A wind tunnel and a novel auto detecting digital video system were used to study the flight behaviour of the nocturnal malaria mosquito Anopheles gambiae s.s. Giles sensu stricto. Females of this anthropophilic mosquito need to locate a host to obtain a blood meal in order to develop eggs. This host-seeking behaviour is mainly guided by olfactory stimuli, but little is known to what extent these stimuli affect flight and landing parameters.

In this study single female mosquitoes were exposed to natural and synthetic human odours in a wind tunnel. Flight behaviour was digitally recorded and with the use of computer programmes the 3-dimensional (3-D) flight track of the mosquito was reconstructed. From the obtained tracks, the amount of plume contact, average flight speed and flight angles were calculated.

Mosquitoes were found to significantly reduce their flight speed, track- and course angle when they were inside a plume of odours. Drift angle was not different inside and outside the odour plume. The flight speed was reduced when the mosquitoes came nearer to the odour source. The observed responses to host-stimuli can be explained by the nocturnal activity pattern of this mosquito. Reducing flight speed and course angle may be an efficient strategy to stay inside the trail of odour molecules when flying in the dark. It is important to know more about the flight behaviour of this malaria vector in response to odours, because it may help in the development or improvement of odour-baited mosquito traps.

Keywords: Anopheles gambiae, 3-D, flight behaviour, odour plumes, auto detection

Appetitive female haematophagous mosquitoes (Diptera: Culicidae) are exposed to a wide variety of visual, olfactory and physical stimuli. These stimuli can be used by the mosquito during host-seeking in order to obtain a blood meal. Olfactory stimuli have been shown to be most important at long ranges from a host (>5 m) (Dekker et al. 2005 and references therein). At smaller distances (<1
Heat and water vapour also become important to get into contact with the host (Healy et al. 2002, Kellogg & Wright 1962, Takken et al. 1997).

Host seeking of a mosquito can be imagined as a journey through an odour trail. But the trail of odour molecules, mostly referred to as plume, is not a cone-shape cloud with straight edges. In a natural windy situation, an odour plume is highly heterogeneous and not perfectly cone-shaped, because of turbulent diffusion. Turbulence separates both odour filaments bundles and filaments within a bundle by regions of clean air (Murlis et al. 1992). Therefore instantaneous wind directions do not necessarily provide reliable information about the direction toward an odour source (Brady et al. 1989).

The source size and shape together with the wind speed and direction will influence the degree of dilution of the odour. Overall it can be said that gaps of clean air will expand as the plume travels away with distance from the source (Murlis et al. 2000). The temporal and spatial fluctuating odour concentrations within the plume body are referred to as fine-scale plume structure and have been shown to be important in instantaneous responses of insects (Dekker et al. 2001, Mafra-Neto & Cardé 1994, 1995b, Murlis et al. 1992).

In order to find an odour source, an insect needs to orientate itself. Kennedy (1939) showed that the mosquito Aedes aegypti (L.) responds well to visual stimuli. From this and many other studies it is believed that flying insects appraise their movement visually by comparing the course heading with the apparent direction of ground movement (Bell & Cardé 1984 and references therein). The visual control of flight speed and course angle by reaction on the flow of ground images is called optomotor anemotaxis.

Flights can be described by looking at specific flight parameters such as flight speed and flight angles. Therefore a flight is simplified as a sequence of coordinates, where each flight coordinate is a vector (motion consisting of both a magnitude and direction) directed to the next coordinate (see Fig. 1). The flight track is the result of the connections between all the courses steered by the mosquito and the wind-drift at any instant.

Flight behaviour has mainly been studied in male moths attracted to pheromone sources (Justus & Cardé 2002, Marsh et al. 1978, Schofield et al. 2003). Flight behaviour of these relatively large insects is generally projected in only two dimensions and thus the flight misses the Z-component in the analysis. However, current techniques facilitates complicated analysis methods and 3-D flight behaviour of even very small insects is reported increasingly: mosquitoes (Cooperband & Cardé 2006, Dekker et al. 2005), aphids (El-Sayed et al. 2000) and fruit-flies (Budick & Dickinson 2006). This paper examines the flight behaviour of the mosquito Anopheles gambiae Giles sensu stricto (herein termed An. gambiae). Its response to natural human odours and the synthetic components lactic acid (LA) and ammonia (NH₃) is tested. Flight parameters inside and outside an odour plume are compared to assess behavioural effects of plume contact.
MATERIAL AND METHODS

Females of the An. gambiae colony at Wageningen University, The Netherlands, of 5-8-days old were used for the experiments. The mosquitoes were non-blood-fed and not previously exposed to host odours in a bioassay set-up. See Smallegange et al. (2005) for mosquito rearing details. Individual mosquitoes were collected randomly from a cage with a suction tube and placed in cylindrical plastic cups (diameter 5 cm, height 3 cm) at least 16 hours prior to testing. Side-walls of the cups were perforated with 12 holes and damp cotton wool was offered to avoid dehydration.

A Lexan polycarbonate wind tunnel (180 x 60 x 60 cm) depicted in Figure 2 was used as flight arena. The side walls and bottom of the flight chamber (120 x 60 x 60 cm) were covered with black polycarbonate. The experimental room was maintained in darkness, but infrared (IR) lights placed at the downwind-side of the wind tunnel were used for illumination.

Air was withdrawn from the experimental room by a fan, led through activated charcoal and a nylon tube (diameter 15 cm) before it entered the wind tunnel. Subsequently, the air-flow passed a humidifying cloth, an aluminium lamination screen and black-painted stainless steel mesh screen. The wind speed was 0.2 m/s, measured right in front of the mesh screen. Immediately behind the screen in upwind direction, a glass funnel was placed, which was used to generate an odour plume. For each treatment, different odour stimuli were placed in the funnel. A worn sock, which is a known attractive natural human odour source to An. gambiae (Pates et al. 2001), was used as a positive control. Ammonia and lactic acid (LA) were tested separately and in combination with each other, where NH₃ was tested in two concentrations (low: 136 and high: 1,363 ppm). Ammonia was transferred from a gas bag to the funnel by silicon tubes using a

Figure 1. Construction of the triangle of velocities between consecutive points of a track \((t, t + 1)\) in the XY plane after Marsh et al. (1978). Thick arrows indicate known vectors (track, wind). The broken arrow indicates the inferred vector (course). Arcs indicate course \((\gamma)\), drift \((\delta)\), and track \((\tau)\) angles (Kerguelen & Cardé 1997).
flow of 230 ml/min. Liquid LA was transferred from a wash bottle to the funnel by a flow of pressurized air (15 ml/min) through silicon tubes. All treatments were run in the presence of an artificial heat source (34°C) in the funnel to simulate heat from a human skin (Clements 1999, Healy et al. 2002).

Tests were performed in the last 3.5 hours of the dark period. Single mosquitoes were released from a release platform 110 cm downwind from the source in the centreline of the wind tunnel. If the mosquito did not take off within two minutes, the experiment was aborted.

The flights were recorded with two monochrome CCD video cameras that were placed in the longitudinal axis of the wind tunnel (parallel to the airstream) with an angle of 40° relative to each other. The effective stereoscopic view was ± 60 x 60 x 60 cm.

The video files were saved as MPEG-2 and 2-D tracks were subtracted using an auto detecting computer program (Ethovision 3.1, Noldus IT, Wageningen, The Netherlands). Subsequently the two track files were exported to a programme written in Matlab 7.0 (Mathworks) that calculated 3-D coordinates of the mosquito. Several parameters were calculated by the programme; flight speed was computed as product of the motion in three dimensions and flight angles were calculated in the XY-plane. Smoke was used to assess the dimensions of the odour plume, and these dimensions were inserted in the Matlab program. In this way, the program calculated for each coordinate of the mosquito’s flight whether it was inside or outside the odour plume.

Statistics were performed with SPSS 12.0.1. Flight analyses were performed over a distance of 50 cm. Upwind flight angles and flight speed inside the plume were compared with the same parameters when flying outside the plume.
Therefore all upwind directed parameters within or outside the plume boundaries were averaged per mosquito. Differences between the averages were checked for normality and tested in a one sample T-test. In this way each mosquito contributed equally to the sample means.

RESULTS AND DISCUSSION

Of the 245 mosquitoes tested in the wind tunnel, 156 (64%) took off and were observed in one of the camera views. From the plume analyses in Matlab it turned out that 156 of the recorded mosquitoes, only 37 had been in contact with the plume. The proportion of plume contact within a flight varied from 0.1-49% between mosquitoes. Flight paths of the mosquitoes were shown to be performed in all three dimensions. When mosquitoes never contacted the plume, flight paths were rather straight. Mosquitoes that flew a considerable proportion of their flight within the plume boundaries, showed longer and more complicated flight paths (Fig. 3).

When mosquitoes were only a short period of time in contact with the plume, they did not seem to show a behavioural response to offered odour. In assessing the effect of plume contact, possible effects could be masked by these weakly or not responding mosquitoes. Therefore the geometric mean of plume contact proportions was calculated (5.6%) and was used as a threshold for analysis. A total of 14 mosquitoes had been within the plume dimensions >5.6% of time. Because these flights were divided over 5 different odour treatments, they were pooled to have sufficient replicates. Of these 14 flight tracks, 7 mosquitoes were exposed to a worn sock, 3 to NH$_3$ at low concentration, 2 to NH$_3$ at high concentration, 1 to LA and 1 to NH$_3$ at low concentration + LA.

Figure 4 shows that the flight speed inside a plume of odours was significantly smaller than outside the plume ($P<0.01$). Track angle and course angle inside the plume were also significantly smaller than outside the plume ($P<0.05$ and

![Figure 3](image)

Figure 3. Two examples of 2D-projected flight tracks of mosquitoes exposed to odours from a worn sock. Flight path of a mosquito that had no contact with the plume (a.), flight in which the plume was repeatedly contacted (b.).
P<0.01, respectively). Drift angle inside the plume was not significantly different from outside the plume (P=0.52). From a study with a different mosquito, *Ae. aegypti*, it was also shown that track angle was decreased inside a plume. Flight speed of this mosquito, however, remained fairly constant (Dekker et al. 2005). This difference may be explained by the difference in activity pattern. *Anopheles gambiae* searches a host during the night and mostly indoors, while *Ae. aegypti* is mainly active during day-time. *Aedes aegypti* responds well to visual cues (Kennedy 1939) and visual cues are used for orientation during host seeking (Bidlingmayer 1994, Muir et al. 1992). *Anopheles gambiae* also seems to use visual cues to orientate (Gibson 1995, Gibson & Torr 1999), even under low light conditions, but possibly to less extent than *Ae. aegypti*. Flying towards a host in dark-

![Graph A](image1.png)

**Figure 4.** Average (+SE) upwind flight speed (**A**) and flight angles (**B**) inside and outside the odour plume (*P<0.05; **P<0.01).
ness may be more efficient for *An. gambiae* when flight speed is lowered and the flight is more aimed at the source upon plume contact and vice versa upon loss of contact. However, the adaptive advantage of a longer and thus more costly path upon plume contact remains unclear. In male moths it was also shown that flight angles became smaller when the plume was contacted (Mafra-Neto & Cardé 1994, 1995a, 1995b, Vickers & Baker 1996). But contrary to *An. gambiae* mosquitoes, flight speed was increased inside an odour plume (Mafra-Neto & Cardé 1998). This variation in behavioural responses of insects with different ecology and phylogeny may indicate differences in the underlying processes at the neural level.

To analyse differences in flight parameters in relation to the mosquito’s distance from the odour/heat source; the plume was divided in four distance classes with a length of 12.5 cm. When a mosquito had >20 upwind coordinates in each distance class, parameter values were averaged and compared between distance classes. This applied for 7 of the 37 mosquitoes of which 4 were exposed to a sock, 2 to NH₃ at high concentration and 1 to the combination of NH₃ at low concentration + LA. With decreasing distance to the source, flight speed decreased, and the difference was significant between class 1 and 3 and between 1 and 4 (P<0.05; see Fig. 5). P-values in comparisons of the other classes were: 1 and 2 (P=0.42); 2 and 3 (P=0.13); 2 and 4 (P<0.16); 3 and 4 (P=0.62). Track angle seemed fairly constant over distance. Only between class 2 and 3 a significant difference

**Figure 5.** Average (+SE) upwind flight speed (A) and track angle (B) inside the odour plume at different distance classes from the source. Class definitions: 1. 0-12.5 cm; 2. 12.6-25 cm; 3. 25.1-37.5 cm; 4. 37.6-50 cm. Bars that share no letters above differ significantly at P<0.05.
was found \((P<0.05)\). P-values in comparisons of the other classes were: 1 and 2 \((P=0.80)\); 1 and 3 \((P=0.35)\); 1 and 4 \((P=0.44)\); 2 and 4 \((P=0.16)\); 3 and 4 \((P=0.54)\). The reduction in flight speed closer to the source may be caused by the sensing of heat emitted by the heat element and considered a response in preparation of a landing. Healy et al. (2002) found that significantly more *An. gambiae* mosquitoes landed on heated cylinders when they were near an object with a temperature similar to that of human skin. The behavioural change may also be caused by a change of plume structure.

As mentioned before, plume dimensions were assessed by artificial smoke. It was seen that the plume increased in turbulence with distance. The observed increase in speed with distance may also be a response to the larger size of air-gaps between odour filaments. Changing speed may provide continuous stimulation of olfactory neurons. In a moth species, *Cadra cautella*, it is also suggested that an extreme flickering signal may be perceived as a fused signal (Justus & Cardé 2002, Justus et al. 2002).

This study shows interesting flight behavioural responses of a malaria mosquito by using novel auto-detecting software. Increased knowledge on the flight behaviour of this insect may help us to develop or improve odour-baited traps. When it becomes clearer how mosquitoes navigate into an odour-baited trap, design of those traps may improve, although attractants that can compete with human odours still need to be discovered. The current set-up may also be also be a useful tool for studying different insects, for instance parasitic wasps using herbivore-damaged plant volatiles to find a host.

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**REFERENCES**


