) noted that those females of the *Culex* spp. with the largest numbers of all olfactory sensilla types coincide with those exhibiting a high degree of ornithophilic behaviors during at least part of their life histories, that is, *Culex pipiens*, *Culex fatigans*, and *Culex restuans*. As *C. quinquefasciatus* is a highly ornithophilic/anthropophilic species, rarely feeding on other vertebrates (Ribeiro 2000; Elizondo-Quiroga et al. 2006; Dennett et al. 2007), we hypothesize that this mosquito will possess, on average, high numbers of olfactory sensilla compared with the other nonornithophilic culicine mosquitoes.

Comprehensive functional and molecular mapping of the peripheral olfactory systems in 2 behaviorally distinct vector mosquitoes: the African malaria mosquito, *Anopheles gambiae* (Meijerink and van Loon 1999; Meijerink et al. 2001;

Hill et al. 2002; Qiu, van Loon, et al. 2006; Lu et al. 2007; Iatrou and Biessmann 2008), and the yellow fever mosquito, *A. aegypti* (Pappenberger et al. 1996; Melo et al. 2004; Bohbot et al. 2007; Ghaninia, Ignell, and Hansson 2007; Ghaninia et al. 2008; Kent et al. 2008) are currently underway. Recent publications have provided functional characterization for >80% of the antennal trichoid sensilla in these 2 vector mosquitoes (Qiu, Smallegange, et al. 2006, Qiu, van Loon, et al. 2006; Ghaninia, Ignell, and Hansson 2007; Ghaninia et al. 2008); and the first phase of olfactory molecular characterization (Fox et al. 2002; Hill et al. 2002; Bohbot et al. 2007; Kent et al. 2008) has lead to the functional characterization of several heterologously expressed mosquito olfactory receptors (Hallem et al. 2006; Bohbot et al. 2007).

The functional characterization of the antennal trichoid sensilla in *C. quinquefasciatus* presented in this report, combined with ongoing research founded on the recent release of the *C. quinquefasciatus* genome (http://cpipiens.vectorbase.org), provides us with an opportunity to compare the peripheral olfactory system of a third vector mosquito species that exhibits more diverse host preferences and host-seeking behaviors, such as host-preference switching between birds and humans (Samuel et al. 2004; Elizondo-Quiroga et al. 2006; Dennett et al. 2007) and diapause-related behaviors, which adapts this species to perpetuating human diseases overwinter without the necessity of relying on an animal reservoir (Bowen 1988, 1991, 1992).

A thorough morphological description of the antennal sensilla and the functional characterization of antennal sensilla trichodea is lacking in *C. quinquefasciatus*. Here, we present a functional and morphological characterization of the antennal sensilla of female *C. quinquefasciatus* by mapping the location of sensilla sensitive to those compounds previously identified as potential kairomones (both host- and oviposition-related volatiles) by behavioral and physiological experiments in *C. quinquefasciatus*, including a few compounds identified as bioactive in *A. aegypti* (Ghaninia, Ignell, and Hansson 2007) and *A. gambiae* (Qiu, van Loon, et al. 2006) for comparative analysis.

Materials and methods

Rearing

Culex quinquefasciatus (Johannesburg strain) were reared in a climate controlled chamber (25 °C, 70% RH, and 12:12 h light:dark with 0.5 h of red light–simulating dawn and dusk). Adults were maintained in mesh and plastic cages (27 × 27 × 30 cm) with ad libitum access to 10% sucrose through a filter paper wick. Larvae were reared in a separate chamber (27 °C, 75% RH, and 12:12 h light:dark) in distilled water filled plastic trays (20 × 30 × 10 cm). Larvae were kept in groups of <500 per tray and fed fish food (Best Friend Flakes Complete, VPG Sweden AB). Pupae were collected daily in 20-ml containers and transferred to the adult cages. Females, sugar fed only, of 3–5 days postemergence were used in all experiments. All physiological experiments were conducted between 6- and 10-h Zeitgeist time. These conditions assured that the females would be in a physiological state conducive to host seeking.

Scanning electron microscopy

Antennae of adult females were dissected and placed directly into ice-cold 95% ethanol and stored overnight at –20 °C. Specimens were mounted on scanning electron microscope stubs and sputter coated with a gold-palladium mix (Jeol JFC-1100). The specimens were visualized using a scanning electron microscope (LEO 435 VP, UK). Image processing was carried out in Adobe Photoshop and MS PowerPoint.

Electrophysiology

Preparation

To obtain a stable preparation for physiological recordings, a female mosquito was anaesthetized (1.5 min at –20 °C) and mounted on a microscope slide (76 × 26 mm) between 2 pieces of double-sided tape. The antennae were fixed by double-sided tape to a coverslip resting on a small ball of dental wax to facilitate manipulation. The coverslip was positioned in parallel with the microscope slide at a right angle to the mosquito head. Once mounted, the mosquito was placed in the single sensillum recording (SSR) rig under a Nikon Eclipse (E600FN) microscope so that the 2 antennal flagella were visible at high magnification (×750). The time of every recording was noted, and there is no apparent correlation between the time of recordings and functional classification during this time period.

Single sensillum recording

Two tungsten microelectrodes (electrolytically sharpened in 10% KNO_2 at 2–10 V to a ~1 μm tip diameter) were created. A reference electrode was connected to the ground and inserted into the eye of the mosquito. The second electrode was connected to a preamplifier (10×, Syntech, Kirchzarten, Germany) and inserted into the shaft of a trichoid sensillum to complete the electrical circuit and to extracellularly record olfactory receptor neuron (ORN) potentials. Controlled manipulation of the electrodes was made possible by 2 micromanipulators (DC-3K, Märzhäuser Wetzlar GmbH & Co. KG, Wetzlar, Germany). The preamplifier was connected to an analog to digital signal converter (IDAC, Syntech) which in turn was connected to a PC computer (DELL, Round Rock, TX) for signal recording and visualization.

Stimuli and stimulus delivery

Forty-four stimuli, for use in sensilla characterization, were chosen from 9 chemical groups (carboxylic acids, alcohols, phenolics, indolics, esters, ketones, terpenoids, aldehydes,

and sulfides) and from 5 physiologically/behaviorally relevant cue groups (blood meal cues from humans/mammals/birds, sugar meal cues, and oviposition cues) (Table 1). As previously described in Ghaninia, Hansson, and Ignell (2007), stimuli were diluted in paraffin oil from neat compounds to an initial concentration of 1:10 v/v with the exception of the phenolics, indolics, hexadecanoic acid, and stearic acid that were diluted to an initial concentration of 1:100 v/v. Aliquots of 10 μl of each diluted compound were dispensed onto a filter paper (10 × 10 mm) inserted in a Pasteur pipette to create the stimulus cartridges. The concentrations applied to the cartridges were chosen to facilitate comparison of response profiles with previous studies in *A. aegypti* (Ghaninia, Ignell, and Hansson 2007) and *A. gambiae* (Qiu, van Loon, et al. 2006).

A constant airflow across the antenna was maintained at 1.5 l/min throughout the experiment. Purified and humidified air was delivered to the preparation through a glass tube (10-mm inner diameter). The glass tube was perforated by a small hole, slightly larger than the tip of a Pasteur pipette 11 cm away from the end of the tube. Stimulation was achieved by inserting the tip of the stimulus cartridge into the hole in the glass tube. A stimulus controller (Syntech) diverted a portion of the air stream (0.5 l/min) to flow through the stimulus cartridge for 500 ms, delivering the stimulus to the sensilla covering the antenna. The distance between the end of the glass tube and the antenna was 4 mm. The total volume of the cylindrical air column from delivery point to the mosquito antenna is 9 ml. At an air speed of 1.5 l/min, this column of air is replaced every 360 ms, which corresponds to the observed delay between the onset of stimulus and the onset of ORN response.

Analysis

To determine the response of an individual ORN to a single compound, the waveforms were analyzed as to spike shape, amplitude, and duration in order to assign each waveform an ORN identity as a spike from an A neuron (by convention the waveform with the larger amplitude), a B neuron, or a double spike consisting of both A and B neuron (Figure 2). All sensilla from which we recorded exhibited two spontaneously active neurons. This does not preclude the possibility of the presence of one or more silent neurons. The assignments were initially made using Autospike (Syntech) and subsequently corroborated through manual observation. Once all spikes were assigned an identity (A or B), the number of spikes of an individual class were counted 500 ms prior to the onset of response and 500 ms following the onset of response. The "onset of response" was determined for each sensillum separately as the shortest duration between the onset of stimulation (the introduction of the volatile compound into the air stream) and the onset of response (the odor-induced activity in an ORN) elicited by 1 of the test volatiles. As stated above, this duration correlates with the amount time it takes to replace the air in the column (360 ms). To control for spontaneous firing, the prestimulus spike counts were subtracted from the poststimulus counts to give the overall change in response to the stimulus. These counts were doubled to generate the conventional units of spikes per second. Spike counts between –10 and +15 spikes/s were deemed not different from controls.

Cluster analysis

To determine whether antennal trichoid sensilla have functionally different response profiles, complete linkage cluster analysis (with squared Euclidean distances) was used to generate a dendrogram (Minitab Release 14.12. 0, Minitab Inc., State College, PA). The dendrogram generated is representative of the relationships between each ORN response profile to all, or a subset, of the stimuli in multidimensional space. ORNs found to cluster together are therefore considered to be a functional class.

Statistical analysis

We analyzed the temporal response of ORNs with a paired Student's *t*-test in which neighboring bins (100-ms duration) were compared with one another. The statistical analysis was performed with GraphPad Prism (version 3.00 for Windows, GraphPad Software, San Diego, CA).

Results

Morphological classification of olfactory sensilla

Each antenna of a female *C. quinquefasciatus* has 13 flagella (F) with a consistent length of 276 ± 2.2 μm, for F1–12, and 371 ± 8.2 μm, for the terminal segment, F13. Each flagellum is covered with sensilla (1362 ± 174, $n = 4$) (Figure 1A). There are 5 morphological types of sensilla found on each antenna: 1 pair of sensilla coeloconica at the distal tip (F13; Figure 1A top inset), 2 subtypes of sensilla chaetica (F1–13; 162 ± 12 in total), 1 subtype of sensilla ampullacea (F1–2; 12 in total; Figure 1A bottom inset), 5 subtypes of sensilla trichodea (F1–13; 1124 ± 153 in total), and 2 subtypes of grooved pegs (F1–13; 238 ± 20 in total; Figure 1B, Table 2). Sensilla chaetica (both long and short), sensilla coeloconica, and sensilla ampullacea are classified as nonolfactory sensilla in mosquitoes (McIver and Hutchinson 1972; McIver 1973, 1982; Boo and McIver 1975, 1976; Davis and Sokolove 1975). Sensilla chaetica are found at the proximal (long) and distal (short) edges of each flagellar segment (Figure 1A,B, Table 2; large and small arrowheads respectively) and are putative mechanoreceptors as evidenced by a flexible socket and thick shaft without perforations. Sensilla coeloconica are short double-walled sensilla found as a pair at the distal tip of F13 (Table 2) and have been putatively classified as hygro- and thermoreceptive (McIver and Hutchinson 1972; McIver 1973, 1982; Boo and McIver 1975; Pitts and Zwiebel 2006). Sensilla ampullacea, found on only the first and second

Table 1 Odorants used as stimuli to characterize *Culex quinquefasciatus* female sensilla trichodea

Stimulus compound	Abbreviations	Purity (%)	Concentration (v/v)	CAS number[a]	Company[b]
Carboxylic acids					
Lactic acid[c]	LA	90	1:10	50-21-5	Fluka
Acetic acid[c]	AA	99	1:10	64-19-7	Fluka
Propionic acid[c,d]	PRA	≥99.8	1:10	79-09-4	Fluka
Butanoic acid[c]	BA	≥99	1:10	107-92-6	Aldrich
Pentanoic acid[c]	PA	≥99.8	1:10	109-52-4	Fluka
Hexanoic acid[d]	HA	99.5	1:10	142-62-1	Aldrich
Heptanoic acid[c,d]	HPA	≥96	1:10	111-14-8	Aldrich
Octanoic acid[b]	OA	≥99.5	1:10	124-07-2	Fluka
Hexadecanoic acid[c,d,e]	HXDA	≥99	1:10	57-10-3	Fluka
Stearic Acid[c,d,e]	SA	≥99.5	1:10	57-11-4	Fluka
Alcohols					
1-Octen-3-ol[c,f]	1-O	≥99	1:10	3391-86-4	Acros
2-Butoxyethanol[f]	2-BE	99.8	1:10	111-76-2	Fluka
4-Methylcyclohexanol[f]	4-MCH	≥98	1:10	589-91-3	Fluka
Benzyl alcohol[d,g]	BZA	>99.5	1:10	100-51-6	Fluka
Ethylene glycol[d]	EG	≥99.9	1:10	107-21-1	Fluka
Phenols					
Phenol[d,f,g]	PH	99	1:100	108-95-2	Aldrich
3-Methylphenol[f]	3-MP	99	1:10	108-39-4	Aldrich
4-Methylphenol[f,g]	4-MP	99	1:100	106-44-5	Aldrich
4-Ethylphenol[f,g]	4-EP	99	1:100	123-07-9	Aldrich
Heterocyclic					
Indole[d,f]	IND	99	1:100	120-72-9	Aldrich
3-Methylindole[d,f]	3-MI	98	1:10	83-34-1	Aldrich
Ketones					
Geranyl acetone[d]	ACE	99	1:10	3796-70-1	Aldrich
2-Tridecanone[f]	2-T	97	1:10	593-08-8	Fluka
2-Undecanone[f]	2-U	99	1:10	112-12-9	Aldrich
Bicyclic monoterpenes					
(−)-α-Thujone[g]	α-T	≥96	1:10	546-80-5	Fluka
(±)-α-Pinene[d,e,g]	α-P	98	1:10	80-65-8	Aldrich
Verbenone[g]	VER	94	1:10	18308-32-5	Aldrich
Monocyclic monoterpenes					
Limonene[g]	LIM	≥99	1:10	5989-27-5	Fluka
Menthone[g]	MEN	≥97	1:10	14073-97-3	Fluka
Eugenol[g]	EUG	≥99	1:10	97-53-0	Aldrich
Thymol[g]	THY	≥99	1:10	89-83-8	Fluka

Table 1 Continued

Stimulus compound	Abbreviations	Purity (%)	Concentration (v/v)	CAS number[a]	Company[b]
Acyclic monoterpenes					
Citral[g]	CIT	>95	1:10	5392-40-5	Fluka
Fatty acid esters					
Methyl butyrate[f]	MB	99	1:10	623-42-7	Acros
Ethyl butyrate[f]	EB	99	1:10	105-54-4	Aldrich
Methyl propionate[f]	MP	99	1:10	554-12-1	Acros
Ethyl propionate[f]	EP	99	1:10	105-37-3	Acros
Isopropyl acetate[f]	IPAA	≥99.8	1:10	108-21-4	Fluka
Esters					
(−)-Ethyl-L-lactate[f]	ELA-	≥99	1:10	687-47-8	Fluka
(+)-Ethyl-D-lactate[f]	ELA+	≥99	1:10	7699-00-5	Fluka
Aldehydes					
Nonanal[d,e]	NON	95	1:10	124-19-6	Acros
Sulfides					
Dimethyl trisulfide[c,f]	DS	98	1:10	3658-80-8	Aldrich
Methylpropyldisulfide[c]	MPD	>90	1:10	2179-60-4	Aldrich
Others					
Linalool (racemic)[g]	LIN	97	1:10	78-70-6	Aldrich
Napthalene[d]	NAP	≥99.7	1:10	91-20-3	Fluka

[a]Chemical Abstract Service registry numbers.
[b]Compounds were ordered from Aldrich, Stockholm, Sweden; Fluka, Stockholm, Sweden; and Acros, Geel, Belgium.
[c]Mammalian emanations.
[d]Human emanations.
[e]Avian emanations.
[f]Oviposition cues.
[g]Plant-related emanations.

flagella, are small, thick-walled peg sensilla protruding perpendicularly to the walls of pits with slit-like openings that have been identified previously as putative hygro- and thermoreceptors (Figure 1A bottom inset; Table 2; Boo and McIver 1975; McIver 1982; Pitts and Zwiebel 2006).

The predominant olfactory sensillum type found on the antenna is sensilla trichodea. There are 5 morphological subtypes of these sensilla trichodea: LST (59 ± 0.7 μm), SST (32 ± 0.6 μm), short sharp-tipped curved (SST-C; 21 ± 0.7 μm), SBT I (18 ± 0.4 μm), and SBT II (19 ± 0.3 μm) sensilla trichodea (Figure 1, Table 2). Each of the trichoid subtypes has distinct spatial distributions across the 13 flagella. The LSTs are evenly distributed among F2–13 but less than half the number of LSTs on each distal flagellum is found on the most proximal flagellum, F1 (Table 2). Most SSTs are found between F1–6, with the lowest number appearing on the terminal segment (Table 2). The SST-C subtype is rare and found on F2-13. The SBT I sensilla are most numerous between F1-9, whereas F10–13 display only a few. The rarest trichoid sensillum subtype is the SBT II which occurs on F1-8 but which is absent on the distal flagella. Most SBT II sensilla on a single flagellum are consistently found on F3 (Table 2). These sensilla are found most often on the lateral margins of the flagella and are the only sensilla trichodeum subtype that appears to be sequestered on a specific region of the flagella. The other morphological olfactory sensillum type, the grooved pegs (short 8.5 ± 0.3 μm; long 9.8 ± 0.2 μm), are most numerous on flagella 12 and 13 but consistently distributed among the other flagella (Figure 1B).

Functional classification of trichoid sensilla

Using the SSR technique, we recorded the spiking activity of individual ORNs from morphologically distinct trichoid sensilla. Each sensillum appeared to be innervated by 2 spontaneously active ORNs discernable by distinct spike shape and amplitude (Figure 2A). These parameters were used to assign each electrical event to either cell A (the larger amplitude class by convention) or cell B (Figure 2B). The activity of

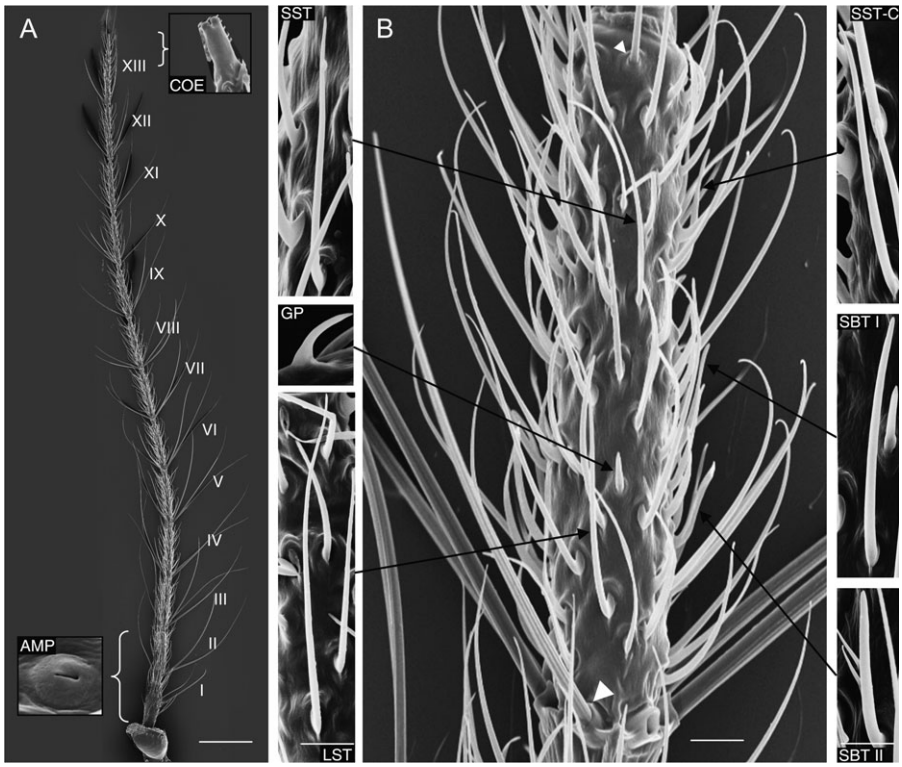


Figure 1 **(A)** A scanning electron micrograph of the antenna of a female *Culex quinquefasciatus* showing all 13 flagellar segments. The top inset shows the distal tip of the antenna with its fused pair of nonolfactory sensilla coeloconica. The bottom inset shows 1 sensillum ampullaceum, also nonolfactory. **(B)** A scanning electron micrograph of a single antennal flagellum (F4) displaying the 5 morphological subtypes of olfactory sensilla trichodea: short sharp-tipped (SST), short sharp-tipped curved (SST-C), short blunt-tipped I (SBT I), short blunt-tipped II (SBT II), and LST as well as grooved pegs (GP). The nonolfactory long and short sensilla chaetica are denoted by large and small white arrowheads, respectively. The scale bar indicates (A) 100 μm, (B) 10 μm and 5 μm for the individual sensilla micrographs.

the various sensilla trichodea demonstrates different functional classes based not only on the sensitivity of the ORNs but also on the activity patterns of the neurons in response to the various synthetic host/oviposition-related volatiles. SSRs enabled us to functionally classify the *C. quinquefasciatus* sensilla trichodea in response to 44 volatiles from a wide variety of chemical groups (Table 1; Table 3). One-hundred and fourteen complete recordings were compiled for this functional classification on 3–5 days postemergence sugar-fed, host-seeking female *C. quinquefasciatus* sensilla trichodea. Based on a cluster analysis of all sensilla trichodea responding to 20 of the most bioactive volatile stimuli (Figures 3 and 4), we were able to identify 17 functional classes (Table 3): 9 for SBT I (Figure 3C,D), 5 for SBT II (Figure 6B), and 3 for SST and SST-C, as distinctions between these morphological classes cannot be confidently assessed under the light microscope (Figure 7B). The LST sensilla (Figure 8B) remain unclassified as this sensillum type did not respond to any of the volatiles tested. Out of the identified 34 ORNs from 17 functional types (with 2 spontaneously active neurons/trichoid sensillum), only 1 did not respond to any of the volatiles in our panel (SST 1A, Table 3), and of all 44 compounds tested, methyl propionate and stearic acid were the only volatiles not to elicit any change in neuronal activity in any sensillum type. The LST sensilla were omitted from the cluster analysis as these sensilla were nonresponsive to odor stimulation and therefore remain functionally unclassified (Figure 8B, Table 3).

We analyzed the 3 main aspects of peripheral coding in ORNs for each of the 5 described morphological types of sensilla trichodea. The response spectra described for each functional class (Figures 3B,C, 4B, 7B, and 8B) represent 1 level of odor coding in the ORNs (selectivity), whereas the intensity of the response of an ORN to various volatiles (sensitivity) represents another (Figure 4). ORNs can use neuronal sensitivity to code information about a stimulus in 2 ways; they can act as presence/absence detectors, in which a small increase in stimulus dose results in a change from a baseline response to a near maximal response (e.g., Figure 4A: indole); or these ORNs can change their response to stimuli in a gradual, dose-dependent manner (e.g., Figure 4A: linalool). The third aspect of peripheral coding is the temporal characteristics of the neuronal response as assessed by the number of events within sequential bins of 100 ms (Figure 5).

Short blunt-tipped sensilla trichodea

Type I. The SBT I sensilla (58.3% of the total responding sensilla) are a functionally heterogeneous group: 6 of the

Table 2 Morphological classification and mapping of olfactory sensilla across the 13 flagellomeres of the female *Culex quinquefasciatus* antenna

	LC	SC	AMP	LST	SST	SST-C	SBT I	SBT II	GP
Segment									
1	5 ± 0.5	10 ± 1.3	5 ± 3.9	10 ± 0.5	8 ± 2.0	0 ± 0	5 ± 2.1	1 ± 0.8	7 ± 0.6
2	3 ± 0.4	4 ± 0.9	1 ± 0	29 ± 7.7	12 ± 2.1	2 ± 0.6	6 ± 0.5	2 ± 1.4	8 ± 4.3
3	4 ± 0	3 ± 0.6	0 ± 0.3	28 ± 2.1	12 ± 3.2	1 ± 0.6	8 ± 3.1	4 ± 1.4	10 ± 1.6
4	4 ± 0.3	3 ± 0.5	0 ± 0	27 ± 3.1	15 ± 2.5	3 ± 1.0	6 ± 0.8	2 ± 0.8	7 ± 1.5
5	4 ± 0.3	2 ± 0.5	0 ± 0	29 ± 2.4	14 ± 1	2 ± 1.4	7 ± 1.3	1 ± 0.4	6 ± 0.3
6	4 ± 0.3	2 ± 0.3	0 ± 0	27 ± 4.1	11 ± 1.3	3 ± 1.7	8 ± 2.7	1 ± 0.5	7 ± 0.5
7	3 ± 0.3	1 ± 0.5	0 ± 0	28 ± 3.3	12 ± 2.5	3 ± 1.1	8 ± 1.1	1 ± 0.3	7 ± 0.8
8	4 ± 0.3	1 ± 0.5	0 ± 0	25 ± 2.9	9 ± 1.0	3 ± 0.5	5 ± 0.9	1 ± 0.3	8 ± 0.9
9	4 ± 0.3	0 ± 0.3	0 ± 0	27 ± 3.5	8 ± 1.1	3 ± 1.1	5 ± 1.7	0 ± 0.3	8 ± 0.3
10	4 ± 0.3	1 ± 0.5	0 ± 0.3	24 ± 3.8	6 ± 1.8	2 ± 1.0	3 ± 1.4	0 ± 0.3	11 ± 1.8
11	4 ± 0.5	1 ± 0.3	0 ± 0.3	27 ± 5.1	7 ± 1.4	2 ± 1.0	2 ± 0.3	0 ± 0	11 ± 1.5
12	3 ± 0.3	1 ± 0.3	0 ± 0	22 ± 3.7	7 ± 1.7	1 ± 0.5	2 ± 0.7	0 ± 0	14 ± 1.7
13	3 ± 1.1	3 ± 1.1	0 ± 0	25 ± 3.9	5 ± 1.7	1 ± 0.5	4 ± 1.8	0 ± 0	15 ± 1.8
T	49 ± 1.7	32 ± 4.3	6 ± 2.8	328 ± 42.2	126 ± 12.4	26 ± 7.9	69 ± 10.8	13 ± 3.2	119 ± 10.4
2T	98 ± 3.4	64 ± 8.6	12 ± 5.7	656 ± 84.4	252 ± 24.8	52 ± 15.9	138 ± 21.7	26 ± 6.4	238 ± 20.8
Length (μm)	264 ± 76	64 ± 1.4	nd	59 ± 0.7	32 ± 0.6	21 ± 0.7	18 ± 0.4	19 ± 0.3	9 ± 0.9

The table also includes the mechano-, hygro-, thermoreceptive long and short sensilla chaetica (LC, SC) for comparison with the LST sensilla trichodea. The other sensilla trichodea present on the antenna are the SST, SST-C, SBT I, and SBT II. The 2 classes of grooved pegs (GP) are considered together as the differences in length between the 2 classes are not consistently distinguishable using 2-dimensional standard error of mean. T represents the total 2-dimensional count of sensilla from each flagellum. 2T represents the doubling of T to more accurately estimate the 3-dimensional number of sensilla on each flagellum. nd, not done.

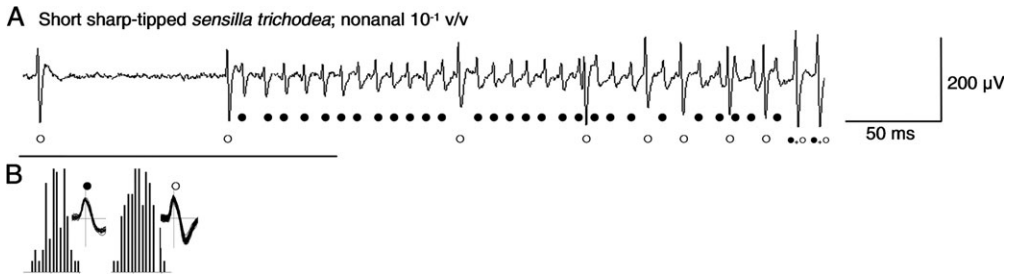


Figure 2 **(A)** Activity of the ORNs housed in a trichoid sensillum reveals differences in spike amplitude between A and B neurons. **(B)** The spike shape and distribution of amplitudes of the 2 neurons are shown. The symbols ○ and ● indicate the large and small spiking potentials from the A and B neurons, respectively. Where a concurrent firing event occurred, it is denoted with ○ + ●. The scale bars indicate 50-ms duration and 200 μV amplitude.

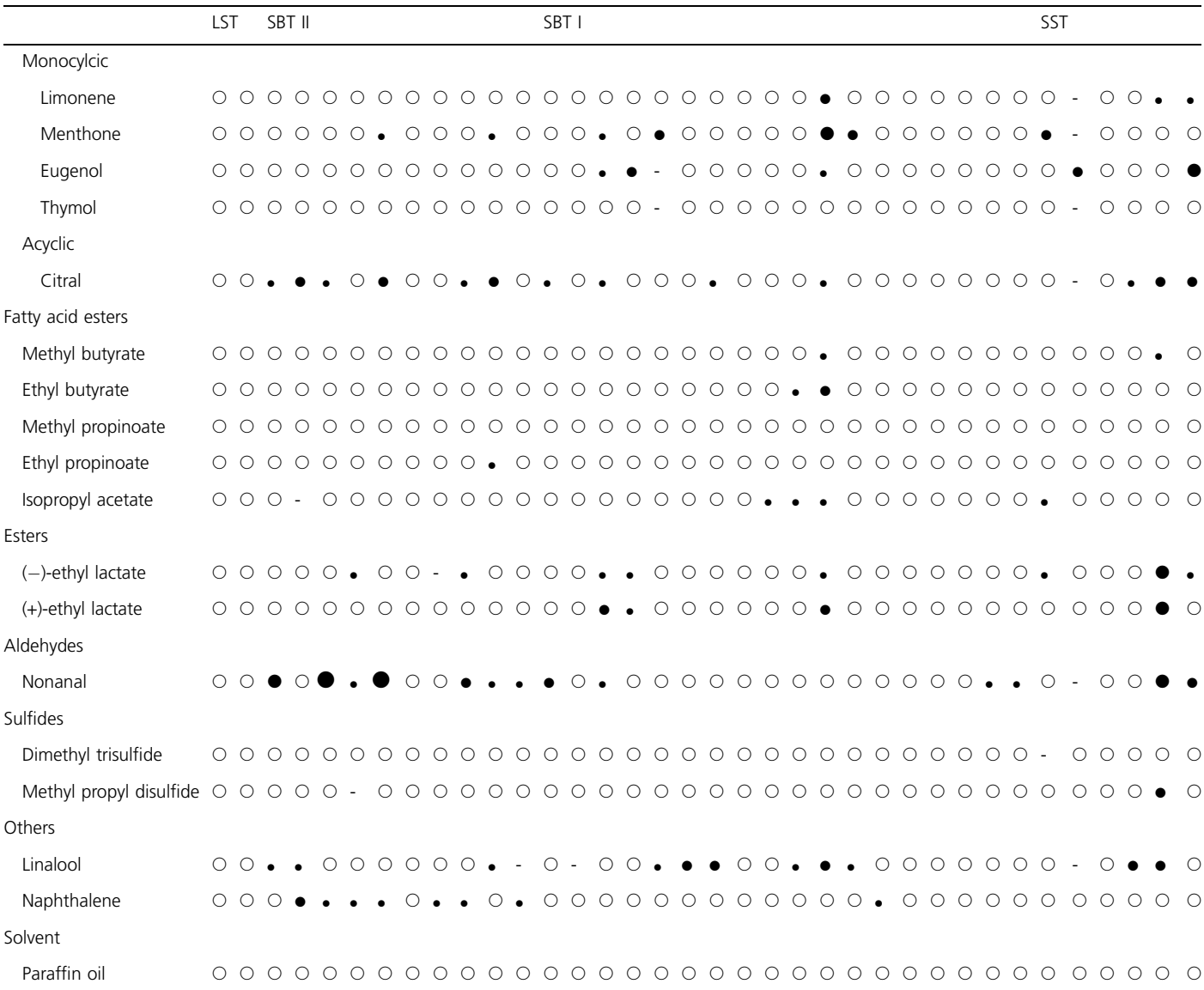
9 functional classes responded to the alcohols, 4 responded to indole and 3-methylindole, and 4 responded to citral, although to a much reduced degree in comparison with those SBT II functional classes responding to citral (Figure 3B, Table 3). The SBT I sensilla includes the only ORN functional type that responded to 3-methylindole without also responding to indole (SBT I 5). The 9 functional classes of SBT I sensilla are divided into 2 subsets based on their spontaneous firing rates: 6 high spontaneous activity classes (22 ± 0.33 spikes/s for both A and B cells; Figure 3B) and 3 low spontaneous activity classes (6.3 ± 0.44 spikes/s; Figure 3C).

The SBT I 3 sensillum functional type demonstrates the 3 main aspects of classical peripheral coding: ORN selectivity, sensitivity, and temporal coding. The ORNs are able to distinguish among many volatile compounds (Figure 3, Table 3). Although both ORNs responded to the alcohols 1-octen-3-ol and 2-butoxyethanol, 1-octen-3-ol appears to be the more effective ligand as demonstrated by both the

Table 3 Specificity of ORNs housed in SST, SBT I, SBT II, and LST sensilla trichodea to a panel of 44 volatile compounds and the solvent paraffin oil based on a total of 116 recordings

	LST	SBT II										SBT I																		SST											
	1	1		2		3		4		5		1		2		3		4		5		6		7		8		9		1		2		3			
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Carboxylic acids																																								
Lactic acid	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○				
Acetic acid	○	○	○	•	•	○	●	○	○	○	●	○	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	-	-	○	○	○	○				
Propionic acid	○	○	•	○	○	○	●	○	○	○	•	-	-	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○	○				
Butanoic acid	○	○	•	●	○	○	●	○	○	•	●	-	-	-	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○	○				
Pentanoic acid	○	○	○	•	○	○	●	○	○	•	●	○	•	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○	○				
Hexanoic acid	○	○	○	●	○	○	●	○	•	•	●	○	●	•	•	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○	○				
Heptanoic acid	○	○	-	●	•	○	●	○	•	○	●	•	●	○	•	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○			
Octanoic acid	○	○	○	●	○	○	●	○	○	●	●	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○				
Hexadecanoic acid	○	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○				
Stearic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○				
Alcohols																																								
1-octen-3-ol	○	○	•	○	○	-	•	-	○	○	•	○	•	○	•	○	●	•	○	•	○	•	●	●	○	○	○	○	•	○	○	-	○	●	●	●				
2-butoxyethanol	○	○	•	•	•	●	○	•	○	●	○	•	●	●	●	•	●	○	○	○	○	○	●	●	○	•	○	○	○	○	●	-	○	●	●	●				
4-methylcyclohexanol	○	○	•	•	○	○	○	○	○	○	○	•	●	○	●	●	○	○	○	○	○	•	○	○	○	○	•	○	•	○	●	○	○	•	●	○				
Benzyl alcohol	○	○	•	○	○	○	○	○	○	○	○	○	•	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○				
Ethylene glycol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	•	•	○	○	•	○				
Phenols																																								
Phenol	○	○	•	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	○	-	○	○	○	○	○				
3-methylphenol	○	○	-	●	○	•	-	○	○	○	•	•	•	○	•	○	-	-	○	○	○	○	○	●	○	○	○	○	○	○	○	-	○	○	○	○				
4-methylphenol	○	○	○	●	○	○	•	○	•	○	○	○	●	○	•	●	○	○	○	○	○	○	○	●	●	○	○	○	○	○	●	●	○	○	○	○				
4-ethylphenol	○	○	•	•	○	○	•	-	•	○	•	○	•	○	○	●	○	○	○	○	○	○	•	●	○	○	○	○	○	○	○	●	○	○	○	○				
Heteroyclics																																								
Indol	○	○	•	●	•	●	•	•	-	●	○	•	○	-	○	○	●	○	●	●	○	•	•	●	○	○	○	○	○	○	○	○	○	○	○	○	○				
3-methylindole	○	○	○	●	○	○	○	○	○	●	○	○	○	○	●	○	●	●	●	●	○	●	○	●	○	○	○	○	○	○	○	●	-	○	○	○	○				
Ketones																																								
Geranyl acetone	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○			
2-tridecanone	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	○	○	○	○	○	○	○			
2-undecanone	○	○	•	•	○	○	●	-	○	○	•	○	○	○	○	○	○	○	○	○	○	○	•	○	○	○	○	○	○	○	○	○	○	○	○	•	●			
Monoterpenes																																								
Bicyclic																																								
(−)-α-thujone	○	○	•	○	○	○	•	○	○	○	○	○	○	○	●	○	-	○	○	○	○	○	•	○	○	○	•	○	○	○	○	○	○	•	●	○				
(±)-α-pinene	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	○	•	-	○	○	○	○				
Verbenone	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	○	-	○	○	○	○	○	○	○	○	○	○	○	○	○	•	-	○	○	●	○				

Table 3 Continued



All stimuli are tested at a concentration of 1:10 (v/v) with the exception of indole, phenol, 4-methylphenol, and 4-ethylphenol, which were tested at 1:100 v/v. An ORN that does not exceed 15 spike/s is considered a nonresponder (○). Responses are excitatory when the increase in firing frequency is less than 20% (•), between 20 and 50% (●), between 50 and 80% (●), and higher than 80% (●) of the highest response, that is, 160 spikes/s, respectively. Responses were considered inhibitory (-) whenever a decrease by ≥10 spikes/s was observed.

earlier onset of activity and its higher response frequency to a similar dose (Figure 3A). The sensilla response to indole was a phasic increase in the firing frequency of the A, but not the B, cell (Figure 3A), whereas citral appears to inhibit the activity of the A cell, whereas not affecting the spontaneous firing rate of the B cell (Figure 3A). Each of these examples of differential responses to volatiles demonstrates a high degree of ORN selectivity.

The sensitivity of these SBT I 3 ORNs is described as the relationship of neuronal activity to the dose of the stimuli applied (Figure 4). In order to better demonstrate the range of ORN sensitivities, 4 volatile stimuli were chosen based on the broad range of response in the A cell to the maximum dose applied in the initial study (Figure 3B); linalool (~100 spikes/s), indole (~75 spikes/s), 1-octen-3-ol (~50 spikes/s), and nonanal (<10 spikes/s) (Figures 3C and 4A, Table 3). Linalool, which elicited a maximal response (109.6 ± 14.4 spikes/s) at 10^{-2} v/v, exhibited the lowest threshold of response (15.2 ± 3.6 spikes/s) to 10^{-4} v/v, thus showing a range of sensitivity to linalool over 2 orders of magnitude. Although the response to 1-octen-3-ol at the highest tested dose (10^{-1} v/v) is not maximal (44.4 ± 1.6 spikes/s), SBT I 3A is able to detect a range of concentrations over at least 2 orders of magnitude. The sensitivity to indole is depicted from below threshold (3.6 ± 3.1 spikes/s at 10^{-3} v/v) to maximal response

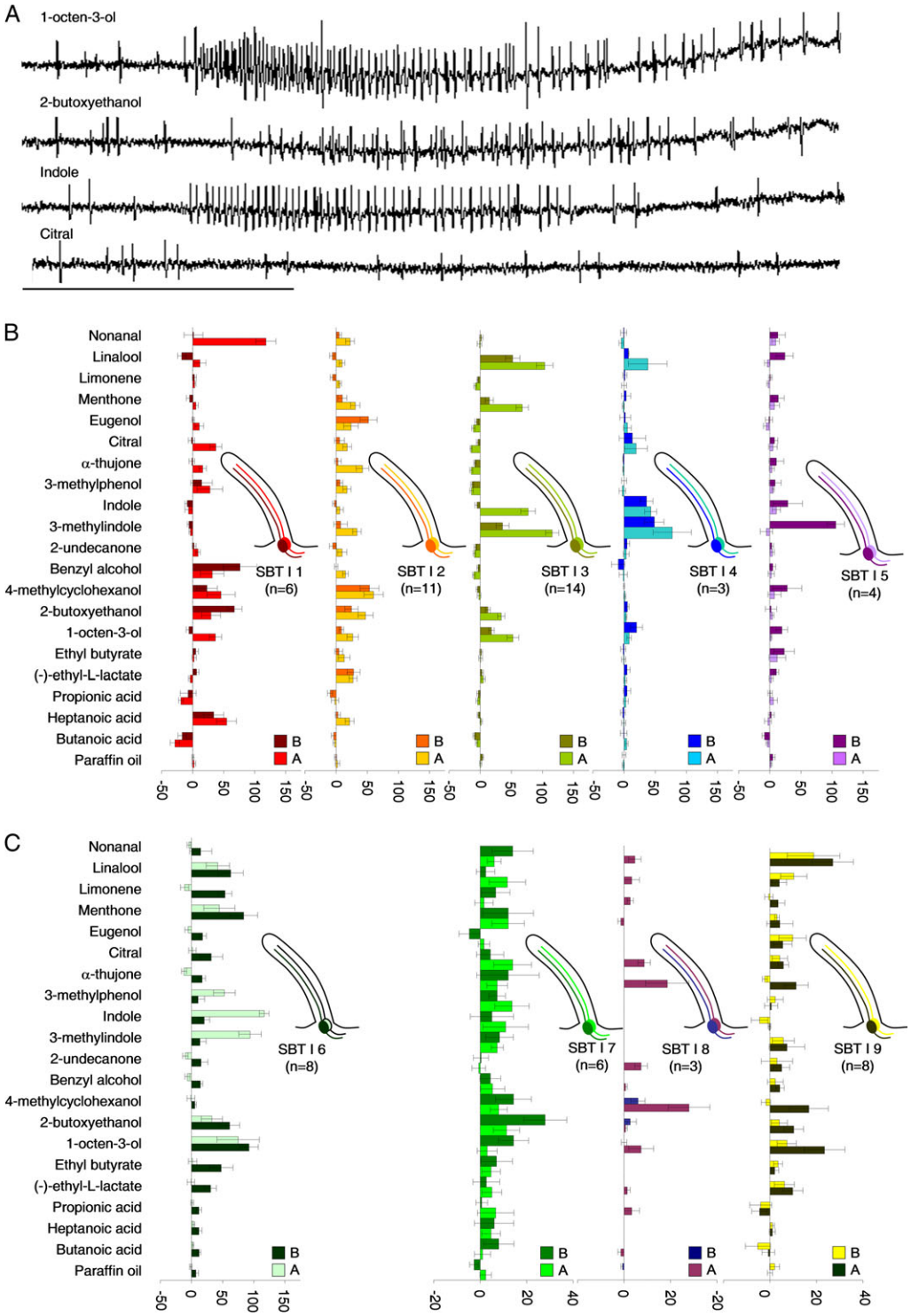


Figure 3 Odor-induced responses from the short blunt-tipped sensillum trichodea type I (SBT I) on the antennae of female *Culex quinquefasciatus*. **(A)** SBT I functional class 3 ORN activity traces in response to a 0.5-s odor stimulation with volatile compounds: 1-octen-3-ol (10^{-1} v/v), 2-butoxyethanol (10^{-1} v/v), indole (10^{-2} v/v), and citral (10^{-1} v/v). Odor-induced response profiles of the 9 functional classes of the of SBT I sensilla, **(B)** 6 exhibiting a high rate of spontaneous activity, and **(C)** 3 exhibiting a low rate of spontaneous activity. The classification is based on a cluster analysis of the ORN responses to the set of 20 odorants presented on the left sides of the histograms (for concentrations, see Table 1). Two spontaneously active neurons are found in all trichoid types (A and B). The neuronal responses of A and B (see color legends) are shown as an average over (*n*) replicates presented. The units for the *x* axis are spikes per second.

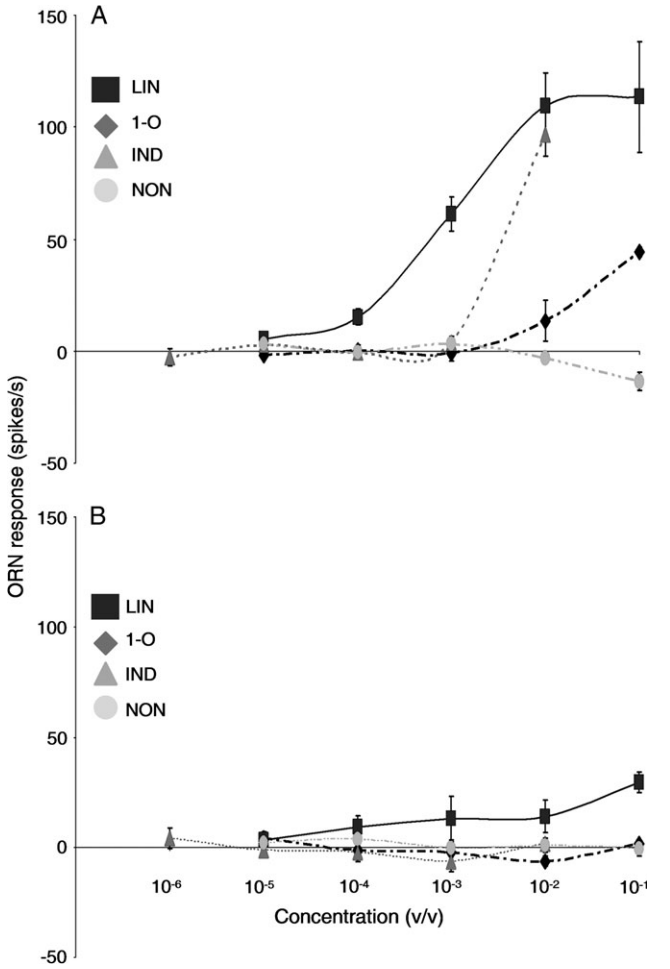


Figure 4 Dose response curves for 5 short blunt-tipped type I sensilla (SBT I 3) to odor stimuli through 6 decadic steps in a dilution series. The *x* axis describes the logarithm dilution series. Each responding ORN is depicted separately: **(A)** A cell and **(B)** B cell. Linalool (LIN, 10^{-5} to 10^{-1} v/v, ■), 1-octen-3-ol (1-O, 10^{-5} to 10^{-1} v/v, ♦), indole (IND, 10^{-6} to 10^{-2} v/v, ▲), and nonanal (NON, 10^{-5} to 10^{-1} v/v, ●) were the odor stimuli used. Error bars represent the standard error of mean.

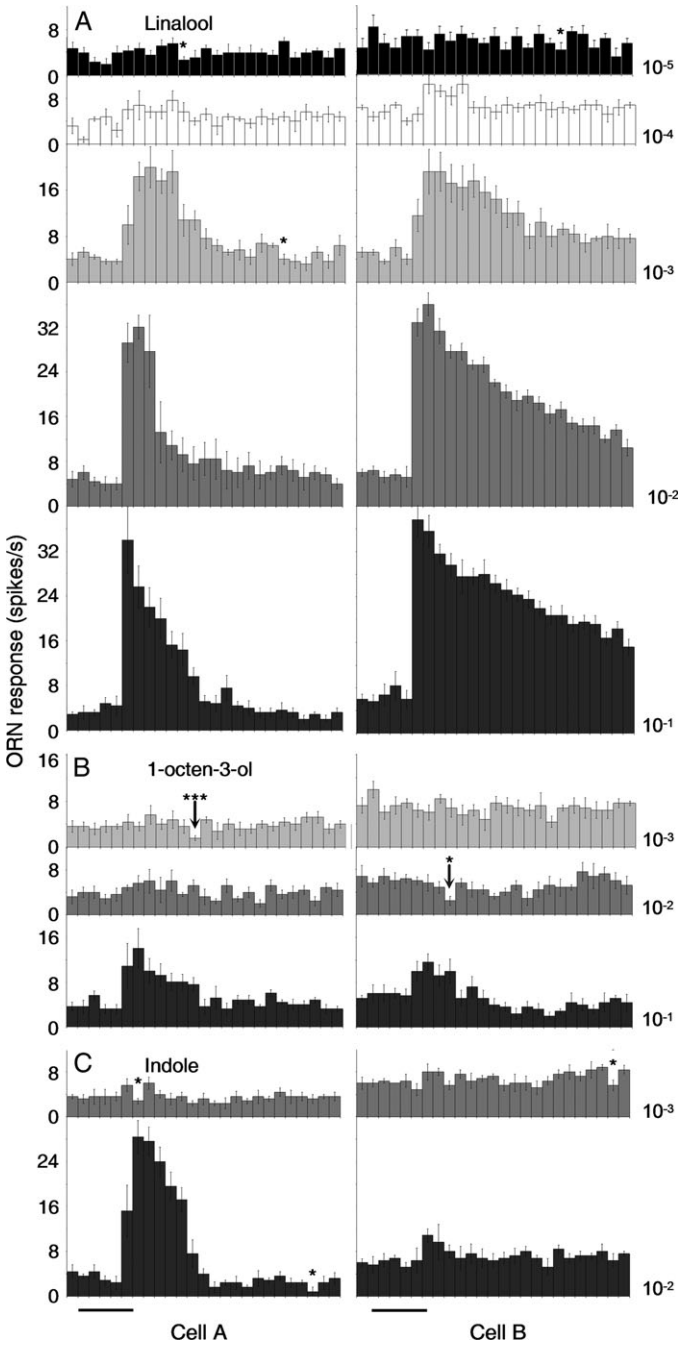


Figure 5 The temporal characteristics of the ORNs in the sensilla trichodea functional type, short blunt-tipped type I 3 (SBT I 3) in response to 3 different volatile stimuli over a range of concentrations (v/v). Each histogram bar represents the number of spikes present within a sampling period of 100 ms. The left column represents the odor-induced responses in the A cell, whereas the right column shows the B cell responses. **(A)** SBT I 3 responds to decadic steps of linalool from $10-^{5}$ to 10^{-1} v/v ($n = 5$). **(B)** These histograms represent the temporal response of SBT I 3 to 1-octen-3-ol over 3 decadic steps, 10^{-3}, 10^{-2}, and 10^{-1} ($n = 5$). The asterisks indicate bins in which a statistically significant inhibition was observed: * $P < 0.5$ and *** $P < 0.0001$. **(C)** SBT I 3 responds in a temporally characteristic manner to indole (10^{-3} and 10^{-2} v/v; $n = 5$). Horizontal bars indicate the duration of stimulation (500 ms). Error bars represent the standard error of mean.

(96.8 ± 9.9 spikes/s at 10^{-2} v/v) in less than 1 order of magnitude (Figure 4A). This response to indole is indicative of an "on/off" type of sensitivity. Nonanal was the only stimulus assayed to which the A cell responded by a reduction in neuronal activity and that only at the highest dose evaluated (10^{-1} v/v; Figure 4A). The only stimulus to which the B cell responded was linalool with a threshold at 10^{-4} v/v (Figure 4B).

The SBT I 3 ORNs also demonstrate the final aspect of peripheral olfactory coding and temporal coding. In these sensilla, the A and B cells respond with different temporal profiles to the same compounds (Figure 5). The response of SBT I 3 to linalool (10^{-1} v/v), when described in bins of 100 ms over a 2-s period, revealed that the A and B cells responded with different temporal characteristics: a predominantly phasic response in the A cell returning to basal activity within 1 s of the onset of response (left column), whereas

the B cell (right column) demonstrated a prolonged phasic–tonic response that lasted longer than 2 s after the onset of response, even at low doses (10^{-3} v/v, $n = 5$, Figure 5A). Both the A and the B cells responded in a phasic manner to 1-octen-3-ol (10^{-1} v/v; Figure 5B). However, there is an interesting and potentially novel aspect of temporal coding described in Figure 5B. In response to an odor concentration low enough not to elicit the phasic response in the ORN (10^{-3} v/v in the A cell and 10^{-2} v/v in the B cell), there appeared to be a transitory inhibition of ORN activity of approximately 100-ms duration. This decrease in the spiking activity occurs at 700 ms and 400 ms after the onset of stimulus response in the A cell and B cell, respectively. In both cells, this phenomenon coincided with the cessation of phasic activity in the ORNs following stimulation with a suprathreshold dose of 1-octen-3-ol (Figure 5B). The SBT I 3 A cell responded to indole in a phasic manner that lasted <1 s at a dose of 10^{-1} v/v. There also seemed to be a slight phasic response in the B cell to indole (10^{-1} v/v), but it was minor and highly transient (Figure 5C).

Type II. Short blunt-tipped type II sensilla made up 25% of the responding sensilla. The most notable features of this morphological class were that all 5 of the functional classes responded to citral, 4 responded to nonanal, 3 responded to indole, and 3 responded to the carboxylic acids (Figure 6B, Table 3).

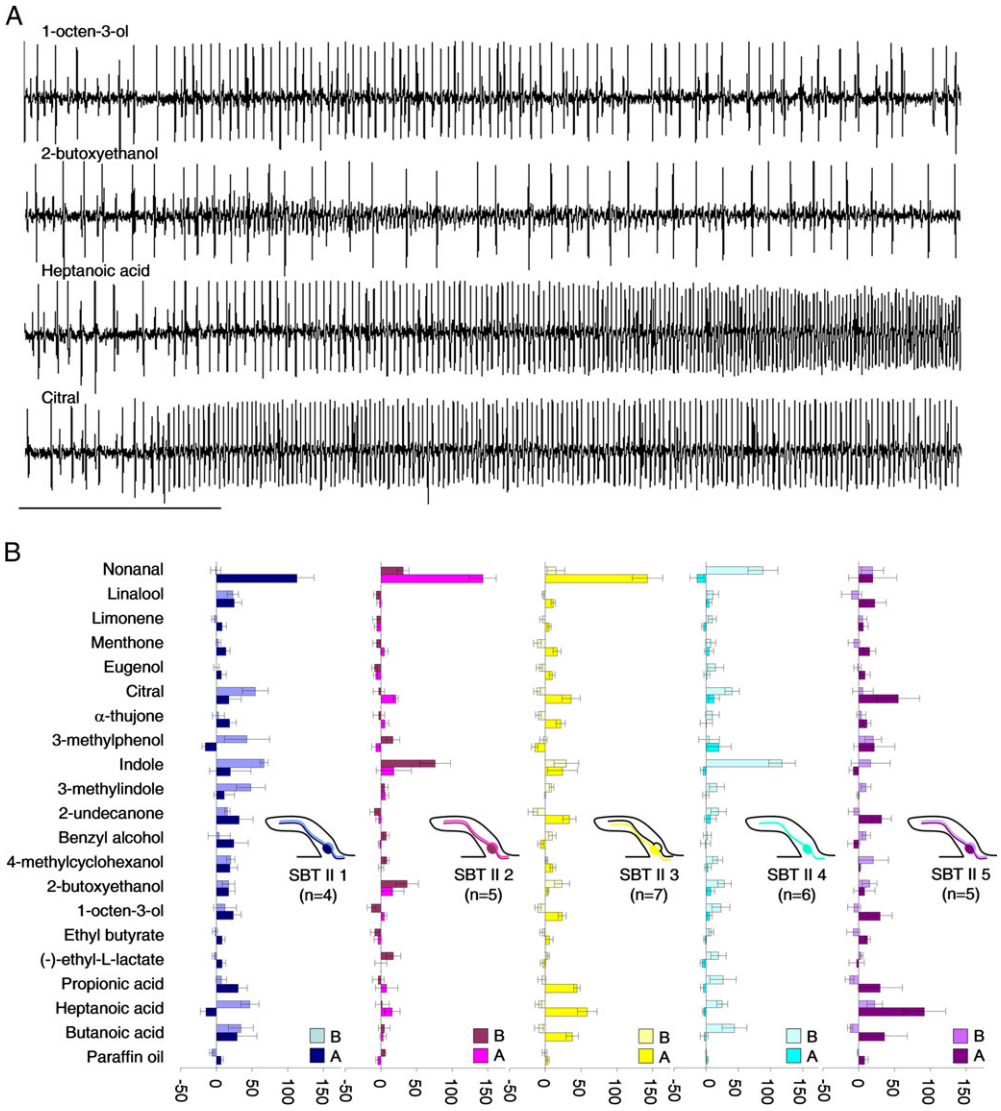


Figure 6 Odor-induced responses from the short blunt-tipped sensillum trichodea type II (SBT II) on the antennae of female *Culex quinquefasciatus*. **(A)** SBT II functional class 3 ORN activity traces in response to a 0.5-s odor stimulation with volatile compounds: 1-octen-3-ol (10^{-1} v/v), 2-butoxyethanol (10^{-1} v/v), heptanoic acid (10^{-1} v/v), and citral (10^{-1} v/v). **(B)** Response profiles of the 5 functional classes of SBT I sensilla. The classification is based on a cluster analysis of the ORN responses to the set of 20 odorants presented on the left sides of the histograms (for concentrations, see Table 1). Two spontaneously active neurons are found in all trichoid types (A and B). The neuronal responses of A and B (see color legends) are shown as an average over (*n*) replicates presented. The units for the *x* axis are spikes per second.

This sensilla type also depicts aspects of classical peripheral coding. The level of spontaneous activity in the SBT II 3 sensillum type (Figure 6A) was moderate for the A cell but remarkably high for the B cell and distinguished this SBT II sensillum functional type from the other SBT II functional types with similar basal rates of firing for the A cell but much lower rates for the B cell (data not shown). The SBT II 3 sensillum type revealed that even compounds with the same chemical moiety (e.g., alcohols) can elicit striking differences in response in the same sensillum clearly demonstrating ligand specificity (Figure 6A). This sensillum type has the ability to distinguish between alcohols in a highly specific manner: 1-octen-3-ol excited only the A cell, 2-butoxyethanol only excited the B cell (Figure 6A), whereas neither 4-methylcyclohexanol and benzyl alcohol nor ethylene glycol elicited a significant change in activity pattern (Figure 6B, Table 3). In temporal characteristics, the SBT II 3 functional type is distinguished among the other SBT II functional classes. Its extended tonic excitatory response to short-chained carboxylic acids (C_4–C_8), as demonstrated here with heptanoic acid (Figure 6A), is unique; the peak frequency of the A cell response occurred ~1 s after the onset of the stimulus and the tonic response lasted for 3–5 s; whereas the A cell response to citral was a relatively high frequency tonic increase from the onset of stimulation, in which an initial phasic period was lacking (Figure 6A).

Short sharp pointed-tipped sensilla trichodea

Straight and curved. As explained above, the function of the straight and curved SST sensilla are described together because light microscopy is not sensitive enough to permit consistent discrimination between these 2 morphological types. All 3 of the SST sensilla functional classes (16.7% of total responding sensilla) responded to the alcohols, 2-butoxyethanol, and 4-methylcyclohexanol (Figure 7B). Whereas we previously have described sensilla which differentiated between the alcohols, 1-octen-3-ol and 2-butoxyethanol, these elicited activity in both the A and B neurons of the SST 3 sensillum in a similar phasic–tonic manner (Figure 7A). The ketone, 2-undecanone, elicited a tonic excitation of the B neuron, whereas the A neuron increased its firing frequency in a weak tonic response 150 ms following the cessation of stimulation (Figure 7A). The SST 3 can be functionally classified as the only trichoid sensillum class in which the B cell is more sensitive to 2-undecanone than the A cell and in which the A cell responds maximally to the ester, (–)-ethyl lactic acid, with a phasic excitatory response (Figure 7A). The response to nonanal is diagnostic in this sensillum as it was the only SST sensillum to respond to this aldehyde. This response was excitatory in both neurons with the onset of phasic–tonic firing in the B cell preceding that of the A cell by ~100 ms (Figure 7A).

Long sharp pointed-tipped sensilla trichodea

The LST type of sensilla remains unclassified as we have found no compound to stimulate a change in firing activity for these sensilla among the 44 volatiles tested. As of yet, no ligand has been identified for any of the LST sensilla in mosquitoes (Qiu, van Loon, et al. 2006; Ghaninia, Ignell, and Hansson 2007). As this sensilla type represents 58% of the total sensilla trichodea present on the antenna, we have expanded our pool of test volatiles, including synthetic compounds and natural extracts, and are continuing to screen this sensillum class for functional types.

Discussion

Using scanning electron microscopy, we provide the first morphological description of the antennal sensillum repertoire of female *C. quinquefasciatus* with special emphasis on sensilla trichodea. Sensilla trichodea are divided into 5 morphological subtypes, and we have differentiated 17 functional types through systematic SSRs based on their response to a panel of behaviorally and ecologically relevant odorants. The described combinatorial coding regime found among the sensilla trichodea suggests that *C. quinquefasciatus* females use across-fiber pattern recognition to discriminate among the complex odor blends signifying potential blood hosts, nectar hosts, and oviposition sites.

Morphological classification of antenna sensilla

The overall morphological organization of the mosquito antenna appears to be conserved: Each antenna is subdivided into 13 flagella (McIver and Charlton 1970; McIver 1982) and the flagella are covered in sensilla (Figure 1), sensory organs responsive to mechanical, thermal, hygro-, and chemical stimuli. The types of sensilla present in culicine mosquitoes are stereotyped as either nonolfactory sensilla, that is, long and short sensilla chaetica, sensilla ampullacea, and a pair of sensilla coeloconica at the distal tip, or olfactory sensilla, that is, sharp and blunt sensilla trichodea and long and short grooved pegs (Ismail 1964; McIver and Charlton 1970; McIver and Hutchinson 1972; McIver 1973, 1978, 1982; Boo and McIver 1975; Pitts and Zwiebel 2006).

Mechanoreceptive sensilla chaetica are long, stiff, ridged hair–like structures without wall pores that are set into a socket at the base and radially distributed in whorls at the flagellar margins: 6 long sensilla chaetica along proximal margins (F2–F13) and 0–8 short sensilla chaetica along the distal margins, decreasing in number from antenna base to tip (F1–F12) (Figure 1A, Table 2, McIver 1982). Sensilla ampullacea in *C. quinquefasciatus* appear on the first and second flagella (Figure 1A; Table 2) as observed in many dipterous insects, including both culicine and anophelines mosquitoes (Boo and McIver 1975; Pitts et al. 2004), biting midges (Felippe and Bauer 1990; Cribb 1996), and blowflies (Fischer 2000). The overall structure of sensilla ampullacea suggests a thermoreceptive function (Ismail 1962; Davis and Sokolove 1976 similar to the pair of sensilla

coeloconica located at the tip of the antenna in all mosquitoes studied (Figure 1A; McIver and Hutchinson 1972; McIver 1973; Boo and McIver 1975).

Culex quinquefasciatus females have the most olfactory sensilla of all mosquitoes previously studied (Table 4). A breakdown of the numbers of each olfactory sensillum type in *C. quinquefasciatus* reveals high numbers of sensilla trichodea, whereas the numbers of grooved pegs are below the average for the *Culex* spp. (Table 4). The grooved pegs we describe for *C. quinquefasciatus* are similar in both shape and size to those described for other culicine mosquitoes (McIver and Charlton 1970; Bowen 1995). Grooved pegs have been shown to be sensitive to those volatiles which are termed "ubiquitous vertebrate host odors," for example, short-chain carboxylic acids, in at least 4 culicine species, including *C. pipiens* (Davis 1988; Bowen 1995), suggesting that grooved pegs may not be of paramount importance in the discrimination between vertebrate blood hosts.

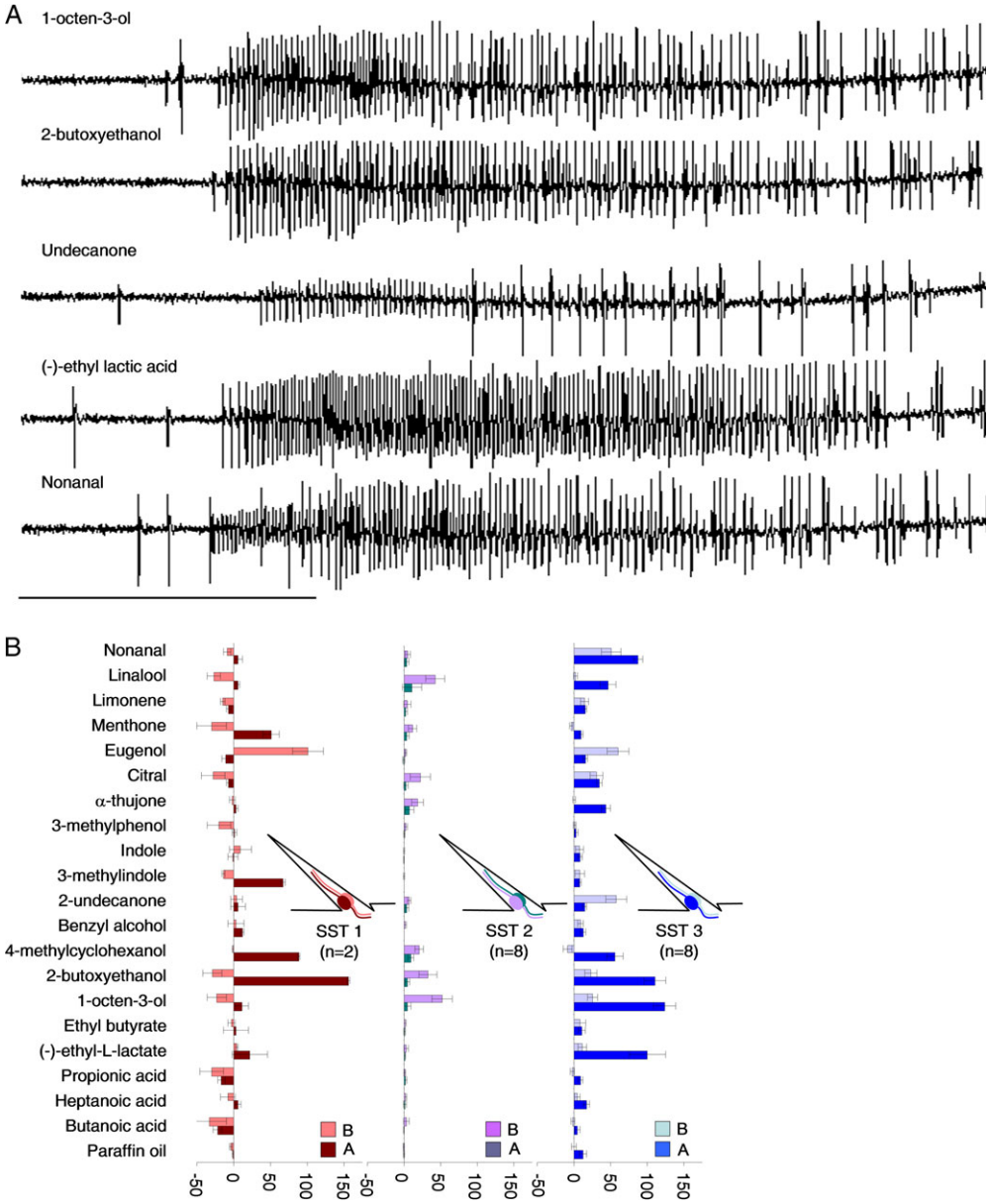


Figure 7 Odor-induced responses from the short sharp-tipped sensillum trichodea (SST) on the antennae of female *Culex quinquefasciatus*. **(A)** SST functional class 3 ORN activity traces in response to a 0.5-s odor stimulation with volatile compounds: 1-octen-3-ol (10^{-1} v/v), 2-butoxyethanol (10^{-1} v/v), 2-undecanone (10^{-1} v/v), (−)-ethyl lactic acid (10^{-1} v/v), and nonanal (10^{-1} v/v). **(B)** Response profiles of the 3 functional classes of SST sensilla. The classification is based on a cluster analysis of the ORN responses to the set of 20 odorants presented on the left sides of the histograms (for concentrations, see Table 1). Two spontaneously active neurons are found in all trichoid types (A and B). The neuronal responses of A and B (see color legends) are shown as an average over (*n*) replicates presented. The units for the *x* axis are spikes per second.

Table 4 Summary of mosquito antennal olfactory sensilla

Species	Olfactory sensilla Total	Trichoids			Grooved pegs	References
		Total	Long	Short		
Culex quinquefasciatus	1340	1124 ± 153	656 ± 84	442 ± 62	234 ± 14	Table 2
Culex pipiens	1300	901	424	480	265	McIver (1970, 1982)
Culex fatigans	1203	970	479	500	233	McIver (1970, 1982)
Culex restuans	1300	902	385	495	348	McIver (1970, 1982)
Culex tarsalis	1025	605	355	250	272	McIver (1970, 1982)
Culex territans	960	537	261	252	275	McIver (1970, 1982)
Aedes aegypti	913	808	245	537	105	Ismail (1964); McIver (1978)
Anopheles gambiae s.s.	709	630 ± 11	np	630 ± 11	79 ± 3.5	Pitts and Zwiebel (2006)
Anopheles quadriannulatus	906	792 ± 24	np	792 ± 24	114 ± 4.8	Pitts and Zwiebel (2006)

np, not present. s.s., sensu stricto.

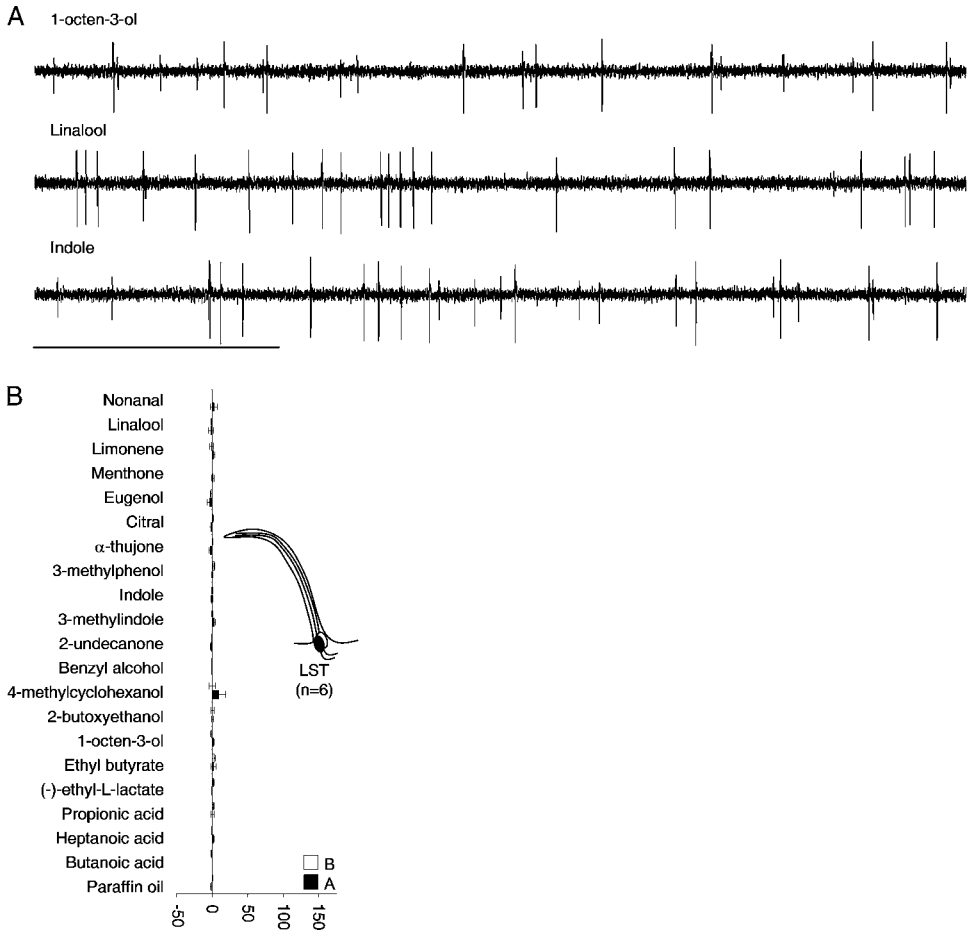


Figure 8 Odor-induced responses from the LST sensillum trichodea on the antennae of female *Culex quinquefasciatus*. **(A)** LST ORN activity traces in response to a 0.5-s odor stimulation with volatile compounds: 1-octen-3-ol (10^{-1} v/v), linalool (10^{-1} v/v), and indole (10^{-2} v/v). **(B)** Response profile of the unclassified of LST sensillum. Two spontaneously active neurons are found in all LSTs assayed (A and B). The neuronal responses of A and B (see color legends) are shown as an average over (*n*) replicates presented. The units for the *x* axis are spikes per second.

The large number of sensilla trichodea present in *C. quinquefasciatus* compared with other *Culex* spp. correlates to its ornithophilic behavior which lead us to the main focus of this study: the morphological and functional characterization of sensilla trichodea in female *C. quinquefasciatus*. Previous studies of sensilla trichodea have been conducted on primarily mammal-feeding mosquitoes, *A. gambiae* (Qiu, van Loon, et al. 2006) and *A. aegypti* (Ghaninia, Ignell, and Hansson 2007). Our study investigates the sensilla predicted to produce the peripheral code to permit the discrimination among vertebrates in a mosquito species that must differentiate between humans and birds and which is known to change its host preference from one to the other in response to environmental cues (Molaei et al. 2007). The morphological classes of sensilla trichodea appear to be conserved among the culicine mosquitoes, with the exception that there is an additional sensilla trichodea subtype found in *C. quinquefasciatus*: SST-C). The long sensilla in *C. quinquefasciatus* appear to be the shortest LSTs compared with the other *Culex* spp. studied to date (Table 2; McIver 1970) and yet the most numerous (Table 4; McIver 1970). The short sensilla, including all 3 short morphological subtypes of sensilla trichodea, range in length in *Culex* spp. from the shortest in *C. quinquefasciatus* to the longest in *C. restuans* (McIver 1970).

It is its ornithophilic/anthropophilic behavior that makes *C. quinquefasciatus* such an effective vector of the West Nile virus (Molaei et al. 2007). The antennal sensilla complement seemingly reflects this selective vertebrate host preference with an increased number of sensilla trichodea, suggested by McIver (1970) to correlate with the ornithophilic behavior (McIver 1970); therefore, it is not a leap to suggest that these classes of morphological sensilla translate into functional olfactory subgroups, denoted by olfactory preference. This is not a new theory: It has been proposed not only for the differences in host preference between *A. gambiae* and *A. aegypti* (Qiu, van Loon, et al. 2006; Ghaninia, Hansson, and Ignell 2007, Ghaninia, Ignell, and Hansson 2007 but also amongst the sympatric *Anopheles* spp., the exclusively zoophilic *Anopheles quadriannulatus* and the severely anthropophilic *A. gambiae* (Pitts and Zwiebel 2006). Although sharing the same types of sensilla on the antennae, *A. quadriannulatus* has approximately one-third more total number of sensilla than *A. gambiae* (Pitts and Zwiebel 2006). The density of sensilla, however, does not appear to differ between these species as *A. quadriannulatus* creates a larger surface area on its antennae compared with *A. gambiae*. This leads us directly to one of the most contentious issues currently in mosquito olfaction, indeed in insect olfaction: whether it is the ratios of the peripheral signals as processed by the antennal lobe (AL), or the absolute magnitude of the peripheral signal that plays the majority role in determining olfactory sensitivity to complex odor blends (e.g., Kazama and Wilson 2008). To address such issues, morphological studies can be indicative, but they are simply insufficient. It appears that morphological descriptions may reflect vector-related behaviors, but sensilla functional class determination is essential if one intends to decode the peripheral olfactory signaling and link it to vector-related behaviors, such as host choice.

Response profiles

Response profiles generated for a population of sensilla using the extracellular electrophysiological technique, SSR, create a means by which a sensillum and by extension the ORNs contained within that sensillum, may be functionally identified. In biological systems, identifiable neurons give us the ability to link peripheral neuronal responses to higher level central nervous system (CNS) activity and ultimately to CNS outputs in the form of fixed pattern behaviors. In the case of mosquitoes, the chief behaviors of interest are those directly related to vectorial capacity (e.g., host preference, host seeking, blood feeding, and oviposition behaviors) and olfaction plays a major role in each of these behaviors. It is the olfactory sensilla housing the ORNs that represent the first interface between the behavior-inducing odor blends and the mosquito.

In assembling SSR response profiles, it is essential to use a behaviorally relevant panel of odors as stimulants because panels composed of behaviorally inert or extraneous compounds do not inform future experiments designed to link a mosquito's behavior to its odor landscape. In the case of *C. quinquefasciatus*, the odor panel (and dosage used) was chosen based on previous behavioral and electrophysiological studies, primarily in *C. quinquefasciatus* but also including some compounds determined to be bioactive in the other mosquito species for which functional maps of antenna sensilla trichodea have been generated: *A. aegypti* and *A. gambiae* (Qiu, van Loon, et al. 2006; Ghaninia, Ignell, and Hansson 2007). The odor panel was constructed to include odors related to each of the main mosquito vector–related seeking behaviors: blood, nectar, and oviposition site seeking.

One area that has been largely neglected in *C. quinquefasciatus* host-seeking behavior is the search for energy flight and metabolic maintenance in the form of nectar and honeydew. In 1992, Bowen reported plant volatile-sensitive sensilla (SBT I) in *C. pipiens*: 34 compounds were assayed including representatives from terpenes, green plant volatiles, fatty acid esters, alcohols, heterocyclics, short chain carboxylic acids, and ketones. Dividing SBT I sensilla into 2 functional groups based on the level of spontaneous activity, Bowen (1992) was able to conclude that those SBT I with a low spontaneous activity responded exclusively to bicyclic monoterpenes and 4-methylcyclohexanol, whereas those with high spontaneous activity were more broadly tuned to all groups tested (Bowen 1992). Our results bear out Bowen's (1992) observations as the functional subtype SBT I 8 has a low spontaneous activity and responds chiefly to α-thujone and 4-methylcyclohexanol; whereas the other 4 terpene-responding sensilla functional subtypes (SBT I 1, 2, 3, and 5) demonstrate high spontaneous activity and broad response profiles (Figure 3).

Functional classification of antennal *sensilla trichodea*

The 5 morphological sensilla trichodea subtypes from female *C. quinquefasciatus* are resolved into 17 functional subtypes, denoting 34 ORN subtypes, in young sugar-fed, host-seeking adult females (3–5 days postemergence; Figures 3 and 6–8, Table 3). *Anopheles gambiae* has at least 6 functional types of sensilla trichodea also determined by using a panel of 44 volatile compounds (Qiu, van Loon, et al. 2006), whereas *A. aegypti* has at least 11 functional types of sensilla trichodea described using 16 compounds (Ghaninia, Ignell, and Hansson 2007). The 16 compounds used for *A. aegypti* functional classification of sensilla trichodea were included in the odor panel of *C. quinquefasciatus* in order that a direct comparison among the functional classes between species could be attempted. There are 3 functional classes of ORNs with similar compound sensitivities (SST 3B with sbtII2B, SBT II 4B with sst2B, and SBT II 4B with sst1B); there are 2 that are close (SST 2B and SBT I 6A with sst1A) and 3/4 (LST AB and SST 2A with lstAB, sbtII1B and sst2A) nonresponding ORNs in *C. quinquefasciatus* and *A. aegypti*, respectively. Therefore, some of the ligand affinities for the odorant receptors (ORs) in both species may be conserved, that is, responding to the same volatile stimuli in a similar manner. These ORs, however, do not appear to be constrained to a single sensillum morphological type.

Of the 44 compounds identified as pertinent to *C. quinquefasciatus*, 19 were directly comparable with those used for *A. gambiae* by Qiu, van Loon, et al. (2006). In order to make such comparisons between our current study and previous mosquito SSR characterization studies, we have used similar dilutions of the volatile compounds (Table 1). The compounds in common include phenols, indoles, alcohols, carboxylic acids, ketones, esters, and sulfides (Table 1; Qiu, van Loon, et al. 2006). There are no sensilla trichodea response profiles completely in common between *C. quinquefasciatus* and *A. gambiae* based on these 19 compounds; however, some similarities appear to exist between these 2 distantly related mosquito species. For example, in *A. gambiae*, ORNs that respond to carboxylic acids invariably respond to phenolic compounds, whereas the same is true for 9 of 13 carboxylic acid-responding ORNs in *C. quinquefasciatus*. In both species, only 1 ORN responds to 3-methylindole by inhibition. The other bioactive volatiles in these response profiles do not, however, overlap. Finally, there are 2 ORNs, pTE1A in *A. gambiae* and SBT II 1B in *C. quinquefasciatus*, which respond to all phenols, both indoles, short-chain carboxylic acids, alcohols excluding 1-octen-3-ol, ketones (either 6-methyl-5-hepten-2-one or 2-undecanone), and no other tested compounds.

From comparisons made between the ORs of the culicine mosquito *A. aegypti* (131) and the anopheline mosquito *A. gambiae* (79), there is minimal conservation in chromosomal arrangement of genes and gene sequence (Bohbot et al. 2007). Twelve chromosome regions were identified as showing significant microsynteny between these 2 species; yet, the gene sequence similarities between these 17 paired ORs share less than 72% identical amino acids and most ORs share less than 20% (Bohbot et al. 2007). It is not surprising therefore that the culicines, *C. quinquefasciatus* and *A. aegypti*, produce ORNs with different response profiles compared with the anopheline, *A. gambiae*. It is more surprising, however, that the response profiles of the culicine mosquitoes do not more significantly overlap. Sequence comparisons between the ORs in *Drosophila melanogaster*, *A. gambiae*, *A. aegypti*, and *C. quinquefasciatus* reveal few conserved or gene lineages at the level of Diptera, most dipteran ORs share less than 20% identity at the amino acid level. Comparisons between the 79 *A. gambiae* and the 131 *A. aegypti* ORs reveal only 18 orthologous subgroups (Bohbot et al. 2007). Ongoing comparisons between *A. aegypti* and *C. quinquefasciatus* reveal that there are culicine-specific OR subgroups; however, there appear to be many more ORs unique to each species than those that are conserved (Hill SR, Hansson BS, and Ignell R, in preparation).

The lack of correlation in functional groups may indicate the necessity to increase the odor panel for *A. aegypti* to include the other compounds studied here. Indeed, for those ORNs of both culicine mosquito species lacking functional classification (3 in *C. quinquefasciatus* and 4 in *A. aegypti*), a larger odor panel is required, as is repeating the current studies with mosquitoes under various physiological states, such as recently blood fed, oviposition site seeking, and, for *C. quinquefasciatus*, during diapause. In fact, a few studies have addressed changes in behavior, gene expression, and ORN sensitivity to odors during various physiological states (Bowen 1988, 1991; Robich and Denlinger 2005; Xia and Zwiebel 2006; Robich et al. 2007). During diapause, the percentage of sensilla trichodea ORNs responding to ethyl propionate or 2-butoxyethanol was 35% lower than nondiapausing females, whereas the percentage of sensilla narrowly tuned to these compounds does not change postdiapause in *Culex pipiens pipiens* (Bowen 1988, 1991), indicating that other functional subtypes increase sensitivities following diapause. The changes in gene expression in response to diapause reported in *C. p. pipiens* (Robich and Denlinger 2005) underlines the ability of these females to modulate gene expression to both induce changes in physiological state and to respond to state changes.

Assuming the one ORN-one glomerulus doctrine (Boeckh et al. 1970; Rospars 1988; Hansson 1995; Couto et al. 2005; Kreher et al. 2005; Ghaninia, Ignell, and Hansson 2007) as an hypothesis, our characterization of 34 functional subtypes of sensilla trichodea ORNs plus the 2 unclassified LST ORNs accords well with expected number of ORN types with projections to the AL in *C. quinquefasciatus*. The number of *C. quinquefasciatus* glomeruli appears to be 50 (Ignell R, personal observation), which is similar to the overall numbers of glomeruli found in other mosquitoes (Ignell

et al. 2005; Ghaninia, Hansson, and Ignell 2007). Syed and Leal (2007) reported 1 morphological and functional type of sensillum on the maxillary palps of female *C. quinquefasciatus*, which agrees with previous studies of maxillary palp sensilla in *A. aegypti* (Grant et al. 1995) and *A. gambiae* (Lu et al. 2007). These capitate peg sensilla are innervated by 3 ORNs with differing odor response profiles, thus reducing the population of 50 glomeruli in the AL available for innervation from the antennal sensilla trichodea to 47. Grooved pegs on the antennae of other mosquito species have been shown to contain 2–5 ORNs (Elizarov and Chaika 1972; Boo and McIver 1976; McIver 1978, 1982; Bowen 1995) for which 2 functional subtypes have been identified in 3 *Aedes* and 1 *Culex* species so far (Bowen 1995) reducing the possible trichoid innervated glomeruli to between 37 and 43. Each trichoid sensillum from which we recorded contained 2 spontaneously active ORNs suggesting that these trichoid ORNs innervate 34 glomeruli, without including the 2 spontaneously active ORNs in LST sensilla. Of course, silent sensilla trichodea ORNs cannot be ruled out because there is currently no transmission electron microscopic analysis of the sensilla trichodea in *C. quinquefasciatus*; however, transmission electron microscopic analyses in other mosquitoes reveal that sensilla trichodea in these species contain the dendrites of only 2 neurons (for review, see McIver 1982). There is support for this neuronal architecture in other mosquitoes: for example, in *A. aegypti*, 14 functionally classified ORNs from 7 of the 11 functional types of sensilla trichodea were stained using neurobiotin anterograde–filling techniques, and it was discovered that each ORN innervated a single, distinct, and identifiable glomerulus (Ghaninia, Ignell, and Hansson 2007).

One odor often activates more than 1 ORN (Hallem and Carlson 2004): for example, 4 of the 5 SBT II sensilla trichodea contain ORNs with sensitivity to nonanal in *C. quinquefasciatus* (Figure 6). The majority of these ORNs are paired within the sensilla with ORNs displaying different odor specificities so that the insect may more accurately measure the ratio between, for example, nonanal and other ligands, which might contain information concerning the blend progenitor (Barata et al. 2002; Qiu, van Loon, et al. 2006). Increasing the complexity of the odor coding, a single ORN may be sensitive to more than 1 volatile compound. In fact, in mosquitoes, this appears to be the more prevalent type of sensilla trichodea*a* ORN (Figures 3–8; Qiu, van Loon, et al. 2006; Ghaninia, Ignell, and Hansson 2007). Both the intensity and the timing of the neuronal response may play a role in identifying the volatile compound within the CNS (Kazama and Wilson 2008). Because the same odor can elicit neuronal responses with different temporal characteristics (Figure 5), it is proposed that phasic activity encodes the rapid changes in odor concentration, whereas a tonic response is a transient form of short-term memory encoding the corpuscular nature of an odor plume during tracking (Takken 1996; De Bruyne et al. 2001; Cooperband and Carde 2006). In the present study, we report a novel phenomenon in temporal characteristics: an inhibition, ≤100 ms in duration, found during subthreshold stimulus that corresponds to the cessation of the phasic response during a suprathreshold stimulus (Figure 5). The short duration inhibition corresponding to the termination of phasic response, revealed at subthreshold stimulations, appears to add to the complexity of odor coding. Higher brain centers have the opportunity to compare among the glomeruli, not only presence or absence of activity but also the intensity and timing of the activity in order to assess the quality of the odor blend stimulus. This form of odor coding is termed across-fiber patterning (Christensen and White 2000) and appears to be an essential component in complex odor discrimination leading to vector-related behaviors.

Conclusions

The sensilla trichodea of young sugar-fed female *C. quinquefasciatus* in a physiological state conducive to host seeking are divided into 5 morphological subtypes and 17 functional groups. Each functional group displays a distinct odor response profile. Thirty-three of the 34 ORN subtypes identified responded to a unique range of volatile compounds. That a particular compound stimulates a response in many functional types suggests the appearance of a combinatorial coding regime among the sensilla trichodea. This in turn suggests that *C. quinquefasciatus* females use this across-fiber pattern recognition to discriminate among the complex odor blends signifying potential blood hosts, nectar hosts, and oviposition sites. Our future studies are focusing on functionally characterizing these sensilla under a variety of physiological states, including gravid and diapausing, and characterizing the ORs for *C. quinquefasciatus* so as to couple volatile ligands to OR and OR to ORN, thereby completing the peripheral functional mapping of *C. quinquefasciatus*. A principal aim of these ongoing studies is to identify potential attractants and repellents for *C. quinquefasciatus* to add to the current methods of ecologically safe control methods.

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References

Allan SA, Bernier UR, Kline DL. 2005. Evaluation of oviposition substrates and organic infusions on collection of Culex in Florida. J Am Mosq Control Assoc. 21:268–273.

Allan SA, Bernier UR, Kline DL. 2006a. Attraction of mosquitoes to volatiles associated with blood. J Vector Ecol. 31:71–78.

Allan SA, Bernier UR, Kline DL. 2006b. Laboratory evaluation of avian odors for mosquito (Diptera: Culicidae) attraction. J Med Entomol. 43:225–231.

Barata N, Mustaparta H, Pickett JA, Wadhams LJ, Araujo J. 2002. Encoding of host and non-host plant odours by receptor neurones in the eucalyptus woodborer, Phoracantha semipunctata (Coleoptera: Cerambycidae). J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 188:121–133.

Bentley MD, Day JF. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annu Rev Entomol. 34:401–421.

Blackwell A, Mordue Luntz AJ, Hansson BS, Wadhams LJ, Pickett JA. 1993. A behavioural and electrophysiological study of oviposition cues for Culex quinquefasciatus. Physiol Entomol. 18:343–348.

Boeckh J, Sandri C, Akert K. 1970. [Sensory inputs and synaptic connections in the insect CNS. Experimental degeneration in the antennal afferent pathway in the supraesophageal ganglia of flies and cockroaches]. Z Zellforsch Mikrosk Anat. 103:429–446.

Bohbot J, Pitts RJ, Kwon HW, Rutzler M, Robertson HM, Zwiebel LJ. 2007. Molecular characterization of the Aedes aegypti odorant receptor gene family. Insect Mol Biol. 16:525–537.

Boo KS, McIver SB. 1975. Fine structure of sunken thick-walled pegs (sensilla ampullacea and coeloconica) on the antennae of mosquitoes. Can J Zool. 53:262–266.

Boo KS, McIver SB. 1976. Fine structure of surface and sunken grooved pegs on the antenna of female Anopheles stephensi (Diptera: Culicidae). Can J Zool. 54:235–244.

Bowen MF. 1988. A behavioural and sensory analysis of host-seeking behaviour in the diapausing mosquito Culex pipiens. J Insect Physiol. 34:805–813.

Bowen MF. 1991. The sensory physiology of host-seeking behavior in mosquitoes. Annu Rev Entomol. 36:139–158.

Bowen MF. 1992. Patterns of sugar feeding in diapausing and non-diapausing Culex pipiens (Diptera: Culicidae) females. J Med Entomol. 29:843–849.

Bowen MF. 1995. Sensilla basiconica (grooved pegs) on the antennae of female mosquitoes: electrophysiology and morphology. Entomol Exp Appl. 77:233–238.

Burkett-Cadena ND, Mullen GR. 2007. Field comparison of Bermuda-hay infusion to infusions of emergent aquatic vegetation for collecting female mosquitoes. J Am Mosq Control Assoc. 23:117–123.

Chavasse DC, Lines JD, Ichimori K, Marijani J. 1995. Mosquito control in Dar es Salaam. I. Assessment of Culex quinquefasciatus breeding sites prior to intervention. Med Vet Entomol. 9:141–146.

Christensen TA, White J. 2000. Representation of olfactory information in the brain. In: Finger TE, Silver WL, Restrepo D, editors. The neurobiology of taste and smell. New York: Wiley-Liss. p. 201–232.

Cooperband MF, Carde RT. 2006. Orientation of Culex mosquitoes to carbon dioxide-baited traps: flight manoeuvres and trapping efficiency. Med Vet Entomol. 20:11–26.

Couto A, Alenius M, Dickson BJ. 2005. Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr Biol. 15:1535–1547.

Cribb BW. 1996. Antennal sensilla of the female biting midge: Forcipomyia (Lasiohelea) townsvillensis (Taylor) (Diptera: Ceratopogonidae). Int J Insect Morphol Embryol. 25:405–425.

Curran AM, Rabin SI, Prada PA, Furton KG. 2005. Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. J Chem Ecol. 31:1607–1619.

Davis EE. 1988. Structure-response relationship of the lactic acid-excited neurones in the antennal grooved-peg sensilla of the mosquito Aedes aegypti. J Insect Physiol. 34:443–449.

Davis EE, Bowen MF. 1994. Sensory physiological basis for attraction in mosquitoes. J Am Mosq Control Assoc. 10:316–325.

Davis EE, Sokolove PG. 1975. Temperature responses of antennal receptors of mosquito, Aedes aegypti. J Comp Physiol. 96:223–236.

Davis EE, Sokolove PG. 1976. Lactic acid-sensitive receptors on the antennae of the mosquito, Aedes aegypti. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 105:43–54.

De Bruyne M, Foster K, Carlson JR. 2001. Odor coding in the Drosophila antenna. Neuron. 30:537–552.

Deane LM, Damasceno RG. 1952. [Filaria bancrofti in Belem, Para, according to the 1951 investigation.]. Rev Bras Malariol Doencas Trop. 4:333–346.

Dennett JA, Bala A, Wuithiranyagool T, Randle Y, Sargent CB, Guzman H, Siirin M, Hassan HK, Reyna-Nava M, Unnasch TR, et al. 2007. Associations between two mosquito populations and West Nile virus in Harris County, Texas, 2003–06. J Am Mosq Control Assoc. 23:264–275.

Du YJ, Millar JG. 1999. Electroantennogram and oviposition bioassay responses of Culex quinquefasciatus and Culex tarsalis (Diptera: Culicidae) to chemicals in odors from Bermuda grass infusions. J Med Entomol. 36:158–166.

Elizarov YA, Chaika SY. 1972. Ultrastructure of olfactory sensillae on antennae and palps of the mosquitoes, Culex pipiens molestus (Diptera, Culicidae). Zool Zhur. 51:1665–1675.

Elizondo-Quiroga A, Flores-Suarez A, Elizondo-Quiroga D, Ponce-Garcia G, Blitvich BJ, Contreras-Cordero JF, Gonzalez-Rojas JI, Mercado-Hernandez R, Beaty BJ, Fernandez-Salas I. 2006. Host-feeding preference of Culex quinquefasciatus in Monterrey, northeastern Mexico. J Am Mosq Control Assoc. 22:654–661.

Felippe ML, Bauer PG. 1990. Sensilla ampullacea on the antennae of culicoides paraensis (Goeldi, 1905) with notes on other Culicoides (Diptera: Ceratopogonidae). Mem Inst Oswaldo Cruz. 85:235–237.

Fischer OA. 2000. Blowflies of the genera Calliphora, Lucilia and Protophormia (Diptera, Calliphoridae) in South-Moravian urban and rural areas with respect to Lucillia bufonivora Moniez, 1876. Acta Vet Brno. 69:225–231.

Fox AN, Pitts RJ, Zwiebel LJ. 2002. A cluster of candidate odorant receptors from the malaria vector mosquito, Anopheles gambiae. Chem Senses. 27:453–459.

Ghaninia M, Hansson BS, Ignell R. 2007. The antennal lobe of the African malaria mosquito, Anopheles gambiae—innervation and three-dimensional reconstruction. Arthropod Struct Dev. 36:23–39.

Ghaninia M, Ignell R, Hansson BS. 2007. Functional classification and central nervous projections of olfactory receptor neurons housed in antennal trichoid sensilla of female yellow fever mosquitoes, Aedes aegypti. Eur J Neurosci. 26:1611–1623.

Ghaninia M, Larsson M, Hansson BS, Ignell R. 2008. Natural odor ligands for olfactory receptor neurons of the female mosquito Aedes aegypti: use of gas chromatography-linked single sensillum recordings. J Exp Biol. 211:3020–3027.

Grant AJ, Wigton BE, Aghajanian JG, O'Connell RJ. 1995. Electrophysiological responses of receptor neurons in mosquito maxillary palp sensilla to carbon dioxide. J Comp Physiol [A]. 177:389–396.

Hallem EA, Carlson JR. 2004. The odor coding system of Drosophila. Trends Genet. 20:453–459.

Hallem EA, Dahanukar A, Carlson JR. 2006. Insect odor and taste receptors. Annu Rev Entomol. 51:113–135.

Hansson BS. 1995. Olfaction in Lepidoptera. J Cell Mol Life Sci. 51: 1003–1027.

Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Cravchik A, Collins FH, Robertson HM, Zwiebel LJ. 2002. G protein-coupled receptors in Anopheles gambiae. Science. 298:176–178.

Iatrou K, Biessmann H. 2008. Sex-biased expression of odorant receptors in antennae and palps of the African malaria vector Anopheles gambiae. Insect Biochem Mol Biol. 38:268–274.

Ignell R, Dekker T, Ghaninia M, Hansson BS. 2005. Neuronal architecture of the mosquito deutocerebrum. J Comp Neurol. 493:207–240.

Ismail IA. 1962. Sense organs in the antennae of Anopheles maculipennis atroparvus (v. Thiel) and their possible function in relation to the attraction of female mosquito to man. Acta Trop. 19:1–58.

Ismail IA. 1964. Comparative study of sense organs in the antennae of culicine and anopheline female mosquitoes. Acta Trop. 21:155–168.

Isoe J, Millar JG, Beehler JW. 1995. Bioassays for Culex (Diptera: Culicidae) mosquito oviposition attractants and stimulants. J Med Entomol. 32:475–483.

Kazama H, Wilson RI. 2008. Homeostatic matching and nonlinear amplification at identified central synapses. Neuron. 58:401. 59:183–413.

Kent LB, Walden KK, Robertson HM. 2008. The Gr family of candidate gustatory and olfactory receptors in the yellow-fever mosquito Aedes aegypti. Chem Senses. 33:79–93.

Kreher SA, Kwon JY, Carlson JR. 2005. The molecular basis of odor coding in the Drosophila larva. Neuron. 46:445–456.

Logan JG, Birkett MA, Clark SJ, Powers S, Seal NJ, Wadhams LJ, Mordue AJ, Pickett JA. 2008. Identification of human-derived volatile chemicals that interfere with attraction of Aedes aegypti mosquitoes. J Chem Ecol. 34:308–322.

Lu T, Qiu YT, Wang G, Kwon JY, Rutzler M, Kwon HW, Pitts RJ, van Loon JJ, Takken W, Carlson JR, et al. 2007. Odor coding in the maxillary palp of the malaria vector mosquito Anopheles gambiae. Curr Biol. 17: 1533–1544.

Mboera LE, Knols BG, Takken W, Huisman PW. 1998. Olfactory responses of female Culex quinquefasciatus Say (Diptera: Culicidae) in a dual-choice olfactometer. J Vector Ecol. 23:107–113.

McIver S, Charlton C. 1970. Studies on the sense organs on the palps of selected culicine mosquitoes. Can J Zool. 48:293–295.

McIver SB. 1971. Comparative studies on the sense organs on the antennae and maxillary palps of selected male culicine mosquitoes. Can J Zool. 49:235–239.

McIver SB. 1969. Antennal sense organs of female Culex tarsalis (Diptera: Culicidae). Ann Entomol Soc Am. 62:1455–1461.

McIver SB. 1970. Comparative study of antennal sense organs of female culicine mosquitoes. Can Entomol. 102:1258.

McIver SB. 1973. Fine structure of antennal sensilla coeloconica of culicine mosquitoes. Tissue Cell. 5:105–112.

McIver SB. 1978. Structure of sensilla trichodea of female Aedes aegypti (L.) with comments on innervation of antennal sensilla. J Insect Physiol. 24:383–390.

McIver SB. 1971. Comparative studies on the sense organs on the antennae and maxillary palps of selected male culicine mosquitoes. Can J Zool. 49:235–239.

McIver SB, Hutchinson SA. 1972. Coeloconic sensilla on the antennae of the yellow fever mosquito, Aedes aegypti (L.). Experientia. 28:323.

Meijerink J, Braks MA, Van Loon JJ. 2001. Olfactory receptors on the antennae of the malaria mosquito Anopheles gambiae are sensitive to ammonia and other sweat-borne components. J Insect Physiol. 47: 455–464.

Meijerink J, van Loon JJ. 1999. Sensitivities of antennal olfactory neurons of the malaria mosquito, Anopheles gambiae, to carboxylic acids. J Insect Physiol. 45:365–373.

Melo AC, Rutzler M, Pitts RJ, Zwiebel LJ. 2004. Identification of a chemosensory receptor from the yellow fever mosquito, Aedes aegypti, that is highly conserved and expressed in olfactory and gustatory organs. Chem Senses. 29:403–410.

Millar JG, Chaney JD, Mulla MS. 1992. Identification of oviposition attractants for Culex quinquefasciatus from fermented Bermuda grass infusions. J Am Mosq Control Assoc. 8:11–17.

Molaei G, Andreadis TG, Armstrong PM, Bueno R Jr, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H, et al. 2007. Host feeding pattern of Culex quinquefasciatus (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris County, Texas. Am J Trop Med Hyg. 77:73–81.

Pappenberger B, Geier M, Boeckh J. 1996. Responses of antennal olfactory receptors in the yellow fever mosquito Aedes aegypti to human body odours. Ciba Found Symp. 200:254–263; discussion 263–256, 281–254.

Pitts RJ, Fox AN, Zwiebel LJ. 2004. A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector Anopheles gambiae. Proc Natl Acad Sci USA. 101: 5058–5063.

Pitts RJ, Zwiebel LJ. 2006. Antennal sensilla of two female anopheline sibling species with differing host ranges. Malar J. 5:26.

Puri SN, Mendki MJ, Sukumaran D, Ganesan K, Prakash S, Sekhar K. 2006. Electroantennogram and behavioral responses of Culex quinquefasciatus (Diptera: Culicidae) females to chemicals found in human skin emanations. J Med Entomol. 43:207–213.

Qiu YT, Smallegange RC, Van Loon JJ, Ter Braak CJ, Takken W. 2006. Interindividual variation in the attractiveness of human odours to the malaria mosquito Anopheles gambiae s. s. Med Vet Entomol. 20: 280–287.

Qiu YT, van Loon JJ, Takken W, Meijerink J, Smid HM. 2006. Olfactory coding in antennal neurons of the malaria mosquito, Anopheles gambiae. Chem Senses. 31:845–863.

Rachou RG. 1956. [Vectors of Bancroft's filariasis in Brazil.]. Rev Bras Malariol Doencas Trop. 8:267–279.

Ribeiro JM. 2000. Blood-feeding in mosquitoes: probing time and salivary gland anti-haemostatic activities in representatives of three genera (Aedes, Anopheles, Culex). Med Vet Entomol. 14:142–148.

Robich RM, Denlinger DL. 2005. Diapause in the mosquito Culex pipiens evokes a metabolic switch from blood feeding to sugar gluttony. Proc Natl Acad Sci USA. 102:15912–15917.

Robich RM, Rinehart JP, Kitchen LJ, Denlinger DL. 2007. Diapause-specific gene expression in the northern house mosquito, Culex pipiens L., identified by suppressive subtractive hybridization. J Insect Physiol. 53:235–245.

Rospars JP. 1988. Structure and development of the insect antennodeutocerebral system. Int J Insect Morphol Embryol. 17:243–294.

Samuel PP, Arunachalam N, Hiriyan J, Thenmozhi V, Gajanana A, Satyanarayana K. 2004. Host-feeding pattern of Culex quinquefasciatus Say and Mansonia annulifera (Theobald) (Diptera: Culicidae), the major vectors of filariasis in a rural area of south India. J Med Entomol. 41:442–446.

Syed Z, Leal WS. 2007. Maxillary palps are broad spectrum odorant detectors in Culex quinquefasciatus. Chem Senses. 32:727–738.

Takken W. 1996. Synthesis and future challenges: the response of mosquitoes to host odours. Ciba Found Symp. 200:302–312; discussion 312–320.

Xia Y, Zwiebel LJ. 2006. Identification and characterization of an odorant receptor from the West Nile virus mosquito, Culex quinquefasciatus. Insect Biochem Mol Biol. 36:169–176.

Zinser M, Ramberg F, Willott E. 2004. Culex quinquefasciatus (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: blood meal analysis indicates feeding on both humans and birds. J Insect Sci. 4:20.

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