

Selection of biting sites on man by two malaria mosquito species

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Abstract. While searching for blood, female mosquitoes pass through a behavioural process involving responses to visual, physical and chemical properties of the host. Temperature and humidity are thought to dominate mosquito orientation near the host. We observed that biting of two malaria mosquito species, i.e. *Anopheles atroparvus* (van Thiel) and *Anopheles gambiae* s.s. (Giles) preferentially occurs on different body regions of a naked motionless human host. Their preference for the head and foot regions respectively correlated with particular combinations of skin temperature and eccrine sweat gland density. Subsequent modification of the host's odour profile by removing exhaled breath and washing feet results in significant changes of these preferences.

Key words. Host-seeking; *Anopheles gambiae*; *Anopheles atroparvus*; chemotaxis; behaviour.

Many investigations have underlined the influence of heat, moisture and, to a lesser extent, carbon dioxide on mosquito behaviour in the close vicinity of the host¹⁻³. These factors are so dominant that odours are believed to be involved only at those distances where heat and moisture are not detectable⁴⁻⁶. However, experimental evidence to confirm this distance-related action of chemotaxis is lacking. Insight into mosquito olfaction at close proximity to the host would provide valuable information about odours involved in host-seeking. Since heat and moisture are reported to govern the biting behaviour of mosquitoes^{4,6-8} one would expect the choice of biting sites on a host to be determined by local variations in body temperature and/or humidity. Induced eccrine sweating, for example, increases the attractiveness of man to mosquitoes⁹. A preference for hands over forearms has been demonstrated for several mosquito species¹⁰⁻¹² but it remains unclear whether this is solely caused by difference in skin temperature and humidity¹⁰. Very few and inconclusive reports of biting site selection by mosquitoes on man exist. Had-dow¹³ found *Aedes simpsoni* mainly biting on the heads of naked individuals. Testing of the subjects in standing, sitting and lying positions gave similar results and therefore excluded the influence of height above the ground. In contrast, biting by the mosquito *Eretmapodites chrysogaster* was confined to a narrow band above ground level, between the ankles and knees of a standing person, and was apparently not influenced by body heat and moisture since biting occurred all over the body when the person was lying down¹⁴. *Culex pipiens fatigans* also bites mainly on the lower half of the

body¹⁵. These scanty records of biting site preferences indicate that several mosquito species use different cues to select biting sites.

Differences in biting site selection may reflect different host-seeking strategies, probably linked with host selection. *Er. chrysogaster*, for example, does not actively search for a bloodmeal but waits until a host enters its visual range¹⁴, a strategy based on visual host finding which supposedly implies a broad host spectrum. Opportunistic feeders may orient towards commonly produced chemical cues, such as carbon dioxide, whereas specialists would require host-specific information.

We studied the biting site selection of two malaria vectors, i.e. *An. atroparvus*, an opportunistic blood-feeder¹⁶, and *An. gambiae* s.s., which is highly anthropophilic^{17,18}, on the same adult male volunteer under controlled laboratory conditions, in an attempt to elucidate which factors govern this process.

Materials and methods

Mosquitoes. Mosquito strains originated from Leiden, The Netherlands (*An. atroparvus*) and Moshi, Tanzania (*An. gambiae* s.s.). Adults were kept in 30 × 30 × 30 cm gauze cages, fed on 6% glucose solution and offered blood (human arm for *An. gambiae* and rat for *An. atroparvus*) twice weekly. Eggs (laid on moist filter paper) were allowed to emerge in water trays and larvae were fed on Tetramin® fishfood. Pupae were collected from the trays daily and placed inside cages. Females used in the experiments were 5-10 days old and had not been given the opportunity to feed on blood. They were starved the night before the experiments by putting them individually into glass vials, closed with a water-moistened plug of cotton wool.

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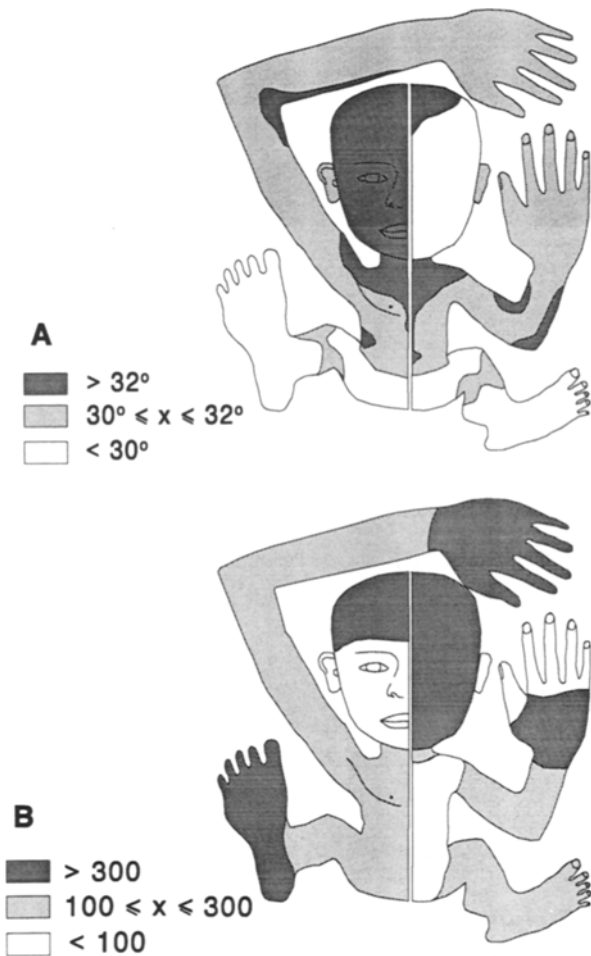


Figure 1. *A* distribution of skin temperatures and *B* eccrine sweat gland densities on the human body (after refs 19 and 21, drawing). Adapted from 'Life on the Human Skin' by M. J. Marples Copyright © igbg by Scientific American, Inc. All rights reserved.

Experimental procedures. The test person, a male Caucasian aged 27 was one of the authors. He was seated on a stool (height 34 cm) inside a 190 cm cubic bed net (mesh width 0.6 mm), in a climate-controlled room. He had not bathed for at least 9 hours before the experiment started. He was only wearing close-fitting underwear, and sat in an upright stationary position with hands resting on the knees and feet (slightly tilted) on the floor. Mosquitoes were released individually through a small hole in the net (95 cm from the side and 27 cm from the top) and were allowed three minutes to bite, after which non-responders were discarded. The site of biting was confirmed by a second person, who entered the net to remove the mosquito, after probing was felt or three minutes had passed. After every ten mosquitoes tested the subject changed position, from facing the release point (head at 140 cm) to facing away from it. Temperature inside the room was $27 \pm 2^\circ\text{C}$, RH at 60–70%, and illumination intensity in the bed net was below 20 Lux. Possible day-to-day variation,

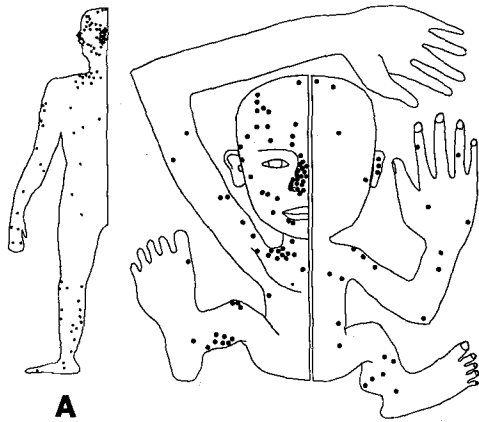
caused for example by variations in diet or physical condition of the test person, were compensated for by testing both species each day for several days. This procedure also excluded the possibility that preferences were 'induced' by previous biting and marking, since the subject bathed between test days. One hundred mosquitoes of each species were tested individually and their biting sites recorded.

Data analysis. We analysed the biting site distributions by comparing relative skin surface areas of various body parts¹⁹ with received and expected number of bites using a G-test²⁰. Thus, chi-squares were computed on the null-hypothesis that the mosquito bites were distributed in proportion to exposed surface areas. Since skin temperature and humidity are believed to be the main, if not the only factors determining short-range host-seeking behaviour of most mosquito species, we correlated the biting site distributions with these skin properties. The body was divided into three temperature¹⁹ and three humidity regions (fig. 1). Since the main discharge of water by the skin is effected by eccrine sweat glands we used their apparent densities to assess humidity levels above the skin²¹.

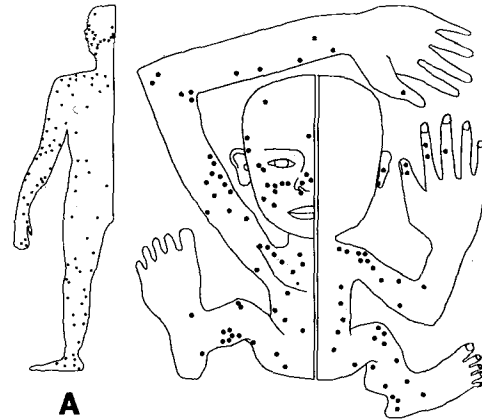
Results and discussion

The choice of biting sites for both species is shown in figure 2. Whether or not the human subject was facing the release point did not influence the biting patterns. Eighteen percent of each species did not attempt to bite within three minutes and were discarded. The data clearly show that biting is non-random and that both species strongly prefer different body regions (table 1A). Biting for both species correlates with particular combinations of skin temperature and eccrine sweat gland density (table 1B). Further analysis (table 1C) shows that both for *An. gambiae* and *An. atroparvus* biting also correlates with both factors separately. However, we cannot exclude that other features of the body influenced the spatial distribution of biting sites.

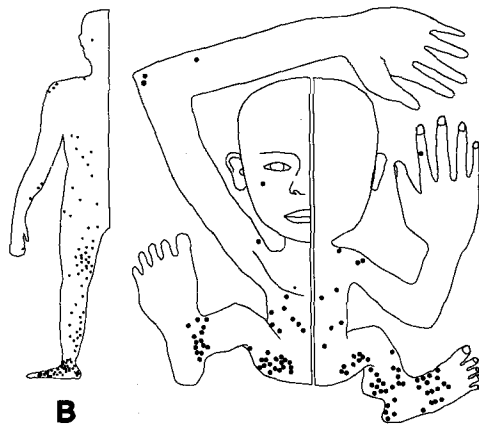
Both mosquito species showed a typical behaviour prior to biting. *An. atroparvus* appeared to be responding to exhaled breath by a sudden reduction of flight speed at ca. 50 cm distance from the face, followed by orientation towards the head. Perception of exhaled breath, therefore, seemed to influence the biting site selection of this species. In olfactometer experiments Laarman⁵ showed that human breath is attractive for *An. atroparvus*. On the other hand, *An. gambiae* was often seen approaching the host in the head region, apparently not responding to breath, and gradually moving downwards to the feet. This species seemed to orient itself by using convection currents along the host^{22,23}, a phenomenon described earlier for other species^{3,34}. Odours emanating from the feet, carried by these convection currents, might have guided *An. gambiae* to this



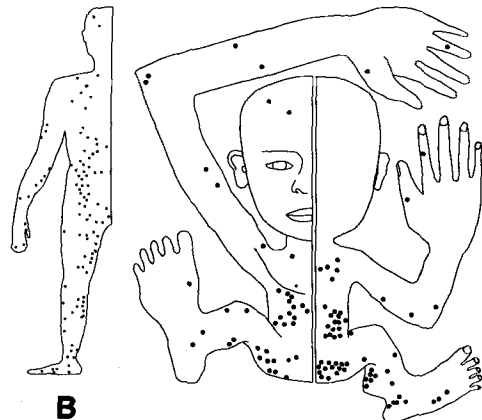
A



A



B



B

Figure 2. Distribution of biting sites of *A. An. atroparvus* and *B. An. gambiae* on a human host. One hundred bites per species are superimposed on a half silhouette (left) and front/back of the body (right).

Figure 3. Distribution of biting sites of *A. An. atroparvus* when breath was removed and *B. An. gambiae* when feet and ankles were washed. One hundred bites per species are superimposed on a half silhouette (left) and front/back of the body (right).

particular odour source. Both species were apparently using other cues—breath for *An. atroparvus* and foot odour for *An. gambiae*—and not only skin temperature and/or humidity at short range. If correct, this hypothesis implies an important role of odours near the host while the actual biting site distributions on the body provide important clues as to which chemical factors govern the host-seeking process.

In order to verify this hypothesis we altered the stimulus from the host which is suspected to be important for *An. atroparvus* by removing exhaled breath from the room with a one-way breathing valve (Harvard Douglas, USA), connected to polythene lay-flat tubing. If breath does not play any role in the mosquito's behaviour close to the body we expect to find a biting pattern similar to that found when the subject's exhaled breath remains in the bed net. However, the results (fig. 3A, and table 2), show a marked change in the choice of biting sites. Biting no longer correlates in the same way with skin temperature and eccrine sweat gland density. The strong preference of *An. atroparvus* for landing and probing on the head can thus be explained by the

known attractiveness of breath for this species. Although the removal of breath altered humidity and temperature levels around the head, which might have influenced the change in selection of biting sites, it is well known that this species responds well to carbon dioxide^{5,25}, an important constituent of breath, and a reliable host-related cue for opportunistic bloodfeeders.

The host's odour profile was changed for *An. gambiae* by washing his feet and ankles with non-perfumed medical soap (Unicura, The Netherlands) containing a bacterial agent (1% 2,4,4'-trichloro-2' hydroxydiphenyl ether). Washing took place at hourly intervals in warm soapy water for a few minutes, followed by rinsing in warm water and drying with a towel. There is no evidence for repellency of this soap. On the contrary, a humidified bar of this soap placed on top of a cage showed attractive properties, inducing landing and active probing responses of females. A dry bar of soap, however, did not elicit any responses. GC-MS analyses failed to detect soap residues after washing, but, instead, showed a marked reduction in the overall amount of volatiles emanating from the subject's feet (Van der Meent, pers.

Table 1. Distribution of mosquito bites *A* on various body parts, *B* for combinations of skin temperature and eccrine sweat gland density, and *C* both factors separately.

<i>A</i>												
Body part	Head ^a			Trunk			Arms			Legs	G-test ^c	
<i>Anopheles atroparvus</i> (n = 100)	50			15			17			18	p < 0.001	
<i>Anopheles gambiae</i> (n = 100)	1			16			7			76	p < 0.001	
Expected ^b	9			32			19			40		
<i>B</i>												
Skin temperature ^d		Low			Medium			High				
Eccrine sweat gland density ^e												
	Low	Medium	High	Low	Medium	High	Low	Medium	High	G-test ^b		
<i>Anopheles atroparvus</i>	1	14	2	8	10	3	35	14	13	p < 0.001		
<i>Anopheles gambiae</i>	1	64	7	8	14	0	3	3	0	p < 0.001		
Expected ^b	5	28	4	9	33	3	6	7	5			
<i>C</i>												
		Skin temperature ^d			G-test ^c			Eccrine sweat gland density ^e			G-test ^c	
	Low	Medium	High		Low	Medium	High		Low	Medium	High	G-test ^c
<i>Anopheles atroparvus</i>	17	21	62	p < 0.001	44	38	18	p < 0.001				
<i>Anopheles gambiae</i>	72	22	6	p < 0.001	12	81	7	p < 0.05				
Expected ^b	37	45	18		20	68	12					

^aHead includes neck region.

^bExpected number of bites, assuming equal numbers per unit skin surface area (19) on all parts of the body.

^cG-test of goodness of fit with expected frequencies based on hypotheses extrinsic to the sample data (ref. 20).

^dCategorised after ref. 19. Low, < 30 °C; Medium, 30–32 °C; High, > 32 °C.

^eCategorised after ref. 21. Low, < 100 glands cm⁻²; Medium, 100–300 glands cm⁻²; High, > 300 glands cm⁻².

Table 2. Distribution of mosquito bites after removal of breath (*An. atroparvus*) or washing of the feet (*An. gambiae*) *A* on various body parts, *B* for combinations of skin temperature and eccrine sweat gland density, and *C* both factors separately.

<i>A</i>												
Body part	Head ^a			Trunk			Arms			Legs	G-test ^b	
<i>Anopheles atroparvus</i> (n = 100)	19			16			39			26	p < 0.001	
<i>Anopheles gambiae</i> (n = 100)	2			48			14			36	p < 0.001	
<i>B</i>												
Skin temperature ^d		Low			Medium			High				
Eccrine sweat gland density ^e												
	Low	Medium	High	Low	Medium	High	Low	Medium	High	G-test ^b		
<i>Anopheles atroparvus</i>	1	18	2	13	33	1	18	11	3	p < 0.001		
<i>Anopheles gambiae</i>	14	26	2	17	27	2	5	5	2	p < 0.001		
<i>C</i>												
		Skin temperature ^d			G-test ^b			Eccrine sweat gland density ^e			G-test ^b	
	Low	Medium	High		Low	Medium	High		Low	Medium	High	G-test ^b
<i>Anopheles atroparvus</i>	21	47	32	p < 0.001	32	62	6	p < 0.001				
<i>Anopheles gambiae</i>	42	46	12	p < 0.001	36	58	6	p < 0.001				

^{a, d, e}see table 1.

^bG-test of goodness of fit with observed data in table 1.

comm.). The washing resulted in a remarkable change in the biting site distribution (fig. 3B, table 2) when related to skin surface areas, skin temperature and sweat gland distribution. The fact that we were able to dramatically divert *An. gambiae* from biting the feet and ankles to other body regions reveals the importance of odours emanating from this region in selection of biting

sites by this species. Since *An. gambiae* is highly anthropophilic we expect these odours to be human-specific. This work shows that different mosquito species exhibit different biting site preferences on one and the same human host and that preferences could be altered by changing the subject's body odour profile. It clearly demonstrates that this process is not entirely governed

by skin temperature, skin humidity and visual cues, but probably also by body odours. Moreover, biting site distributions on the body give important information about kairomones used in host seeking and host selection by mosquito species. Knowledge about these chemical cues opens up possibilities for the development of odour-baited traps which could find wide application in epidemiological studies of mosquito-borne diseases and their control.

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