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RESEARCH ARTICLE

Smelling your way to food: can bed bugs use our odour?

V. Harraca[1,2], C. Ryne[1], G. Birgersson[2] and R. Ignell[2,*]

[1]Division of Chemical Ecology, Department of Ecology, Ecology Building, Lund University, SE-223 62 Lund, Sweden and [2]Division of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Box 102, SE-230 53 Alnarp, Sweden

*Author for correspondence (rickard.ignell@slu.se)

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SUMMARY

The resurgence in developed countries of the common bed bug, *Cimex lectularius*, has led to a search for new sustainable methods to monitor and control this human ectoparasite. Because of increased resistance to insecticides, traps baited with attractive cues are considered a promising method to be developed into efficient monitoring tools for bed bugs. Despite their potential as attractants, only a few studies have investigated the odorant cues implicated in the attraction of bed bugs to human hosts. In this study, we used aeration extracts from human volunteers to assess the role of olfaction in host searching by bed bugs. By coupled gas chromatography and single sensillum recordings on all the antennal sensilla, we measured the electrophysiological response elicited by the compounds present in our human odour extracts. Only five compounds were clearly detected by the olfactory receptor neurons housed in the smooth-peg sensilla of the bed bugs. We tested the behavioural effect of these extracts in a still-air arena and showed a gradient of repellence linked to the dose, as well as a higher propensity of local search behaviour associated with human odours containing a lower ratio of 6-methyl-5-hepten-2-one to C_7–C_{10} aldehydes. We conclude that human odour alone has a weak influence on the behaviour of *C. lectularius* and we propose that human kairomones may have a significant impact on bed bug behaviour in combination with heat and carbon dioxide, the only two currently known attractive vertebrate cues used by bed bugs for host seeking.

Key words: chemoreception, electrophysiology, host cue, single sensillum recording, behaviour, *Cimex lectularius*.

INTRODUCTION

The recent resurgence of the common bed bug, *Cimex lectularius* Linnaeus (Heteroptera: Cimicidae), as a public health concern in the developed world has driven an increased interest in the research on this insect pest species (Reinhardt and Siva-Jothy, 2007). Because of the development of insecticide resistance (Romero et al., 2007) and the removal of dangerous non-specific but effective insecticides from the market (Reinhardt and Siva-Jothy, 2007), there is now a pressing need for new bed-bug-specific tools and techniques to monitor and control infestations. For this purpose, odorant cues used by the bed bugs are considered novel constituents in the integrated bed bug management. Consequently, odours emitted by the bed bugs themselves, such as alarm pheromones and aggregation semiochemicals, have been recently investigated (Siljander et al., 2008; Harraca et al., 2010a; Liedtke et al., 2011; Weeks et al., 2011). In spite of the potential of host attractants for both targeted control treatments and surveillance, there is limited knowledge of the identity of these semiochemicals (e.g. Anderson et al., 2009; Wang et al., 2009).

Early behavioural experiments concluded that bed bugs both perceive and orientate toward their hosts at a close range (a few centimetres) using carbon dioxide (CO_2) (Marx, 1955) and heat (Kemper, 1929; Rivnay, 1932; Marx, 1955). Recently, attraction to these cues was confirmed in trapping experiments (Anderson et al., 2009; Wang et al., 2009). In 1932, Rivnay observed that bed bugs were attracted to cold mouse and rabbit skins, and suggested that volatiles associated with these host skins are part of the mechanism regulating host seeking behaviour (Rivnay, 1932). Observational studies have shown that beg bugs generally have a preference for human odour over the odour of other potential hosts (Aboul-Nasr and Erakey, 1968), and intimate relationships with humans have been reported for more than four millennia (Reinhardt and Siva-Jothy, 2007).

Human skin emanates contain up to 400 chemical compounds (e.g. Bernier et al., 1999; Penn et al., 2007; Gallagher et al., 2008), some of which are used by blood-feeding insects in their search for blood. Of these compounds, 1-octen-3-ol, L-lactic acid and C_3–C_5 carboxylic acids are known to elicit attraction in blood-feeding insects such as mosquitoes, biting midges, kissing bugs and tsetse flies (reviewed in Lehane, 2005). However, these volatiles are not behaviourally (Anderson et al., 2009; Wang et al., 2009) or physiologically (Harraca et al., 2010b) active in bed bugs. This suggests that, in their search for a blood meal, bed bugs use a different odour spectrum in the human odour profile compared with other haematophagous insects.

In this study, we used gas-chromatography coupled single sensillum recordings (GC-SSR) to identify biologically active compounds in human body volatiles, and thereby provide a first glimpse into the chemical basis of host detection in the common bed bug. Furthermore, we conducted behavioural experiments on the most divergent individual human odour extracts in order to assess a behavioural effect of the compounds detected by the bed bug antennal olfactory system.

MATERIALS AND METHODS

Insects

Bed bugs *Cimex lectularius* were reared in an incubator (KB8400 FL, Termaks, Bergen, Norway) under a 12 h:12 h light:dark cycle at

25°C and 70% relative humidity (RH). Colonies were kept in plastic jars [40 mm, 30 mm internal diameter (i.d.)] covered by nylon netting (0.5 mm mesh); jars contained a folded filter paper to allow the insects to walk and lay eggs. Bed bugs were fed once every 1–2 weeks on defibrinated chicken or sheep blood using an *in vitro* feeding system (Montes et al., 2002). After a blood meal, last instar nymphs were isolated individually until they moulted as adults. One-to-five week old unmated males and females were used for the experiments, at least 1 week post blood meal.

Collection of human body volatiles

Twenty-four hours prior to odour collection, volunteers were asked to avoid alcohol and spicy food, and to wash with a non-perfumed soap (Lactacyd, GlaxoSmithKline, Solna, Sweden). Whole-body volatiles were collected by placing volunteers in customized heat-sealed oven bags (2×1.75 m; Stekpåsar, Pingvin, Sweden), with only their heads protruding; an empty bag of the same size was used as a control. Synthetic air (20.9% O_2 79.1% N_2; Strandmöllen AB, Ljungby, Sweden) was introduced into the test bag (~20 cm from the top) at a rate of 3 l min^{-1}, and eight pumps (Rena 301, Rena, US) extracted the air (~50 cm from the bottom of the bag) through the same number of adsorbent columns at a rate of ~0.8 l min^{-1}. Odour collection was made in a similar way for the control bag. In this case, however, a single pump was used to extract the air through two adsorbent columns, by way of a Y-tube, at a rate of 1.6 l min^{-1}. Adsorbent columns were made of Teflon tubes (7 cm long, 0.5 cm i.d.), four containing 40 mg of Tenax GR (TGR; 60/80 mesh, Alltech, Deerfield, IL, USA) and four containing 40 mg of Porapak Super Q (PQ; 80/100 mesh, Alltech), between glass wool plugs. The columns were rinsed with 1 ml each of methanol, acetone and pentane before use. An equal proportion of each column type was used for both test and control bags. Aeration extracts of eight volunteers and eight control bags were run in parallel for 150 min.

Adsorbed volatiles were desorbed by eluting each column with 400 µl of pentane (≥99.9%, Merck KGaA, Darmstadt, Germany). For each volunteer and for each type of adsorbent column used, three of the four eluted extracts were pooled. In the remaining extract, as well as in the ones from the control bag, 1 µg of heptyl acetate was added as an internal standard. These extracts were concentrated under N_2 to reach a final volume of ~20 µl and then used for chemical analysis.

Chemical analysis of human whole-body volatiles

The extracts of the volatiles collected were analyzed on a Hewlett-Packard 6890 gas chromatograph (GC) combined with a Hewlett-Packard 5973 MSD (Palo Alto, CA, USA) or an Agilent Technologies 5975MS mass spectrometer (MS; Santa Clara, CA, USA). The GC was equipped with either a non-polar HP-5MS capillary column or a polar InnoWax capillary column (both 30 m×0.25 mm i.d.; d.f.=0.25 µm; Agilent Technologies) with helium as a mobile phase (35 cm s^{-1}). Aliquots of each sample (2 µl) were injected into the GC splitless injector kept at 225°C. The GC oven temperature was programmed from 40 or 30°C (3 min hold), followed by a ramp of 8°C min^{-1} to 230 or 225°C, and held isothermal for 5 min, when equipped with the HP-5MS column or the InnoWax column, respectively. In order to compare volatile chemical profiles of the eight individuals, all sample chromatograms were compared with their corresponding controls and any background compounds were subtracted from the sample data after quantification using the internal standard.

Retention times and GC peak areas were obtained by integration of peaks using Agilent ChemStation software (Agilent Technologies), and their mass spectra were compared with the Wiley275 and NIST05 reference databases. Compounds eliciting electrophysiological activity in the GC-SSR analysis were confirmed with retention times and mass spectra of synthetic reference compounds.

Gas chromatography coupled single sensillum recordings

For single sensillum recordings (SSR), a bed bug was dorsally restrained under a Nikon Eclipse microscope (E600-FN8, Tokyo, Japan), which allowed us to identify individual sensilla at high magnification (×750). Using a piezoelectric micromanipulator (DC-3K, Märzhauser, Wetzlar, Germany), an electrolytically sharpened tungsten microelectrode was introduced into the shaft or base of a sensillum and the reference tungsten electrode was inserted into the head capsule through the neck region. The recording electrode was connected to a preamplifier (×10, Syntech, Kirchzarten, Germany) and the electrical signals were fed through an analogue-digital signal converter (IDAC-4, Syntech) and then visualized and recorded on a computer using Autospike software (v3.3, Syntech). The insect antenna was placed in a continuous humidified charcoal-filtered airstream delivered at 1 m s^{-1} *via* a glass tube (6 mm i.d.).

Prior to GC-SSR analysis, 10 µl of the extract to be injected onto the GC was loaded onto a filter paper (~5×15 mm) placed inside a Pasteur pipette. A stimulus controller (CS-SS, Syntech) diverted a 2 ml l^{-1} airflow through the Pasteur pipette for 500 ms into the airstream flowing over the antenna. All previously characterized functional types of sensilla (Harraca et al., 2010b) were tested for electrophysiological activity. An HP 6890 GC fitted with a non-polar HP-5MS capillary was used for the GC-SSR analysis, using the same program as for the GC-MS analysis (see above). The extracts used included the pooled human body aeration extracts, and a blend of synthetic compounds at purum grade [heptanal, octanal, nonanal, decanal, undecanal, tridecanal, 6-methyl-5-hepten-2-one (hereafter sulcatone), 2,6-dimethyl-2,6-undecadien-10-one (hereafter geranyl acetone), cinnamaldehyde and dodecanol] diluted 10^3, 10^4 and 10^5 times in hexane.

Behavioural assays

The behavioural assay used to test the human extracts was constructed of two superimposed plastic Petri dishes (100 mm in diameter, 15 mm height; Fig. 1A). Each division of the bottom Petri dish, a segmented I-plate (BD Falcon, San Jose, CA, USA), contained a filter paper (~5×15 mm) soaked with either 10 µl of a human whole-body extract or the solvent (pentane) as a control. The top Petri dish served as an arena. Two half circles were cut out from the bottom (75 mm in diameter) and covered by a metallic 1 mm mesh grid. The half circles were lined up with the compartments in the bottom dish. The top dish was covered by a lid with a central hole (20 mm in diameter), which prevented odour accumulation and allowed for the introduction of individual bed bugs (Fig. 1A). To further prevent odour contamination, the behavioural assay was placed under an air exhaust. As bed bugs are known to be more active at night, the behavioural experiments were conducted during scotophase and inside a red-light illuminated climatic chamber at 25°C and 70% RH.

The behaviour of the bed bugs was recorded with a camera (DCR-TRV30, Sony, Tokyo, Japan) placed ~250 mm above the behavioural assay. Recordings started 30 s after bed bud introduction and lasted 7 min. The video was then analyzed using EthoVision software (v3.0, Noldus, Wageningen, The Netherlands) which measured the bed bug position every second, based on the mean position among five

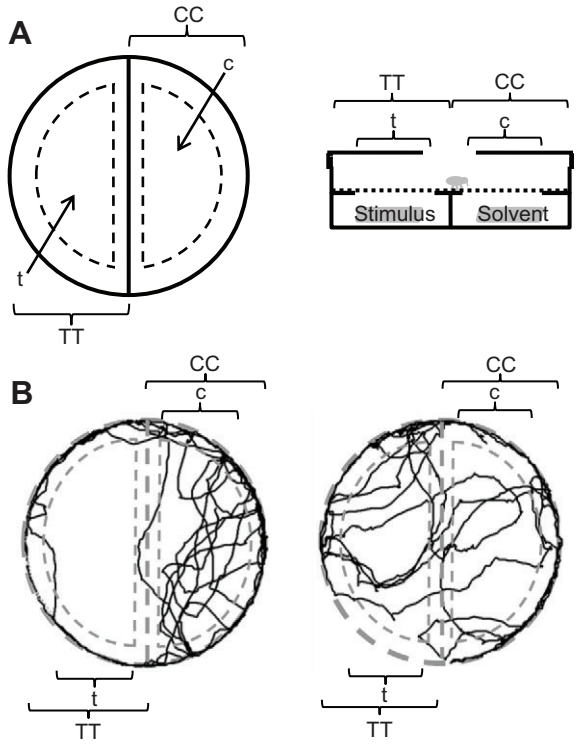

Fig. 1. Top and side illustrations of the still-air olfactometer used in the tracking behavioural experiments (A) and examples of recorded bed bug tracking races (B). The letters correspond to the zones aligned with the control (c and CC) or the test (t and TT) chambers of the Petri dish below. Double uppercase letters refer to the zone half of the top Petri dish (90 mm diameter) whereas single lowercase letters are the smaller half circle zones (75 mm diameter) excluding the edge of the Petri dish. In B, the bed bug movements (one point captured every 200 ms) are shown over a 7 min test period in response to neat extract of volunteer A (left) and 100-times-diluted extract of volunteer A (right). The grey dashed lines represent the edges of the four zones, with chambers containing the solvent placed on the right.

intervals of 200 ms (Fig. 1B). The total area of the Petri dish was delimited into two half-circle zones lined up with the control (CC) and test (TT) chambers (Fig. 1). Within each of these zones, a smaller half circle zone (75 mm diameter) was defined, which corresponded to the pierced open zone and excluded the edges (c and t; Fig. 1). Based on the position of the insect, the time spent in each zone was measured. In addition, by considering that bed bugs were moving with a displacement of more than 5 mm between two consecutive points, the time spent in movement, the total distance and the mean speed were also calculated for each zone. Unmated female and male bed bugs were starved for 10 to 20 days and 10 insects of each sex were individually tested once with each stimulus. In order to reduce the variation between individuals, distance and time values were standardized in percentage. In addition, the ratio of time spent and distance run between test and control zones or between open and total zones was quantified.

Based on the odour profile (Fig. 2) and the SSR (Figs 3–5), we selected the extracts of volunteers A, B, C and D as stimuli for this behavioural analysis (Table 1). In order to achieve a sufficient testing volume, the samples collected on the two adsorbents (PQ and TGR) for each of these volunteers were pooled. Despite the fact that PQ captured higher quantities, similar compounds at similar ratios were captured on both absorbents (as shown in Fig. 2). In addition, the pooled extract from all volunteers, used for the GC-SSR analysis, was also tested. These five samples were diluted at three logarithmic dilutions, 10^0, 10^1 and 10^2 times diluted, giving a total of 15 samples tested in the behavioural assay. In addition, a control with solvent on both sides of the compartmented Petri dish confirmed that there was no position effect.

Data and statistical analyses

To compare similarities between human whole-body odour, relative quantities of chemicals were used because the total amount of emitted volatiles varied between samples and adsorbents. Cluster analysis and non-metric multidimensional scaling (MDS) were performed on the data using the software Primer (v6.0, Primer-E, Plymouth, UK). MDS objectively separated and classified the different samples by constructing a two-dimensional configuration, using the rank of similarity between the different samples (Clarke and Warwick, 2001) (Fig. 2).

The antennal olfactory system of bed bugs is composed of three functional types of smooth-peg sensilla (Dα, Dβ and Dγ), a single type of grooved-peg sensilla (C) and two morphological types of trichoid sensilla (E) (Steinbrecht and Müller, 1976; Harraca et al., 2010b). These sensilla contain large numbers of olfactory receptor neurons (ORNs), which prevents the differentiation of individual ORN responses (Harraca et al., 2010b). For this reason, the total number of all spikes was manually counted for each of the 3015 stimulations, allowing us to assess the overall ORN activity elicited by stimulation within a specific sensillum. The mean temporal pattern of response was assessed by counting the total number of spikes within 250 ms duration bins (in SSR) or 500 ms duration bins (in GC-SSR). For this, the mean spike frequency per bin was calculated using the values recorded 2 s before stimulation. This frequency was then subtracted from each bin in order to obtain the net firing rate change per bin. The total number of SSR using the

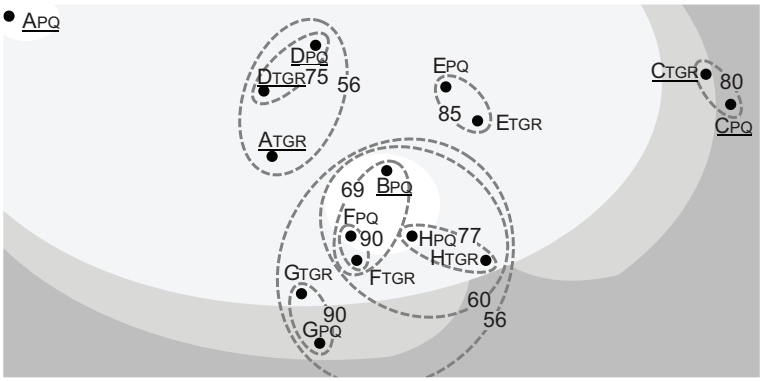

Fig. 2. Non-metric multidimensional scaling of the human scent profiles, with indications of similarity between subjects and superposition of the presence of some relevant compounds. The aeration extracts of eight individuals (volunteers A–H) were collected simultaneously with Tenax GR (TGR) and Porapack Super Q (PQ). Because of variations between the quantities of the two adsorbents, percentage of compounds was used in the classification. Underlined letters are extracts used in the behavioural tracking experiments (Fig. 6). Dashed circles represent the similarity between samples measured with a cluster analysis; associated numbers indicate the percentages of homology. The shaded surfaces indicate, from dark grey to white, the cumulative presence of nonanal and decanal, +sulcatone, +octanal, +heptanal, respectively. The extract H_{TGR} does not contain sulcatone.

Table 1. Quantity of the five compounds detected by olfactory receptor neurons housed in the smooth-peg sensilla of *Cimex lectularius* as well as the total amount of these five compounds

	Quantity (ng)					
Sample	Heptanal	Sulcatone	Octanal	Nonanal	Decanal	Total
A	12	176	59	259	436	942
B	15	106	29	140	202	491
C	–	49	–	113	217	379
D	–	209	62	250	415	936
MIX	79	1133	347	1983	2921	6463

Samples A, B, C and D correspond to the mix extracts collected on the two adsorbents of volunteers A, B, C and D, respectively. Sample MIX is the pooled whole-body human odour extract.
A blank cell (–) means the compound was not detected during the gas chromotography-mass spectrometry analysis.

pooled human whole-body odour extract was 25 for the E sensilla, 48 for the C sensilla, and 28, 29 and 35 for the Dα, Dβ and Dγ sensilla, respectively; only ORNs housed in D sensilla responded to the extract. Two to five GC-SSR replicates with the synthetic blend were made for each functional type of D sensilla.

Parameters computed by EthoVision software were analyzed using a cluster analysis based on the rank similarity matrix and a principal component analysis (PCA), using the Euclidean distance between the standardized mean values of the behavioural parameters (Primer v6.0). Based on the GC-SSR results, the amounts of the relevant compounds were then overlapped on the PCA mapping in order to predict links between the detection of compounds and the different behavioural response measured.

RESULTS

Single sensillum recording

The pooled whole-body human odour extract induced a differential increase in activity in ORNs housed in the three functional types of smooth-peg sensilla (D sensilla): Dα<<Dβ<Dγ sensilla (Fig. 3). However, the same extract did not elicit any response in ORNs housed in either the grooved-peg (C) or the trichoid (E) sensilla.

Analysis of the whole-body human odour extract by GC-SSR and GC-MS revealed that sulcatone and C_7–C_{10} aldehydes elicited responses in Dβ and Dγ sensilla (Fig. 4). However, none of the eluted compounds elicited a detectable response in the Dα sensilla, possibly because of thermolability (Figs 4, 5). The identity of the bioactive compounds was confirmed through injecting synthetic standards onto the GC (Fig. 5). Both C_7–C_{10} aldehydes and sulcatone elicited a response in ORNs housed in Dβ and Dγ sensilla, but ORNs in the Dγ sensilla responded more strongly to sulcatone (Fig. 4). In both Dβ and Dγ sensilla, these responses increased with dose, revealing that heptanal, octanal and sulcatone were the most potent stimuli, with a response already recorded as low as 10^5 times dilution (Fig. 5).

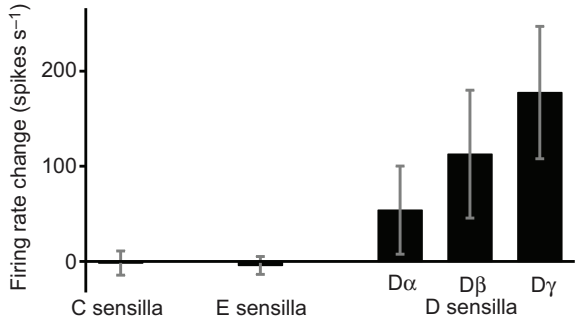

Fig. 3. Mean firing rate change of olfactory receptor neurons housed in antennal olfactory sensilla of *Cimex lectularius* due to stimulation with human body aeration extracts. Trichoid (E) and grooved-peg (C) sensilla do not show any change during the 500 ms stimulation, in contrast to the three functional subtypes of smooth-peg (Dα, Dβ and Dγ) sensilla.

Individual human-body volatile emissions

The samples collected on the two adsorbent types for each volunteer were pooled as the inter-individual variation was higher than the intra-individual variation (hierarchical cluster analysis superposed to the MDS, stress=0.04; Fig. 2).

Based on the detection ability of *C. lectularius* to individual odorants in the human odour, we were able to distinguish three different chemical fingerprints within our volunteer population: (1) all five compounds detected by the bed bugs were present in the odour profile of volunteers A, B, F and H; (2) all compounds except heptanal were present in the odour profile of volunteers D, E and G; and (3) all compounds except heptanal and octanal were present in the odour profile of volunteer C (Table 1). Based on these results, we decided to investigate the behavioural response of the bed bugs to extracts of volunteers A, B, C and D, as well as to the pooled whole-body human odour extract (MIX). The odour profile of both volunteers A and B included all the five detected compounds, but were present in much larger amounts in volunteer A compared with volunteer B (Table 1).

Behavioural assays

A PCA allowed a clear separation of the behavioural responses of the bed bugs to the 15 human extracts, four volunteers and one pooled extract in three dilutions, with the two major axes explaining 90.6% of the data separation (Fig. 6). The first axis accounted for 69.1% of the separation, and was linked to the time spent in the control zone (Fig. 6). For this reason, we associated this component as an index of repulsion, which was significantly correlated with the total amount of compounds present in the treatment zone (R^2=0.523, P<0.05; graph on top in Fig. 6). The second axis accounted for 21.5% of the dispersion, and allowed the separation of behaviours elicited by the extract of volunteer C and the pooled human odour extracts from behaviours elicited by the extracts of the three other volunteers (Fig. 6). The parameter accounting mostly for this axis was the presence near the edge of the arena, giving an index of displacement in open space zones (Fig. 6). The ratio of sulcatone to the total amount of compounds detected by the bed bugs is the most likely to be connected to this axis, as a higher proportion of sulcatone corresponds to an increase in the displacement to near the edges of the arena (graph on the right in Fig. 6).

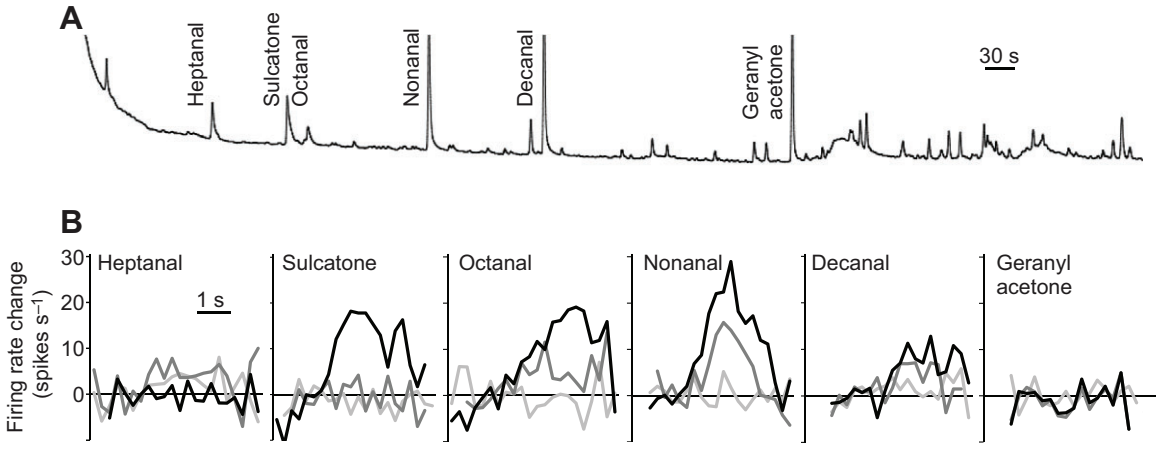

Fig. 4. Gas chromatography fractionation of pooled whole-body human odour extract coupled to single sensillum recordings. (A) Flame ionisation detector response to the mixture of human body aeration extracts. (B) Temporal response of olfactory receptor neurons housed in Dα (light grey lines), Dβ (dark grey lines) and Dγ (black lines) sensilla during individual stimulation by six compounds of human body aeration extracts fractionated by gas chromatography (see Materials and methods for further explanation).

To complement the PCA analysis, a Bray–Curtis similarity test with a hierarchical cluster analysis (Clarke and Warwick, 2001) constructed four distinct clusters with similar behavioural responses (dashed circles in Fig. 6). The undiluted extracts of volunteers A and C as well as the pooled extract were grouped as having a similar repellent effect because of the high amounts of sulcatone and C_7–C_{10} aldehydes (Fig. 6). In contrast, the undiluted extracts of volunteers B and D and the 10-times-diluted extracts of volunteers A and D were associated with the control, suggesting that these extracts elicited no specific behavioural choices (Fig. 6). The two remaining groups, mostly composed of the 100-times-diluted extracts, elicited similar slight attraction, but in one case the bed bugs stayed near the edges of the arena, whereas in the other group they ventured more into the open space (Fig. 6).

DISCUSSION

Bed bugs are able to detect C_7–C_{10} aldehydes and sulcatone present in human emanates, similar to other blood-feeding insects (Gikonyo et al., 2002; Birkett et al., 2004; Ghaninia et al., 2008; Logan et al., 2008; Logan et al., 2009; Syed and Leal, 2009; Logan et al., 2010). Quantitative differences in the amount of these compounds in headspace volatile extracts collected from a group of volunteers modulate the behavioural response of bed bugs. The bed bugs were more repelled by highly concentrated extracts of human volatile emanation

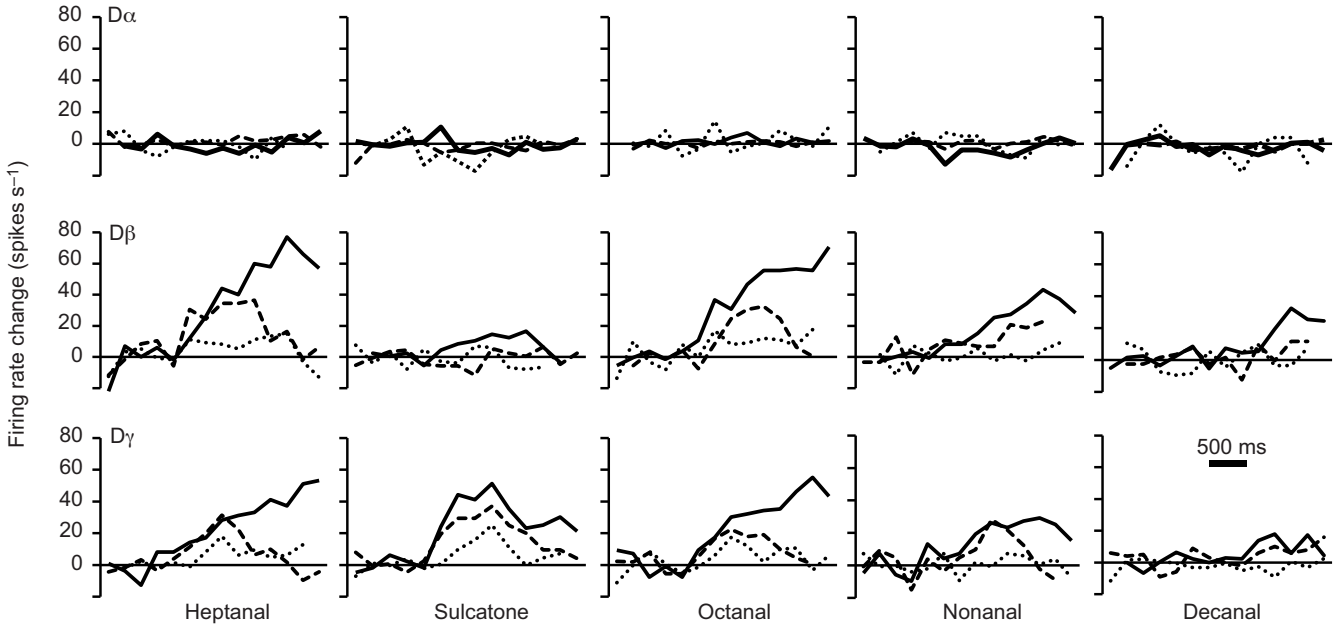

Fig. 5. Temporal response of *Cimex lectularius* olfactory receptor neurons (ORNs) housed in the three subtypes of smooth-peg sensilla to synthetic compounds eluted from a gas chromatographic column. Each graph represent the temporal response of ORNs housed in Dα, Dβ and Dγ sensilla stimulated with synthetic compounds diluted 10^3 (solid lines), 10^4 (dashed lines) and 10^5 (dotted lines) times. Each point of the graph represents an average change of action potentials over two to five replicates within sampling period of 500 ms (see Materials and methods for further explanation).

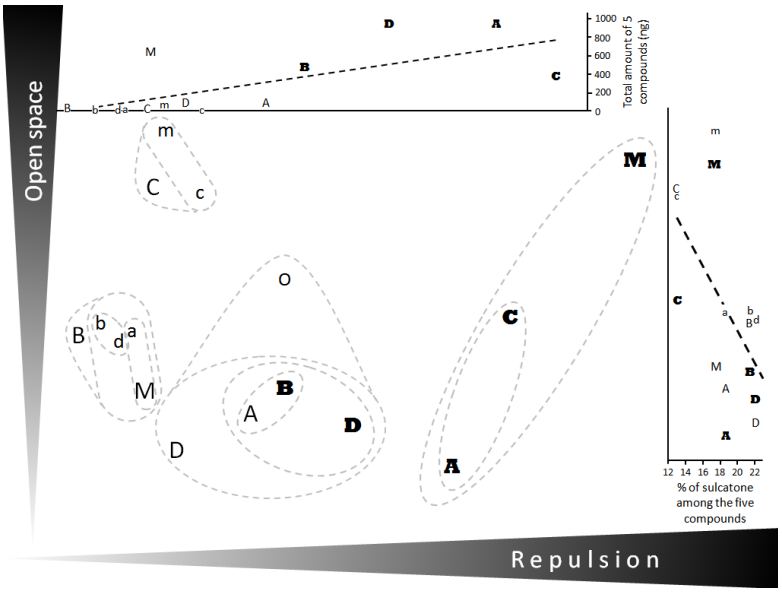

Fig. 6. Principal component analysis of *Cimex lectularius* behaviour elicited by 15 extracts of human odours. Four human body aeration extracts (A, B, C and D) and the pooled extract of all human samples (M) were used neat (bold uppercase letters), diluted 10^1 times (uppercase letters) or diluted 10^2 times (lowercase letters) as stimuli. O represents the control behaviour with solvent placed in both chambers. The dashed circles indicate the similarity between samples, based on the behaviour they elicited. The resemblance was measured by Bray–Curtis similarity with a hierarchical cluster analysis. The horizontal axis representing 69.1% of the separation is linked to the presence of the bed bug in the control zone (right) or in the test zone (left), giving an index of repulsion. It can be associated, as shown by the graph on top, with the total amount of compounds detected by the bed bugs. The vertical axis, accounting for 21.5% of the separation, is linked to the presence of the bed bug in the zones without edges (c and t), and is associated with the ratio of sulcatone among the five compounds detected by bed bugs, as shown by the graph on the right.

than by diluted ones. In addition, we observed that a low ratio of sulcatone compared with aldehydes in the human emanates may increase the bed bugs' local search as the bed bugs moved further away from the edges of the arena.

Aldehydes are part of the complex mixture of volatiles in human odour (e.g. Bernier et al., 1999; Penn et al., 2007; Gallagher et al., 2008), and are metabolized from long-chain carboxylic acids by cutaneous aerobic bacteria (Gallagher et al., 2008). Among the several aldehydes identified in human emanates, only C_7–C_{10} aldehydes elicited a clear electrophysiological response in ORNs housed in smooth-peg sensilla of *C. lectularius*. Other blood-feeding insects such as the mosquitoes *Culex quinquefasciatus* (Hill et al., 2009; Syed and Leal, 2009) and *Aedes aegypti* (Ghaninia et al., 2008; Logan et al., 2008), tsetse flies (Gikonyo et al., 2002), the kissing bug *Triatoma infestans* (Guerenstein and Guerin, 2001) and the biting midge *Culicoides impunctatus* (Logan et al., 2009) are also able to detect the same range of aldehydes. Therefore, there is a similarity of detection among the blood-feeding arthropods for this class of chemicals regularly emitted by vertebrate hosts. Sulcatone, present in all of the individual extracts of human volatile emanation, elicited an electrophysiological response in the antennal olfactory system of *C. lectularius*. Similar responses have been observed in other blood-feeding insects, e.g. *A. aegypti* (Logan et al., 2008), *C. impunctatus* (Logan et al., 2009) as well as different cattle fly species (Birkett et al., 2004). This ketone is one of the prominent compounds commonly recovered in human emanations along with geranyl acetone, L-lactic acid and carboxylic acids (e.g. Bernier et al., 1999; Dekker et al., 2002; Penn et al., 2007; Gallagher et al., 2008). Although the latter compounds are detected by most of the free-living blood-feeding insects (e.g. Knols et al., 1997; Guerenstein and Guerin, 2001; Barrozo and Lazzari, 2004; Logan et al., 2008; Ghaninia et al., 2008; Harraca et al., 2009; Logan et al., 2009; Syed and Leal, 2009), bed bugs appear to have a more restricted detection range. Some authors (Steinbrecht and Müller, 1976; McIver, 1987; Harraca et al., 2010b) have suggested that the close association between the obligatory blood-feeding bed bugs and their host may be the cause of this narrowed response. Indeed, bed bugs do not need to cover large distances to seek their meal. Bed bugs are repulsed by air movement and therefore do not use coupled anemotactic–chemotactic responses to direct their path (Aboul-Nasr and Erakey, 1968; Weeks et al., 2011) (V.H., personal observation on servosphere). Instead, bed bugs seem to use their antennal olfactory system to detect odorant cues at close range, such as during their intraspecific interactions (Siljander et al., 2008; Harraca et al., 2010a; Liedtke et al., 2011; Weeks et al., 2011).

Our human odour extracts contained different amounts of C_7–C_{10} aldehydes and sulcatone, and elicited different behavioural responses of the bed bugs. The major conclusion drawn from our behavioural experiments is that highly concentrated odour extracts are repellent, and that bed bugs become more attracted at lower concentrations. Similar results has been demonstrated by Knols et al., who showed that the attraction or repulsion of the malaria mosquito *Anopheles gambiae* is dependent on the concentration of carboxylic acids employed (Knols et al., 1997). Similarly, Logan et al. (Logan et al., 2008) showed that *A. aegypti* was less attracted by human extracts with an increased amount of an average of eight compounds, among which were the C_8–C_{10} aldehydes and sulcatone. Variability in the confinement of the bed bug near the edge of the arena was also observed and seemed to be associated with the ratio between aldehydes and sulcatone in our five different human extracts. Aldehydes alone appear to elicit attraction in various blood-feeding insects when presented at relevant doses. For example, tsetse flies, *Glossina morsitans*, fly upwind in presence of a synthetic blend of six aldehydes (Gikonyo et al., 2003), *C. quinquefasciatus* is caught in traps baited with nonanal (Syed and Leal, 2009) and the addition of C_8–C_{10} aldehydes significantly increases the number of *A. gambiae* and *C. quinquefasciatus* landing on a human arm (Logan et al., 2010). In contrast, sulcatone has been shown to inhibit the final approach and landing of different haematophagous insects such as cattle flies (Birkett et al., 2004), and the mosquitoes *A. aegypti*, *A. gambiae* and *C. quinquefasciatus* (Logan et al., 2008; Logan et al., 2010).

To conclude, the common bed bug *C. lectularius* responds to only a few stimuli that are ubiquitously produced by vertebrates, such as common volatile compounds emanating from the human body (present study), heat (Kemper, 1929; Rivnay, 1932; Marx, 1955) and CO_2 (Marx, 1955). The hypothesis that the bed bugs' reduction of their olfactory range is linked to the close association of the host is supported by behavioural observations of many authors who have reported a mere orientation towards different host cues

(Rivnay, 1932; Johnson, 1941; Marx, 1955; Aboul-Nasr and Erakey, 1968; present study). As heat and CO_2 are emitted in parallel to human odours, adding heat and/or CO_2 to the extracts may have created a synergistic behavioural effect, as observed for *T. infestans* (Barrozo and Lazzari, 2004) and *Culex* spp. (Syed and Leal, 2009). This was, however, not the focus of the present study. Moreover, behavioural experiments using different synthetic blends and concentrations of sulcatone and C_8–C_{10} aldehydes will be required to confirm the modification of local search observed with our human body extracts alone. In all, this might lead to the improvement of monitoring traps (Anderson et al., 2009; Wang et al., 2009).

These results improve our knowledge about the chemical ecology of bed bugs and their relation to their human host. Indeed, even if humans are reported as their preferred host, it seems that the bed bug olfactory system is not specifically tuned to detect our odour. To corroborate this statement, lactic acid, which is a human-signifying host cue (Dekker et al., 2002), is not perceived by the reduced bed bug antennal olfactory system (Harraca et al., 2010b). Therefore, and as mentioned by Johnson, we may represent one of the safest blood meals for the bed bugs, as other potential vertebrate hosts such as birds or rodents prey on them (Johnson, 1941).

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