INFLUENCE OF ODOUR PLUME STRUCTURE ON UPWIND FLIGHT OF MOSQUITOES TOWARDS HOSTS

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Summary

Both the concentration and the fine-scale plume structure of host odours influence the upwind flight of female mosquitoes *Aedes aegypti* (L.) (Diptera: Culicidae) in a wind tunnel. The attractive effects of carbon dioxide, human skin odour and L-(+)-lactic acid were tested in homogeneous, turbulent and filamentous odour plumes. With carbon dioxide, the percentage of upwind-flying mosquitoes increased with the increasing fluctuations in concentration that occur in turbulent and filamentous plumes. In homogeneous plumes, an initial activation effect was observed, but sustained upwind flights were less frequent than in the other plumes. The opposite was found with plumes of human skin odour: the highest number of mosquitoes flew upwind in the homogeneous plume, whereas in turbulent or filamentous plumes their numbers were significantly lower. Regardless of plume type, the percentage of upwind-flying mosquitoes increased with increasing concentrations of carbon dioxide and of skin odour. With L-(+)-lactic acid, the dose–response characteristics were not consistent, and the relative effects of different plume types upon upwind flights differed within different ranges of concentration. Even maximum reactions to this compound were modest compared with those to carbon dioxide or to skin odour. Our findings demonstrate (1) that mosquitoes are able to orient upwind under continuous odour stimulation and (2) that upwind flight is dependent upon plume structure in different ways for different host odour components.

Key words: *Aedes aegypti*, mosquito, host-finding, upwind flight, odour plume, attraction, carbon dioxide, lactic acid.

Introduction

The orientation of insects towards attractive odour sources depends upon both the temporal and spatial distribution of odorants downwind from the source. This was first investigated by Wright (1958) and studied in further detail using male moths, which are attracted by female pheromones over long distances (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Murlis et al., 1992; Kaissling and Kramer, 1990). An odour evaporating from a small source such as a female pheromone gland remains confined to a thin filament of pheromone-laden air near the source. During downwind transport, however, the filament becomes increasingly disrupted and diluted due to turbulence in the air. This results in a highly intermittent signal, whose temporal and structural characteristics vary with distance from the source (Murlis, 1997; Murlis and Jones, 1981). For the males of several moth species, such intermittence has been demonstrated to be necessary for sustained upwind progress (Kennedy et al., 1980, 1981; Willis and Baker, 1984; Baker et al., 1985; Kramer, 1986, 1992; Kaissling and Kramer, 1990). More recent experiments demonstrate that the brief moments of perception of short pheromone pulses dictate subsequent flight manoeuvres (Mafra-Neto and Cardé, 1994, 1995, 1996;

Vickers and Baker, 1994; Baker and Vickers, 1997). Although many aspects of odour-mediated orientation have been studied in other flying insects, such as parasitoid wasps (Kaiser et al., 1994; Kerguelen and Cardé, 1997) and haematophagous Diptera (Brady et al., 1989, 1995; Bursell, 1984, 1990; Griffiths and Brady, 1995), we know little about the influence of the fine-scale structure of odour plumes in these cases. It would be interesting to learn whether different adaptations exist in other cases of odour-guided orientation that occur in different contexts and under different stimulus conditions, such as the host-finding behaviour of bloodsucking insects. There are no quantitative data regarding the spatial/temporal distribution of odours emitted by the potential hosts of such insects. However, we can assume that, in contrast to the typical pheromone plume arising downwind from a small source such as a female moth's pheromone gland, the odours released from a larger source such as a human body will form an initially large and probably less disrupted plume with different fine-scale characteristics (Cardé, 1996). Other characteristic features of the distribution of odours around or downwind of larger animals result because odours are emitted in two different ways (1) with the breath, which is exhaled

periodically, and (2) from the skin, which gives off volatile substances continuously by convection currents.

As a first step in exploring whether the plume structure influences the odour-mediated orientation of mosquitoes, we investigated how differences in the spatial/temporal distribution of odour molecules downwind from an odour source affect the upwind flight of female *A. aegypti* towards different components of host odour in a wind tunnel. We used carbon dioxide, L-(+)-lactic acid and a natural blend of human skin components as test odours. The latter has repeatedly been shown to attract mosquitoes in different types of olfactometers (Rahm, 1958; Schreck et al., 1981; summary in Takken, 1991; Geier et al., 1996). Carbon dioxide, a major component of exhaled breath, is known to increase the probability of take-off and to prolong the duration of flight in mosquitoes; it is also an attractant (Geier et al., 1996; Eiras and Jepson, 1991; Gillies, 1980). L-(+)-Lactic acid can be detected both in human breath and on human skin (Acree et al., 1968; Smith et al., 1970). As a single stimulus, this compound is only slightly attractive for *A. aegypti*, but in combination with carbon dioxide it acts as a synergist by increasing the total attractiveness (Acree et al., 1968; Smith et al., 1970; Eiras and Jepson, 1991). L-(+)-Lactic acid also plays an essential role in the attractiveness of human skin odour since without this compound the remaining volatiles from the skin are not effective (Geier et al., 1996).

Odour plumes of three different structures were generated by varying the mode of odour inlet into the air current of the wind tunnel. The resulting distribution of odours in the plumes was simulated and visualised by introducing smoke from $TiCl_4$ sources instead of odorants into the wind tunnel.

Materials and methods

Animals

10- to 40-day-old female *Aedes aegypti* (L.) from cultures raised at the Centre for Plant Research (*Pflanzenschutzzentrum*) at Bayer AG, Monheim, Germany, were used in our experiments. They were reared as larvae and fed with Tetramin fish food. Adults (300–500) were kept in containers (50 cm×40 cm×25 cm) at 26–28 °C, 60–70 % relative humidity and on a 12 h:12 h L:D photoperiod. In the containers, the animals had access to a 10 % glucose solution on filter paper.

Wind tunnel

The wind tunnel consisted of an 800 mm long transparent Plexiglas tube (thickness 5 mm). The tube was 70 mm in diameter, representing approximately seven *A. aegypti* wingspans. A gauze screen situated 150 mm from the upwind end of the tunnel could be rotated by hand to open or close the upwind chamber (Fig. 1A). The wind tunnel connected with a polyvinylchloride tube (stimulus chamber, length 200 mm, inner diameter 100 mm) at the upwind end, where the odour plumes were generated. The release chamber (length 150 mm; inner diameter 70 mm) containing the mosquitoes was attached

to the downwind end of the wind tunnel. A constant air stream (flow rate 58 l min^{-1}) from the Institute's pressurised air system was purified using an activated charcoal filter, heated and humidified before being passed through the wind tunnel. Further details of this experimental arrangement are described elsewhere (Geier, 1995). The temperature in the wind tunnel was 28±1 °C, the relative humidity 70±5 % and the wind speed 0.25 m s^{-1}.

The wind tunnel was placed on a white table, and a white cardboard shield (height 20 cm) covered both sides of the wind tunnel. Overhead illumination was provided by two 40 W light bulbs.

Plume generation

Different patterns of odour distribution in the wind tunnel air stream were generated by injection of odorants into the stimulus chamber at different positions (Fig. 1B). Stimulus-laden air was injected through Pasteur pipettes (tip i.d. 1.1 mm, o.d. 1.4 mm, total length 150 mm, length of tip 60 mm) at flow rates of 1600 ml min^{-1} at position 1 and 200 ml min^{-1} at positions 2 and 3 (see Fig. 1B). In this way, three distinctly different patterns of odour distribution were produced, which were simulated and visualised (see Fig. 4A) by introducing smoke into the tunnel instead of odours. To generate smoke, 0.1 ml of $TiCl_4$ (99 %, Merck) was placed on a strip of filter
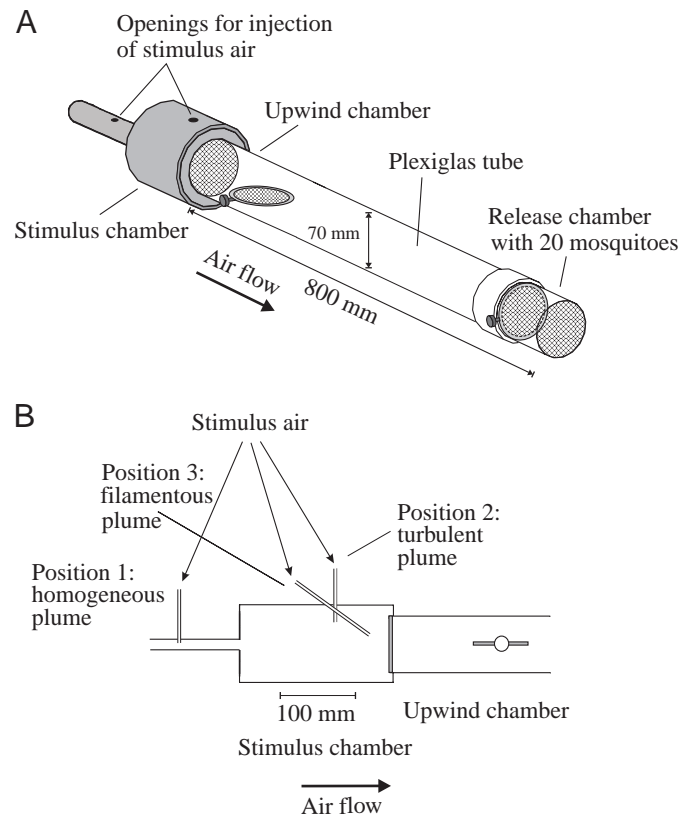

Fig. 1. (A) Diagram of the wind tunnel. (B) Section through the stimulus chamber. Different plume structures were produced by injecting stimulus air *via* Pasteur pipettes at three different positions.

paper (0.5 cm×5 cm), which was then introduced into the pipette. When air was passed through the pipette, plumes of smoke, whose structures varied considerably according to the different positions of the pipettes and the different flow rates, appeared in the wind tunnel (Fig. 1B, see Fig. 4A). To estimate the temporal distribution of odours within the different plume types, the smoke density was measured using small light barriers placed in the middle of the wind tunnel at a distance of 10 cm and 70 cm, respectively, from the upwind end. These light barriers consisted of an infrared light-emitting diode facing an infrared phototransistor (distance 15 mm). The diameter of the infrared beam was 1.5 mm. Smoke blown across the beam changed the voltage produced by the phototransistor, and this was taken as a measure of smoke density. The voltage signals were amplified, fed into an analogue-to-digital (A/D) converter (Syntech IDAC-01) at rate of 6944.4 samples s^{-1}, stored on a personal computer and analysed using Autospike software (Syntech, Hilversum, The Netherlands). The number of smoke peaks per unit time and their amplitude were evaluated. Peaks were counted where the amplitude was 50 % higher than the background level and where they occurred more than 25 ms apart.

Odour stimuli

Three different odour stimuli were used; in addition, compressed air purified with a charcoal filter was used as control stimulus to check for contamination effects. L-(+)-Lactic acid at varying concentrations was produced with an olfactometer based on the design of Ough and Stone (1961). Charcoal-filtered compressed air at flow rates from 0.16 ml min^{-1} to 1000 ml min^{-1} was passed through a 250 ml Erlenmeyer flask filled with 10 ml of L-(+)-lactic acid solution (90 % aqueous solution; Merck). The air stream passed over the surface of the solution. To determine the output of lactic acid, the air stream was passed at various flow rates through solutions of 0.01 mol l^{-1} sodium hydroxide and titrated with 0.01 mol l^{-1} HCl solution (Ough and Stone, 1961). Fig. 2 shows the relationship between the flow rate through the flask and the amount of lactic acid trapped per minute. To ensure that the total output of lactic acid was collected, four sodium hydroxide traps were used in series, and the amount in each trap was determined. We never found significant amounts of lactic acid in the last trap. Carbon dioxide was taken from a gas cylinder with a trade-standard purity of 99.9 % (Linde, Germany). Various volumes of the gas were mixed with clean air to produce different concentrations in the stimulus air. According to the method of Schreck et al. (1981, 1990), skin odours from a volunteer subject were transferred to the surface of a glass test tube (o.d. 16 mm, length 160 mm) by intensely rubbing it in the hands for 5 min. The hands were rinsed with tap water 2 h before the experiment, and care was taken to avoid any contact with cosmetics or perfumes prior to the rubbing. The glass tube with attached skin residues was then inserted into a Teflon tube (i.d. 17 mm, length 150 mm), and charcoal-filtered air was passed through the narrow space between the Teflon tube and the glass tube at 1600 ml min^{-1}
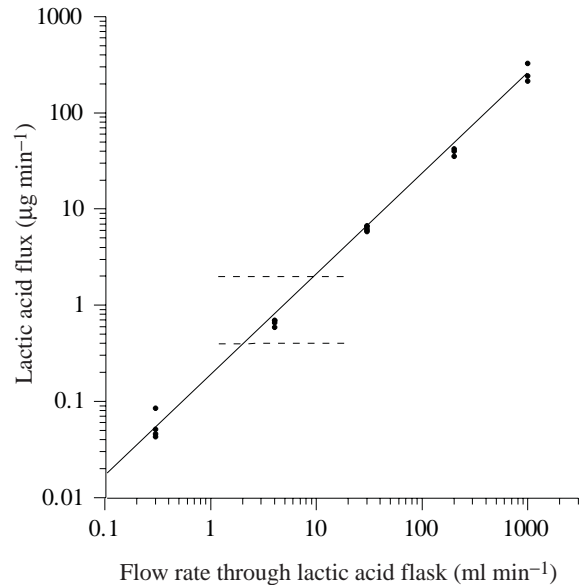

Fig. 2. Calibration of the lactic acid output from the stimulus flask. Each point shows the amount of lactic acid trapped per minute in a solution of 0.01 mol l^{-1} sodium hydroxide at a given flow rate. Flow rates between 0.3 and 1000 ml min^{-1} were used. The regression line was used to calculate the lactic acid flux at flow rates lower than 0.3 ml min^{-1} by extrapolation. The equation for the regression line was log$y$=−0.757+1.041log$x$ (r^2=0.996, P<0.0001). The broken horizontal lines indicate the lowest and the highest rates of lactic acid output from human hands measured by Smith et al. (1970).

(Fig. 3). To vary the odour concentration in the plumes, the odour-laden air was mixed with clean air. The concentration of the skin odour in the plume was defined as the ratio of air loaded with skin odour to the volume of the total air stream forming the plume.

Flow meters (for flow rates over 3 ml min^{-1}) or a precision tubing pump (Masterflex, Novodirect GmBH, Kehl/Rhein, Germany; flow rates 0.1–3 ml min^{-1}) were used to control the gas flow.

To estimate the odour concentration in the plumes, the maximum possible concentration of the generated odour filaments was used as a reference value. For the turbulent and filamentous plumes, this concentration was considered to be the same as in the injected stimulus-laden air. For homogeneous plumes, in which the injected odours are evenly distributed, the concentration was calculated from the injected dose and the total volume of air in the wind tunnel.

Bioassay

Groups of 18–22 female mosquitoes were used for the tests. The experimenter's hand was used to lure them out of their cage into the release chamber, which then was attached to the downwind end of the wind tunnel. Before odour stimulation, the mosquitoes were given 20 min of acclimation time in the wind tunnel air stream. Upon stimulus onset, the release chamber was opened and the mosquitoes were allowed to enter the wind tunnel. After 30 s of stimulation, the rotating screens
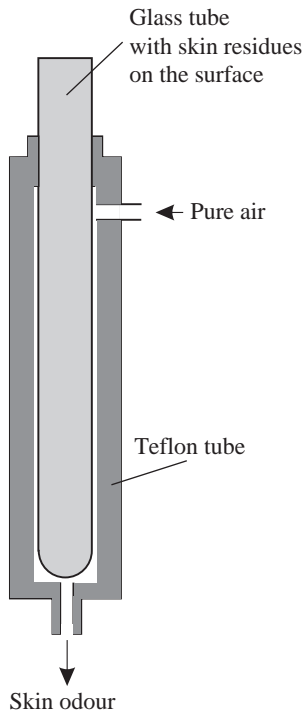
Fig. 3. Apparatus for generating skin odour stimuli. A glass test tube, which had been rubbed intensively in the hands of a volunteer subject, was placed into a Teflon tube. An airflow of $1600\,ml\,min^{-1}$ was passed through the gap (0.5 mm) between the glass tube and the wall of the Teflon tube. The exiting air contains skin odour, which can be diluted with clean air to provide various stimulus concentrations.

of both the upwind and the release chamber were closed, and the number of mosquitoes that had left the release chamber and the number of mosquitoes that had reached the upwind end were counted. After the experiment, the wind tunnel was connected to a fan which reversed the airflow (wind speed approximately $0.3\,m\,s^{-1}$). The mosquitoes were then lured back into the release chamber using the hand as an attractant. The wind tunnel was then reattached to the purified air stream. Each group of mosquitoes was tested up to 10 times with an interval of at least 20 min between each test.

Each odour was tested in a separate block of experiments. Within such a block, both stimulus concentration and plume type were varied. Each stimulus treatment and pure air as a control were tested using 20 different groups of mosquitoes, each group being exposed to every treatment in random order. Between the tests, a constant flow of fresh air was passed through the wind tunnel. The experiments were carried out between 09:00 h and 18:00 h.

Evaluation

In each test, we distinguished two behavioural categories of responses: (1) the percentage of mosquitoes found outside the release chamber was taken as a measure of activation, which included flight initiation and short-duration upwind progress;

(2) the percentage of mosquitoes trapped at the upwind end after 30 s was taken as a measure of sustained upwind flight. The values for both activation and upwind flight were averaged from the 20 experiments. Since the data are percentages, they were transformed using angle transformation (Sokal and Rohlf, 1981) for further statistical analysis. The transformed means were analysed independently using a one-way analysis of variance (ANOVA) with the least-squares difference (LSD) method as a *post-hoc* test for comparison of treatments. All calculations and statistics were carried out using SPSS 6.0 for Windows.

Results

Features of odour plumes

The different types of odour plumes generated in the wind tunnel were visualised using plumes of TiCl$_4$ smoke (Fig. 4A). The distribution of smoke was verified several times with consistent results during the course of the study. The smoke pattern shows that the plume structure is mainly dependent upon (1) the position of the injection pipette and (2) the injected volume of air. We found empirically that injection of $1600\,ml\,min^{-1}$ at position 1 (Fig. 1B) resulted in the formation of a homogeneous plume (Fig. 4A), uniformly distributed over the whole cross section of the wind tunnel. No gradient appeared along the wind tunnel, as documented by the photographs and by the measurements of smoke density (Fig. 4B). This indicates that odours applied in this way were evenly distributed in the wind tunnel. Injection of smoke at $200\,ml\,min^{-1}$ at position 2 (Figs 1B, 4) generated a turbulent plume, which is unevenly distributed, with filaments or areas of higher smoke density alternating with areas of lower density. These variations are smaller than those found in the filamentous plume, with the plume relatively dispersed in the wind tunnel. Measurements of smoke density at both ends of the wind tunnel revealed that the plume became less disrupted and more evenly distributed towards the downwind end. A filamentous plume type was produced by the injection of $200\,ml\,min^{-1}$ at position 3 (Fig. 1B). The oblique position of the glass pipette resulted in the formation of a meandering filament, which breaks down into filaments and packets (Fig. 4). Such filaments of high smoke density alternate with pure air, and the distribution of smoke is highly intermittent. As in the turbulent plume, the filaments were more dispersed and diluted towards the downwind end. To quantify the difference in the plume structures, the smoke density was measured for 20 s in three experiments using the infrared light-emitting diodes. In the homogeneous plume, no peaks of high smoke density were detected. The turbulent plume yielded peaks with a mean amplitude of $4.7\pm1.0\,mV$ ($N=96$). The mean frequency of pulses was $1.6\pm1.1\,Hz$ ($N=3$) and the mean duration of pulses was $55.3\pm26.8\,ms$ ($N=96$). The mean peak amplitude in the filamentous plume was $35.4\pm35.4\,mV$ ($N=339$), the frequency $5.7\pm2.3\,Hz$ and the mean duration of pulses $83.8\pm50.6\,ms$ ($N=339$) (means $\pm$ S.D.). The turbulent and filamentous plumes differed significantly in pulse duration

A

Upwind chamber   Release chamber

Homogeneous

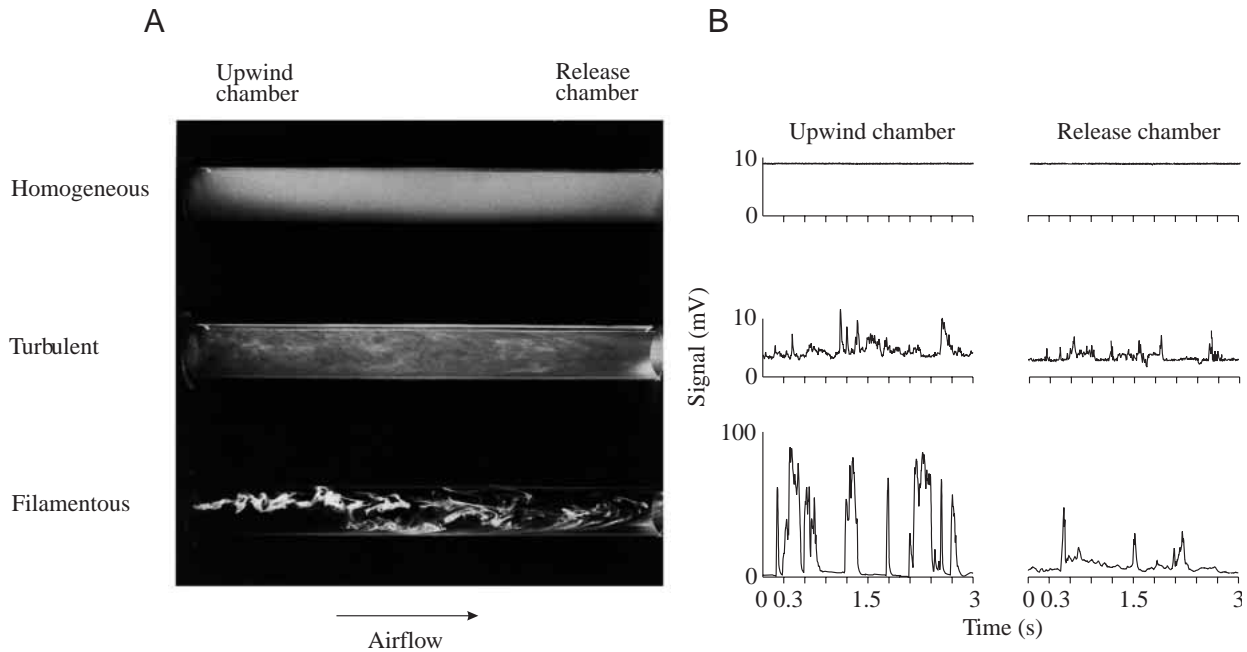Turbulent

Filamentous

Airflow

B

Fig. 4. Smoke visualisation of odour plumes in the wind tunnel. (A) Photographs of the wind tunnel from above during the generation of homogeneous, turbulent and filamentous plumes (see Fig. 1B) using TiCl$_4$ smoke in place of an odour stimulus. The photographs were taken 10–15 s after 'stimulus' onset. The wind tunnel was illuminated from the left side. (B) Local smoke densities measured in each plume type using two small light-emitting diodes and transistors placed in the centre of the wind tunnel, one 10 cm from the upwind end (upwind chamber) and one 70 cm from the upwind end (release chamber). Changes in smoke density caused changes in the voltage output of the phototransistor. Note that the voltage scale differs for the filamentous plume.

(t=3.06; P=0.003; t-test for unpaired samples) and pulse amplitude (t=7.65; P<0.0001; t-test for unpaired samples).

Behaviour of the mosquitoes in the wind tunnel without odour stimuli

During the first minutes after attaching the release chamber to the wind tunnel, most of the mosquitoes flew around in the chamber. They gradually became more stationary, and after 20 min of acclimation they were positioned on the gauze or on the wall of the release chamber, with flights occurring only sporadically. After opening the rotating screen, 20–40 % of the mosquitoes left the chamber during the 30 s test period in control tests with pure air (Fig. 5). These mosquitoes mostly flew a short distance upwind, turned back, flew upwind again, and so on. Sometimes they landed on the wall of the wind

tunnel, and in a few cases they flew back into the release chamber. The mean percentage of mosquitoes trapped after 30 s in the upwind chamber was 10 % or less (Fig. 5). The activation response did not differ significantly between the different plume types, but the percentage of mosquitoes that reached the upwind end was slightly, but significantly, greater for the filamentous plume than for the homogeneous or turbulent plume.

After all the tests, the mosquitoes could easily be lured back

Fig. 5. Responses of mosquitoes to pure air. Three plume types (homogeneous, turbulent and filamentous) of pure air were tested by injecting air without addition of odours into the stimulus chamber (see also Fig. 1B). The shading of the columns indicates the plume type. 'Activated' represents the mean percentage of mosquitoes that left the release chamber. 'Upwind flight' represents the mean percentage of mosquitoes trapped in the upwind chamber of the wind tunnel after 30 s. Values are means + S.E.M. of 20 experiments. In each experiment, 18–22 mosquitoes were tested. Means within each category were compared using an LSD *post-hoc* test and a one-way ANOVA; different letters above columns indicate significant (*P*<0.05) differences within a category.
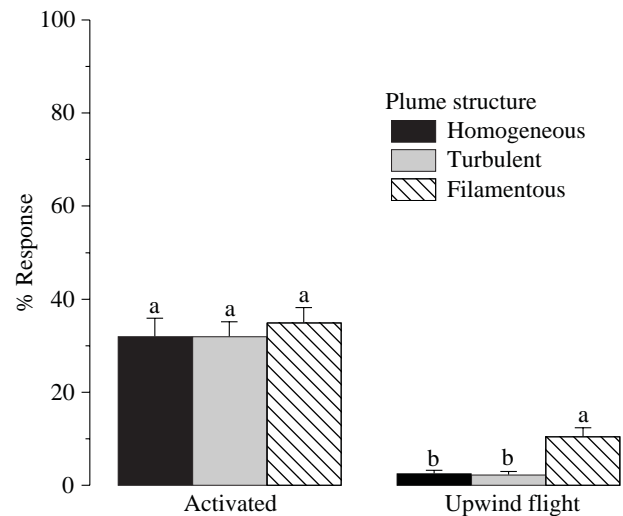
into the release chamber by using the experimenter's hand as an attractant and reversing the airflow in the wind tunnel. The initially increased flight activity that occurred immediately after exposure to the hand odour decreased during the following rest period of 20 min, during which the mosquitoes were exposed to clean wind tunnel air only. Using such 'recovery' intervals, we found no indication that repeated stimulus exposure affected the responses of mosquitoes in subsequent tests.

Response to carbon dioxide

To investigate the effects of carbon dioxide, 20 groups of mosquitoes were tested with homogeneous, turbulent and filamentous plumes of carbon dioxide varying in concentration from 0.05 to 100 vol%. The maximum concentration tested in the homogeneous plume structure was 4 % to avoid toxic effects. At all concentrations, a significantly higher percentage of mosquitoes reached the upwind chamber in the filamentous plume than in the other plume types, and the turbulent plume also evoked a significantly higher upwind-flight response than the homogeneous plume at a concentration of 4 % (Fig. 6A). Two trends are obvious for sustained upwind flight: (1) in all plume types, the response increased with increasing concentration of carbon dioxide; (2) the response also increased with increasing fluctuation of carbon dioxide concentration within the wind tunnel air. These trends are not consistent for the activation response, with the response to the homogeneous plume being significantly greater at low levels of carbon dioxide (Fig. 6A). The homogeneous carbon dioxide plume appeared to stimulate the mosquitoes to take flights with a short-duration subsequent upwind progress. However, they did not fly persistently upwind, often changed their flight direction and flew around irregularly. Some mosquitoes flew into the upwind chamber within the first few seconds, but then flew out again during the experiment. This was rarely observed with turbulent or filamentous plumes. In general, the carbon dioxide stimuli increased the percentage of activated and of upwind-flying mosquitoes compared with the control experiments with pure air (Figs 5, 6A).

Response to lactic acid

Compared with the control experiments, the lactic acid odour slightly increased the percentage of upwind-flying mosquitoes, but an influence of plume structure on activation and upwind flight was less obvious (Fig. 6B). At lower doses, the homogeneous plume stimulated a similar percentage of upwind-flying mosquitoes to the turbulent or filamentous plumes at higher doses. Whereas approximately 60 % of the mosquitoes flew upwind in the filamentous carbon dioxide plume at the highest concentrations (Fig. 6A), no more than 20 % flew upwind in lactic acid plumes regardless of the plume structure. Homogeneous plumes were more effective than turbulent or intermittent ones for lower concentrations of lactic acid. The responses to the more fluctuating plumes increased with increasing concentration of lactic acid; however, a significant decline in the number of upwind-flying mosquitoes

occurred at the highest lactic acid concentration in homogeneous plumes. This decline was not seen in the turbulent or filamentous plumes, even though the maximum concentration used was approximately six times higher than in the homogeneous plume. In general, lactic acid at the tested concentrations activated the mosquitoes to a lesser degree than did carbon dioxide, and the plume structure did not consistently affect this behavioural response.

Response to odours from skin residues

The upwind-flight response to skin odour depends significantly both on the odour concentration and on the fine-scale structure of the plume (Fig. 6C). The percentages of activated and of upwind-flying mosquitoes were significantly higher for the homogeneous skin odour plume than for the turbulent and filamentous plumes. This effect was also apparent in the different slopes of the dose–response relationships between the homogeneous plume and the turbulent or filamentous plume. For a 100-fold increase in odour concentration, the behavioural responses increased from 40 % to 95 % (activation) and from 10 % to 87 % (upwind flight) in the homogeneous plume. For a 200-fold increase in concentration, however, filamentous and turbulent plumes evoked only an increase of the behavioural responses from 40 % to 60 % (activation) and from 10 % to 30 % (upwind flight). The responses in turbulent and filamentous plumes did not differ significantly.

Discussion

Plume features

Our findings regarding the influence of the distribution pattern of host odours upon the upwind flight of *A. aegypti* rely upon the applicability of the patterns visualised using $TiCl_4$ smoke instead of odour. The use of smoke to visualise odour patterns is generally accepted, since it is assumed that the plume's physical dimensions and structure are essentially independent of the chemical and physical properties of the material within it (Murlis et al., 1992). In wind-tunnel experiments with male gypsy moths *Lymantria dispar*, however, Charlton et al. (1993) obtained different estimates of the size of a pheromone plume at high concentrations from behavioural responses (wing-fanning) and smoke visualisation. Therefore, we should not overestimate the precision of the patterns derived from smoke visualisation when correlating plume features with behavioural responses. Nevertheless, smoke visualisation provides at least a qualitative measure of the distribution of odours and permits differentiation between homogeneous, turbulent and filamentous plumes.

Since the odour filaments in turbulent or filamentous plumes were not precisely defined and were dispersed irregularly, the odour intensity perceived by a mosquito flying through the wind tunnel might vary considerably and cannot be determined exactly. The resolution of the smoke densities measured with the light-emitting diode does not permit an estimation of the odour density in the filaments, because the density is averaged
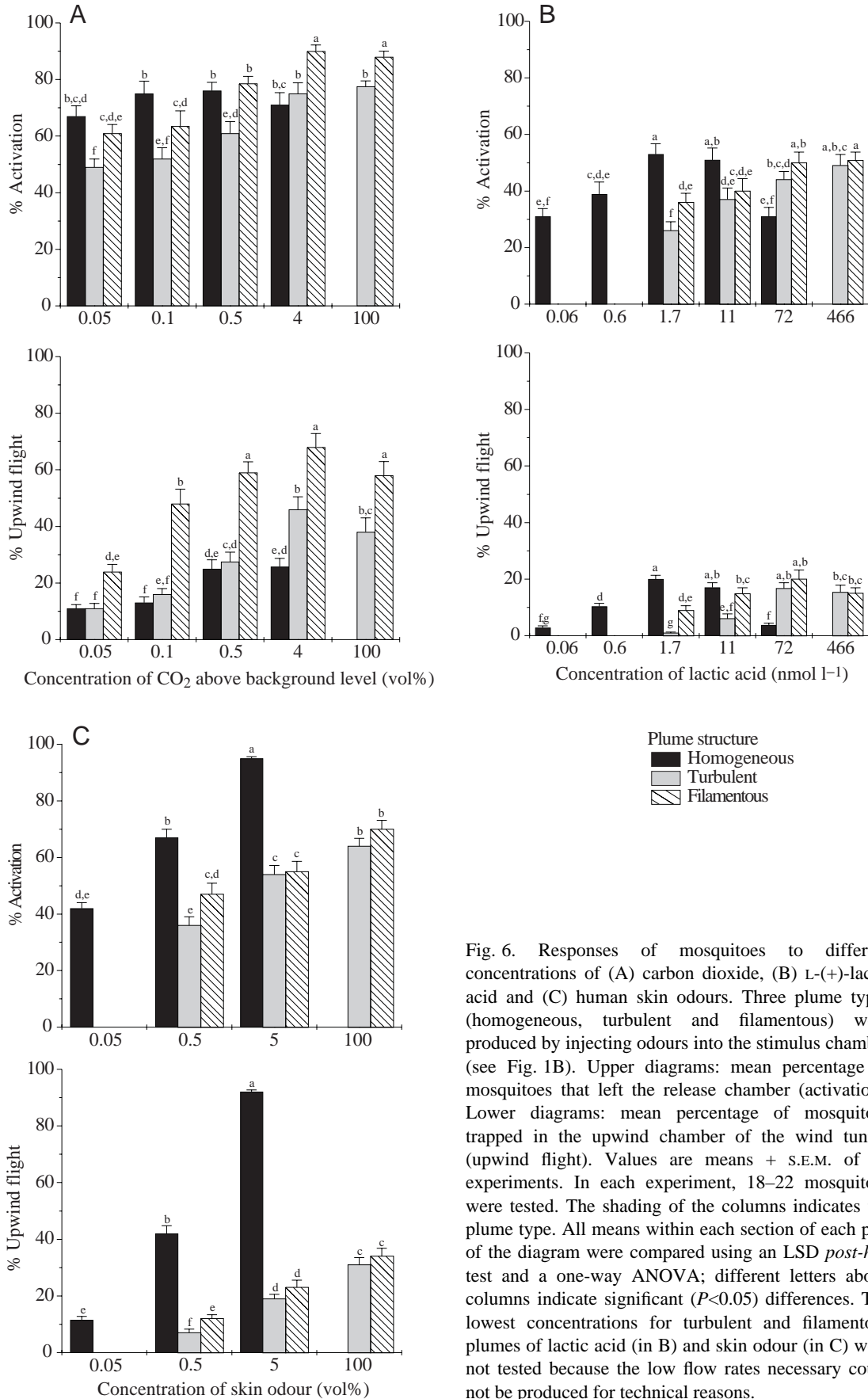
Fig. 6. Responses of mosquitoes to different concentrations of (A) carbon dioxide, (B) L-(+)-lactic acid and (C) human skin odours. Three plume types (homogeneous, turbulent and filamentous) were produced by injecting odours into the stimulus chamber (see Fig. 1B). Upper diagrams: mean percentage of mosquitoes that left the release chamber (activation). Lower diagrams: mean percentage of mosquitoes trapped in the upwind chamber of the wind tunnel (upwind flight). Values are means + S.E.M. of 20 experiments. In each experiment, 18–22 mosquitoes were tested. The shading of the columns indicates the plume type. All means within each section of each part of the diagram were compared using an LSD *post-hoc* test and a one-way ANOVA; different letters above columns indicate significant ($P<0.05$) differences. The lowest concentrations for turbulent and filamentous plumes of lactic acid (in B) and skin odour (in C) were not tested because the low flow rates necessary could not be produced for technical reasons.

over a volume of $0.4\,cm^3$. Furthermore, differentiation between a thin smoke filament with high density and a thicker one with lower density is not possible. However, the smoke distribution shown in Fig. 4A and the peak values of the smoke density measurements plotted in Fig. 4B suggest that odour clusters of higher concentration exist more often in filamentous than in turbulent plumes. In homogeneous plumes, odours are equally distributed, and the actual odour concentration is the same throughout the wind tunnel. However, because a flying insect is not stationary relative to the air, the flux of odour molecules measured by the sensory receptors will vary depending on the direction and speed of flight with respect to the air currents even at a constant wind velocity and stimulus concentration (Kaissling and Kramer, 1990; Elkinton and Cardé, 1984). To compare concentration effects within the three plume types, the maximum concentration that mosquitoes could encounter within a plume was taken. In the homogeneous plume, this value is constant throughout the wind tunnel, whereas in filamentous and turbulent plumes, the peak concentration decreases during downwind transport. Since it is difficult to define the exact stimulus strength detected by the mosquito during an experiment, this variable was changed for all three plume types by changing the concentration of the injected stimulus air. This allowed discrimination between the effects of odour concentration and the influence of short-term fluctuations in the signal within the odour plume.

Effects of different plume structures

The tests using plumes of carbon dioxide demonstrate that increased intermittence of the signal increased the probability of sustained upwind flight, irrespective of concentration over a wide range. The large difference between the percentage of activated mosquitoes and the percentage of mosquitoes found in the upwind chamber in homogeneous plumes indicates that upwind progress is reduced under continuous stimulation with carbon dioxide. This is further confirmed by our observations of irregular up- and downwind flights in the homogeneous plume. These findings are in agreement with the proposed orientation mechanisms of male moths following upwind a plume of sex pheromone released from a calling female. The intermittence of the pheromone signal is essential for the sustained upwind flight of male moths (Kennedy et al., 1980, 1981; Willis and Baker, 1984; Baker et al., 1985; Kaissling and Kramer, 1990). Recent studies have shown that the fine-scale structure of a pheromone plume influences instantaneous flight manoeuvres (Mafra-Neto and Cardé, 1994, 1995, 1996; Vickers and Baker, 1994; Baker and Vickers, 1997). Omer (1979) has demonstrated that carbon dioxide elicits upwind flight of *Anopheles arabiensis* and *Culex pipiens fatigans* only if presented intermittently. In contrast to our experiments, he used a low repetition rate and long-lasting pulses (20 s on, 20 s off). Bowen (1991) argued that it is not the absolute level but the change in concentration of the gas that is the important factor for eliciting behavioural responses. This was confirmed in the present experiments.

Our most surprising result was for stimulation with skin odours. With this stimulus, the percentage of mosquitoes flying persistently upwind was significantly higher in a homogeneous plume than in an intermittent one. This was consistent over a wide range of concentration and was different from the responses to carbon dioxide. These findings provide the first evidence for an alternative orientation mechanism to that proposed in male moths, which need intermittent signals for sustained upwind flight towards a pheromone source. In moths, this is likely to be an adaptation to the special plume pattern that occurs downwind from a calling female (Cardé, 1996). The different responses of mosquitoes to the plumes of carbon dioxide and skin odours might reflect different release patterns of these stimuli from the host. Humans give off carbon dioxide by periodically expiring air through the mouth or nose. It can be assumed that, in the immediate vicinity of such a host, this periodicity and also the turbulence of expired air, which contains 4–5 % carbon dioxide, cause strong concentration fluctuations against the atmospheric background level of 0.03–0.05 %. Accordingly, we found the strongest upwind flight responses in plumes with filaments of concentrations between 0.5 % and 4 %. The odours from the skin, however, are probably given off more continuously and are dispersed by the convection currents produced by a warm-blooded animal (Willemse and Takken, 1994). Moreover, the source size of the odour will have a considerable effect on plume structure: the larger the source, the lower will be the intermittence and the concentration fluctuations (Murlis et al., 1992). Therefore, the distribution of skin odours may be rather homogeneous close to the host and may become more dispersed and intermittent as a result of air turbulence with increasing distance. The observed differences in upwind flight tendency between plumes of carbon dioxide and skin odour may reflect this situation, and two different modes of orientation could be proposed. (1) Far from the host, the mosquitoes perceive a turbulent and intermittent plume, in which both carbon dioxide and skin odours are present. In this disrupted and incompletely mixed plume, synergistic effects of skin odours and carbon dioxide may be important for orientation. The underlying mechanisms could be similar to those found in moths. (2) Near the host, a uniform cloud of skin odours indicates to the mosquitoes that they are near a host and they can then follow the homogeneous plume produced by the skin odour and by the convection currents of a warm-blooded host. It can be assumed that different orientation mechanisms underlie this short-range attraction, and these need to be studied in more detail.

Lactic acid is a major component of both human skin odour and human breath and acts as a synergist together with carbon dioxide and odour components on human skin (Acree et al., 1968; Smith et al., 1970; Geier et al., 1996). According to Smith et al. (1970), the rate of emission of L-(+)-lactic acid from human hands ranges from 0.38 to $2.22\,\mu g\,min^{-1}$. These doses correspond to lactic acid concentrations of $0.08–0.36\,nmol\,l^{-1}$ in the homogeneous plume. We found that the most effective lactic acid concentration was $1.7\,nmol\,l^{-1}$, giving 20 % upwind-flying mosquitoes in the homogeneous

plume. Similar responses were also elicited in turbulent or intermittent plumes, but with filaments of higher stimulus intensity. Homogeneous plumes of these high concentrations of lactic acid resulted in a significant decrease in activation as well as in upwind flights. To understand the behavioural relevance of lactic acid in different plume structures, quantitative investigations of the release patterns in the breath and from the skin should accompany future studies to explore the effects of lactic acid, carbon dioxide and other skin odour components individually and in combination. The responses of *A. aegypti* to different plume structures suggest that the host-finding behaviour of mosquitoes is affected not only by the odour composition but also by the characteristics of the plume structure, which may depend on the shape and body size of the host, on physical variables such as temperature or humidity and on the distribution of distinct components on the body surface. Unfortunately, no quantitative data exist regarding the typical features of odour plumes generated from large vertebrates by breathing or convection currents. The present study was instigated as a first step in the exploration of these aspects of the odour-mediated orientation behaviour of mosquitoes, and we have demonstrated that fine-scale plume structure has a strong influence on the upwind flight behaviour of these insects. Subsequent quantitative behavioural analysis of individual mosquitoes flying in defined odour plumes will be conducted to study their orientation behaviour in detail. In addition to further investigations of the orientation mechanisms, studies must be undertaken of the typical features of plumes generated from the respective hosts of the mosquito species investigated. These findings could also be important for developing traps for mosquito control. For this purpose, different types of odour-release equipment should be tested, which imitate the natural plume pattern given off by a host and thereby enhance the trapping effect of the artificial baits used.

Sensory aspects of the perception of intermittent and continuous stimuli

The peripheral sensory equipment of *A. aegypti* for odour perception has been studied repeatedly (Lacher, 1967; Pappenberger et al., 1996; Davis and Sokolove, 1976; Davis and Bowen, 1994), but we know little about receptor responses with respect to temporal changes of odour stimuli. In addition, the 'follower' capabilities of the carbon dioxide receptors on the maxillary palps to pulsed stimuli have not been studied in detail. Their strong phasic on-response, however, should enable them to follow rapid changes in carbon dioxide concentration (Kellogg, 1970; Grant et al., 1995), as do moth pheromone-specific cells, which can follow up to 10 odour pulses per second (Kaissling, 1986, 1997; Rumbo and Kaissling, 1989). These insects seem to sustain upwind progress only if there is phasic input from the receptor cells. Continuous pheromone stimulation fails to evoke persistent upwind flight probably because of the tonic response of the pheromone chemoreceptor cells or their adaptation (Baker et al., 1988; Kennedy et al., 1980; Willis and Baker, 1984;

Kaissling and Kramer, 1990). This might also hold true for the upwind flight of mosquitoes in response to carbon dioxide; for skin odours, the situation is probably different. In response to such odours, *A. aegypti* orient upwind in homogeneous plumes, and it can be assumed (1) that the relevant receptor cells do not adapt quickly to continuous stimulation, and (2) that the tonic responses of these cells are sufficient to evoke sustained upwind flight.

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