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Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours

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Summary

Female mosquitoes are noted for their ability to use odours to locate a host for a blood meal. Two sensory organs contribute to their sense of smell: the maxillary palps, which measure the level of CO_2, and the antennae, which detect other host-released odours. To establish the relative importance and interactions of CO_2 and other body emissions in freely flying mosquitoes, we presented female yellow fever mosquitoes *Aedes aegypti* L. with broad plumes of human skin odour and CO_2 at natural concentrations and dilutions thereof in a wind tunnel. 3-D video-recorded flight tracks were reconstructed. Activation, flight velocity, upwind turning and source finding waned quickly as skin odours were diluted, whereas in the presence of CO_2 these parameters remained unchanged over more than a 100-fold dilution from exhaled concentrations. Although mosquitoes were behaviourally less sensitive to skin odours than to CO_2, their sensitivity to skin odours increased transiently by at least fivefold immediately following a brief encounter with a filament of CO_2. This sensitization was reflected in flight velocity, track angle, turning rate upon entering and exiting the broad odour plume and, ultimately, in the source-finding rate. In *Ae. aegypti*, CO_2 thus functions as a 'releaser' for a higher sensitivity and responsiveness to skin odours. The initially low responsiveness of mosquitoes to skin odours, their high sensitivity to CO_2, and the sensitization of the olfactory circuitry by CO_2 are ecologically relevant, because rapidly fluctuating CO_2 levels reliably signal a potential host. Possible mechanisms of the instantaneous sensitization are considered.

Key words: *Aedes aegypti*, mosquito, orientation, sensitisation, host odour, carbon dioxide, human skin odour.

Introduction

Mosquitoes are remarkable for their ability to locate a blood meal using host-emitted odour. However, their orientation behaviour in response to these complex blends of host odours has not been analyzed in detail. In contrast, the orientation of male moths to female pheromone has been well studied. Pheromones are unique, simple messages of one or several components, often in a specific ratio, which are used in conspecific mate recognition and orientation. Flying male moths respond to single, very brief (10 ms) encounters with pheromone by surging upwind (Mafra-Neto and Cardé, 1994, 1998; Vickers and Baker, 1994; Justus et al., 2002), and are able to tell the difference between pheromone and antagonists (pheromone components of another species that reduce attraction) encountered within as little as 1 ms of each other (Baker et al., 1998). Male moths also respond to their pheromone over a large concentration range (Cardé and Charlton, 1984), which is ecologically relevant as the presence of pheromone reliably indicates the presence of an upwind, calling female.

In mosquitoes, however, many different classes of sensory neurons are involved in host odour detection, and the odour mix that emanates from a host is very complex, consisting of several hundred compounds (Bowen, 1996; Meijerink and Van Loon, 1999; Pappenberger et al., 1996). The cues picked up by the antenna are ambiguous, as many odours are not specific to a vertebrate host, remain present in the absence of a live host (such as inside houses), and vary among individuals of the same host species and from day to day (Bernier et al., 2001). Filtering and integration of odour mixtures should be exceedingly important for host recognition by mosquitoes.

In contrast, exhaled CO_2, a nearly universal mosquito activator and attractant (Rudolfs, 1922; Reeves, 1953; Gillies, 1980), is diluted against atmospheric CO_2 levels (background around 0.035%, *vs* 4% for exhaled CO_2). Fluctuating levels of CO_2 therefore invariably signify a nearby living vertebrate. We tested the sensitivity of mosquitoes to CO_2 and skin odours, and whether, besides being a strong activator and attractant, CO_2 can influence the mosquito's olfactory response to skin odours, thereby acting as a 'releasing stimulus'. We used the synanthropic subtropical mosquito *Aedes aegypti* L., an important vector of dengue and yellow fever.

Methods

Mosquitoes

We used the Rockefeller strain of *Aedes aegypti* L. Mosquitoes were reared at 80% RH and a 14 h:10 h L:D photoperiod. The 14 h 'day' included a 1 h artificial dusk period. Adults were kept in screen cages (30 cm×30 cm×30 cm) and provided with a 6% glucose solution. Larvae were reared on Tetramin® Fish Food (Melle, Germany). We tested 10–20 day-old, non-blood-fed, mated female mosquitoes that had not had prior exposure to host odours in a bioassay. Mosquitoes were transferred to release cages 12 h before testing. Each release cage contained four mosquitoes. Release cages were purged with clean air, covered and kept overnight. Care was taken not to expose the mosquitoes to odours for at least 12 h prior to testing. Experiments were conducted during the first 5 h of photophase.

Experimental setup and testing procedures

We recorded the flight behaviour of mosquitoes in a wind tunnel (Fig. 1) with a transparent Plexiglas flight chamber of 150 cm (length)×50 cm (width)×50 cm (height), the sides of which were covered with Medium Red light filter (Roscolux, Rosco Laboratories, Stamford, CT, USA, which blocks light <600 nm) to prevent orientation of the mosquitoes to visual cues outside of the tunnel. Transparent red dots (6 cm diameter, approx. 100 m^{-2}, Medium Red, Roscolux, Stamford, CT, USA) randomly arranged on the floor of the wind tunnel provided 'non-directional' optomotor cues. Outside air from 8 m above ground was pushed by a centrifugal in-line duct fan through the wind tunnel; air flowing through the tunnel was free of human odours. The air was filtered by an activated charcoal filter, humidified to 70±5% RH, and adjusted to 27±2°C. A turbulence-free airflow of 30 cm s^{-1} was created by passing the air through an activated carbon air filter, an aluminum honeycomb laminizer (consisting of cells of 1.5 cm diameter and 15 cm long), and two stainless steel screens (150 mesh). Mosquitoes were illuminated against the background by four infrared lights (UFL 694, Rainbow, Irvine, CA, USA) at the tunnel's downwind end. Each had 60 LEDs (940 nm), equipped with cut-off filters permeable to light of >950 nm. During the experiments the light level in the human visual spectrum from diffuse fluorescent lights was approximately 15 lux.

A release cage was transferred to the wind tunnel and the covers removed. The cage was positioned on a release platform on the wind-tunnel floor 130 cm downwind from the upwind screen. The opening of the cage faced downwind and was placed against a screen, which prevented mosquitoes from initiating flight before the start of the experiment. After 3 min the platform was lifted and turned slowly upwind, such that the cage was inside the broad odour plume. We observed mosquitoes for 3 min and recorded their behaviour using two video cameras equipped with 6 mm lenses, one from the side and one from the bottom of the wind tunnel. The camera views were synchronized with an Event & Video Control Unit (Peak Performance Technologies, Centennial, CO, USA), overlaid, and recorded on one videotape. The 3-D flight coordinates of the flight tracks were obtained using Motus (Peak Performance) at 30 frames s^{-1} and salient flight parameters were calculated (see Data analysis).

Odours

We tested CO_2 and human skin odour at various concentrations. CO_2 was obtained from 100% (99.9% purity) and 4% pressurized cylinders. Skin odour was obtained by inserting a human arm (belonging to T.D.) in a 10 cm diameter glass tube and pushing 30 l min^{-1} clean air from a pressurized

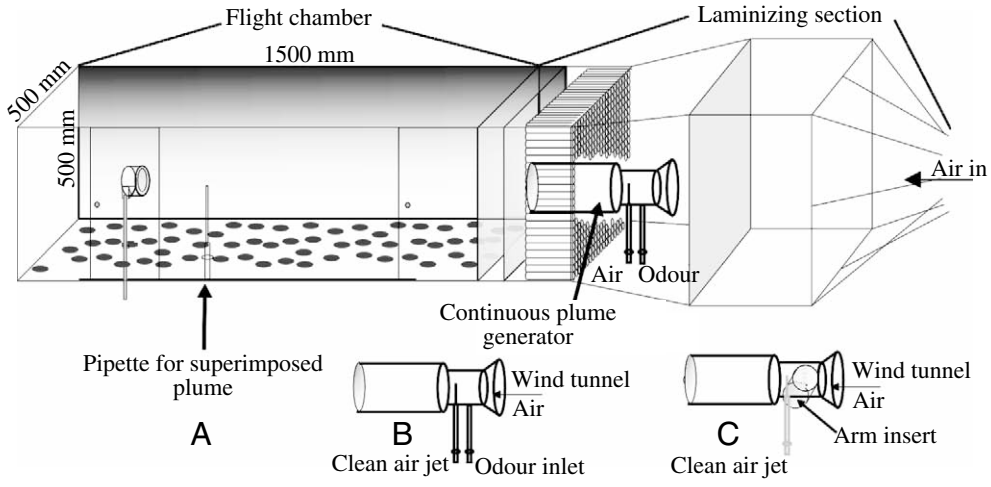

Fig. 1. Wind tunnel setup and plume generators. Superimposed ribbon plume generator (A), and the continuous (see also Fig. 2) plume generators (B,C). The superimposed plume was generated by a pipette positioned 100 cm downwind and 30 cm upwind of the release cage. This configuration created a ribbon CO_2 plume that passed through the centre of the release cage. The continuous plume generator (B) was placed behind the stainless steel laminising screens. It had two inlets, one for the odour, and the other for a 4 l min^{-1} clean air 'jet' to mix the mixture. We also tested a continuous skin odour plume by inserting a hand from outside the wind tunnel directly in the continuous plume generator through a tube (C) upwind from the laminising screens.

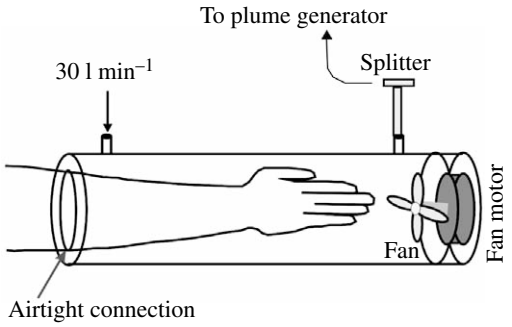

Fig. 2. Skin-odour tube. The experimenter's arm was inserted in one end. An airtight connection prevented leakage of air. A fan at the end of the tube created a flow over the arm and ensured high skin-odour uptake. The air stream could be split to create skin-odour plumes of lower concentration. Flow rates were verified 'off-line' using a bubble flow meter.

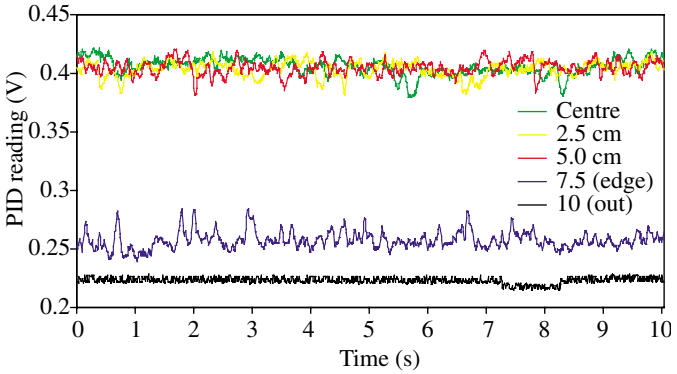

Fig. 3. Sample propylene density plots measured using the photoionization detector (PID) from the centre to outside of the continuous broad plume at 50 cm from the source. The sampling rate was 100 Hz, and the distance of the measuring point from the centre of the plume was 0–10 cm. Only small concentration fluctuations were observed within the plume. The values given by black trace '10' reflect background (no detectable propylene) values.

cylinder through the tube (Fig. 2). A stainless steel fan was mounted at one end of the tube to create a strong air movement over the hand, ensuring a high uptake of skin odour and thorough mixing of the odour-laden air. We used Teflon® tubing (0.3 cm i.d.) from the skin-odour tube to the plume generators. The tubes were 15 cm long, thereby minimizing odour adsorption in the tubes. Except for the hand used in the skin-odour tube, we used Fisherbrand® (Pittsburgh, PA, USA) latex examination gloves during the experiments to avoid contamination with any experimental device. 3 h before the experiments the arm was washed with tapwater for 1 min. The laminizing screens of the wind tunnel were replaced whenever the concentration of skin odour of a new experiment was lower than used in the previous experiment. Screens were washed thoroughly with water and soap.

Plumes

Two kinds of plumes were used.

Broad plume

We created a broad continuous plume by pushing the odour-laden air into a 14 cm diameter plume generator placed upwind from the two laminizing screens (Fig. 1B,C). The resulting flow was isokinetic to the main flow through the wind tunnel. To ensure vigorous mixing, clean air was blown at 4 l min^{-1} into the plume generator, immediately downwind of the point where the odour entered (see Fig. 1B). To obtain the desired concentration of CO_2, we adjusted the flow rate from a cylinder of 100% or 4% CO_2, with a background CO_2 concentration of 350 p.p.m. and a flow of 360 l min^{-1} through the plume generator. Lower concentrations of skin odour were obtained by taking a fraction of the skin-odour-laden (see above) air (verified 'offline' using bubble flow meters with a negligible resistance). As a control in the broad-continuous skin-odour experiments, we also created a homogeneous skin-odour plume by directly inserting a hand (belonging to T.D.) in the plume generator upwind from the laminising screens (Fig. 1C).

The plume structure was analyzed for homogeneity by simulating the plume structure with air containing 1000 p.p.m. propylene as a chemical tracer gas (Justus et al., 2002). We analyzed the plume structure at 50 cm from the source, at various points along the lateral and vertical axes using a mini-photoionization detector (mini-PID, Aurora Scientific, Aurora, ONT, Canada) at a sampling rate of 100 Hz. This demonstrated that the plume was fairly homogeneously distributed (Fig. 3).

The laminar flow through the wind tunnel allowed for accurate estimation of the plume's diameter and the position. The plume had a diameter of approximately 15 cm. The centre of the plume was 25 cm from the side and 17 cm above the wind tunnel floor. By turning the release cage at the start of the experiment, the cage was centred in the plume. Mosquitoes exiting the cage once it was turned were in contact with the broad plume.

Superimposed ribbon CO_2 plume

We created a ribbon plume of 4% CO_2 from a pipette 100 cm downwind and 30 cm upwind of the release cage (Fig. 1A). The ribbon plume had a diameter of 0.5 cm. The plume passed through the centre of the release cage and was superimposed within a broad homogeneous skin odour plume. Most mosquitoes leaving the release cage contacted the ribbon plume.

Experiments

(1) Effect of odour concentration on activation, upwind flight and source finding

The following series were performed with broad continuous plumes:

(A) Skin-odour series, included the following treatments:

(a) 100% skin odour (see description above)
(b) 20% skin odour
(c) 4% skin odour
(d) 'hand' (inserted in the plume generator, Fig. 1C)
(e) clean air.

(B) CO_2 series, included the following treatments:

(a) 1% CO_2

(b) 0.3% CO_2

(c) 0.1% CO_2

(d) 0.05% CO_2

(e) 'hand' (inserted in plume generating device, Fig. 1C).

We determined the percentage of mosquitoes activated and the percentage reaching the source with each treatment. Tracks were analyzed for other behavioural parameters (see below). Treatments were randomized within each experimental day.

(2) Effect of exposure to CO_2 filaments on sensitivity to skin odour

In this experimental series we presented the mosquitoes with broad continuous skin-odour plumes of various concentrations, with or without a ribbon plume of 4% CO_2 superimposed within the downwind end of the skin odour plume (see Fig. 1A). We tested 100% (i.e. the full skin-odour-laden air) skin odour and fivefold steps dilutions thereof. The following treatments were tested:

(a) 100% skin odour

(b) 20% skin odour

(c) 20% skin odour + exposure to CO_2 filament (20% skin odour^{CO_2})

(d) 4% skin odour

(e) 4% skin odour + exposure to CO_2 filament (4% skin odour^{CO_2})

(f) 1.25% skin odour

(g) 1.25% skin odour + exposure to CO_2 filament (1.25% skin odour^{CO_2})

(h) CO_2 only.

We scored source finding for all treatments, and reconstructed the flight tracks for treatments a, b, c and h. We analyzed the flight tracks upwind from the ribbon CO_2 plume. These tracks therefore represent flight tracks in the absence of a fluctuating CO_2 signal. Treatments were randomized within each experimental day.

Data analysis

A Weibull distribution was used to characterise the activation rate of *Ae. aegypti* (Crawley, 1993). The shape parameter α allows the activation rate ('hazard') to increase ($\alpha>1$) or decrease ($\alpha<1$) over experimental time, starting with a constant rate (exponential distribution, $\alpha=1$). Mosquitoes that left the release cage before the cage was turned into the plume were excluded from further analysis. We used a censoring indicator for mosquitoes that did not take off within the experiment, allowing for 'non-responders' to contribute to the survivorship function. Differences between the survivorship curves were assessed with χ^2-values (Aitkin et al., 1989; Crawley, 1993).

The percentage of mosquitoes that left the release cage and reached the source (the section of the upwind screen from where the plume entered the wind tunnel) was arcsine square-root transformed and analyzed by a two-way analysis of variance (ANOVA), followed by an LSD *post hoc* test to assess the significance of differences between the means.

Mosquitoes flying along the side of the wind tunnel were excluded from track analysis. In the sensitization experiments, those parts of the flight tracks downwind from the ribbon CO_2 plume were excluded from analysis. The subsequent tracks represented tracks in response to skin odour only. The data were smoothed with the cubic spline algorithm, a method that is particularly well suited for data that are parabolic in nature (Jackson, 1979).

Custom-made programs in Visual Basic® for Applications were employed to analyze the flight tracks with respect to the mosquito's position relative to the plume. We verified the plume's position visually using $TiCl_4$ 'smoke,' and chemically using surrogate odour propylene in conjunction with the photoionization detector (mini-PID; for details, see above). We averaged flight parameters over 100 ms (3 frames) and scored how the flight parameters changed over time after flying into or out of the plume, first within each flight track followed by between flight tracks. Here we present only two flight parameters, the track angle and the flight speed. The track angle is defined as the angle of the insect with respect to wind, with upwind at 0°. The flight speed (in mm s^{-1}) is the velocity of the insect in 3-D.

The data were log transformed, checked for normality and day effects, and analyzed in Statistica (StatSoft, Inc., Tulsa, OK, USA) using a repeated-measure ANOVA, followed by an LSD *post hoc* test. Contrasts were used to test for significant changes in a parameter within a treatment after plume contact (repeated measures).

Results

(A) Skin-odour series

Activation

Fig. 4A shows the activation rate for the treatments within the skin-odour series. Activation rates with 100% skin odour and 'hand' were similar. Activation rates with dilutions of skin odour were lower than with 100% skin odour, and did not differ from the clean air.

Flight velocities

100% skin odour and 'hand' elicited faster flight than 20% and 4% skin odour or clean air ($P<0.001$ in all comparisons, Table 1).

Track angle

Mosquitoes headed more due upwind within 300 ms after entering a 100% skin odour or 'hand' plume (Fig. 5A); mosquitoes leaving the plume headed more crosswind within 300 ms (skin odour and 'hand'). No significant change in track angle was observed after mosquitoes had entered or exited a plume of 20% skin odour, 4% skin odour or clean air.

Source finding

The percentage of mosquitoes that reached the source was comparable between 100% skin odour and 'hand' (Table 1). Source finding with 20% skin odour was lower than with 100%

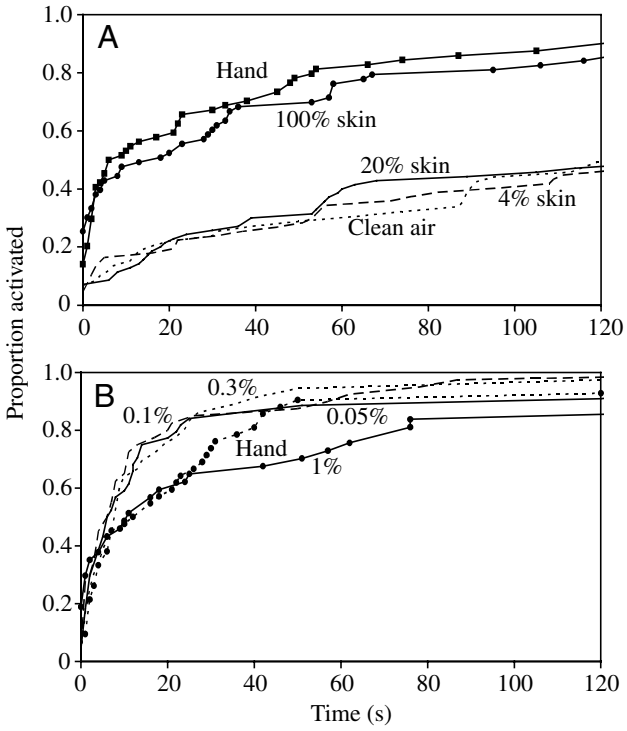

Fig. 4. Activation by skin odour and CO_2 (A) skin odour series: 100% skin odour (N=64), 20% skin odour (N=67), 4% skin odour (N=70), 'hand' (N=64), clean air (N=59); (B) CO_2 series: 1% CO_2 (N=40), 0.3% CO_2 (N=40), 0.1% CO_2 (N=55), 0.05% CO_2 (N=55), 'hand' (N=46). The shape parameter α was in all cases <1 (between 0.5 and 0.6), which implies a higher activation rate at the beginning of the experiment.

skin odour, but higher than with clean air. Source finding was similar with 4% skin odour and clean air.

(B) CO_2 series

Activation

Fig. 4B shows the activation rate for the treatments within the CO_2 series. Activation was lower for 1% CO_2 and 'hand' compared to the other treatments (P<0.02 and <0.005, respectively). Activation rates with 1% CO_2 and 'hand' were comparable.

Flight velocities

Flight velocities were similar for all treatments (Table 1).

Track angle

Upon entering the plume, the track angle (Fig. 5B) changed to more due upwind within 200 ms (all concentrations of CO_2) and 300 ms (skin odour). Mosquitoes exiting the plume projected their tracks more crosswind within 200 ms (skin odour, 0.05% and 0.1% CO_2), 300 ms (0.3%), or 600 ms (1% CO_2) of exiting the plume.

Source finding

Source finding was comparable for all treatments (Table 1). The lower source finding rate with 'hand' was not significant (P=0.06).

(C) Sensitization series

Fig. 6 shows representative tracks in response 20% skin odour, 20% skin odour^{CO_2}, 100% skin odour, and to CO_2 only (ribbon plume). In the analysis, the first part of the tracks downwind from the CO_2 source (i.e. 100 cm and further downwind) were excluded, such that the data represent responses to skin odour alone.

Flight velocities

CO_2, 100% skin odour and 20% skin odour^{CO_2} elicited higher flight velocities than 20% skin odour (Table 1).

Track angle

After entering a plume of 100% skin odour, mosquitoes aimed their thrust more due upwind within 200 ms, and more crosswind within 200 ms after exiting the plume (Fig. 5C). No changes were observed with CO_2 only and 20% skin odour. Mosquitoes that intercepted one or more CO_2 filaments, however, changed their flight paths upon entering and exiting the 20% skin odour plume in a similar fashion to mosquitoes responding to 100% skin odour.

Source finding

Table 1 shows that the percentage of mosquitoes that reached the source was lower with 20% skin odour than with 100% skin odour (P<0.001). However, the percentage of mosquitoes that reached the source of 20% skin odour^{CO_2} was comparable to the 100% skin-odour plume. After CO_2 filament encounters, mosquitoes showed increased orientation (i.e. upwind turning when entering the plume) to 20% skin odour for a period of over 10 s. Source finding was comparable for 20% skin odour and 4% skin odour^{CO_2}, and higher than for 0.8% skin odour, 0.8% skin odour^{CO_2} or CO_2 only.

Discussion

Sensitivity to skin odour and CO_2

The relative importance of host stimuli and the range over which they act on mosquitoes have been much debated (Gillies, 1980; Mboera and Takken, 1997; Takken and Knols, 1999). Stimuli have been categorized as long range (>5 m) if sensed well downwind of the host (e.g. most attractants and activators), and close range (<1 m) if sensed near the host (e.g. cues inducing landing and biting; Daykin et al., 1965; Gillies, 1980; Sutcliffe, 1987; De Jong and Knols, 1995; Dekker et al., 1998). For hematophagous insects, CO_2 is generally considered to be an attractant only when close to the host (e.g. Brady and Griffiths, 1993; Paynter and Brady, 1993; Schofield and Brady, 1997). However, in our experiments, *Ae. aegypti* appeared several orders of magnitude more sensitive to dilutions of human-emitted levels of CO_2 than to dilutions of skin odours, as reflected in activation, orientation and source finding (Figs 4, 5A,B, Table 1).

Table 1. *Average values for flight speed and percent source finding*

		Flight speed			Source finding		
Series	Treatment	mm s^{-1}	S.E.M.	*N*	%	S.E.M.	*N*
Skin odor	'Hand'	41[a]	1.1	36	85[a]	2.1	55
	100% skin	43[a]	1.4	28	88[a]	2.0	30
	20% skin	35[b]	2.6	22	19[b]	4.1	39
	4% skin	35[b]	4.4	49	0[c]	0	57
	Clean air	33[b]	1.6	17	1[c]	1.5	31
CO_2	1% CO_2	39[a]	1.2	31	93[a]	1.3	43
	0.4% CO_2	40[a]	1.1	26	98[a]	1.9	48
	0.1% CO_2	40[a]	1.5	30	97[a]	2.3	55
	0.05% CO_2	41[a]	1.7	25	96[a]	3.5	43
	'Hand'	39[a]	1.1	23	82[a]	1.3	43
Sensitization	100% skin	42[a]	1.7	24	93[a]	3.1	54
	20% skin	35[b]	1.1	38	19[b,c]	3.4	43
	20% $skin^{CO_2}$	45[a]	1.8	40	97[a]	3.0	52
	4% skin				3[c,d]	2.4	34
	4% $skin^{CO_2}$				29[b]	4.7	49
	0.8% skin				0[d]	0	26
	0.8% $skin^{CO_2}$				8[b,c,d]	4.2	44
	Ribbon CO_2	45[a]	1.6	19	3[c,d]	1.4	25

Values within the same column and experiment that do not have a letter in common are significantly different at $P<0.05$.
$Skin^{CO_2}$, skin odour + exposure to CO_2 filament.

Activation

CO_2 is a very potent activator. *Ae. aegypti* was activated by highly diluted, close to background concentrations of CO_2 (0.05%, background 0.035%). High activation rates by CO_2 have been frequently reported (e.g. Gillies, 1980; Geier and Boeckh, 1999). After the onset of the experiment, activation rates with 1% CO_2 declined over time, which may have been caused by sensory adaptation. CO_2 receptor cells adapt rapidly under continuous stimulation (Grant and O'Connell, 1996; Grant et al., 1995). In contrast to CO_2, our data suggest that skin odour is only activating at concentrations that occur near a human host. Although we scored an initially higher activation rate at the start of the experiment (the Weibull shape parameter, or 'hazard,' $\alpha<1$ for all treatments), probably through a slight mechanical stimulation during turning of the cage (e.g. vibration and reversal of the wind flow), this should have influenced all treatments equally. Historically, activation and orientation have been considered separate steps in the host-seeking process. We found a similarly strong effect of CO_2 on parameters of mosquito flight, including speed and kinetic responses.

Flight speed

The flight speed increased with high concentrations of skin odour and with all concentrations of CO_2. Such orthokinetic responses have been reported for moths upon repetitive interception of pheromone filaments (e.g. Mafra-Neto and Cardé, 1994, 1995; Vickers and Baker, 1994) and for tsetse and stable flies in response to CO_2, acetone and octenol (Paynter and Brady, 1993; Schofield and Brady, 1997). However, mosquito flight speed remained fairly constant over time, irrespective of flying in or outside of the plume. In contrast, male moths increased their flight speed by twofold or more after contacting pheromone filaments (Mafra-Neto and Cardé, 1994, 1998). The relatively constant flight speed in mosquitoes may be a requirement when flight stability depends on haltere input (Chan et al., 1998). Alternatively, mosquito flight may be characterized by close to maximum flight muscle output, such that mosquitoes cannot increase flight speed much without compromising flight stability (Lehmann and Dickinson, 2001).

Track angle

Within about 200 ms of encountering a single filament of pheromone, moths aim their tracks more due upwind (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Quero et al., 2001). Upwind turning in odour plumes also has been recorded for tsetse and stable flies (Colvin et al., 1989; Paynter and Brady, 1993; Schofield and Brady, 1997) in response to CO_2, octenol and acetone odours. In a different bioassay setup using vertical, odour-laden convection currents, Daykin et al. (1965) reported increased turning of *Ae. aegypti* when leaving but not when entering a skin-odour plume. Our averaged flight tracks revealed a robust response of *Ae. aegypti* to entering and to exiting a plume of CO_2 and high concentrations of skin odour. Entering such a plume resulted in a rapid (i.e. within 200 ms) change of the track to more due upwind. Conversely, exiting the plume resulted in a similarly rapid increase to more due crosswind. At 1% CO_2, mosquitoes exiting the plume increased their angle more slowly than at lower CO_2

concentrations. This may be caused by sensory adaptation of CO_2 olfactory sensory neurons, of which the phasic response portion quickly adapts upon stimulation (Grant and O'Connell, 1996). In contrast, with a high skin-odour concentration, a fivefold dilution of skin odour evoked only a weak and irregular average upwind turning upon entering the plume, and no change upon leaving the plume. Often this resulted in mosquitoes not regaining contact with a lost skin-odour plume.

Source finding

Source finding is the most commonly used measure of 'attractiveness.' Our source-finding data parallel the activation and track analysis data by showing a high source-finding rate at all CO_2 concentrations and at 100% skin odour, and a rapid loss of sensitivity at dilutions of skin odour. Our source-finding results also suggest that *Ae. aegypti* mosquitoes are more sensitive to CO_2 than to skin odour. Although mosquitoes may respond to highly diluted filaments of CO_2, other environmental factors, such as the constancy of wind direction, atmospheric stability and habitat (Brady et al., 1990; Murlis et al., 1992), may limit the range over which a CO_2 plume can be followed to a potential host.

In summary, our flight track analysis shows that upwind flight and source finding of *Ae. aegypti* in response to skin odour wanes quickly with dilution. Because undiluted skin odour was in the range of naturally encountered concentrations, we conclude that *Ae. aegypti* may be less sensitive to skin odours than previously supposed (e.g. Gillies, 1980; Takken and Knols, 1999). In contrast, our study shows that the sensitivity and the range of attraction of CO_2 may be greater than assumed. The previous conjecture of a limited range of attraction for CO_2 may partly have been caused by the assumption that as CO_2 is transported downwind in a plume, it rapidly decreases to background concentrations (Gillies, 1980). In reality, CO_2 follows an asymptotic rather than linear dilution curve, i.e. a 100× dilution of 4% CO_2 against a background of 0.035% yields a concentration of 0.075% (asymptotic dilution) instead of 0.04% (linear dilution). Moreover, filaments of odour can be transported in an odour plume many meters downwind without appreciable dilution (Murlis et al., 1992). Because a concentration difference of only 0.005% (i.e. an 800× dilution from 4%) changes the firing rate of CO_2-sensitive cells for several mosquito species (Grant and O'Connell, 1996; Grant et al., 1995), and the mosquito shows behavioural sensitivity to slight elevations in CO_2 levels (this study), the range over which CO_2 can potentially attract mosquitoes may have been underestimated. Zöllner et al. (2004) released CO_2 from a point source into two types of woodland habitats at a rate equivalent to that emitted by a typical bovid host and measured CO_2 levels at various distances downwind. Fluctuations in CO_2 levels could be readily detected over background at distances up to 64 m (the maximum distance sampled) in a riverine habitat.

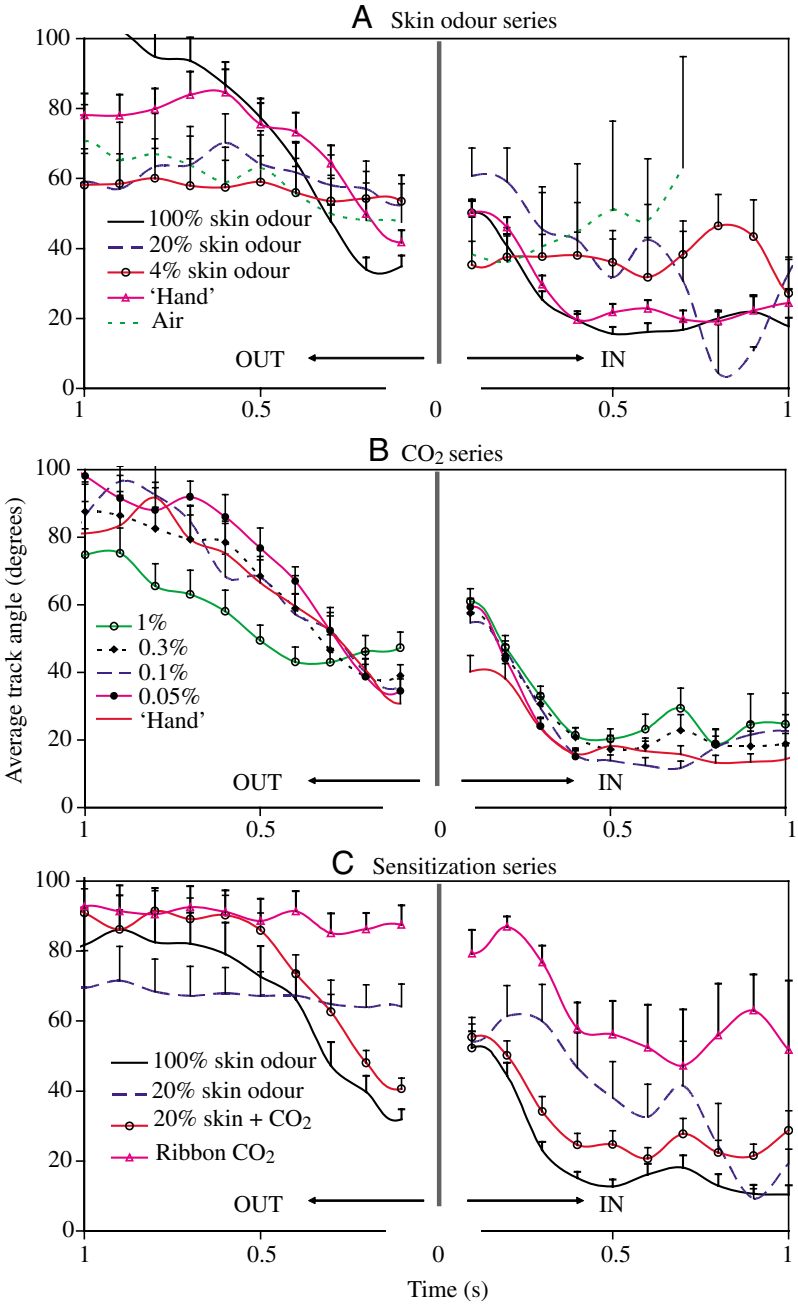

Fig. 5. Average change in track angle over time when flying in (right; mosquitoes entering the plume) or out (left; mosquitoes leaving the plume) of a plume consisting of skin odour or CO_2. Straight upwind flight would be 0°. The grey vertical line represents the plume boundary. Values are means ± S.E.M., averaged over all mosquito tracks (see *N* values) (A) Skin odour series: 100% skin odour (*N*=36), 20% skin odour (*N*=28), 4% skin odour (*N*=22), 'hand' (*N*=49), clean air (*N*=17); (B) CO_2 series: 1% CO_2 (*N*=31), 0.3% CO_2 (*N*=26), 0.1% CO_2 (*N*=30), 0.05% CO_2 (*N*=25), 'hand' (*N*=23); (C) sensitization series: 100% skin odour (*N*=24), 20% skin odour (*N*=38), 20% skin odour + CO_2 (*N*=40), CO_2 (*N*=19).

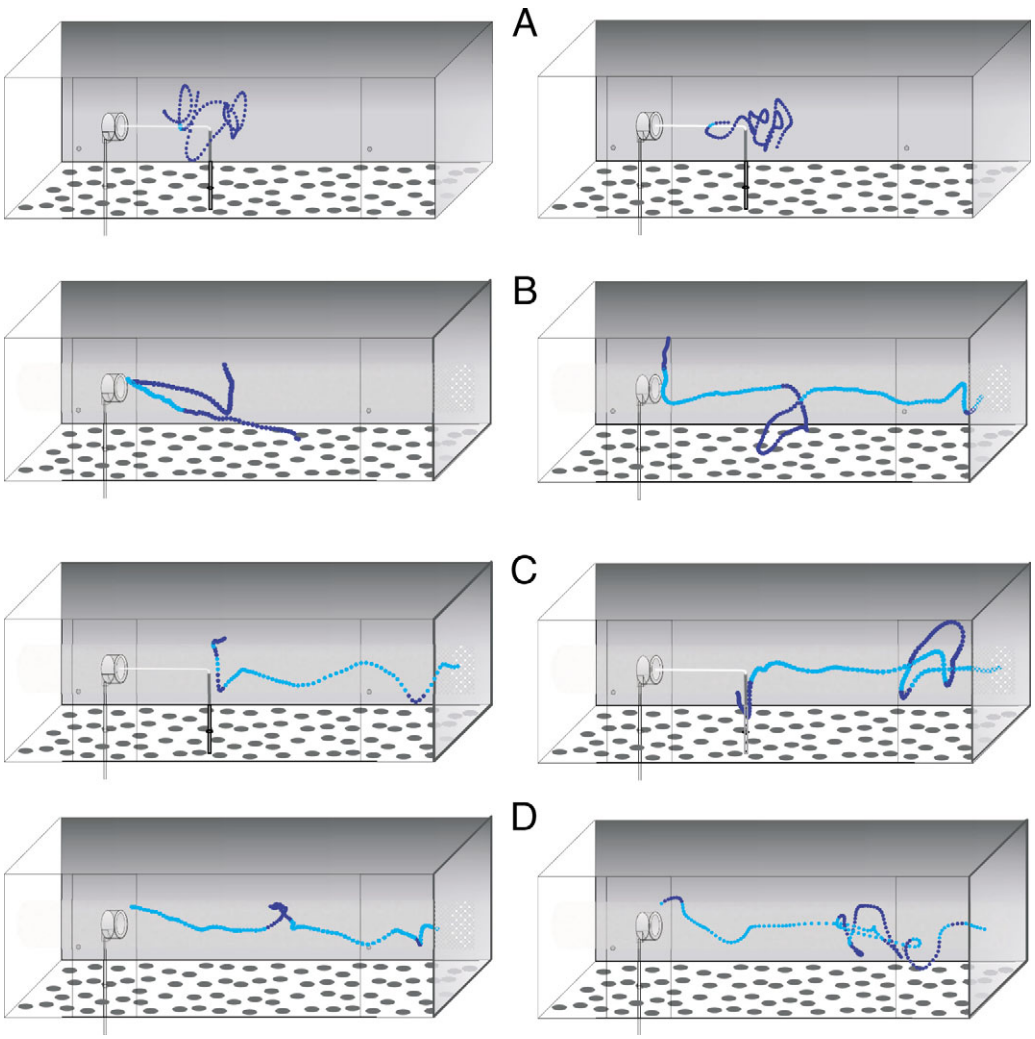

Fig. 6. Sample tracks of mosquitoes in response to (A) a ribbon CO_2 plume, (B) 20% skin odour, (C) 20% skin odour after contact with a ribbon plume of CO_2, and (D) undiluted skin odour. Dots represent the mosquito's position at intervals of 100 ms. Contact with the odour plume is indicated by a light blue colour. B (left) shows two typical tracks of mosquitoes flying through a diluted skin odour plume. Only rarely did 20% skin odour induce sustained upwind flight to the odour source (right).

This indicates that intermittent bursts of CO_2 from a vertebrate host could be used as an orientation cue over distances much greater than previously assumed.

Although CO_2 does not signify a host-specific cue for mosquito species exhibiting a preference to bite certain vertebrate species (Mboera et al., 1998; Takken and Knols, 1999; Pates et al., 2001), it does invariably indicate a live potential host. The behavioural sensitivity to CO_2 and innate olfactory sensitivity for certain odours may be different for other mosquito species. The fact, however, that CO_2 is the only odour that increases capture rates of many (Mboera and Takken, 1997) mosquito species in the field, and the fact that CO_2-sensitive cells exhibit a similar sensitivity across various species (Grant and O'Connell, 1996), suggest that CO_2 is key in the host orientation of most mosquito species.

Sensitization

Our results show that CO_2 also modulates the female *Ae. Aegypti*'s threshold of sensitivity to skin odours. A brief encounter with a CO_2 filament instantaneously increased their sensitivity by at least fivefold. Such sensitization persisted for at least 10 s (the time between the last CO_2 encounter and the tracks recorded). To our knowledge, this is the first example of an instantaneous behavioural sensitization of the olfactory circuitry. It differs from classic synergistic responses (Acree et al., 1968; Eiras and Jepson, 1994; Geier and Boeckh, 1999) in that the stimuli are separated in time. Because we analyzed flight tracks upwind of the CO_2 source, our results demonstrate that the increased response to diluted skin odour is caused by true sensitization, i.e. increased sensitivity of the olfactory system to skin odour. CO_2-induced sensitization may be more pronounced after deprivation of fluctuating CO_2 signals, such as in our study (for 12 h).

CO_2-sensitive sensory cells of mosquitoes are located on the maxillary palps, whereas skin odours are perceived by sensory cells on the antennae (Kellogg, 1970; Meijerink and Van Loon,

1999). This implies that sensitization takes place either at the level of the olfactory lobe or at higher brain centres. In honeybees, a slow-acting modulation of sensitivity of the antennal lobe *via* a protocerebral feedback loop has been implied in olfactory learning (Iwama and Shibuya, 1998; Faber et al., 1999; Sachse and Galizia, 2002). In locusts, repeated stimulation with the same odour enhanced oscillatory synchronization of projection neurons (Stopfer and Laurent, 1999), and induced a higher sensitivity of individual projection neurons (Bäcker, 2002). Other potential underlying neuronal mechanisms of the observed behavioural sensitization may involve a general neuromodulatory network in the antennal lobe (e.g. Sachse and Galizia, 2002), or feedback neurons from the antennal lobe to the OSNs, a unique neuromodulatory pathway in mosquitoes (Meola et al., 2000; Meola and Sittertz-Bhatkar, 2002; Bäcker, 2002).

Although the proximate cause of sensitization for skin odour by CO_2 remains to be established, such rapid sensitization may be adaptive, because in a miasma of potential host odours, a fluctuating CO_2 signal reliably signifies a warm-blooded vertebrate. However, as CO_2 is not a host-specific cue, the question arises whether such a high sensitivity to CO_2 is indeed an adaptation by mosquitoes that prefer to bite humans. This raises the issue of what portion of host preference is determined by odours, and what by other factors, such as resting behaviour. Host preference in *Ae. aegypti* is dependent in part on one or a few genes that determine its propensity to either rest inside or outside houses (Trpis and Hauserman, 1978). Similarly, blood meal analysis shows that the host preference of *Ae. aegypti* is strongly influenced by the relative availability of host species inside dwellings (Tandon and Ray, 2000). Furthermore, *Ae. aegypti* oviposits predominantly close to or inside dwellings, which implies that adults may not have to orient from far away to their preferred host. In such habitats a high sensitivity to fluctuations in CO_2 levels would indeed help to alert the mosquito to the presence of a host. The question of how these and other ecologically important cues mediate host finding in the field necessitates further study.

Finally, the observed CO_2-induced activation, sensitization and orientation to a potential host may be more important for day-active mosquitoes such as *Ae. aegypti*, which bite when the host may be moving. Whether other mosquito species and genera with other feeding habits, such as the nocturnal anopheline mosquitoes, which typically feed when the host is stationary, have a similar organisation of response to host odours remains to be determined.

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References

Acree, F., Jr, Turner, R. B., Gouck, H. K., Beroza, M. and Smith, N. (1968). L-Lactic-acid: A mosquito attractant isolated from humans. *Science* **161**, 1346-1347.

Aitkin, M., Anderson, D., Francis, B. and Hinde, J. (1989). *Statistical Modeling in GLIM*. Oxford: Clarendon Press.

Bäcker, A. (2002). Pattern recognition in locust early olfactory circuits: priming, gain control and coding issues. PhD thesis, California Institute of Technology, USA.

Baker, T. C. and Vickers, N. J. (1997). Pheromone-mediated flight in moths. In *Insect Pheromone Research: New Directions* (ed. R. T. Cardé and A. K. Minks), pp. 248-264. New York: Chapman and Hall.

Baker, T. C., Fadamiro, H. Y. and Cosse, A. A. (1998). Moth uses fine tuning for odour resolution. *Nature* **393**, 530.

Bernier, U. R., Kline, D. L., Barnard, D. R., Schreck, C. E. and Yost, R. A. (2001). Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for the yellow fever mosquito (*Aedes aegypti*). *Anal. Chem.* **72**, 747-756.

Bowen, M. F. (1996). Sensory aspect of host location in mosquitoes. In *CIBA Foundation Symp., **200**, Olfaction in Mosquito-Host Interactions* (ed. G. R. Bock and G. Cardew), pp. 196-208. New York: John Wiley and Sons.

Brady, J. and Griffiths, N. (1993). Upwind flight responses of tsetse flies (Glossina spp.) (Diptera: Glossinidae) to acetone, octenol and phenols in nature: A video study. *Bull. Entomol. Res.* **83**, 329-333.

Brady, J., Packer, M. J. and Gibson, G. (1990). Odour plume shape and host finding by tsetse. *Insect Sci. Appl.* **11**, 377-384.

Cardé, R. T. and Charlton, R. E. (1984). Olfactory sexual communication in Lepidoptera: strategy, sensitivity and selectivity. In *Insect Communication* (ed. T. Lewis), pp. 241-265. New York: Academic Press.

Cardé, R. T. and Mafra-Neto, A. (1997). Mechanisms of flight of male moths to pheromone. In *Insect Pheromone Research: New Directions* (ed. R. T. Cardé and A. K. Minks), pp. 275-290. New York: Chapman and Hall.

Chan, W. P., Prete, F. and Dickinson, M. H. (1998). Visual input to the efferent control system of a fly's 'gyroscope'. *Science* **280**, 289-292.

Colvin, J., Brady, J. and Gibson, G. (1989). Visually-guided, upwind turning behaviour of free-flying tsetse flies in odour-laden wind: a wind-tunnel study. *Physiol. Entomol.* **14**, 31-39.

Crawley, M. J. (1993). *GLIM for Ecologists*. London: Blackwell Science.

Daykin, P. N., Kellogg, F. E. and Wright, R. H. (1965). Host-finding and repulsion of *Aedes aegypti*. *Can. Entomol.* **97**, 239-263.

De Jong, R. and Knols, B. G. J. (1995). Selection of biting sites on man by two malaria mosquito species. *Experientia* **51**, 80-84.

Dekker, T., Takken, W., Knols, B. G. J., Bouman, E., Laak, S. V. D., Bever, A. D. and Huisman, P. W. T. (1998). Selection of biting sites on a human host by *Anopheles gambiae s. s*, *An arabiensis* and *An-quadriannulatus*. *Entomol. Exp. Appl.* **87**, 295-300.

Eiras, A. E. and Jepson, P. C. (1994). Responses of female *Aedes aegypti* (Diptera: Culicidae): to host odours and convection currents using an olfactometer bioassay. *Bull. Entomol. Res.* **84**, 207-211.

Faber, T., Joerges, J. and Menzel, R. (1999). Associative learning modifies neural representations of odors in the insect brain. *Nature Neurosci.* **2**, 74-78.

Geier, M. and Boeckh, J. (1999). A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. *Entomol. Exp. Appl.* **92**, 9-19.

Gillies, M. T. (1980). The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bull. Entomol. Res.* **80**, 525-532.

Grant, A. J. and O'Connell, R. J. (1996). Electrophysiological responses from receptor neurons in mosquito maxillary palp sensilla. In *Ciba Foundation Symposium: Olfaction in Mosquito-Host Interactions* (ed. G. R. Bock and G. Cardew), pp. 233-248. New York: John Wiley and Sons.

Grant, A. J., Wigton, B. E., Aghajanian, J. G. and O'Connell, R. J. (1995). Electrophysiological responses of receptor neurons in mosquito maxillary palp sensilla to carbon dioxide. *J. Comp. Physiol. A* **177**, 389-396.

Iwama, A. and Shibuya, T. (1998). Physiology and morphology of olfactory neurons associating with the protocerebral lobe of the honeybee brain. *J. Insect Physiol.* **44**, 1191-1204.

Jackson, K. M. (1979). Fitting of mathematical functions to biomechanical data. *IEEE Trans. Biomed. Eng.* **26**, 122-124.

Justus, K. A., Murlis, J., Jones, C. and Cardé, R. T. (2002). Measurement of odor-plume structure in a wind tunnel using a photoionization detector and a tracer gas. *Environ. Fluid Mech.* **2**, 115-142.

Kellogg, F. E. (1970). Water vapor and carbon dioxide receptors in *Aedes aegypti*. *J. Insect Physiol.* **16**, 99-108.

Lehmann, F. and Dickinson, M. H. (2001). The production of elevated flight

force compromises manoeuvrability in the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* **204**, 627-635.

Mafra-Neto, A. and Cardé, R. T. (1994). Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. *Nature* **369**, 142-144.

Mafra-Neto, A. and Cardé, R. T. (1995). Influence of plume structure and pheromone concentration on upwind flight of *Cadra cautella* males. *Physiol. Entomol.* **20**, 117-133.

Mafra-Neto, A. and Cardé, R. T. (1998). Rate of realized interception of pheromone pulses in different wind speeds modulates almond moth orientation. *J. Comp. Physiol. A* **18**, 563-572.

Mboera, L. E. G. and Takken, W. (1997). Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. *Rev. Med. Vet. Entomol.* **85**, 355-368.

Mboera, L. E. G., Knols, B. G. J., Takken, W. and Huisman, P. W. T. (1998). Olfactory responses of female *Culex quinquefasciatus* Say (Diptera: Culicidae) in a dual-choice olfactometer. *J. Vector Ecol.* **23**, 107-113.

Meijerink, J. and Van Loon, J. J. A. (1999). Sensitivities of antennal olfactory neurons of the malaria mosquito, *Anopheles gambiae*, to carboxylic acids. *J. Insect Physiol.* **45**, 365-373.

Meola, S. M. and Sittertz-Bhatkar, H. (2002). Neuroendocrine modulation of olfactory sensory neuron signal reception *via* axo-dendritic synapses in the antennae of the mosquito, *Aedes aegypti*. *J. Mol. Neurosci.* **18**, 239-245.

Meola, S. M., Sittertz-Bhatkar, H., Pendleton, M. W., Meola, R. W., Knight, W. P. and Olson, J. (2000). Ultrastructural analysis of neurosecretory cells in the antennae of the mosquito, *Culex salinarius* (Diptera: Culicidae). *J. Mol. Neurosci.* **14**, 17-25.

Murlis, J., Elkinton, J. S. and Cardé, R. T. (1992). Odor plumes and how insects use them. *Annu. Rev. Entomol.* **37**, 505-532.

Pappenberger, B., Geier, M. and Boeckh, J. (1996). Responses of antennal olfactory receptors to odours from the human body in the yellow fever mosquito, *Aedes aegypti*. In *Ciba Foundation Symposium* vol. 200 (ed. G. R. Bock and G. Cardew), pp. 254-266. New York: John Wiley and Sons.

Pates, H. V., Takken, W., Stuke, K. and Curtis, C. F. (2001). Differential behaviour of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) to human and cow odours in the laboratory. *Bull. Entomol. Res.* **91**, 289-296.

Paynter, Q. and Brady, J. (1993). Flight responses of tsetse flies (Glossina) to octenol and acetone vapour in a wind-tunnel. *Physiol. Entomol.* **18**, 102-108.

Quero, C., Fadamiro, H. Y. and Baker, T. C. (2001). Responses of male *Helicoverpa zea* to single pulses of sex pheromone and behavioural antagonist. *Physiol. Entomol.* **26**, 106-115.

Reeves, W. C. (1953). Quantitative field studies on a carbon dioxide chemotropism of mosquitoes. *Am. J. Trop. Med. Hygiene* **2**, 325-331.

Rudolfs, W. (1922). Chemotropism of mosquitoes. *Bull. NJ Agric. Exp. Stn.* **367**, 4-23.

Sachse, S. and Galizia, C. G. (2002). Role of inhibition for temporal and spatial odor representation in olfactory output neurons: A calcium imaging study. *J. Neurophysiol.* **87**, 1106-1117.

Schofield, S. and Brady, J. (1997). Effects of carbon dioxide, acetone and 1-octen-3-ol on the flight responses of the stable fly, *Stomoxys calcitrans*, in a wind tunnel. *Physiol. Entomol.* **22**, 380-386.

Stopfer, M. and Laurent, G. (1999). Short-term memory in olfactory network dynamics. *Nature* **402**, 664-668.

Sutcliffe, J. F. (1987). Distance orientation of biting flies to their hosts. *Insect Sci. Appl.* **8**, 611-616.

Takken, W. and Knols, B. G. J. (1999). Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annu. Rev. Entomol.* **44**, 131-157.

Tandon, N. and Ray, S. (2000). *Dengue Bulletin* **24**, http://w3.whosea.org/en/Section10/Section332/Section522_2537.htm.

Trpis, M. and Hauserman, W. (1978). Genetics of house entering behaviour in East African populations of *Aedes aegypti* (L.) (Diptera: Culicidae) and its relevance to speciation. *Bull. Entomol. Res.* **68**, 521-532.

Vickers, N. J. and Baker, T. C. (1994). Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proc. Natl. Acad. Sci. USA* **91**, 5756-5760.

Zöllner, G. E., Torr, S. J., Ammann, C. and Meixner, F. X. (2004). Dispersion of carbon dioxide plumes in African woodland: implications for host-finding by tsetse flies. *Physiol. Entomol.* **29**, 381-394.