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Location of and landing on a source of human body odour by female *Culex quinquefasciatus* in still and moving air

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Abstract

The orientation to and landing on a source of human odour by female *Culex quinquefasciatus* Say (Diptera: Culicidae) is observed in a wind tunnel without an airflow or with a laminar airflow of 0.2 m s^{-1} . Odours from human feet are collected by 'wearing' clean glass beads inside a stocking and presenting beads in a Petri dish in a wind tunnel. Mosquitoes are activated by brief exposure to a 1 L min^{-1} jet of 4% CO_2 positioned 10 cm from the release cage. In moving air at 0.2 m s^{-1} , a mean of 3.45 ± 0.49 landings are observed in 10 min trials (5 mosquitoes per trial), whereas 6.50 ± 0.96 landings are recorded in still air. Furthermore, 1.45 ± 0.31 mosquitoes are recorded on beads at any one time in moving air (a measure of individuals landing versus one landing multiple times) compared to 3.10 ± 0.31 in still air. Upwind flight to beads in moving air is demonstrated by angular headings of flight immediately prior to landing, whereas approaches to beads in still air are oriented randomly. The mean latency until first landing is $226.7 \pm 17.98 \text{ s}$ in moving air compared to 122.5 ± 24.18 in still air. Strategies used to locate a prospective host at close range in still air are considered.

Keywords

Anemotaxis; klinotaxis; orientation; static-air olfactometer; wind tunnel

Introduction

Female *Culex quinquefasciatus* Say (Diptera: Culicidae) feed on the blood of many vertebrates, with a preference for birds, but also opportunistically on humans (Clements 1999), evidently without a preference for any particular region of the human body (de Jong & Knols, 1996). Many studies have documented field attraction of female *C. quinquefasciatus* to traps baited with carbon dioxide (CO_2) and human skin odours (see Clements, 1999; Mboera *et al.*, 2000; Syed & Leal, 2009), but relatively few studies have examined the host-seeking behaviour of *C. quinquefasciatus* in well defined odour plume structures and airflow regimes (Mboera *et al.*, 1998; Allan *et al.*, 2006; Cooperband & Cardé, 2006; Puri *et al.*, 2006; Cooperband *et al.*, 2008; Lacey & Cardé, 2011). Digital video and tracking software allow ready visualization of mosquito flight in response to chemical cues, from activation to location of the odour source (Takken *et al.*, 1997; Dekker

et al., 2005; Cooperband & Cardé, 2006; Beeuwkes *et al.*, 2008; Spitzen *et al.*, 2008a; Lacey & Cardé, 2011; Dekker & Cardé, 2011). Digitized in 3-D, flight tracks of upwind orientation demonstrated differences in flight behaviour by *C. quinquefasciatus* in response to CO₂ plumes emitted by different designs of mosquito traps in a large 'field' wind tunnel (Cooperband & Cardé, 2006). Another study utilizing 3-D tracking found relatively rapid activation of *C. quinquefasciatus* after exposure to a plume of air enriched to 4% CO₂, preferential landing on glass beads coated with human foot odour, relatively fast flight upwind when exposed to a plume of CO₂ or clean air, and flight that was slower and less directly upwind when exposed to a plume of odour from a human foot (Lacey & Cardé, 2011). Wind tunnel studies of *Anopheles gambiae sensu stricto* using digital tracking similarly did not reveal strong evidence of upwind orientation along plumes of skin odour by this species (Takken *et al.*, 1997; Beeuwkes *et al.*, 2008; Spitzen *et al.*, 2008a), despite unequivocal evidence that traps baited with CO₂ and some human skin odours catch both species (Clements, 1999; Cardé & Gibson, 2010).

In contrast, female *Aedes aegypti* readily activate and orient along turbulent plumes of CO₂ in concentrations as little as 0.05% above ambient, and also activate and orient directly towards a source of human body odour (Dekker *et al.*, 2005; Dekker & Cardé, 2011). Moreover, a transient burst of CO₂ enhanced the sensitivity of the mosquito to human body odours (Dekker *et al.*, 2005). Female *A. aegypti* turned upwind and increased speed after leaving clean air and entering odour plumes of CO₂ or human skin odour and began counterturning without upwind displacement after losing the plume; these manoeuvres are consistent with orientation by optomotor anemotaxis (Kennedy, 1940; Cardé & Willis, 2008).

Once a mosquito reaches the immediate vicinity of a prospective host, it must land before it can commence feeding. One prelude to landing is a change in the flow of the visual field, from a generally forward movement which produces a front-to-rear image flow, to a manoeuvre that expands the apparent size of an object directly in front of the mosquito, rather than changing direction to avoid a collision (Cardé & Gibson, 2010). Although landing of mosquitoes has been used as a proxy for attraction (Dia *et al.* 2005; Kweka & Manhande, 2009, among others), the specific cues inducing mosquitoes to land remain poorly understood. Oxocarboxylic acids in human sweat were identified as chemical cues associated with landing in *A. gambiae s.s.* (Healy & Copland, 2000; Healy *et al.*, 2002). Visual cues (Haddow 1954, 1956; see also Maimon *et al.*, 2008) and also heat and humidity (Kellogg & Wright, 1962) are other cues likely to induce landing by mosquitoes.

Location of an odour source in a still-air environment should be a difficult task as even close to an odour source the gradient of odour concentration should be fairly shallow and therefore difficult to use as a reliable orientation cue (Cardé & Willis, 2008). Nevertheless, some flying insects are capable of locating odours at relatively close range by using concentration gradients (Duistermars *et al.*, 2009; Gomez Marin *et al.*, 2010) and female *C. quinquefasciatus* often locate hosts for a blood meal in locations where wind flow would presumably be very low or absent such as inside human dwellings (Clements, 1999). In this study, we compared how well female *C. quinquefasciatus* located and landed on a nearby

source of human skin odour with and without airflow as a guide. The specific cues that induce *C. quinquefasciatus* to land on a host remain to be established.

Materials and methods

Mosquitoes

A colony of *C. quinquefasciatus* was maintained at University of California, Riverside at LD 14 : 10 h photocycle, ~27 °C, and 70% relative humidity (r.h.) (Cooperband & Cardé, 2006). Once a week, 1-d-old chickens *Gallus gallus domesticus* were provided overnight to adult mosquitoes as a blood source to propagate the colony (Animal Use Protocol #A-0303007-1). Larvae were reared in plastic containers filled with deionized water and fed rabbit pellets (Purina Mills, LLC, St. Louis, Missouri). Pupae were transferred to screen-cages (30 × 30 × 30 cm) prior to eclosion and adults of both sexes were enclosed together and provided a 10% solution of sucrose in deionized water *ad libitum*. Females used in experiments were 6-12 d post-eclosion, assumed to be mated, and not blood-fed.

Mosquitoes were transferred to release cages 4 hours before bioassays. Release cages consisted of cylinders of clear acrylic (~7 cm long and 8 cm i.d.) covered on one end with insect screen and open on the other. A slit was cut through the side of the cylinder near the open end and a card-stock partition was inserted to confine mosquitoes. Release cages were covered with damp paper towels and housed in a vented cage illuminated by a fluorescent light under same light /dark schedule as above until used in bioassays. Bioassays were conducted 2-4 hours after the onset of scotophase.

Wind tunnel

Bioassays were conducted in a wind tunnel constructed with transparent Plexiglas (Altuglas International, Philadelphia, Pennsylvania), 1.5 m long × 0.5 m height × 0.5 m width (see Dekker *et al.*, 2005; Lacey & Cardé, 2011). Air was pushed into the tunnel from outside the building by an in-line duct fan (Kanalflokt, Inc. Sarasota, Florida), steam humidified (IMBA9, Sussman® electric boilers, Long Island City, New York), and passed through an activated charcoal filter, an aluminium honeycomb laminizer (cells 1.5 cm diameter × 15 cm long; HoneyCommCore LLC, Cattaraugus, New York) and two fine-mesh wire screens before entering the arena. The airflow (0.2 m s⁻¹, 26-29°C, 60-70% r.h.) was even throughout the tunnel (measured with an Omega HHF 52 anemometer; Omega Engineering, Stamford, Connecticut). Air was exhausted into the room. A wall-mounted exhaust fan was operated between assays to remove contaminated air from the room. To prevent surface contamination, latex gloves were worn whenever handling objects in the wind tunnel.

Light for video recording was provided by four infrared light arrays (60 LEDs / light, 940 nm) directed upwind from the downwind end of the tunnel. Visible light for mosquito orientation was provided by one 8 w white incandescent bulb reflected against the wall at the downwind end of the tunnel. Light levels in the arena were low but some visible light was present (< 0.007 W m⁻² Gossen Ultra-Pro light meter; Gossen Foto und Lichtmesstechnik, Nurnberg, Germany). Video was recorded from above the wind tunnel by two CCTV video cameras (ICD 48; Ikegami Electronics, Maywood, New Jersey) with 6 mm

lenses in audio-video-interleave (AVI) format directly onto a computer at 30 frames-per-second by video capture software (Noldus MPEG4 recorder; Noldus Information Technology, Wageningen, The Netherlands).

Mosquitoes were released from an open-ended acrylic cylinder (15 cm long, 9.5 cm Ø) fitted with a swinging screen door that opened remotely without evident disturbance of the mosquitoes. The release cage was along the midline of the tunnel 120 cm from the upwind screen and 20 cm above the floor.

Landing bioassay

Black, 2 mm glass beads (Darice Inc., Strongsville, Ohio) were used to collect human foot odour (see, Bernier *et al.*, 2000; Qui *et al.*, 2006; Lacey & Cardé, 2011) and as a source of odour in the wind tunnel. Beads were cleaned by agitating in a sonication bath in deionized (d.i.) water and detergent (Sparkleen; Fisher Scientific, Pittsburgh, Pennsylvania), rinsed thoroughly in d.i. water, rinsed twice in acetone, and heated to 250°C for > 12 hr. Beads (20 g) were held in contact with the toes and sole of a human foot (author E.S.L.) by 'wearing' them inside a clean cotton stocking (Hanesbrands Inc., Winston-Salem, North Carolina) and athletic shoe (Converse Inc., North Andover, Massachusetts). Feet were cleaned with bar soap (Unilever, Rotterdam, The Netherlands) ~1 h prior to insertion and beads were 'worn' for four hours prior to bioassays.

To activate mosquitoes, a plume of 4% CO₂ was presented from a glass tube (2 mm Ø) bent at 90° (Healy & Copland, 1995; Dekker *et al.*, 2005; Lacey & Cardé, 2011). The jet was oriented at 45° to the long axis of the wind tunnel and 10 cm from the opening of the release cage. The 4% CO₂ was produced by mixing air (medical grade; 0.03% CO₂; Air Gas, Inc., Lakewood, California) and CO₂ (100%; Air Gas, Inc., Lakewood, California) in a 1 L side-arm flask containing 100 ml of d.i. water heated to 100°C. Flow rates were regulated with flow meters (Cole-Parmer Instrument Company, Vernon Hills, Illinois) to produce flow of 1 L min⁻¹ at ~32°C and 85% r.h. To prevent mosquitoes from congregating in the downwind section of the wind tunnel and to prevent accumulation of CO₂ in the wind tunnel during trials with no airflow (see below), the flow of CO₂ was shut off 1 min after a bioassay was begun.

In each trial, two 7 cm Petri dishes containing 20 g of beads each were presented adjacent to each other on the floor of the wind tunnel, 16 cm apart, 30 cm from the upwind screen, and 90 cm upwind from the release cage in every trial. One dish held beads coated with foot odour and the second clean beads as a control. Beads were not heated. The location of treatment and control beads was rotated between trials. To clear odours from the arena, beads were removed from the wind tunnel while airflow of ~0.2 m s⁻¹ was maintained for 5 min between each trial. In trials with no airflow, beads were not replaced until airflow was terminated to reduce any residual plume. No prominent optomotor cues (e.g., coloured dots on the floor or ceiling) were present in the wind tunnel as no effect on orientation or numbers landing was observed in preliminary trials (data not presented) and excess objects in the wind tunnel confound digital tracking of mosquitoes (see below). Trials were conducted in either laminar flow of air at ~0.2 m s⁻¹ or in still air. Turning the fan off produced the still-air environment. Flow of air (or lack thereof) was tested with an

anemometer and further confirmed with a visual tracer, TiCl_4 'smoke'. With airflow, this tracer produced a plume ~ 11 cm in diameter and 6 cm above the floor (see Lacey & Cardé, 2011). In still air, the tracer did not rise more than 1 cm above the beads and did not expand over the edge of the petri dish within 10 s.

For each trial, five female *C. quinquefasciatus* were released into the wind tunnel. Trials were terminated after 10 min. We recorded numbers of mosquitoes activated, total number landing on beads, maximum number on beads at anytime during a trial (as an approximation of number of individuals landing vs. one individual landing multiple times) and time until the first mosquito landed. Data were recorded manually by viewing the raw video. In total, 11 trials in moving air and 10 trials in still air were analysed. On several days, landing rates were low (< 2 landings / trial) for both treatments and data from these days were not included in the analysis for statistical simplicity. Data were compared with the Kruskal-Wallis nonparametric analysis of variance (Statistix, v. 7.1, Analytical Software, Tallahassee, Florida).

For all landings, a segment of flight prior to landing was evident wherein the mosquito turned and flew a direct path to the beads. We used Ethovision XT (v 7.1, Noldus Information Technology, Wageningen, The Netherlands) to track individual mosquitoes for their last segment of flight prior to landing and to calculate the angular heading and distance travelled. Distribution of angular headings and sum of vectors (r) were tested with Rayleigh test (Oriana 3, v. 3.21, Kovach computing Services, Wales, United Kingdom). Distances travelled in this segment of orientation were compared with the Kruskal-Wallis nonparametric analysis of variance.

Results

In 21 trials with 110 *C. quinquefasciatus*, all but 9 mosquitoes were activated. Statistics for activation time and initial flight performance were not measured; however, regardless of conditions of air flow in the wind tunnel, most mosquitoes readily activated (< 30 s), flew "upwind" directed along the x-axis briefly after activation and reached the far half ("upwind" end) of the wind tunnel. Flights were never oriented directly to the beads after activation, but rather were meandering; mosquitoes often traversed the length of the wind tunnel several times before landing. For all trials, 104 landings occurred on the beads with foot odour and only 5 landings were observed on the control beads. There was a significant difference in the number of total landings between the laminar airflow and still air treatments (Kruskal-Wallis $_{1,110} = 6.41$, $P = 0.011$, Fig. 1a) with a mean of 3.45 ± 0.49 (S.E.) landings per trial on beads in a laminar air flow and a mean 6.50 ± 0.96 landings in still air. Individual mosquitoes landed more than once in most trials, as evidenced by a mean of 6.50 landings by 5 mosquitoes in trials with still air. However, there was also a significant difference in the maximum number of mosquitoes on the beads at any one time during 10 min (Kruskal-Wallis $_{1,47} = 10.58$, $P = 0.001$, Fig. 1b), with a mean of 1.45 ± 0.31 per trial on beads in a laminar air flow and a mean 3.10 ± 0.31 in still air. Latency to first landing was shorter in trials with still air (Kruskal-Wallis $_{1,22} = 9.60$, $P = 0.002$, Fig. 1c), with a mean latency of 226.73 ± 17.98 s in air flow and 122.50 ± 24.18 s in still air.

Because Ethovision permits tracking of only one mosquito at a time, a few landings where more than one was in the field of view could not be tracked; however, 73 of 104 landings provided continuous tracks. The headings of flight prior to landing on the beads differed in wind and still air. Angular headings of flight prior to landing were not randomly distributed in laminar air flow ($Z = 26.95$, $P < 0.0001$, Fig. 2a) with a mean heading of $353.93 \pm 23.75^\circ$ ($\pm$ circular S.D.; $0 / 360^\circ$ is due upwind). Angular headings in still air were randomly distributed ($Z = 1.43$, $P = 0.24$, Fig. 2b) with a mean heading of $204.50 \pm 110.04^\circ$. The distances from the beads where mosquitoes changed angular heading, flew directly, and landed were not significantly different between laminar air flow and still air (Kruskal-Wallis $_{1,89} = 0.93$, $P = 0.33$). The mean distances were 154 ± 15.61 mm and 142 ± 13.71 mm for air flow and still air respectively, with a maximum distance of 327 mm and a minimum of 18 mm.

Discussion

Many studies have attempted to describe the host-seeking behaviours of mosquitoes and identify host odours that modulate mosquito orientation, usually in an olfactometer or in the field with variable environmental conditions. One common feature of most studies is an assumption of air flow dispersing the odour downwind to the mosquito. This study compared host seeking behaviour of female *C. quinquefasciatus* in environments with still or moving air. Perhaps surprisingly, a higher proportion of female *C. quinquefasciatus* landed on a source of human body odour in still air than in a laminar air flow and they did so more rapidly.

Female *C. quinquefasciatus* certainly located and landed on a source of human body odour in moving air. The results demonstrate that anemotactic orientation upwind was used to locate the human odour source in moving air, but overall *C. quinquefasciatus* located the human odour most rapidly in still air. Our study employed wind speeds much lower than wind speeds that would limit the flight capabilities of female *C. quinquefasciatus* (Hoffman & Miller, 2003), and similar to many studies of mosquitoes in olfactometers (Mboera *et al.*, 1998; Pates *et al.*, 2001; Healy & Copland, 2000; Dekker *et al.*, 2005; Smallegange *et al.*, 2009). How moving air interfered with host location in the present study is unknown.

The odour sources in this study were not heated to near the temperature of human skin (as in Lacey & Cardé, 2011) to prevent the upward movement of semiochemicals in convective currents (Eiras & Jepson, 1994). A jet of CO₂-laden air was used to activate mosquitoes but, its flow rate was low enough to have a negligible effect on odour movement at the far end of the wind tunnel and, in any case flow was terminated 1 min. after the start of each trial. There was no evidence of response to visual cues from the control beads alone as few mosquitoes landed on them.

In still air, flight segments prior to landing were short and random headings are suggestive of location of the odour source using a concentration gradient (Fig. 2b). Not only did mosquitoes land on beads more often in still air, but latency to first landing was much shorter. This suggests that *C. quinquefasciatus* has an effective strategy for host location in still air at distances in the range of centimetres. Still-air olfactometers have been used

numerous times in studies of small or flightless insects orienting to odours over short distances (< 10 cm) by chemotaxis (Vet, 1983; Collatz *et al.*, 2010; Weeks *et al.*, 2011 among others), but few studies have used still-air olfactometers to examine orientation over longer distances.

Recent studies have demonstrated the ability of *Drosophila melanogaster* to locate readily an attractive odour in still air (Reynolds & Frye, 2007; Stewart *et al.*, 2010). Other studies have employed an olfactometer with very little air flow to study port-entering behaviour by *A. gambiae* (Pates *et al.*, 2001; Smallegange *et al.*, 2010; Qui *et al.*, 2011 among others) and *C. quinquefasciatus* (Mboera *et al.*, 1998). This olfactometer employs a wind tunnel-sized arena comparable to the one employed in the present study. Odours were delivered into the arena by two 4 cm ports with air flows of 0.2 m sec⁻¹ and to be trapped, mosquitoes must fly upwind into these ports. The methods employed in the present study allow for tracking of mosquitoes throughout most of the arena and visualization of activation, orientation, and landing behaviours and could help elucidate the roles of semiochemicals that modulate host location but do not necessarily lead a mosquito to a landing site on a host. For instance, CO₂ undoubtedly plays a role in activation and navigation along the host's odour plume in female *C. quinquefasciatus*, but its role in close-range orientation and ultimately landing requires is not fully understood (Dekker *et al.*, 2005; Spitzen *et al.*, 2008b; Lacey & Cardé, 2011). In *A. aegypti*, a species with high affinity for humans, females were more likely to locate an upwind source of human skin odour in a wind-tunnel assay when the plume structure was homogeneous rather than when it was filamentous (Dekker & Cardé, 2011). Similarly, an odour plume emanating from a glass bead dish in still air would have a relatively homogeneous structure, possibly contributing to enhanced orientation in *C. quinquefasciatus*. The interactions of factors such as odours from the host, plume structure, visual cues, and airflow in mediating orientation to a host and landing by *C. quinquefasciatus* and other mosquito species remain largely unknown and require further exploration.

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References

- Allan SA, Bernier UR, Kline DL. Laboratory evaluation of avian odors for mosquito (Diptera: Culicidae) attraction. *Journal of Medical Entomology*. 2006; 43:225–231. [PubMed: 16619603]
- Beeuwkes, J.; Spitzen, J.; Spoor, CW.; van Leeuwen, JL.; Takken, W. 3-D flight behaviour of the malarial mosquito *Anopheles gambiae* s.s. inside an odour plume. *Proceedings of The Netherlands Entomological Society Meeting*; 2008. p. 137-146.
- Bernier UL, Kline DL, Barnard DR, Schreck CE, Yost RA. 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*). *Analytical Chemistry*. 2000; 72:747–756. [PubMed: 10701259]
- Cardé, RT.; Gibson, G. Host finding by female mosquitoes: mechanisms of orientation to host odours and other cues. In: Takken, W.; Knols, BGJ., editors. *Ecology of Vector-Borne Diseases, Olfaction*

- in Vector-Host Interactions. Vol. 2. Wageningen Academic Publishers; Wageningen, The Netherlands: 2010. p. 115-141.
- Cardé RT, Willis MA. Navigational strategies used by insects to find distant, wind-borne sources of odor. *Journal of Chemical Ecology*. 2008; 34:854–866. [PubMed: 18581182]
- Clements, AN. *The Biology of Mosquitoes, Sensory Reception and Behaviour*. Vol. 2. CAB International; Wallingford, Oxon, UK: 1999.
- Collatz J, Fuhrmann A, Selzer P, Oehme RM, Hartelt K, Kimmig P, Meiners T, Mackenstedt U, Steidle JLM. Being a parasitoid of parasites: host finding in the tick wasp *Ixodiphagus hookeri* by odours from mammals. *Entomologia experimentalis et applicata*. 2010; 134:131–137.
- Cooperband MF, Cardé RT. Orientation of *Culex* mosquitoes to carbon dioxide-baited traps: flight manoeuvres and trapping efficiency. *Medical and Veterinary Entomology*. 2006; 26:11–26. [PubMed: 16608486]
- Cooperband MF, McElfresh JS, Millar JG, Cardé RT. Attraction of *Culex quinquefasciatus* Say (Diptera: Culicidae) to odours from chicken feces. *Journal of Insect Physiology*. 2008; 54:1182–1194.
- de Jong, R.; Knols, BGJ. Selection of biting sites by mosquitoes. *Olfaction in Mosquito-Host Interactions*; Ciba Foundation Symposium; 1996. p. 89-108.
- Dekker T, Cardé RT. Moment-to-moment flight manoeuvres of the female yellow fever mosquito (*Aedes aegypti* L.) in response to plumes of carbon dioxide and human skin odour. *Journal of Experimental Biology*. 2011; 214:3474–3479. [PubMed: 21957111]
- Dekker T, Geier M, Cardé RT. Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. *Journal of Experimental Biology*. 2005; 208:2963–2972. [PubMed: 16043601]
- Dia I, Diallo D, Duchemin J, Ba Y, Konate L, Costantini C, Diallo M. Comparison of human-landing catches and odor-baited entry traps for sampling malaria vectors in Senegal. *Journal of Medical Entomology*. 2005; 42:104–109. [PubMed: 15799517]
- Duistermars BJ, Chow DM, Frye M. Flies require bilateral sensory input to track odor gradients in flight. *Current Biology*. 2009; 19:1301–1307. [PubMed: 19576769]
- Eiras AE, Jepson PC. Responses of female *Aedes aegypti* (Diptera: Culicidae) to host odors and convection currents using an olfactometer. *Bulletin of Entomological Research*. 1994; 84:207–211.
- Gomez Marin A, Duistermars BJ, Frye MA, Louis M. Mechanisms of odor-tracking: multiple sensors for enhanced perception and behavior. *Frontiers in Cellular Neuroscience*. 2010; 4
- Haddow AJ. Studies on the biting-habits of African mosquitoes. An appraisal of methods employed, with special reference to the twenty-four-hour catch. *Bulletin of Entomological Research*. 1954; 45:199–242.
- Haddow AJ. Observations on the biting-habits of African mosquitoes in the genus *Eretmapodites* Theobald. *Bulletin of Entomological Research*. 1956; 46:761–772.
- Healy TP, Copland MJW. Activation of *Anopheles gambiae* mosquitoes by carbon dioxide and human breath. *Medical and Veterinary Entomology*. 1995; 9:331–336. [PubMed: 7548953]
- Healy TP, Copland MJW. Human sweat and 2-oxopentanoic acid elicit a landing response from *Anopheles gambiae*. *Medical and Veterinary Entomology*. 2000; 14:195–200. [PubMed: 10872864]
- Healy TP, Copland MJW, Cork A, Przyborska A, Halket JM. Landing responses of *Anopheles gambiae* elicited by oxocarboxylic acids. *Medical and Veterinary Entomology*. 2002; 16:126–132. [PubMed: 12109705]
- Hoffmann EJ, Miller JR. Reassessment of the role and utility of wind in suppression of mosquito (Diptera: Culicidae) host finding: stimulus dilution supported over flight limitation. *Journal of Medical Entomology*. 2003; 40:607–614. [PubMed: 14596273]
- Kellogg FE, Wright RH. Studies in mosquito repellency. IV. The effect of repellent chemicals. *The Canadian Entomologist*. 1962; 94:1017–1021.
- Kennedy JS. The visual responses of flying mosquitoes. *Proceedings of the Zoological Society of London Series A, General and Experimental*. 1940; 109:221–242.

- Kweka EJ, Manhande AM. Comparative evaluation of four mosquitoes sampling methods in rice irrigation schemes of lower Moshi, northern Tanzania. *Malaria Journal*. 2009; 8:149–154. [PubMed: 19580663]
- Lacey ES, Cardé RT. Activation, orientation and landing of female *Culex quinquefasciatus* in response to carbon dioxide and odour from human feet: 3-D flight analysis in a wind tunnel. *Medical and Veterinary Entomology*. 2011; 25:94–103. [PubMed: 21118282]
- Maimon G, Straw AD, Dickinson MH. A simple vision-based algorithm for decision making in flying *Drosophila*. *Current Biology*. 2008; 18:463–470.
- Mboera LEG, Knols BGJ, Takken W, Huisman PWT. Olfactory responses of female *Culex quinquefasciatus* Say (Diptera: Culicidae) in a dual-choice olfactometer. *Journal of Vector Ecology*. 1998; 23:107–113. [PubMed: 9879067]
- Mboera LEG, Takken W, Sambu E. The response of *Culex quinquefasciatus* (Diptera: Culicidae) to traps baited with carbon dioxide, 1-octen-3-ol, acetone, butyric acid and human foot odour in Tanzania. *Bulletin of Entomological Research*. 2000; 90:155–159. [PubMed: 10948375]
- Pates HV, Takken W, Stuke K, Curtis CF. Differential behaviour of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) to human and cow odours in the laboratory. *Bulletin of Entomological Research*. 2001; 91:289–296. [PubMed: 11587625]
- Puri SN, Mendki MJ, Sukumaran D, Ganesan K, Prakash S, Sekhar K. Electroantennogram and behavioral responses of *Culex quinquefasciatus* (Diptera: Culicidae) females to chemicals found in human skin emanations. *Journal of Medical Entomology*. 2006; 43:207–213. [PubMed: 16619600]
- Qiu YT, Smallegange RC, Van Loon JJA, Ter Braak CJF, Takken W. Interindividual variation in the attractiveness of human odours to the malaria mosquito *Anopheles gambiae s. s.* *Medical and Veterinary Entomology*. 2006; 20:280–287. [PubMed: 17044878]
- Qiu YT, Smallegange RC, Van Loon JJA, Ter Braak CJF, Takken W. Behavioural responses of *Anopheles gambiae sensu stricto* to components of human breath, sweat and urine depend on mixture composition and concentration. *Medical and Veterinary Entomology*. 2011; 25:247–255. [PubMed: 21108650]
- Reynolds AM, Frye MA. Free-flight odor tracking in *Drosophila* is consistent with an optimal intermittent scale-free search. *Plos One*. 2007; 2:e354. [PubMed: 17406678]
- Smallegange RC, Qiu YT, Bukovinszky Kiss G, Van Loon JJA, Takken W. The effect of aliphatic carboxylic acids on olfaction-based host-seeking of the malaria mosquito *Anopheles gambiae sensu stricto*. *Journal of Chemical Ecology*. 2009; 35:933–943. [PubMed: 19626371]
- Smallegange RC, Knols BGJ, Takken W. Effectiveness of synthetic versus natural human volatiles as attractants for *Anopheles gambiae* (Diptera: Culicidae) *sensu stricto*. *Journal of Medical Entomology*. 2010; 47:338–344. [PubMed: 20496580]
- Spitzen, J.; Spoor, CW.; Kranenbarg, S.; Beeuwkes, J.; Grieco, F.; Noldus, LPJJ.; van Leeuwen, JL.; Takken, W. Track 3D: Visualization and flight track analysis of *Anopheles gambiae s.s.* mosquitoes. In: Spink, AJ.; Ballintijn, MR.; Bogers, ND.; Grieco, F.; Loijens, LWS.; Noldus, LPPJ.; Smit, G.; Zimmerman, PH., editors. *Proceedings of Measuring Behavior 2008*. Noldus Information Technology; Wageningen, The Netherlands: 2008a. p. 133–135.
- Spitzen J, Smallegange RC, Takken W. Effect of human odours and positioning of CO₂ release point on trap catches of the malaria mosquito *Anopheles gambiae sensu stricto* in an olfactometer. *Physiological Entomology*. 2008b; 33:116–122.
- Stewart FJ, Baker DA, Webb B. A model of visual-olfactory integration for odour localization in free-flying fruit flies. *Journal of Experimental Biology*. 2010; 213:1886–1900. [PubMed: 20472776]
- Syed Z, Leal WS. Acute olfactory response of *Culex* mosquitoes to a human- and bird-derived attractant. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:18803–18808. [PubMed: 19858490]
- Takken W, Dekker T, Wijnholds YG. Odor-mediated flight behavior of *Anopheles gambiae* Giles *sensu stricto* and *An. stephensi* Liston in response to CO₂, acetone, and 1-octen-3-ol (Diptera: Culicidae). *Journal of Insect Behavior*. 1997; 10:395–407.
- Vet LEM. Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym.: Eucoilidae), a parasitoid of fungivorous *Drosophila* – the influence of conditioning. *Netherlands Journal of Zoology*. 1983; 33:225–248.

Weeks ENI, Logan JG, Gezan SA, Woodcock MA, Birkett MA, Pickett JA, Cameron MM. A bioassay for studying behavioural responses of the common bed bug *Cimex lectularius* (Hemiptera: Cimicidae) to bed bug derived volatiles. Bulletin of Entomological Research. 2011; 101:1–8. [PubMed: 20102661]

Figure 1.

Performance of female *Culex quinquefasciatus* in a wind tunnel under conditions of still air (dark grey bars) or a 0.2 m sec^{-1} laminar air flow (light grey bars): a.) mean ($\pm$ SE) number of landings on glass beads coated in human foot odour per 10 min trial, b.) mean ($\pm$ SE) maximum number of *C. quinquefasciatus* on beads at any time during trials (evidence of multiple individuals landing), and c.) mean ($\pm$ SE) latency between opening of release cage and first landing. All data significantly different between treatments of still-air and laminar air flow (Kruskal-Wallis nonparametric analysis of variance, $P < 0.05$).

Figure 2.

Planar depiction of angular flight heading of female *Culex quinquefasciatus* immediately prior to landing on beads coated with foot odour. Each dot represents angular heading of an individual mosquito in 10° bins: a.) flight heading in laminar air flow with 0° being due upwind, distribution of headings significantly different from evenly distributed (Rayleigh's test, $P < 0.0001$), b.) flight headings in still-air, distribution evenly distributed (Rayleigh's test, $P > 0.05$). Arrows represent mean vector length (r) of the corresponding mean direction.