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Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components

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Abstract. Afrotropical malaria vectors of the *Anopheles gambiae* complex (Diptera: Culicidae), particularly *An. gambiae sensu stricto*, are attracted mainly to human hosts. A major source of human volatile emissions is sweat, from which key human-specific components are the carboxylic acids (*E*)- and (*Z*)-3-methyl-2-hexenoic acid and 7-octenoic acid. Electrophysiological studies on the antennae of *An. gambiae s.s.* showed selective sensitivity to these compounds, with a threshold at 10^{-6} g comparable to that of known olfactory stimulants 1-octen-3-ol, *p*-cresol, isovaleric acid, and lower than threshold sensitivity to L-lactic acid and the synthetic mosquito repellent *N*,*N*-diethyltoluamide (DEET). A combination of the acids released at concentrations $> 10^{-5}$ g in wind tunnel bioassays significantly reduced the response to CO_2, the major attractant released by human hosts, for strains of *An. gambiae s.s.* originating from East and West Africa. Field trials with odour-baited entry traps (OBETs) in Burkina Faso showed that 7-octenoic acid significantly increased (by 1.7-fold) the catch of females of *An. gambiae sensu lato* (comprising two sibling species: *An. arabiensis* Patton and *An. gambiae s.s.*) in OBETs baited with CO_2, whereas combinations of the acids significantly reduced the catch in CO_2-baited traps (by 2.1-fold) and in whole human odour-baited traps (by 1.5-fold). The pure (*E*) and (*Z*) geometric isomers of 3-methyl-2-hexenoic acid gave comparable results to the (*E*/*Z*) isomer mixture. These results provide the first experimental evidence that human-specific compounds affect the behaviour of highly anthropophilic *An. gambiae s.l.* mosquitoes. The compounds appear to inhibit the 'upwind flight' response to known long-range attractants, and may serve either to 'mask' the attractants present or, more probably, to 'arrest' upwind flight when mosquitoes arrive at a host under natural conditions. In the final approach to hosts, vectors are known to reduce their flight speed and increase their turning rate, to avoid overshooting the source. In our experimental apparatus, these changes in flight behaviour would reduce the number of mosquitoes entering the ports of the collection devices.

Key words. *Anopheles gambiae*, 7-octenoic acid, behaviour, (*E*)-3-methyl-2-hexenoic acid, electroantennogram, odour-baited entry trap, malaria mosquito, (*Z*)-3-methyl-2-hexenoic acid, semiochemical, wind tunnel, Burkina Faso.

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Introduction

Malaria causes high levels of human suffering and mortality, with over one million deaths per year in sub-Saharan Africa alone. The tremendous transmission potential of some members of the Afro tropical *Anopheles gambiae* species complex (Diptera: Culicidae), namely *An. gambiae* Giles s.s. and *An. arabiensis* Patton, contributes to the maintenance of high levels of endemicity across most of the African continent. The high vectorial capacity of these mosquitoes is especially due to their strong preference for human hosts (with exceptions, cf. Duchemin *et al*., 2001). The mechanisms by which these vectors select human hosts, as distinct from other warm-blooded vertebrates, and why individuals vary in their attractiveness, are largely unknown.

Many aspects of mosquito behaviour, including host location and oviposition, are mediated by detection of volatile semiochemicals (Pickett & Woodcock, 1996; Gibson & Torr, 1999; Takken & Knols, 1999). Blood-feeding mosquitoes generally show a marked attraction to carbon dioxide (CO_2), universally present in mammalian breath. For highly anthropophilic *An. gambiae* mosquitoes, however, CO_2 accounts for only approximately 50% of the attractancy of a human host (Costantini *et al*., 1996), and it is thought that other cues emanating from the host are also involved (Costantini *et al*., 1998). The exact role and chemical nature of these cues remain largely undefined. *Anopheles gambiae* s.s. respond to volatiles reminiscent of human foot odour (de Jong & Knols, 1995), to Limburger cheese (perceived to have a similar odour; de Jong & Knols, 1995) and to a synthetic blend of the co-occurring fatty acid components when diluted (Knols *et al*., 1997), but are repelled by the same blend when tested undiluted (Knols *et al*., 1997). Repeat experiments using the same experimental apparatus and stock solutions, however, did not confirm the 'attractiveness' of the diluted synthetic blend (Braks, 1999). Some components from human sweat which are electrophysiologically active for *An. gambiae* have been identified (Cork & Park, 1996; Meijerink *et al*., 2000) and in-depth studies on single sensillum recordings have been made (Meijerink & van Loon, 1999; van den Broek & den Otter, 2000). Also, human sweat incubated for 1–2 days, but not fresh, has been shown to be attractive (Braks & Takken, 1999; Meijerink *et al*., 2000). From these studies, however, only compounds that are released generally by warm-blooded vertebrates have been identified and studied in the laboratory, thus it is unlikely that these chemical cues alone fully account for the highly anthropophilic behaviour of *An. gambiae s.s*. in the field.

The human body surface excretes many compound types, a proportion of which contribute to overall human volatile emissions (Sastry *et al*., 1980). The excreted compounds are modified through microbial action, particularly on the essentially odourless sweat secreted from the apocrine glands situated in the axillary regions (e.g. armpits) (Stoddart, 1990). The most abundant components that are specific to human beings have been identified during studies relating to human hygiene as (*E*)- and (*Z*)-3-methyl-2-hexenoic acid and 7-octenoic acid (Leyden *et al*., 1981; Zeng *et al*., 1991). Since these carboxylic acids are specific to human volatile emissions, they would present anthropophilic mosquitoes with human-specific cues. Thus, the aim of this study was to synthesize these compounds and investigate their role as components of human-specific kairomones, initially by electrophysiological studies on the antennae of *An. gambiae s.s*. Specific electrophysiological sensitivity suggested a behavioural role, which was investigated further, first with an established wind tunnel laboratory assay (Miller & Gibson, 1994; Mohammed, 1997), comparing the response to a known attractant, CO_2, with the response to the test chemicals, and then in the field with odour-baited entry traps (OBETs), as for previous dual-choice protocols (Costantini *et al*., 1998; Duchemin *et al*., 2001).

Materials and methods

Mosquitoes

For electroantennogram (EAG) studies, *An. gambiae s.s*. mosquitoes (strains unspecified) were obtained from the European Molecular Biology Laboratories, Heidelberg, Germany, or Wageningen Agricultural University, Netherlands. Recordings were made from 2- to 14-day-old females that had previously shown feeding attempts. For wind tunnel assays, two strains of *An. gambiae s.s*. were obtained from the London School of Hygiene and Tropical Medicine: 'G3' originating from The Gambia type-locality, and 'KIL' from Marangu, Kilimanjaro, Tanzania. The colonies were maintained at 27–28°C and 80–90% relative humidity with LD 12 : 12 h photo/scotophase. Eggs were laid on wet filter paper, hatched in trays of dilute saline solution and larvae were fed Tetramin™ fish food. Pupae were collected from the trays and allowed to emerge in netted cages. Adults were kept in the cages and fed on 10% (v/v) sucrose solution. Females were offered sterile defibrinated horse blood (TCS microbiology, Botolph Claydon, Buckinghamshire, U.K.) every 2–3 days for egg production. Females aged 3–7 days that had been denied a bloodmeal, but had access to sucrose, were assayed in the wind tunnel.

Chemicals

3-Methyl-2-hexenoic acid, as a 3 : 1 mixture of (*E*) and (*Z*) isomers with > 99% purity was synthesized via condensation of triethylphosphonoacetate and 2-pentanone, followed by saponification (Zeng *et al*., 1991). (*E*)-3-Methyl-2-hexenoic acid (> 99% purity) was isolated by recrystallization of the (*E*/*Z*) mixture from petroleum ether (boiling point 60–80°C). (*Z*)-3-Methyl-2-hexenoic acid (> 99% purity) was synthesized following the method of Ramsby (1971), but with appropriate materials for (*Z*)-isomer synthesis, i.e. reaction of lithium dimethyl cuprate with ethyl 3-hexynoate, followed by saponification. 7-Octenoic acid (> 99% purity) was synthe-

sized by condensation of acetic acid with 6-bromohex-1-ene (Zhang *et al*., 1991). Other chemicals used in electroantennogram studies were obtained commercially (Aldrich Chemical Company, Gillingham, Kent, U.K.) and all were diluted in pentane or diethyl ether prior to use.

Electroantennography

Whole female insects were immobilized using CO_2 and permanently fixed with Spectra 360 electrode gel (Parker Laboratories Inc., Orange, NJ, U.S.A.) directly onto the reference electrode. The gel covered most of the head and thorax, immobilizing them completely, but leaving the antennae free to move. The tips of both antennae were pushed into a small drop of gel on the recording electrode. This immobilized the antennae and allowed stable electrical contact. Electrodes were silver wire (0.2 mm diameter) coated with silver/silver chloride. The preparations were held in a continuous air stream (600 mL/min, 1.5 m/s) originating from a Teflon tube (0.7 cm internal diameter), consisting of 500 mL/min humidified carrier stream and 100 mL/min dry air, both charcoal filtered. During stimulation, the tip of a pipette was inserted into the Teflon tube through a small hole, and the dry air was switched to the pipette with the sample. Signals were passed through an amplifier and analysed using a customised software package (Syntech, Hilversum, Netherlands). To confirm electrophysiological activity of antennae, 1-octen-3-ol, 4-methylphenol (*p*-cresol), isovaleric acid, and L-lactic acid, all known olfactory stimulants (Cork & Park, 1996), were used. Samples of standard solutions of the known stimulants and the human-specific acids (10^{-5}–10^{-9} g per 10 μL) were applied to filter paper squares (1 cm^2) and the filter paper placed in a glass Pasteur pipette. For the known stimulants, all results were expressed as a mean percent response to the control (room air, mean response = 100 ± 10%, $n = 8$ preparations). The amplitude of the responses of individual mosquitoes to human-specific acids was extremely variable when compared to repeated stimulations with one mosquito, so these results were expressed as a mean percent response to *p*-cresol (10^{-6} g) [mean response to control (room air) = 71 ± 14%, $n = 24$ preparations]. Results were analysed for significant differences by Student's *t*-test ($P < 0.01$).

Wind tunnel bioassays

A dual choice wind tunnel bioassay was used to evaluate the response of *An. gambiae s.s*. to the human-specific acids. The general protocol was to release a group of mosquitoes at the downwind end of a wind tunnel in the air-stream coming from two upwind chambers, each of which emitted different stimuli. After 20 min, the numbers of mosquitoes in each of the two upwind chambers were counted. The mosquitoes were then drawn back to the downwind end of the tunnel and the odour cues were changed. Again, the mosquitoes were left to respond to the new cues and the number in each upwind chamber was counted after 20 min, and the mosquitoes were drawn back to the downwind end.

Anopheles gambiae generally fly upwind in wind tunnels, even in the presence of only clean air. Thus, the 'control' stimuli in these choice tests was always clean air and the 'treatment' stimuli, either (i) 'CO_2 on its own', or (ii) 'CO_2 plus the human-specific acids', were released from the other chamber. The difference in the number of mosquitoes entering each chamber was a measure of the degree to which the 'test stimuli' altered the inherent tendency for mosquitoes to fly upwind.

Since CO_2 is a known attractant (Gillies, 1980), the response to 'CO_2 on its own' was used as the measure of a standard response to a known attractive stimulus, and tested both before and after the response to 'CO_2 plus human-specific acids' was tested, to obtain a baseline response.

The wind tunnel, similar to the one described previously (Miller & Gibson, 1994), consisted of a flight area (172 cm long, 45 cm wide, 45 cm high) with a gentle flow of clean air (0.1 m/s) which had been passed through a charcoal filter and an air-conditioning unit to bring the air to 25 ± 1°C and 70 ± 10% humidity. The last 6 cm of the upwind end was divided into two collecting chambers by a vertical wall of clear perspex. Test stimuli were released from either of the two upwind chambers, alternating for each run. Carbon dioxide was released through tubing from a pressurized cylinder at a level similar to that found in human breath (400 mL/min). The acids solutions (10 μL each per individual filter paper) were presented on a filter paper attached to one end of a metal rod, then inserted through an opening into the collection chambers and allowed to hang at the level of CO_2 release (i.e. 25 cm above the floor). A filter paper (solvent only) was inserted into the other, control chamber, through which only clean air flowed.

KIL strain mosquitoes were tested across a range of dilutions (10^{-5}–10^{-11} g in diethyl ether), whilst the G3 strain was tested at 10^{-5} g only. Before each run, a new batch of 25 female mosquitoes was released into the downwind end of the wind tunnel, and left for 20 min to acclimatize. Stimulus (i) 'CO_2 on its own', was added to the air-stream of one of the upwind chambers, and the numbers in each chamber were counted at the end of 20 min. The mosquitoes were drawn back down the wind tunnel with a light and left for 20 min. The filter papers were added to the chambers, as described above for stimulus (ii) 'CO_2 plus acids', and again the numbers in each chamber were counted at the end of 20 min, and the mosquitoes drawn downwind as before. Finally, to ensure that the acids had not altered the mosquitoes' sensory responsiveness or behavioural output, the response of the same mosquitoes to stimulus (i) 'CO_2 on its own' was measured again. It was clear from subsequent analysis that there was no significant difference between the response to CO_2 before and after exposure to the acids, so these data were pooled, to arrive at a mean response to CO_2.

The difference in the number of mosquitoes in each chamber is the response variable, as this single number reflects the behaviour of all the responding mosquitoes, thus providing an overall estimate of 'responsiveness'. Fortunately, there was no

Table 1. Response of female *Anopheles gambiae s.l.* mosquitoes (*An. arabiensis* + *An. gambiae s.s.*) to human-specific acids in a dual-choice field assay (n = number of nights, i.e. replicates). A random choice is indicated by a 0.5 mean proportion, with a higher or lower figure indicating a greater or lesser response to the acids, respectively; NS = $P > 0.05$.

Treatment	Control	n	Total catch	Mean proportion (95% confidence limits)	P
(*E*/*Z*)-3-Methyl-2-hexenoic acid + CO_2	CO_2	15	420	0.47 (0.37–0.57)	NS
(*E*/*Z*)-3-Methyl-2-hexenoic acid + human odour	Human odour	12	2817	0.43 (0.34–0.52)	NS
7-Octenoic acid + CO_2	CO_2	13	550	0.63 (0.53–0.73)	< 0.02
7-Octenoic acid + human odour	Human odour	12	5296	0.51 (0.42–0.60)	NS
(*E*/*Z*)-3-Methyl-2-hexenoic acid + 7-octenoic acid + CO_2	CO_2	14	4047	0.36 (0.28–0.45)	< 0.01
(*E*/*Z*)-3-Methyl-2-hexenoic acid + 7-octenoic acid + human odour	Human odour	10	3272	0.40 (0.31–0.49)	< 0.03
(*E*)-3-Methyl-2-hexenoic acid + 7-octenoic acid + CO_2	CO_2	10	685	0.34 (0.28–0.40)	< 0.001
(*Z*)-3-Methyl-2-hexenoic acid + 7-octenoic acid + CO_2	CO_2	12	344	0.27 (0.21–0.35)	< 0.001

significant effect of the odours on the proportion of mosquitoes flying upwind in these experiments, with a mean of 20.5 out of 25 responding, so the number of mosquitoes found downwind of the chambers at the end of each assay was disregarded.

For the statistical analysis of the difference in response to 'CO_2 on its own' with the response to 'CO_2 plus acids', the following calculations were performed:

x = mean difference in the number of mosquitoes in the 'clean air' control and 'CO_2 on its own' chambers (mean of the two CO_2 tests).

y = difference in the number of mosquitoes in the 'clean air' control and 'CO_2 plus acids' chambers.

If the acids do not alter the response to CO_2, then $(y - x) = 0$ is expected. If the acids cause a greater number of mosquitoes to enter the 'CO_2 plus acids' chamber than enter the 'CO_2 on its own' chamber, then $(y - x)$ would be > 0, and if the acids cause fewer mosquitoes to enter the 'CO_2 plus acids' chamber than enter 'CO_2 on its own' chamber, then $(y - x)$ would be < 0.

Each dilution was tested 10 times, giving a mean difference in response of $\overline{(y - x)}$, with $n = 10$, for each dilution. The data were subjected to a Student's t-test of the null hypothesis that the acids had no effect, i.e. $\overline{(y - x)} = 0$.

Field bioassays

Field assays using odour-baited entry traps (OBETs) as for previous dual-choice protocols (Costantini *et al.*, 1998; Duchemin *et al.*, 2001) were set up at the end of the rainy season in proximity to compounds of the rural village of Goden (12°25′N, 1°21′W), near Ouagadougou, the capital of Burkina Faso. Each of the human-specific acids (20 μL) were placed in separate borosilicate glass vials (Chromacol Ltd, Welwyn Garden City, Hertfordshire, U.K.) fitted with polyethylene caps with 1 mm diameter holes. These lures gave evaporation rates similar to those found for human beings (Zeng *et al.*, 1992). The lures were then presented either individually or in various combinations (see Table 1). The acid(s) were released with either 'pure CO_2' (flow-rate was 2000 mL/min), or 'whole human odour' (which includes the natural release rate of CO_2 with breath) provided by an individual adult male sleeping inside a tent. The controls were 'pure CO_2' or 'whole human odour', respectively. Control odours were released from a host-holding tent and delivered into both the 'control' and 'treatment' OBETs, while the acids were released by putting the borosilicate vials into the air-stream of the polythene tubing leading to the 'treatment' OBET. Empty control borosilicate vials were put into the other compartment leading to the 'control' OBET. The mean speed of the air currents coming out of both OBETs was set at 50 cm/s with a hot-wire anemometer. The relative abundance of *An. gambiae sensu lato* mosquitoes (comprising two species, *An. gambiae s.s.* and *An. arabiensis* in roughly equal frequencies) caught in OBETs was assessed as the mean proportion collected in the 'treatment' OBET, where a random choice was expressed by a 0.5 mean proportion, with a higher or lower figure indicating a greater or lesser response to the acids, respectively. In this way, the air currents from the left and right OBETs differed only for the presence/absence of the acids. The number of replicates (i.e. the number of trapping nights) for each experiment ranged 12–15 (Table 1). Anopheline mosquitoes were identified using the key of Gillies & Coetzee (1987). Statistically significant departures from the random choice were tested for by Student's t-tests on the parameter estimates of a logistic regression model using the software GLIM (Payne, 1987).

Results

Electrophysiological activity

Initially, EAG responses of female *An. gambiae* s.s. to known mosquito olfactory stimulants 1-octen-3-ol, 4-methylphenol (*p*-cresol), isovaleric acid and L-lactic acid were measured. Dose-response curves to these compounds are shown in Fig. 1. All compounds led to significant responses with respect to room air (mean response = 100 ± 10%), with a threshold dose of 10^{-6} g for 1-octen-3-ol and *p*-cresol, and 10^{-5} g for isovaleric acid and L-lactic acid. Both of the human-specific acids showed significant activity with respect to room air (mean response = 71 ± 14%), with a threshold dose of 10^{-5} g, with 7-octenoic acid giving a slightly greater response than

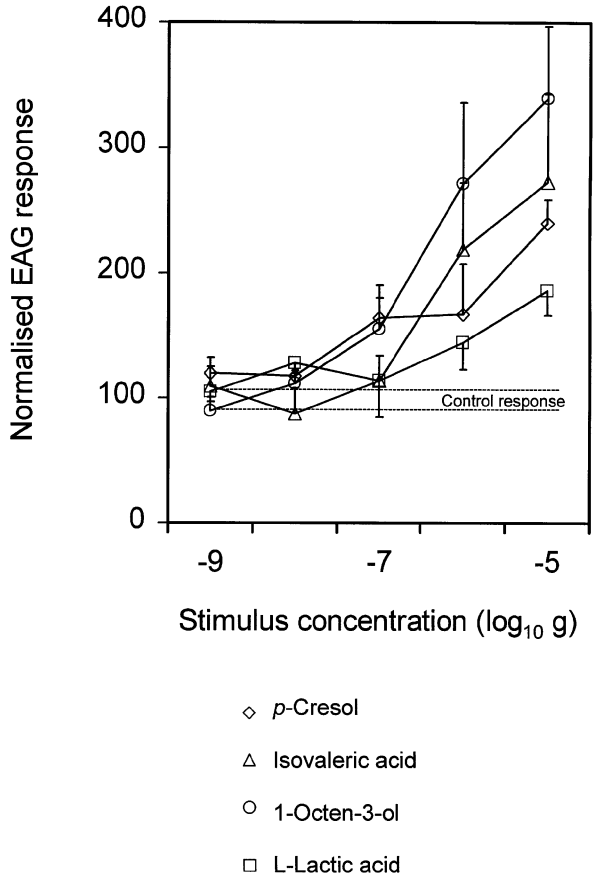

Fig. 1. Electroantennogram (EAG) responses of female *Anopheles gambiae* s.s. mosquitoes to known mosquito semiochemicals (means of eight preparations ± SE). Results expressed as a percentage of the response to the control (room air; 100 ± 10%).

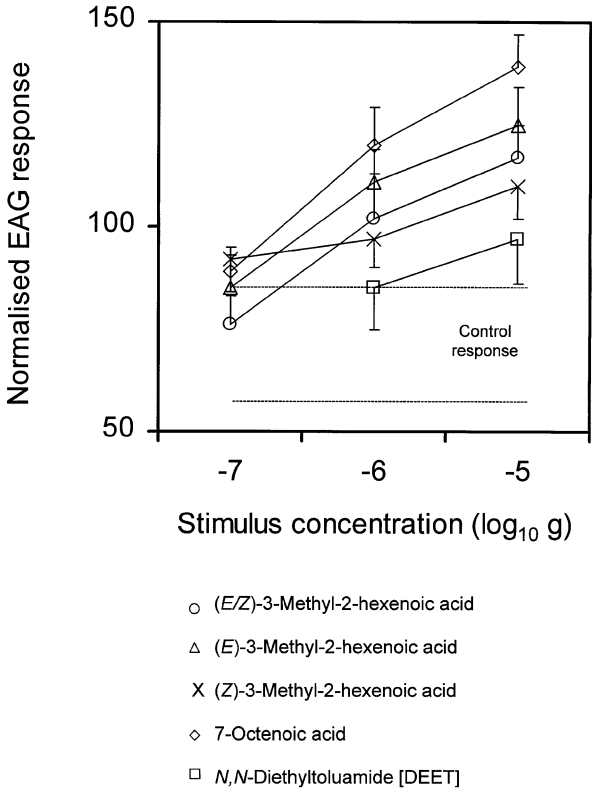

Fig. 2. Electroantennogram (EAG) responses of female *Anopheles gambiae* s.s. mosquitoes to human-specific acids (means of 24 preparations ± SE). Results expressed as a percentage of the response to *p*-cresol (10^{-6} g). Response to control (room air) = 71 ± 14%.

the (*E*/*Z*) isomer mixture of 3-methyl-2-hexenoic acid (Fig. 2). Responses to the two pure (*E*) and (*Z*) isomers were of similar magnitude to the isomer mixture. The synthetic mosquito repellent *N*,*N*-diethyltoluamide (DEET) gave lower responses than the acids, with a higher threshold dose of 10^{-4} g.

Behaviour in the laboratory

In wind tunnel bioassays, significantly fewer female *An. gambiae* s.s. mosquitoes chose the chamber releasing CO_2 plus the human-specific acids [a combination of the (*E*/*Z*)-3-methyl-2-hexenoic acid isomer mixture and 7-octenoic acid], when offered undiluted and in doses down to 10^{-5} g (Table 2). Similar responses were found at the 10^{-5} g dose for two strains of the mosquito, 'KIL' (East Africa) and 'G3' (West Africa). Although the proportion of mosquitoes responding by flying upwind towards the odour sources was not affected by the chemicals, it appears that the ability of the mosquitoes to fly upwind towards the source of CO_2 plus acids was adversely affected.

Behaviour in the field

In the dual-choice field assays, 7-octenoic acid significantly increased the trap catch of CO_2, but no other individual treatments gave significant differences between the treatment and control OBETs (Table 1). When 7-octenoic acid was presented with the (*E*/*Z*)-3-methyl-2-hexenoic acid isomer mixture, however, the treatment trap catch was significantly less than the control trap catch for both 'pure CO_2' and 'whole human odour' treatments. The pure (*E*) and (*Z*) isomers of 3-methyl-2-hexenoic acid gave similar results to the (*E*/*Z*) isomer mixture (Table 1).

Discussion

Detection of volatile semiochemicals by mosquitoes occurs mainly via the olfactory neurones on the antenna (Pickett & Woodcock, 1996). In earlier studies on *An. gambiae* s.s., electroantennogram (EAG) responses of whole antennae to compounds identified from human sweat and carboxylic acids identified from Limburger cheese, were recorded (Cork & Park, 1996; Knols *et al*., 1997; Meijerink *et al*., 2000). At the

Table 2. Response of female *Anopheles gambiae* s.s. mosquitoes to (*E*/*Z*)-3-methyl-2-hexenoic acid and 7-octenoic acid mixtures in a dual-choice wind-tunnel bioassay ($n = 10$). Control = clean air. Results are expressed as the mean difference in response to 'CO_2 alone' and 'CO_2 plus human-specific acids' = $(\overline{y - x})$. NS = $P > 0.05$

Mosquito strain	Dose (g)	$(\overline{y - x})$	SE	*P*
KIL	10^{-3}*	–2.90	1.00	< 0.02
KIL	10^{-5}	–2.50	0.93	< 0.03
KIL	10^{-7}	–0.25	0.78	NS
KIL	10^{-9}	–0.30	1.18	NS
KIL	10^{-11}	–0.05	0.83	NS
G3	10^{-5}	–2.75	1.13	< 0.04

*Undiluted.

single cell level, recordings for receptors responding to aliphatic carboxylic acids were reported (Meijerink & van Loon, 1999; van den Broek & den Otter, 2000). Although Healy & Copland, 2000) recently reported a study of human sweat composition and landing responses by *An. gambiae* s.s., human-specific compounds were not identified, and a synthetic mixture of 22 of the identified carboxylic acids was inactive. 2-Oxopentanoic acid was shown to elicit a landing response, but the relationship between host acceptability and this particular compound was not discussed, and was only tested because of its structural similarity to L-lactic acid (Carlson *et al*., 1973), and the identification of certain oxoacids found in human blood and urine (Hoffman *et al*., 1971; Chalmers & Lawson, 1982). In our study, significant EAG responses were first obtained for the known olfactory stimulants 1-octen-3-ol, 4-methylphenol (*p*-cresol), isovaleric acid and L-lactic acid (Fig. 1). Having confirmed the validity of the EAG technique, antennal detection of human-specific axillary volatile components (*E*) and (*Z*)-3-methyl-2-hexenoic acid and 7-octenoic acid was exhibited (Fig. 2). The results from the laboratory behavioural (Table 2) and field bioassays (Table 1) show that the host-seeking behaviour of female *An. gambiae* s.l. mosquitoes is significantly affected by (*E*/*Z*)-3-methyl-2-hexenoic acid and 7-octenoic acid, the two major volatile components produced in the axillary region in human beings. This is the first demonstration of human-specific acids significantly influencing the behaviour of *An. gambiae* in the field. It is worth noting that the behavioural effects observed were dependent on the combination of odours released, demonstrating the capacity of *An. gambiae* s.l. to modulate its behaviour in response to blends of odours. This is particularly relevant when interpreting tests with single compounds; the activity of (*E*/*Z*)-3-methyl-2-hexenoic acid would have been missed if it had not been tested in combination with 7-octenoic acid.

Clearly, it is at first sight disappointing that the effect of the human-specific compounds was apparently either to repel *An. gambiae*, or perhaps to 'mask' the effect of the known attractants 'pure CO_2' and 'whole human odour'. There are examples of insects avoiding energy-wasting attempts to feed on, or colonize, hosts upon which they cannot successfully develop, by responding to components of unsuitable hosts (Pickett *et al*., 1998). It has been hypothesized that this may be a mechanism by which haematophagous insects avoid nonhosts, and there is evidence that cattle odour reduces the response of *An. gambiae* s.s. and *An. arabiensis* to CO_2 (Costantini *et al*., 1998). Thomas *et al*. (1987, 1989) have shown that individual animals within a breed of cattle are less attractive to flies (Muscidae) that cause a nuisance to cattle and are vectors for some cattle pathogens (e.g. summer mastitis). The horn fly, *Haematobia irritans*, and the head fly, *Musca autumnalis*, detect a number of cues present in the volatile profiles of individual cattle, and differences in volatile profile are responsible for the differential attractiveness of hosts (Birkett *et al*., 1998). This selectivity of individual hosts could be accomplished by either outright repellency of some components, or by reducing sensitivity (modification of input signal) or responsiveness (modification of outputs) to the normal spectrum of volatiles emitted by individual hosts, thus 'masking' the presence of a host. The volatile profile of human beings constitutes a complex range of organic compounds, dominated by the components produced in the axillary regions, with the exact odour profile of individuals varying like fingerprints. Mosquitoes may be able to use the differences in the odour profile of individuals to determine the suitability of a particular odour source, and alter their responses to the odours accordingly.

There is a risk, however, of misinterpreting the behavioural responses observed in the wind tunnel experiment and the OBET dual-choice tests. Interpretation of results derived from behavioural assays must consider the range of behavioural repertoires that can be potentially elicited, compared with the observed behaviour under specific experimental conditions (Costantini, 1996). Both assays were related to the strength of the anemotactic response (i.e. the tendency of mosquitoes to fly against an air current), which is normally strengthened when appropriate host cues are encountered. Under natural conditions, this is the usual response of *An. gambiae* at a distance from the host. When in close proximity to the source of odours, however, host-related cues must antagonize this anemotactic response and 'arrest' upwind progress, if the mosquito is not to overshoot the source of semiochemicals. This 'arrestment' behaviour has been recorded for tsetse flies, and includes measurable changes in flight behaviour, such as a reduction in flight speed and an increase in the strength of its klinokinetic responses to host cues, until landing responses are triggered (Warnes, 1990; Gibson *et al*., 1991). In particular, 1-octen-3-ol (an ox-emitted chemical) is attractive to tsetse when used at the appropriate doses in traps or on treated targets (Hall *et al*., 1984), although, unlike CO_2, at concentrations at or above those found in ox breath, it significantly reduces the flight speed and increases the flight sinuosity of tsetse, with virtual loss of upwind anemotaxis at very high concentrations (Paynter & Brady, 1993). Thus, the decrease in catch for *An. gambiae* in the traps/chambers releasing human-specific acids could be related to an increase in negative orthokinetic and positive klinokinetic components of flight, consequently deviating some of the approaching mosquitoes away from

the ports of the collection apparatus. Hence, the two human-specific acids may well play an essential part in the positive identification of appropriate hosts for anthropophilic mosquitoes, such as *An. gambiae*. Laboratory experiments expressly aimed at measuring short-range behavioural responses are needed to resolve this issue.

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