Chemical profiling of the human skin surface for malaria vector control via a non-invasive sorptive sampler with GC×GC-TOFMS

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ABSTRACT

Volatile organic compounds (VOCs) and semi-VOCs detected on the human skin surface are of great interest to researchers in the fields of metabolomics, diagnostics, skin microbiota and in the study of anthropophilic vector mosquitoes. Mosquitoes use chemical cues to find their host, and humans can be ranked for attractiveness to mosquitoes based on their skin chemical profile. Additionally, mosquitoes show a preference to bite certain regions on the human host. In this study, the chemical differences in the skin surface profiles of 20 human volunteers were compared based on inter-human attractiveness to mosquitoes, as well as inter- and intra-human mosquito biting site preference. A passive, non-invasive approach was followed to sample the wrist and ankle skin surface region. An in-house developed polydimethylsiloxane (PDMS) passive sampler was used to concentrate skin VOCs and semi-VOCs prior to thermal desorption directly in the GC inlet with comprehensive

gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS). Compounds from a broad range of chemical classes were detected and identified as contributing to the differences in the surface skin chemical profiles. 5-Ethyl-1,2,3,4-tetrahydro-naphthalene, 1,1'-oxybisoctane, 2-(dodecyloxy)-ethanol, α,α-dimethylbenzene methanol, methyl salicylate, 2,6,10,14-tetramethylhexadecane, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, 4-methylbenzaldehyde, 2,6-diisopropylnaphthalene, *n*-hexadecanoic acid, and γ-oxo-benzenebutanoic acid, ethyl ester were closely associated with individuals who perceived themselves as attractive for mosquitoes. Additionally, biological lead compounds as potential attractants or repellants in vector control strategies were tentatively identified. Results augment current knowledge on human skin chemical profiles and show the potential of using a non-invasive sampling approach to investigate anthropophilic mosquito-host interactions.

4.1. Introduction

The use of volatile organic compounds (VOCs) emanating from the human skin surface presents great promise in the fields of metabolomics [1], diagnostics [2, 3], skin microbiota [4], and in the investigation into novel mosquito attractants and repellents [5, 6]. The latter application is of great importance in the ongoing battle against malaria. Insecticide resistance and changes in mosquito-host biting behaviour have prompted the need for new vector control strategies [7, 8]. Anthropophilic mosquitoes are guided by human odours to find their host. These mosquitoes are important vectors for human diseases, such as malaria, due to their preference to blood-feed on humans [9]. The difference in mosquito attractiveness between different individuals has been ascribed to differences in skin-odour profiles specifically differences in human skin microbial flora [10-12]. Skin bacteria metabolise the components of sweat, thus giving sweat its characteristic odour. The amount of certain skin bacteria is directly related to the intensity and type of odour released from the human skin [13]. The volatile chemicals released by skin microorganisms have thus become a major focal point for studying how mosquitoes distinguish between hosts [13]. The skin surface chemical profile can consequently be used to find specific mosquito attractiveness biomarkers. These biomarkers can be used as attractants or repellents in push-and-pull vector control strategies.

A vast range of compounds is associated with the human skin chemical profile. Adding to the complexity is the non-homogenous characteristics of the skin surface and the distribution of different gland types and bacterial flora across the skin. A single individual's chemical profile can vary with age, diet, emotional state and sleep patterns. Furthermore, personal care products often interfere with skin secretion studies [1]. It is, however, important to consider all the chemicals emitted by the host, which may include primary odours that do not change with diet, secondary odours that are dependent on the diet and various environmental interactions, and lastly, tertiary odours that come from the application of, for example, lotions and make-up when investigating mosquito-host attraction [14]. Verhulst et al. found that when volunteers stopped using skincare products prior to skin sampling no difference in attraction of *An. coluzzi* to different body parts was detected, leading to the conclusion that skincare products may affect a person's mosquito attractiveness [6]. The vast amount of skin

associated volatiles makes bioassays aimed at determining the behavioural responses of mosquitoes almost impossible. Fortunately, sophisticated analytical techniques are being applied for elucidating the identity of mosquito semiochemicals and potential semiochemical blends. Gas chromatography-mass spectrometry (GC-MS) is mostly used in skin chemical profiling due to its ability to identify compounds by spectral matching [1, 3]. De Lacy Costello et al. recommend retention time matching and using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) to avoid misidentification of compounds due to peak coelution [1].

Skin volatiles are present in low concentrations and require a preconcentrating step [3]. Furthermore, human skin VOCs comprise of a broad range of chemicals from various chemical classes with different characteristics. The development and use of a non-invasive sampler suitable for various chemical compounds are of paramount interest [2]. Various sampling procedures for skin surface chemicals have been reported. These include passive sampling procedures such as wiping the skin surface with organic solvents, thermal or solvent desorption of cotton pads or glass beads used to swab the sample, dynamic headspace adsorption onto various polymers, solid phase microextraction (SPME) [15], and polydimethylsiloxane (PDMS) samplers such as stir bars [16], thin films [2], and loops [17]. Active sampling approaches are usually more invasive and often cause discomfort for the individual sampled. These techniques usually involve the placement of a body part, or whole body in the case with a body chamber [18], into a plastic or cellulose bag and passing air over the skin. Skin volatiles are collected on polymer filters and analysed by GC-MS [19]. These sampling methods often yield qualitative information, however, abundances of skin VOCs have been reported for glass beads, cotton patches, and PDMS loops [6, 17, 20]. The abundance of chemical compounds on the skin is of importance when investigating mosquito-host attractiveness. For example, it is widely reported that quantitative differences in CO_2 output will influence mosquito attractiveness between individuals [5].

Polydimethylsiloxane (PDMS) passive samplers were used in this study due to their reproducibility and versatility [2, 17, 21-23]. The PDMS samplers were fashioned into anklets and bracelets for ease-of-use and comfort. Polydimethylsiloxane can

easily be paired with thermal desorption (TD) as the material is thermally stable and breakdown products are known [24]. The hydrophobicity of PDMS enables high recovery of hydrophobic compounds. However, polar compounds have shown lower recoveries [24]. The ability of the material to concentrate a broad range of chemical compound and its increased sensitivity when paired with TD make it an ideal option for sampling skin VOCs and semi-VOCs. Furthermore, Roodt et al. demonstrated the ability of using PDMS samplers in determining relative abundances for skin VOCs [17]. The comparison of skin chemical profiles is accordingly possible. In this study, a passive PDMS sampler is used for the non-invasive sampling of the human skin surface. Twenty volunteers were assessed based upon their perceived mosquito attractiveness. Different skin regions were also explored due to differences in mosquito host biting site preference [25-27]. The PDMS sampler was used with TD thereof directly in the inlet liner of a GC for analysis with GC×GC-TOFMS. This approach enabled the comparison of the skin surface chemical profile for perceived mosquito attractive and non-attractive individuals enabling the identification of biomarkers which can be used in future push-and-pull vector control strategies.

4.2. Materials and methods

4.2.1. Reagents and chemical standards

4.2.1.1. Reagents

Toluene, acetone, methanol (MeOH), *n*-hexane, acetonitrile (ACN) and isopropanol were all purchased from Merck, South Africa. For linear retention index determination *n*-alkanes C_8-C_{28} were used (Merck, Pretoria, South Africa).

4.2.1.2. Chemical standards

Heptanal, phenylethyl alcohol (Fluka), (*R*)-(+)-β-citronellol, (-)-carvone, octanal, eucalyptol (1,8-cineole) (Fluka), nonanal, (*E*)-2-octenal, (*E*)-2-nonenal, linalool, (*E*)-2-

decenal, 3-methyl-2-butenal, tetradecanoic acid, propanoic acid, 2-tridecanone, butanoic acid, indole, terpineol (mixture of isomers), and 2-octanone analytical standards were purchased from Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa. NLEA FAME mixture, containing hexadecanoic acid, methyl ester, was purchased from Restek (Bellefonte, PA, USA).

4.2.1.3.   Standard solutions

A 100 µg/ml stock solution mixture of the liquid standards was made in toluene, whilst individual stock solutions of 100 µg/ml were prepared for each of the two solid standards (tetradecanoic acid and indole) in toluene. Individual stock solutions of 300 µg/ml FAME mixture and 652 µg/ml eucalyptol were prepared in hexane. The working standard solution comprised of a 1 µg/ml mixture of all the target analytes in hexane. An 0.1 µg/ml eucalyptol internal standard (IS) solution was prepared in isopropanol. All the stock solutions were stored in glass vials and kept at 4 °C.

4.2.2.   Non-invasive sampler

Passive PDMS samplers were made in-house for the sorptive extraction of VOCs and semi-VOCs from the human skin surface. The samplers (0.074 ± 0.0026 g) were each manufactured from a 25 cm length of a silicone elastomer medical grade tubing (0.64 mm OD x 0.3 mm ID, Sil-Tec®, Technical Products, Georgia, USA). The tubing was formed into loops as anklets and bracelets by joining the ends with a 1 cm piece of uncoated silica capillary column (250 µm ID) (SGE Analytical Science, Separation Scientific (Pty) Ltd, Roodepoort, South Africa) [28]. The sampler loop size was chosen to fit a range of individuals. The sorption volume of the loop was 62.75 µl and the internal volume was 17.67 µl. The sampler was initially developed for solvent free extraction of soil [29, 30]. It was employed as a passive sampler to concentrate pollutants from surface water due to its ability to exclude water during sampling [28, 31, 32]. The sampler has also proved reliable for the quantitative analysis of endocrine disrupting compounds in water [31, 32] and the determination of relative abundances of VOCs on the human skin surface [17]. The PDMS samplers were cleaned and

conditioned before sampling using the method outlined by Triñanes et al. for cleaning silicone sampling disks [33]. This entails sonicating the samplers three times for 5 minutes each with a MeOH:acetone (1:1, v/v) mixture followed by overnight conditioning in a 17.8 cm long glass desorption tube (4 mm ID, 6 mm OD) from Gerstel™ (Chemetrix, Midrand, South Africa) at 250 °C using a Gerstel™ tube conditioner with 100 mL/min hydrogen gas flow. The samplers were then sonicated three times for 5 minutes each with ACN after conditioning, pat dry with a lint free tissue and stored in a glass vial.

4.2.3.   Cohort selected for the study

A cohort of 20 volunteers participated in this study. Written consent was given by all 20 volunteers to partake in the study. Ten percent of the volunteers smoked tobacco products. Half of the participants were male; half was White and the other half Black. Participants spanning a range of ages, 20 – 59, were sampled. Twelve of the participants were between the ages of 20 – 29, seven between 30 – 49, and one between 50 – 59. The volunteers were asked to complete a questionnaire pertaining to their diet, medicinal usages, skincare routine, perceived mosquito attractiveness and mosquito biting site preference. No dietary or special hygiene requirements were made before sampling. The study was approved by the ethics committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria, South Africa (Reference number EC171109-159).

4.2.4.   VOC and semi-VOC sampling

Volatile organic compounds and semi-VOCs were collected from the wrist and ankle skin surface region of all 20 volunteers. To correct for any variation in the sampler size (wrist vs. ankle), sampling duration and instrumental analysis when comparing skin chemical profiles an eucalyptol IS was added to the PDMS samplers before sampling. The IS was added in accordance with the method outlined by Wooding et al. [34]. The authors demonstrated that the addition of an organic solvent such as adding an IS made no significant impact on the sampler's performance. The

uncoated capillary coupling was removed from the sampler loop ends and the open samplers were then sonicated in 12 ml of the 0.1 μg/ml eucalyptol IS isopropanol solution [34]. A stainless-steel tweezer was used to remove the samplers after which the samplers were wiped dry with a lint free tissue and formed back into a loop by re-joining the sampler loop ends with the uncoated capillary coupling. Medical grade alcohol cleansing pads (70% isopropanol, Dischem, South Africa) were used to clean the skin surface area prior to sampling. The volunteers' right wrists and ankles were sampled in a single sampling event, using two loops per skin surface area, sampling for 1 hour. The samplers were worn as anklets (n=2) and bracelets (n=2) by the volunteers (Fig. 1). The samplers were placed in direct contact with the skin surface to facilitate the sampling process with reduced invasiveness [17]. Mylar® reflective sheeting (Hydroponic, South Africa), 35 cm x 3cm, was used to cover the samplers. The sheeting was secured in place using 3M Micropore medical dressing tape (Dischem, South Africa). The samplers were carefully removed from each participant with a clean stainless-steel tweezer and separately stored in aluminium foil at 4 °C for no more than 48 hours before GC analysis. The individual sampler was transferred into the inlet liner of a GC followed by TD directly in the GC inlet (Fig. 1).

The volunteers continued with their daily routine during the one-hour sampling period. No effort was made to control the environmental parameters during the sampling. Two method blanks (samplers placed in a Schott glass bottle in a water bath at 31 °C to simulate human skin temperature) were analysed to account for laboratory background (see Supplementary Material Figs. S1 and S2).
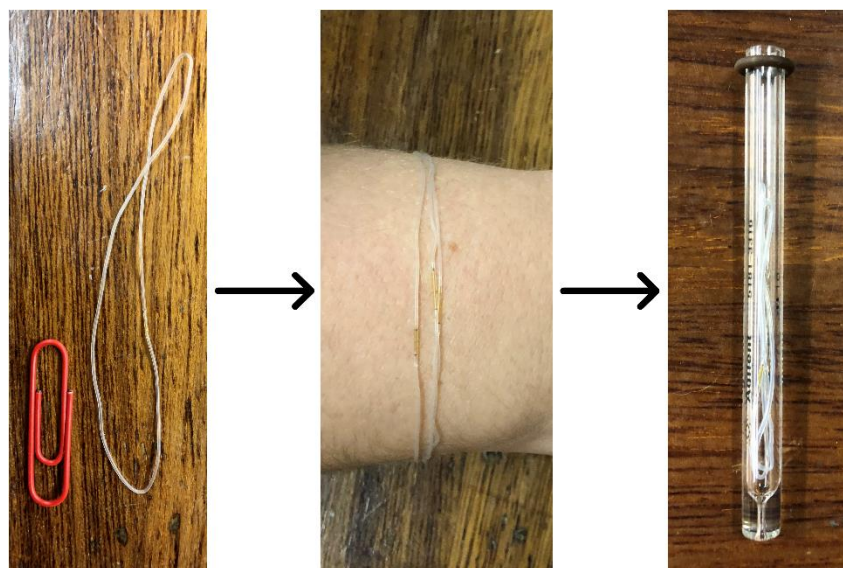
Fig. 1 PDMS sampler fashioned into a loop to be used as either anklet or bracelet (left), wrist skin surface sampling (middle) without a Mylar® cover for illustration purposes, and the PDMS sampler placed in the GC inlet liner for direct TD in the GC inlet (right)

4.2.5. GC×GC-TOFMS analysis

Separation of compounds was performed on a LECO Pegasus® 4D GC×GC-TOFMS system. The system consists of an Agilent© 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa) modified to contain a dual stage modulator and secondary oven. Nitrogen gas was used for the cold jets (cooled with liquid nitrogen) and for the hot jets. The primary column was connected to the secondary column with a presstight column connector (Restek, Bellefonte, PA, USA). ChromaTOF® software (version 4.50.8.0 optimised for Pegasus®, LECO Africa (Pty) Ltd.) was used to operate the instrument and for data capturing and processing. Tentative identification of compounds for untargeted analysis was based on a comparison of sample mass spectra to that of the NIST14 library (version 2.2). A spectral match quality of ≥ 80% was reported.

The column set consisted of a Rxi-5Sil MS 30 m x 0.25 mm ID x 0.25 µm film thickness as the primary column (1D) joined to a Rxi-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness secondary column (2D) (Restek, Bellefonte, PA, USA). The primary oven temperature programme was 40 °C (hold for 1.5 min) at 10 °C/min to

280 °C (hold for 3 min). The GC run time was 28.5 min. The secondary oven was offset by + 5 °C relative to the primary oven. The modulator temperature was offset 15 °C relative to the second oven temperature. The modulation period was 3 s with a hot pulse time of 0.8 s. The carrier gas (helium 5.0, Afrox, South Africa) flow rate was 1.4 mL/min in the constant flow mode. The MS transfer line temperature was set at 280 °C. The ion source temperature was 230 °C, the electron energy was 70 eV in the electron ionisation mode (EI+), the data acquisition rate was 100 spectra/s, the mass acquisition range was 35 – 500 Daltons (Da), and the detector voltage was set at 1586 V. The PDMS sampler was inserted into a splitless glass inlet liner (Agilent Chemetrix, Midrand, South Africa) and desorbed in a GC inlet at 250°C with a splitless time of 30 s. The gas flow was shut off prior to opening of the GC inlet, followed by manual removal of the hot inlet liner from the GC inlet using a pair of tweezers. The PDMS sampler was folded in half and was then inserted into the inlet liner, the liner was placed back into the GC inlet, the gas flow was restored and the run was started.

Linear retention indices were determined by analysing a mixture of *n*-alkanes (C_8-C_{28}). Experimental linear retention indices were calculated for non-target compounds according to the method of van den Dool and Kratz [35]. Compounds having a match of within ±35 RI units to the literature values were reported. Peak areas were calculated on the total ion chromatogram (TIC).

4.2.6.   Data processing

Chromatographic data generated during the initial processing were aligned using ChromaTOF Statistical Compare software (LECO (Pty.)). Statistical Compare software employs Fisher ratios as a simplified approach to identify significant differences between classes investigated. The mass spectral threshold was set at 800, and the first (1D) and second dimension (2D) retention time deviations were set to 3 and 0.1 s, respectively, for peak alignment. The S/N cut-off was set at 50 for initial peak finding and a secondary cut-off was set at 20 for peaks not aligned during the initial alignment. Principle component analysis (PCA) using JMP® Pro 14 a statistical software package from the SAS® Institute Inc. (Cary, North Carolina, USA) was employed to visually demonstrate variance between the skin surface chemical profiles.

The mean peak area of the two biological replicates per wrist or ankle was used throughout the study. Peak areas were normalised using the TIC area of the eucalyptol IS. Background laboratory compounds as obtained from the method blanks were normalised using the IS and then subtracted from the normalised peak areas of the human skin volatile samples.

4.3. Results and discussion

4.3.1. Comparison of skin chemical profiles

4.3.1.1. Perceived mosquito attractiveness

Volunteers were assessed based on their perceived attractiveness for mosquitoes with the aid of a questionnaire. The volunteers were asked: (1) *Would you say that when outdoors you are the person who preferentially gets bitten by mosquitoes?* and (2) *How attractive do you consider yourself to be for mosquitoes?* In the case of the first question, the volunteers were asked to provide a simple *yes* or *no* answer. With the second question, mosquito attractiveness was rated using a response scale: *1*, not attractive, to *5*, highly attractive. ChromaTOF Statistical Compare software was used to identify chemical features contributing to the difference in mosquito attraction between the two groups using the ankle skin surface data. In the first instance, the *yes* (10 individuals) vs *no* (10 individuals) groups were compared. In the second case *1 – 2* responses were grouped as not attractive (6 individuals) and *4 – 5* responses were grouped as attractive (9 individuals) and consequently compared; the 5 individuals indicating a score of *3* on the attractiveness scale, were not considered. The analyses yielded 39 compounds that contributed to the difference in perceived mosquito attractiveness for the 20 individuals sampled (Table 1). Exemplary contour plots of two individuals, one who self-perceived as attractive and another as not attractive for mosquitoes, are given in Fig. 2. Visual inspection reveals striking differences in the skin chemical profiles between the two individuals using ankle skin surface data.
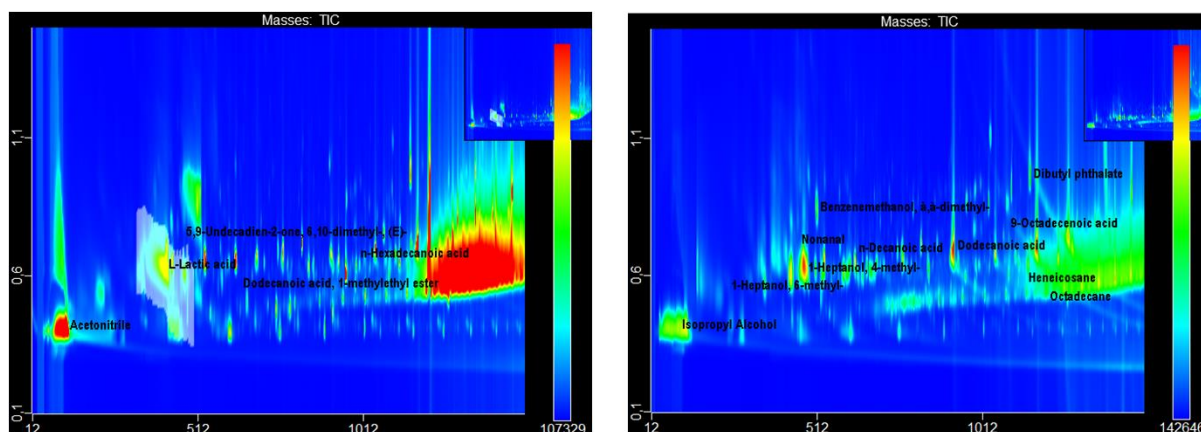
Fig. 2 Contour plots (TIC) (GC×GC-TOFMS) from the analysis of the human ankle skin surface using a non-invasive PDMS sampler. The contour plot on the left shows the chemical profile of an individual who classified themself as highly attractive for mosquitoes and the contour plot on the right is the chemical profile of an individual who perceived themself as not attractive for mosquitoes

The 39 compounds were tentatively identified based on mass spectral library matches (≥ 80%) and further confirmed by corresponding experimental first dimension linear retention indices to that of the NIST14 mass spectral library. Most of the compounds were alcohols, carboxylic acids and esters. Exogenous skin compounds such as polycyclic aromatic hydrocarbons (PAHs) and phthalates (1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester) were also classified as contributing to differences in perceived mosquito attractiveness. Exogenous compounds are included in this study as mosquito attraction to its blood-host is known to be influenced by deodorant compounds and plant volatiles such as limonene [6, 36]. 5-Ethyl-1,2,3,4-tetrahydro-naphthalene, 1,1'-oxybisoctane, 2-(dodecyloxy)-ethanol, α,α-dimethylbenzene methanol, methyl salicylate, 2,6,10,14-tetramethylhexadecane, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, 4-methylbenzaldehyde, 2,6-diisopropylnaphthalene, *n*-hexadecanoic acid, and γ-oxo-benzenebutanoic acid, ethyl ester were more abundant (greater than double the mean peak area) in individuals who perceived themselves as mosquito attractive. Conversely, dodecanoic acid, pentadecanoic acid heptadecanoic acid, phenanthrene, butyrolactone, 2-undecanone, and 6-methyl-1-heptanol were more abundant (greater

than double the mean peak area) in individuals who perceived themselves as not attractive to mosquitoes. These compounds provide the potential to disrupt mosquito behaviour in malaria vector control programs.

Octanal and tetradecanoic acid are associated with highly mosquito attractive individuals [36]. However, no notable differences were found for these two compounds between the groups investigated. Of interest is the bacterial volatile, 2-undecanone, which was more abundant (in terms of peak area where present) in unattractive individuals, however, it was present on more (7 vs 5) individuals in the attractive group. This volatile was previously detected in the headspace of *Staphylococcus* and *Corynebacterium* species [37] and it is reported that these bacteria species produce volatiles that attract anthropophilic mosquitoes [12]. Further investigation about the influence of compound concentration levels on mosquito response is needed. Another compound worth noting is methyl salicylate, a mosquito-plant semiochemical, which was found in higher mean abundance in the attractive group. This compound elicited an antennal response in *Cx. pipiens* and *Ae. aegypti* mosquitoes [38]. This lead compound can potentially be used in future lure-and-kill vector control strategies. L-lactic acid, a well-documented mosquito-host semiochemical [10], was not included in the study as identification could not be confirmed with retention indices (RI<800) and derivatisation is required for separation and unambiguous identification of the enantiomers [39]. This compound, tentatively identified based on a mass spectral library match (≥ 80%), is present in higher abundance when viewing the contour plot (Fig. 2) for the perceived mosquito attractive individual. The compound is used synergistically with ammonia and tetradecanoic acid in a 3-compound mosquito lure [40, 41]. Further investigation is needed for the unambiguous detection of L-lactic acid using GC-MS.

Table 1 Compounds tentatively identified during an untargeted analysis of the human ankle skin surface using a non-invasive PDMS sampler with inlet TD-GC×GC-TOFMS. The compounds listed were classified by ChromaTOF Statistical compare software to contribute to the difference, using Fisher ratios, between perceived mosquito attractiveness of 20 volunteers

#	Compound	CAS Number	1D RT^a (s)	2D RT^b (s)	1D RI$_{exp}$	1D RI$_{Lit}$ NIST14	Previously reported on skin	Response reported in mosquitoes	Subject Count^c	Mean^d	Median	Range Min	Range Max	Subject Count^c	Mean	Median	Range Min	Range Max
	Would you say that when outdoors you are the person who preferentially gets bitten by mosquitoes?								Yes (m=10)^e					No (m=10)^e				
Alkanes																		
1	Pentadecane, 2-methyl-	1560-93-6	922	0.51	1565	1564	[34]	n/a^f	8	0.2426	0.2310	0.0803	0.4423	8	0.2971	0.1790	0.0532	0.9694
Alkenes																		
2	Squalene	111-02-4	1586	0.86	2808	2791	[1]	n/a	3	0.7101	0.5253	0.1684	1.4366	5	0.7192	0.3063	0.1061	2.1381
Alcohols																		
3	1-Heptanol, 6-methyl-	1653-40-3	431	0.59	995	977	n/a	n/a	7	1.4442	0.6534	0.4391	5.2335	9	2.7273	1.9107	0.1747	9.2375
4	Ethanol, 2-(2-ethoxyethoxy)-	111-90-0	432	0.80	984	979	n/a	n/a	5	0.5880	0.4282	0.0185	1.4047	5	0.5067	0.5764	0.0342	1.0344
5	Benzenemethanol, α,α-dimethyl-	617-94-7	511	0.84	1057	1062	[17]	n/a	7	2.9513	2.5986	0.8175	6.6873	9	1.4673	1.4790	0.0777	4.2009
6	1-Nonanol	143-08-8	602	0.61	1157	1160	[17]	n/a	3	0.2077	0.2085	0.1658	0.2487	8	0.3275	0.2384	0.0301	0.8599
7	1-Octanol, 2-butyl-	3913-02-8	810	0.52	1393	1408	n/a	n/a	5	0.1993	0.1594	0.0922	0.3420	5	0.3337	0.3177	0.1214	0.6690
8	Ethanol, 2-(dodecyloxy)-	4536-30-5	1016	0.66	1731	1704	[34]	n/a	5	0.2455	0.3388	0.0716	0.3931	4	0.1119	0.1044	0.0541	0.1847
9	Hexadecen-1-ol, *trans*-9-	64437-47-4	1120	0.66	1862	1867	n/a	n/a	7	0.7733	0.3418	0.0103	3.2720	7	0.6804	0.5307	0.1527	1.7702
10	1-Octadecanol	112-92-5	1240	0.68	2066	2074	[1]	n/a	6	2.1379	1.4362	0.5250	4.2432	7	1.4938	1.4115	0.6551	2.3734
PAHs																		
11	Naphthalene, 5-ethyl-1,2,3,4-tetrahydro-	42775-75-7	756	0.80	1357	1342	n/a	n/a	3	0.0237	0.0262	0.0157	0.0291	2	0.0016	0.0016	0.0012	0.0020
12	Phenanthrene	85-01-8	1047	1.23	1758	1751	n/a	n/a	7	0.0022	0.0017	0.0005	0.0046	9	0.0046	0.0033	0.0003	0.0172
Aldehydes																		
13	Octanal	124-13-0	435	0.64	982	981	[1, 3, 17, 42]	[36, 43-45]	6	0.0099	0.0072	0.0015	0.0269	7	0.0115	0.0097	0.0030	0.0176
Ethers																		
14	Octane, 1,1'-oxybis-	629-82-3	987	0.57	1659	1659	[1]	n/a	9	0.8062	0.1561	0.0912	4.9517	8	0.2243	0.1494	0.0277	0.6259

Carboxylic acids

#	Compound	CAS							n					n				
15	Dodecanoic acid	143-07-7	921	0.64	1556	1563	[1, 3, 34]	[6, 41, 46, 47]	6	3.2070	2.8139	1.8836	5.1142	3	9.3084	5.1502	2.5468	20.2283
16	Tetradecanoic acid	544-63-8	1049	0.67	1752	1754	[1, 17, 34]	[6, 36, 41, 46, 48-55]	10	0.0117	0.0106	0.0016	0.0222	10	0.0097	0.0055	0.0017	0.0358
17	Pentadecanoic acid	1002-84-2	1110	0.68	1849	1850	[17]	n/a	8	0.7881	0.6603	0.1971	1.4919	9	0.6623	0.3681	0.1337	2.5107
18	Heptadecanoic acid	506-12-7	1230	0.61	2039	2056	[17]	n/a	6	0.3036	0.2348	0.1614	0.7094	3	0.6893	0.7823	0.1557	1.1298
19	9,12-Octadecadienoic acid (*Z,Z*)-	60-33-3	1269	0.76	2113	2128	[17]	n/a	7	3.3809	2.9915	0.1841	10.0796	3	5.5173	4.7895	0.7057	11.0566

Acid esters

#	Compound	CAS							n					n				
20	Methyl salicylate	119-36-8	611	0.87	1174	1170	[34]	[56]	6	0.4760	0.0270	0.0021	2.6650	7	0.0761	0.0309	0.0047	0.2216
21	Acetic acid, octyl ester	112-14-1	633	0.62	1193	1194	n/a	n/a	3	0.0827	0.0866	0.0009	0.1605	8	0.1174	0.1074	0.0291	0.2479
22	Isopropyl palmitate	142-91-6	1207	0.63	2012	2013	[1, 58]	n/a	10	3.1759	1.7978	0.7152	10.3164	10	1.6597	1.1518	0.1558	5.0716

Nitrogen containing volatiles

#	Compound	CAS							n					n				
23	Cyclobutylamine	2516-34-9	75	0.91	684	<800	n/a	n/a	6	0.9371	0.4156	0.0483	3.6844	5	0.9621	0.5495	0.0063	2.8251
24	4-Cyanocyclohexene	100-45-8	429	1.04	1008	975	[1]	n/a	4	0.1055	0.0863	0.0584	0.1911	2	0.1706	0.1706	0.0559	0.2852

How attractive do you consider yourself to be for mosquitoes?									**4 – 5 attractive (m=9)[e]**					**1-2 not attractive (m=6)[e]**				

Alkanes

#	Compound	CAS							n					n				
25	Hexadecane, 3-methyl-	6418-43-5	999	0.52	1673	1677	n/a	n/a	5	0.2500	0.1723	0.0343	0.7078	4	0.2029	0.2027	0.1095	0.2968
26	Hexadecane, 2,6,10,14-tetramethyl-	638-36-8	1090	0.52	1813	1817	[17]	n/a	6	0.5097	0.0258	0.0118	2.8790	4	0.2234	0.0537	0.0307	0.7556

Alkenes

#	Compound	CAS							n					n				
27	1,3,5,7-Cyclooctatetraene	629-20-9	330	0.66	880	872	[17]	n/a	3	0.3192	0.3259	0.3012	0.3306	1	0.5068	0.5068	0.5068	0.5068

Alcohols

#	Compound	CAS							n					n				
3	1-Heptanol, 6-methyl-	1653-40-3	431	0.59	995	977	n/a	n/a	6	0.8127	0.6168	0.4391	1.3721	6	3.0787	1.9821	0.6195	9.2375
28	1-Octanol	111-87-5	507	0.62	1057	1058	[1, 3]	n/a	3	0.2285	0.2329	0.0618	0.3909	5	0.3648	0.2443	0.0049	1.0354
5	Benzenemethanol, α,α-dimethyl-	617-94-7	511	0.84	1057	1062	[17]	n/a	6	2.7481	2.5026	0.8175	6.6873	5	1.0394	1.1804	0.0777	1.9693
29	1-Eicosanol	629-96-9	1346	0.64	2273	2278	[17]	n/a	3	0.2733	0.2495	0.1152	0.4553	1	0.2645	0.2645	0.2645	0.2645

PAHs

#	Compound	CAS							n					n				
30	2,6-Diisopropylnaphthalene	24157-81-1	1016	0.84	1727	1703	n/a	n/a	7	0.0564	0.0284	0.0038	0.1620	3	0.0058	0.0055	0.0012	0.0106

Aldehydes

13	Octanal	124-13-0	434	0.64	982	980	[1, 3, 17, 42]	[36, 43-45]	5	0.0065	0.0057	0.0015	0.0109	5	0.0094	0.0088	0.0030	0.0167
31	Benzaldehyde, 4-methyl-	104-87-0	501	0.94	1069	1051	n/a	n/a	2	1.4645	1.4645	0.1230	2.8059	5	0.0547	0.0115	0.0015	0.2308

Ketones

32	2-Undecanone	112-12-9	702	0.64	1273	1276	[1, 3, 34]	n/a	7	0.0253	0.0117	0.0068	0.0690	5	0.0607	0.0496	0.0117	0.1411

Carboxylic acids

17	Pentadecanoic acid	1002-84-2	1113	0.71	1849	1855	[17]	n/a	8	0.8344	0.7071	0.2551	1.5986	4	2.1425	2.0689	0.2750	4.1573
33	*n*-Hexadecanoic acid	57-10-3	1171	0.68	1954	1951	[1, 17]	[6]	6	3.1040	2.9439	0.5879	5.6328	2	0.8059	0.8059	0.1243	1.4875

Cyclic esters

34	Butyrolactone	96-48-0	333	1.27	867	875	[42]	n/a	2	0.0182	0.0182	0.0071	0.0293	1	0.1065	0.1065	0.1065	0.1065

Acid esters

35	2-Propenoic acid, octyl ester	2499-59-4	701	0.62	1273	1275	n/a	n/a	0	n.d.[g]	-	-	-	2	0.1558	0.1558	0.0759	0.2356
36	Benzenebutanoic acid, γ-oxo-, ethyl ester	6270-17-3	953	1.07	1594	1607	n/a	n/a	7	0.0299	0.0017	0.0007	0.1988	4	0.0006	0.0005	0.0002	0.0012
37	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	84-69-5	1098	0.94	1850	1831	[17]	n/a	8	0.8599	0.1555	0.0127	4.4308	6	0.2894	0.2025	0.0088	0.6232
22	Isopropyl palmitate	142-91-6	1207	0.62	2012	2013	[1, 57]	n/a	9	2.3825	1.7246	0.7152	6.8223	6	1.3651	1.0684	0.1558	3.8061
38	Octadecanoic acid, 2,3-dihydroxypropyl ester	123-94-4	1536	0.94	2681	2680	n/a	n/a	4	0.0415	0.0394	0.0145	0.0729	0	n.d.	-	-	-

Nitrogen containing volatiles

39	Benzonitrile, 3, 5-dimethyl-	22445-42-7	597	0.95	1185	1155	n/a	n/a	2	0.0455	0.0455	0.0181	0.0728	4	0.0388	0.0175	0.0040	0.1164
24	4-Cyanocyclohexene	100-45-8	429	1.04	1008	975	[1]	n/a	4	0.1055	0.0863	0.0584	0.1911	1	0.0559	0.0559	0.0559	0.0559

[a]First dimension retention time
[b]Second dimension retention time
[c]Number of subjects the compound was detected on
[d]Peak areas were normalised using the TIC of the eucalyptol IS
[e]Number of subjects within a group
[f]n/a = not available
[g]Not detected in subjects within a group

Mosquito-host biting site preference was assessed by asking the volunteers: *Which part of your body gets bitten most by mosquitoes?*. The volunteers were asked to choose between four options namely, (1) *I get bitten all over my body*, (2) *Hands, wrists and arms*, (3) *Face and neck*, and (3) *Feet, ankles and legs*. Twenty-five percent of the volunteers responded that they get bitten all over their body; the data from this group was consequently not considered for comparison. Five percent of the participants selected *Face and neck* and 15% chose *Hands, wrists and arms*; the data from these two responses was pooled to represent the upper body part preferred group (4 individuals). Data from the cohort *Feet, ankles and legs* (55 % of participants) represented the lower body part mosquito biting site preference group (11 individuals). ChromaTOF Statistical Compare software was used to compare the two groups, namely upper vs lower body skin surface area. Compounds perceived to contribute to the difference in mosquito-host biting site preference are given in Table 2. The analyses yielded 16 compounds that contributed to the difference in mosquito-host biting site selection for the 15 individuals considered.

The 16 compounds detected are from a broad range of chemical classes and were tentatively identified based on mass spectral library matches (≥ 80%) and further confirmed by corresponding first dimension linear retention indices. The compounds included exogenous skin compounds such as undecylbenzene, PAHs and Propoxur. Of interest is the detection of Propoxur a carbamate insecticide used for the control of household pests, fleas and *Anopheles* mosquitoes [58]. Propoxur was present on the wrist and ankle skin surface of three of the volunteers with a higher abundance on the wrist surface area (Fig. 3). These volunteers indicated that mosquitoes preferred biting them on the upper body parts, however, the volunteers rated themselves as *1* not attractive to mosquitoes, *3* no preference, to *5* highly attractive to mosquitoes. Further investigation is needed to determine the origin of exposure to the insecticide, as none indicated that they used an insecticide on the day of sampling, and also whether insecticide resistance to this ubiquitous insecticide accounts for the range of mosquito attractiveness responses. Benzoic acid, pentadecyl ester, triethyl citrate and dodecyl acrylate, not previously reported on the human skin surface to the author's knowledge, were detected using the non-invasive PDMS sampler.

Carboxylic acids are often used synergistically with lactic acid and ammonia in mosquito lures [41, 48]. Octanoic acid was present in higher abundance in the upper body part preference group when considering the ankle skin surface area data. However, the compound was detected on more individuals in the lower body part preferred group (Fig. 3). This is of interest as concentration can influence the attractiveness of a compound and it is known that a mixture of carboxylic acids on their own, without lactic acid and ammonia, has a repellent effect [41]. The mosquito oviposition semiochemical, hexadecanoic acid, methyl ester [59], showed the same trend as octanoic acid (Fig. 3). The compound was detected on double the number of individuals from the lower extremities group than the upper extremities group when considering the ankle skin surface data. However, the abundance was once again higher for the upper extremities group. The compound is reported to have a deterrent ovipositional effect on *Aedes aegypti* mosquitoes [59]. The results indicate that there is no correlation between the abundance of compound detected and the number of individuals the compound was detected on (refer to Fig. 3). The effect of compound concentration on mosquito response is thus of importance when developing attractants and repellents for push-and-pull vector control strategies.
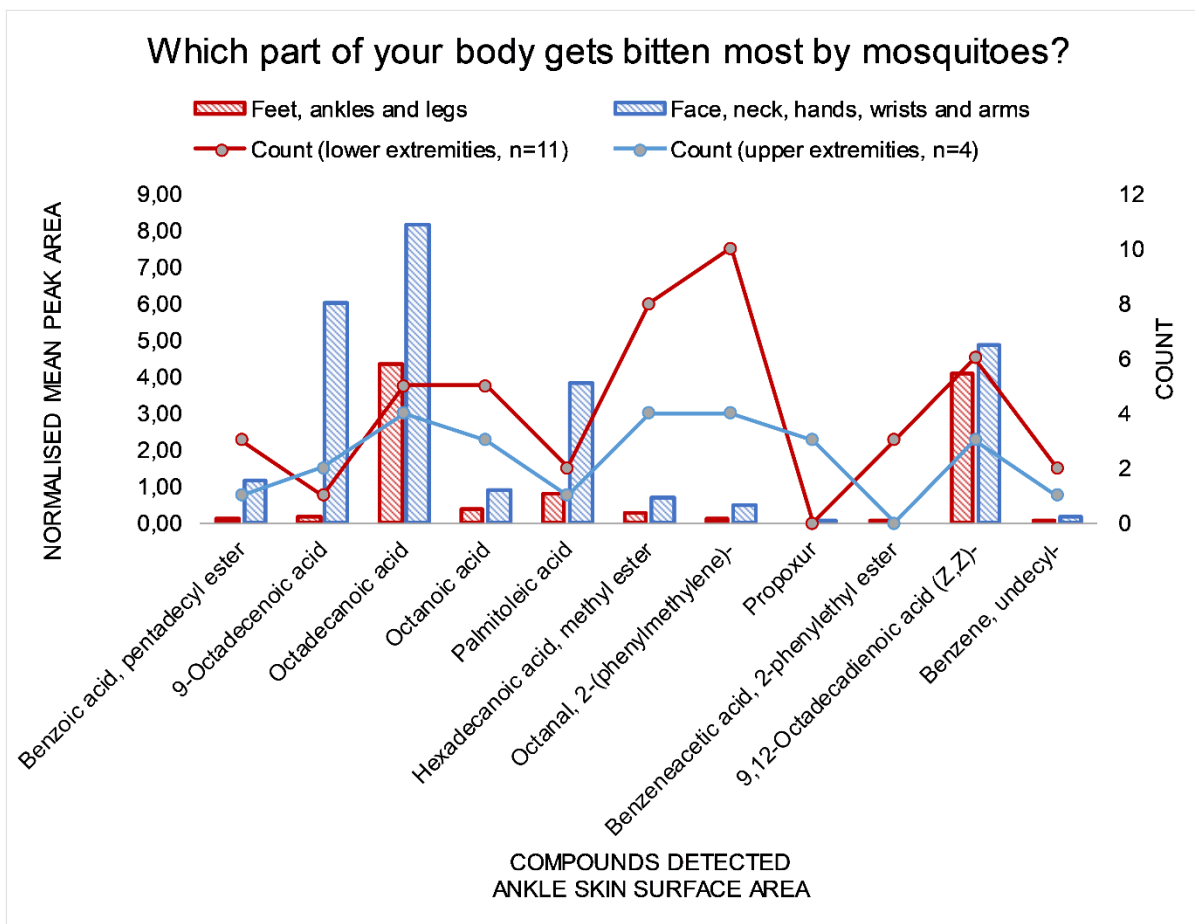
Fig. 3 Combination bar-and-line chart of the 11 chemical compounds identified by ChromaTOF Statistical Compare software to contribute to the difference in mosquito-host biting site selection using ankle skin surface data. Upper (blue) and lower (red) body part preference was compared for 15 participants. The bar chart gives the normalised mean peak area whilst the line chart gives the count, i.e. the number of individuals the compounds was detected on

Table 2 Compounds tentatively identified during an untargeted analysis of the human ankle skin surface using a non-invasive PDMS sampler with inlet TD-GC×GC-TOFMS. The compounds listed were classified by ChromaTOF Statistical Compare software to contribute to the difference in mosquito-host biting site preference for 15 volunteers. Mosquito biting site preference for different body regions, namely feet, ankles and legs (lower) vs face, neck, arms and wrist (upper), was compared first using (1) ankle skin surface data and then by (2) wrist skin surface data. The first part of the table lists compounds detected using ankle skin surface data and the second part of the table gives compounds detected on the wrist skin surface area

#	Compound	CAS Number	1D RT[a] (s)	2D RT[b] (s)	1D RI_exp	1D RI_Lit NIST14	Previously reported on skin	Response reported in mosquitoes	Subject Count[c]	Mean[d]	Median	Range Min	Range Max	Subject Count[c]	Mean	Median	Range Min	Range Max
	Which part of your body gets bitten most by mosquitoes?								Feet, ankles and legs (m=11)[e]					Face, neck, hands, arms and wrist (m=4)[e]				
	Ankle skin surface area																	
Benzyl and phenyl hydrocarbons																		
1	Benzene, undecyl-	6742-54-7	1059	0.67	1764	1768	n/a[f]	n/a	2	0.0304	0.0304	0.0259	0.0349	1	0.1783	0.1783	0.1783	0.1783
Aldehydes																		
2	Octanal, 2-(phenylmethylene)-	101-86-0	1030	0.86	1728	1723	[1]	n/a	10	0.0946	0.0516	0.0037	0.3328	4	0.4975	0.4879	0.2514	0.7627
Carboxylic acids																		
3	Octanoic acid	124-07-2	608	0.65	1165	1166	[1, 3, 34]	[41, 46, 48-50]	5	0.3487	0.4546	0.0538	0.4593	3	0.8953	0.3794	0.1781	2.1282
4	Palmitoleic acid	373-49-9	1167	0.71	1963	1944	[1, 34]	[59]	2	0.7629	0.7629	0.6617	0.8641	1	3.8207	3.8207	3.8207	3.8207
5	9,12-Octadecadienoic acid (*Z,Z*)-	60-33-3	1265	0.75	2113	2121	[17]	n/a	6	4.0920	2.6217	1.4099	10.0796	3	4.8768	3.4000	2.5992	8.6313
6	9-Octadecenoic acid	2027-47-6	1277	0.75	2141	2142	[1]	n/a	1	0.1561	0.1561	0.1561	0.1561	2	6.0133	6.0133	5.0824	6.9441
7	Octadecanoic acid	57-11-4	1286	0.69	2153	2160	[1, 17]	n/a	5	4.3132	2.4682	0.1867	13.2577	4	8.1357	6.2029	3.4455	16.6916
Esters																		
8	Benzeneacetic acid, 2-phenylethyl ester	102-20-5	1125	1.13	1882	1875	[1]	n/a	3	0.0651	0.0539	0.0014	0.1402	0	n.d.[g]	-	-	-
9	Hexadecanoic acid, methyl ester	112-39-0	1147	0.64	1909	1911	[34, 57]	[59]	8	0.2415	0.1930	0.0611	0.5778	4	0.6940	0.7080	0.5468	0.8133
10	Benzoic acid, pentadecyl ester	68411-27-8	1417	0.79	2452	2421	n/a	n/a	3	0.1267	0.0914	0.0880	0.2008	1	1.1556	1.1556	1.1556	1.1556
Nitrogen containing volatiles																		
11	Propoxur	114-26-1	928	1.15	1578	1572	n/a	n/a	0	n.d.	-	-	-	3	0.0046	0.0043	0.0035	0.0060
	Wrist skin surface area																	

Benzyl and phenyl hydrocarbons

12	Benzene, (1-ethyldecyl)-	2400-00-2	1053	0.64	1756	1760	n/a	n/a	8	0.2461	0.1752	0.0161	0.8005	4	0.0851	0.0717	0.0049	0.1922

PAHs

13	Naphthalene, 2,6-dimethyl-	581-42-0	789	0.89	1388	1382	n/a	n/a	7	0.0063	0.0016	0.0001	0.0323	4	0.0102	0.0053	0.0031	0.0271
14	Phenanthrene	85-01-8	1047	1.23	1758	1750	n/a	n/a	9	0.0014	0.0012	0.0002	0.0034	4	0.0056	0.0051	0.0045	0.0076

Esters

15	Triethyl citrate	77-93-0	963	0.91	1655	1623	n/a	n/a	1	0.0272	0.0272	0.0272	0.0272	3	0.3906	0.4058	0.2204	0.5455
16	Dodecyl acrylate	2156-97-0	1002	0.65	1670	1682	n/a	n/a	4	0.1264	0.1218	0.0255	0.2367	3	0.7542	0.4118	0.2619	1.5887

Nitrogen containing volatiles

11	Propoxur	114-26-1	927	1.16	1578	1571	n/a	n/a	1	0.0041	0.0041	0.0041	0.0041	3	0.0093	0.0102	0.0069	0.0109

[a]First dimension retention time
[b]Second dimension retention time
[c]Number of subjects compound was detected on
[d]Peak areas were normalised using the TIC of the eucalyptol IS
[e]Number of subjects within a group
[f]n/a = not available
[g]Not detected on subjects within a group

21

The ankle and wrist skin surface data sets of all 20 volunteers were compared using ChromaTOF Statistical Compare software. It is known that African malaria vector mosquitoes show a biting preference towards the lower parts of their human hosts with the selection of biting sites mediated by host odour cues [26, 27, 60, 61]. The comparison yielded a list of 29 lead compounds to be investigated as potential repellents and attractants in vector control strategies (Table 3). A PCA score plot was used to visually demonstrate the differences in the chemical profile between the ankle and wrist skin surface areas for 20 individuals sampled using a non-invasive PDMS sampler with GC inlet TD and GC×GC-TOFMS (Fig. 4). The compounds are from a broad range of chemical classes and were tentatively identified based on mass spectral library matches (≥ 80%) and further confirmed by corresponding first dimension linear retention indices. Of interest, is the detection of the exogenous compound caprolactam, a compound used in the manufacture of Nylon 6, on the wrist skin surface region of 8 individuals. 1-Cyclopentyleicosane was only detected on the wrist skin surface area, whilst 2-ethylhexyl acrylate, *cis*-7-decen-1-al, 2,2'-oxybisethanol, and 2-(dodecyloxy)-ethanol were only detected on the ankle skin surface region, thereby providing potential lead compounds for repellents and attractants.
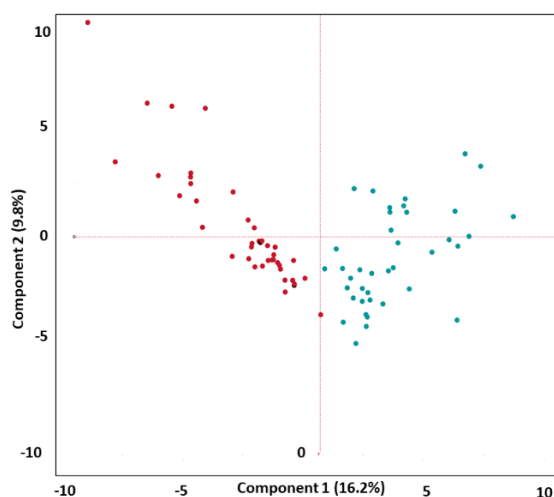
Fig. 4 A principal component score plot graphically demonstrating the variance in skin surface chemical profiles for different skin surface regions of the 20 individuals sampled. Red dots indicate the score for the ankle skin chemical profiles (n=20, m=39) and blue dots indicate the score for the wrist skin chemical profiles (n=20, m=40). Sampling was performed using a passive PDMS sampler and analysed with a GC×GC-TOFMS

Table 3 Compounds tentatively identified during an untargeted analysis of the human ankle skin surface using a non-invasive PDMS sampler with inlet TD-GCxGC-TOFMS. The compounds listed were classified by ChromaTOF Statistical Compare software to contribute to the difference in the ankle and wrist skin surface area of 20 volunteers

#	Compound	CAS Number	1D RT[a] (s)	2D RT[b] (s)	1D RI_exp	1D RI_Lit NIST14	Previously reported on skin	Response reported in mosquitoes	Subject Count[c]	Mean[d]	Median	Range Min	Max	Subject Count[c]	Mean	Median	Range Min	Max
									Ankles (m=20)[e]					Wrists (m=20)[e]				
Alkanes																		
1	Pentadecane, 2-methyl-	1560-93-6	924	0.51	1565	1566	n/a[f]	n/a	16	0.2699	0.2138	0.0532	0.9694	3	2.6588	1.9714	1.4771	4.5279
2	1-Cyclopentyleicosane	0-00-0	1492	0.65	2549	2582	n/a	n/a	0	n.d.[g]	-	-	-	12	2.8610	2.7936	0.2326	6.7467
Alkenes																		
3	Heptacosane	593-49-7	1541	1.16	2700	2690	n/a	n/a	1	0.0568	0.0568	0.0568	0.0568	13	0.0072	0.0037	0.0001	0.0323
Benzyl and phenyl hydrocarbons																		
4	Benzene, (1-butylhexyl)-	4537-11-5	897	0.63	1526	1530	n/a	n/a	12	0.0412	0.0362	0.0053	0.1284	2	0.0998	0.0998	0.0994	0.1002
5	Benzene, (1-ethyldecyl)-	2400-00-2	1053	0.64	1756	1759	n/a	n/a	11	0.2101	0.0716	0.0141	0.7183	12	0.7554	0.1371	0.0198	6.6356
6	Benzene, undecyl-	6742-54-7	1059	0.67	1764	1769	n/a	n/a	4	0.0761	0.0500	0.0259	0.1783	1	0.0709	0.0709	0.0709	0.0709
PAHs																		
7	Naphthalene, 2,6-dimethyl-	581-42-0	790	0.89	1388	1383	n/a	n/a	10	0.0061	0.0033	0.0003	0.0349	9	0.2510	0.0840	0.0128	1.5052
Alcohols																		
8	1-Nonanol	143-08-8	600	0.63	1157	1158	[17]	n/a	11	0.2948	0.2204	0.0301	0.8599	3	0.4125	0.4001	0.1597	0.6777
9	Ethanol, 2,2'-oxybis-	111-46-6	403	0.93	927	947	n/a	n/a	6	0.0968	0.0955	0.0437	0.1636	0	n.d.	-	-	-
10	Ethanol, 2-(dodecyloxy)-	4536-30-5	1015	0.66	1731	1701	n/a	n/a	9	0.1862	0.1546	0.0541	0.3931	0	n.d.	-	-	-
11	Geraniol	106-24-1	671	0.70	1237	1238	[1, 57]	[56]	4	0.9425	0.4488	0.3056	2.5669	12	2.8610	2.7936	0.2326	6.7467
12	Hexadecen-1-ol, *trans*-9-	64437-47-4	1119	0.65	1862	1866	n/a	n/a	14	0.7268	0.4363	0.0103	3.2720	13	0.0262	0.0174	0.0049	0.0818
Aldehydes																		
13	2-Octenal, (*E*)-	2548-87-0	486	0.70	1035	1035	[17]	n/a	18	0.0166	0.0055	0.0015	0.1100	16	0.0236	0.0068	0.0014	0.1565
14	2-Nonenal, (*E*)-	18829-56-6	570	0.70	1135	1126	[1, 3, 34]	n/a	19	0.0063	0.0032	0.0008	0.0302	20	0.0085	0.0061	0.0016	0.0485
15	*cis*-7-Decen-1-al	21661-97-2	615	0.69	1197	1175	n/a	n/a	4	0.0725	0.0476	0.0410	0.1536	0	n.d.	-	-	-

			a	b					c	d				e				
16	Decanal	112-31-2	625	0.65	1185	1185	[1, 17]	[6, 40, 43, 44]	13	0.4939	0.3285	0.0110	1.7216	16	0.1733	0.1258	0.0334	0.5380
17	2-Dodecenal, (E)-	20407-84-5	837	0.68	1448	1446	n/a	n/a	6	0.0466	0.0463	0.0228	0.0752	8	1.3195	1.3731	0.2058	3.0222
Carboxylic acids																		
18	Nonanoic acid	112-05-0	702	0.65	1272	1276	[1, 17]	[41, 46, 47]	3	0.4569	0.2615	0.0793	1.0300	16	2.7452	2.4036	0.4149	5.7651
19	n-Decanoic acid	334-48-5	766	0.66	1362	1354	[1, 17, 34]	[41, 46, 47]	4	0.5564	0.3578	0.2530	1.2569	9	0.7283	0.4776	0.0823	2.0129
20	Dodecanoic acid	143-07-7	919	0.70	1556	1560	[1, 3]	[6, 41, 46, 47]	8	3.7722	2.6782	1.8836	10.1327	3	0.0364	0.0441	0.0113	0.0539
21	Tetradecanoic acid	544-63-8	1053	0.71	1752	1760	[1, 17, 34]	[6, 36, 41, 46, 48-55]	20	0.0107	0.0097	0.0016	0.0358	11	0.4277	0.2245	0.0571	1.5997
22	Pentadecanoic acid	1002-84-2	1112	0.68	1849	1854	[17]	n/a	16	1.2391	0.6662	0.1142	4.1573	10	3.0773	3.1625	0.7721	5.8610
23	n-Hexadecanoic acid	57-10-3	1172	0.71	1954	1953	[1, 17]	[6]	11	2.4025	1.4875	0.1243	5.6328	16	3.8136	3.0388	0.9747	9.5866
24	Heptadecanoic acid	506-12-7	1222	0.69	2039	2041	[17]	n/a	6	0.3583	0.3257	0.0777	0.6647	7	0.2227	0.1920	0.0153	0.4569
25	Octadecanoic acid	57-11-4	1283	0.70	2153	2153	[1, 17]	n/a	16	2.4463	1.4606	0.1855	11.4781	13	0.1311	0.0904	0.0343	0.5123
Esters																		
26	Acetic acid, octyl ester	112-14-1	601	0.61	1193	1159	n/a	n/a	4	0.4313	0.3935	0.2000	0.7379	1	1.0710	1.0710	1.0710	1.0710
27	2-Ethylhexyl acrylate	103-11-7	649	0.61	1215	1212	n/a	n/a	9	0.1890	0.1326	0.0109	0.5425	0	n.d.	-	-	-
Nitrogen containing volatiles																		
28	Cyclobutylamine	2516-34-9	68	0.35	684	700	n/a	n/a	10	0.2262	0.1636	0.0281	0.8945	3	0.7196	0.7756	0.3665	1.0166
29	Caprolactam	105-60-2	671	1.19	1255	1238	n/a	n/a	1	0.0319	0.0319	0.0319	0.0319	8	0.4384	0.0905	0.0042	1.2155

[a]First dimension retention time
[b]Second dimension retention time
[c]Number of subjects compound was detected on
[d]Peak areas were normalised using the TIC of the eucalyptol IS
[e]Number of subjects within a group
[f]n/a = not available
[g]Not detected on subjects within a group

4.3.2.   Targeted analysis

Nineteen VOCs and semi-VOCs were unequivocally identified on the human skin surface using analytical reference standards and a non-invasive sampling technique confirming the presence of alkanes, alkenes, alcohols, aldehydes, carboxylic acids, esters, ketones, and nitrogen containing compounds on the human skin surface. All of the target analytes were previously detected on the human skin surface and 15 of these target analytes, namely phenylethyl alcohol, octanal, (*E*)-2-nonenal, nonanal, (*E*)-2-decenal, propanoic acid, butanoic acid, tetradecanoic acid, hexadecanoic acid, methyl ester, 2-octanone, 2-tridecanone, indole, terpineol, linalool, citronellol, were reported to elicit a response in mosquitoes [6, 7, 12, 36, 40, 41, 43, 44, 46-48, 54, 59, 62-67]. A box-and-whisker plot (Fig. 5) was used to investigate the differences between the wrists and ankle skin surface area for the target analytes detected on the 20 volunteers.

Octanal and tetradecanoic acid, both associated with highly mosquito attractive individuals [36], were more abundant on the wrist skin area. The higher abundance of tetradecanoic acid on the wrist skin surface area is in agreement with findings from Verhulst et al., Roodt et al. and Wooding et al. [6, 17, 34]. The mosquito oviposition volatile, hexadecanoic acid, methyl ester [59], was also more abundant in the wrist region. The mosquito semiochemical phenylethyl alcohol was more abundant on the ankle skin regions compared to the wrist skin regions. This compound is reportedly associated with mosquito unattractive individuals, specifically for *An. gambiae* mosquitoes [36]. However, in this current study phenylethyl alcohol was almost three times more abundant on the ankle skin surface area of perceived attractive individuals. Furthermore, the plant mosquito semiochemicals, linalool, terpineol and citronellol [56], were more abundant on the ankle skin surface area, with linalool and citronellol 2.4 and 3.7 times, respectively, more abundant on perceived attractive individuals. Conversely, terpineol was 2.4 times more abundant on the ankle skin surface area for perceived non-attractive individuals. Terpineol and citronellol elicited an antennal sensilla response in *Ae. aegypti* mosquitoes, but whether the response was positive, i.e. attractive, was not investigated [56]. To note is the plant compound linalool which when used on its own is attractive to *Ae. aegypti* mosquitoes, however, when combined with CO_2 or with CO_2 and octenol reduced mosquito catches

thus acting as a spatial repellent [68]. These findings highlight the necessity of investigating synergism and concentration effects when studying mosquito-host semiochemicals. The large variation, not only in terms of the number of compounds but also in the levels at which compounds are present, between the 20 individuals underpin the intra-species complexity of the skin surface, which is not surprising given the complexity of the skin microbiome.
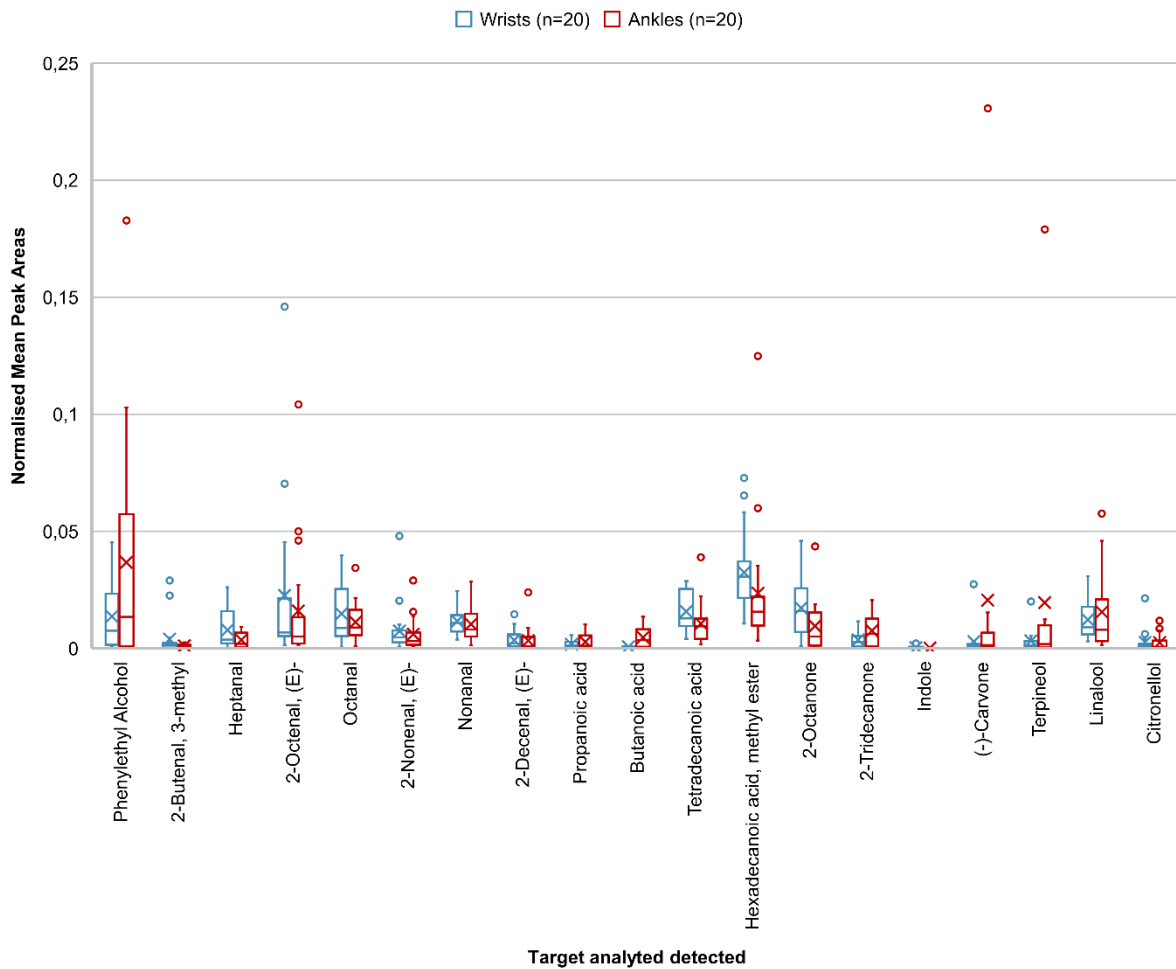
Fig. 5 Box-and-whisker plot of the mean peak areas (TIC) of the 19 target analytes detected on the wrist (blue) and ankle (red) skin surface area of 20 volunteers during a one-hour sampling period. Mean peak areas (indicated by a cross) were normalised using the TIC of an eucalyptol IS. Outliers are represented by circles

4.4. Conclusion

Volatile human skin compounds are used by female anthropophilic mosquitoes to locate their blood-host. This study showed the considerable capability of a non-invasive passive sampling approach as a tool to investigate mosquito-host relationships and consequently the ability to discover new biomarkers to be used in novel vector control strategies. The sampling procedure proved non-invasive, simple and easy when compared to obtrusive and cumbersome active sampling approaches. The complexity of the skin volatolome, further complicated by the skin microbiome, was addressed by employing an in-house constructed PDMS sampler followed by direct TD in the GC inlet liner with GC×GC-TOFMS. Comprehensive two-dimensional gas chromatography can separate thousands of chromatographic peaks making it ideal for the analysis of complex biological matrices. By employing statistical software the skin chemical profiles of 20 volunteers were compared based on their perceived mosquito attractiveness, mosquito biting-site preference, and the different skin regions sampled. A broad range of chemical compounds (69 compounds in total), amenable to PDMS extraction, was detected and tentatively identified on the human skin surface. Thirty-one compounds detected have not been previously reported on the human skin surface to the authors' knowledge. Nineteen compounds were unequivocally identified on the human skin surface of the 20 volunteers. Identification of chemical compounds that contributed to the differences in inter- and intra-human surface skin regions sampled was demonstrated. Thirteen of the skin surface compounds detected are known mosquito semiochemicals, confirming the potential of the method to benefit the development of attractants and repellents in push-and-pull vector control strategies. The ability of the analytical approach in identifying new mosquito attractants and repellents was shown, paving the way for future work on larger sample sets to expand on these current findings, and for exploring alternative means to confirm the degree of mosquito attractiveness of volunteers.

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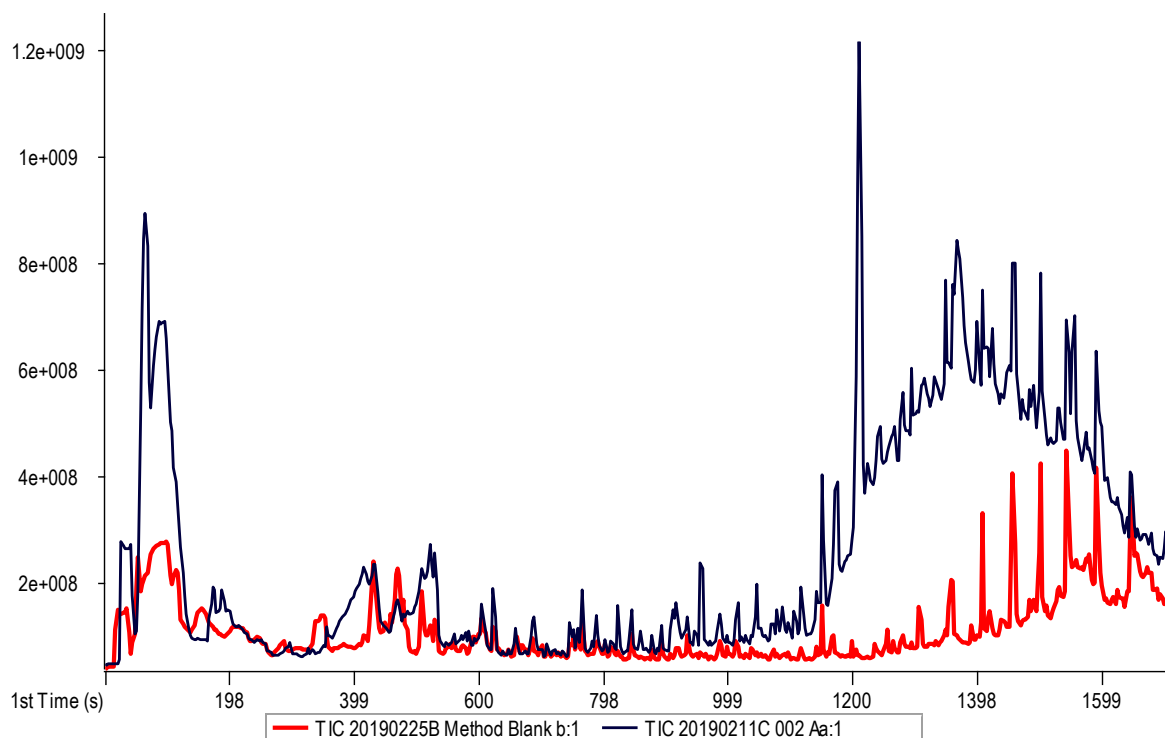
Fig. S1 1D total ion chromatogram. Representative ankle surface skin sample (black trace) overlaid with method blank sample (red trace)
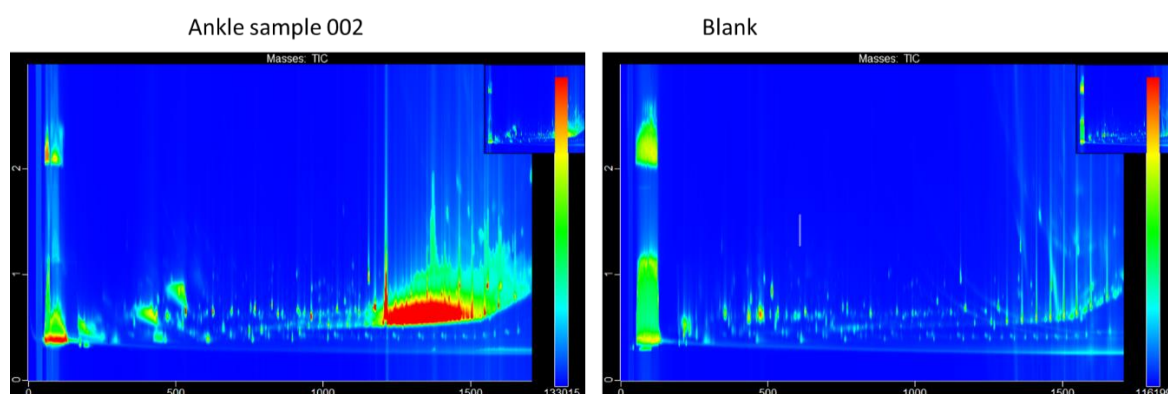
Fig. S2 Contour plot of a total ion chromatogram of a representative ankle surface skin sample (left) and a method blank sampler (right). All data reported have been background subtracted to account for any potential laboratory background compounds