

INFLUENCE OF HUMAN BREATH ON SELECTION OF BITING SITES BY *ANOPHELES ALBIMANUS*

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ABSTRACT. The selection of biting sites by *Anopheles albimanus* on a human host was studied in the laboratory. The perception of exhaled breath guided the mosquitoes towards the head region, and was associated with a preference ($P < 0.001$) for biting this part of the body. Subsequent removal of breath resulted in a strong reduction of the number of bites on the head (from 49 to 20%). The significance of these findings in relation to host location is discussed.

INTRODUCTION

In the process of host location, bloodfeeding mosquitoes orient by means of various host-related stimuli, that is, visual, physical, and chemical cues (Clements 1963, Gillies 1980, Takken 1991). For nocturnally active mosquito species, the long-distance orientation behavior is thought to be governed by olfaction (Gillies and Wilkes 1972). It has long been assumed that in the vicinity of the host, body heat and humidity dominate this process (Burgess 1959, Friend and Smith 1977) but De Jong and Knols (1994) demonstrated that body odors play an important additional role in the selection of biting sites by mosquitoes. It was shown that both *Anopheles atroparvus* van Thiel and *Anopheles gambiae* Giles s.s. have a significant preference for biting on a particular site of the body, the head and feet, respectively. By altering the host's odor profile through breath removal or washing of the feet, both species were diverted from biting the previously preferred sites to other parts of the body. Thus, an analysis of how biting sites are selected by mosquitoes provides important information about the origin of cues involved in host location. Moreover, selection of biting sites might be an indication of the range of hosts fed upon. Biting by the opportunistic species *An. atroparvus* (Swellengrebel and De Buck 1938), for example, is strongly influenced by breath, and its major component, carbon dioxide, is a known attractant for this species (Van Thiel and Weurman 1947) and represents a reliable kairomone for opportunistic feeders.

Anopheles albimanus Wied., an important malaria vector in Mexico and Central America (Ramsey et al. 1986), is reported to be opportunistic (Breeland 1972, Loyola et al. 1993). On the basis of blood meal analyses, Loyola et al. (1993) found high forage ratios for bovines and equines but also reported bloodfeeding on humans, pigs, and chickens. Carbon dioxide could well play a role in host location by *An. albimanus*, as shown by Wilton (1975), who found a

2.2-fold increase in *An. albimanus* catch when CDC traps were baited with dry ice. Therefore, we expect that the selection of biting sites by this species is similar to that of *An. atroparvus*. Here we report on the selection of biting sites by *An. albimanus* on human bait in an attempt to determine whether breath indeed plays a role in the host-location process.

MATERIALS AND METHODS

Mosquitoes: The *An. albimanus* strain used originated from El Salvador. Adults were kept in gauze-covered cages (38 × 39 × 46 cm) at 27°C and ca. 80% RH, and fed on cotton wool soaked with a 10% glucose solution. Experimental females were 2-3 days old, had not received a blood meal, and were selected for experimental use with an apparatus as described by Posey and Schreck (1981). These females were then transferred individually into glass vials using a suction tube where they were kept prior to testing.

Experimental design: A male Caucasian volunteer (28 years old) was seated on a 36-cm-high stool inside a rectangular bednet (80 × 200 × 145 cm; mesh width 2 mm), wearing only tight underwear. He sat upright, hands on the knees and feet on the floor, and was facing the release point (130 cm above floor, in the short side of the net) for the mosquitoes. The head of the subject was thus 150 cm from the release point. Mosquitoes were released individually by a 2nd person (outside the bednet) and after probing was felt, their biting sites were recorded, and the mosquito removed from the net. This was repeated until 100 bites were scored. Mosquitoes not biting within a 3-min period were removed and discarded in the analyses. Temperature in the room was 27°C and relative humidity was ca. 80%. In a 2nd experiment, to assess the importance of breath in the selection of biting sites, breath was removed from the bednet by exhaling into 60-cm-long plastic tubing (internal diam 2 cm) and the nostrils were closed by a nose clip.

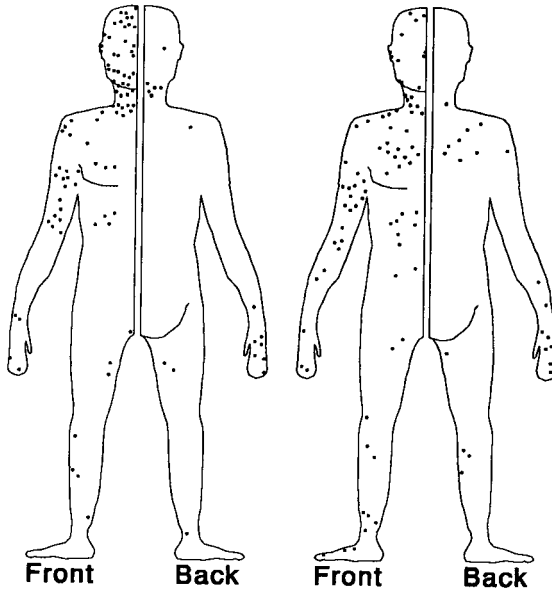
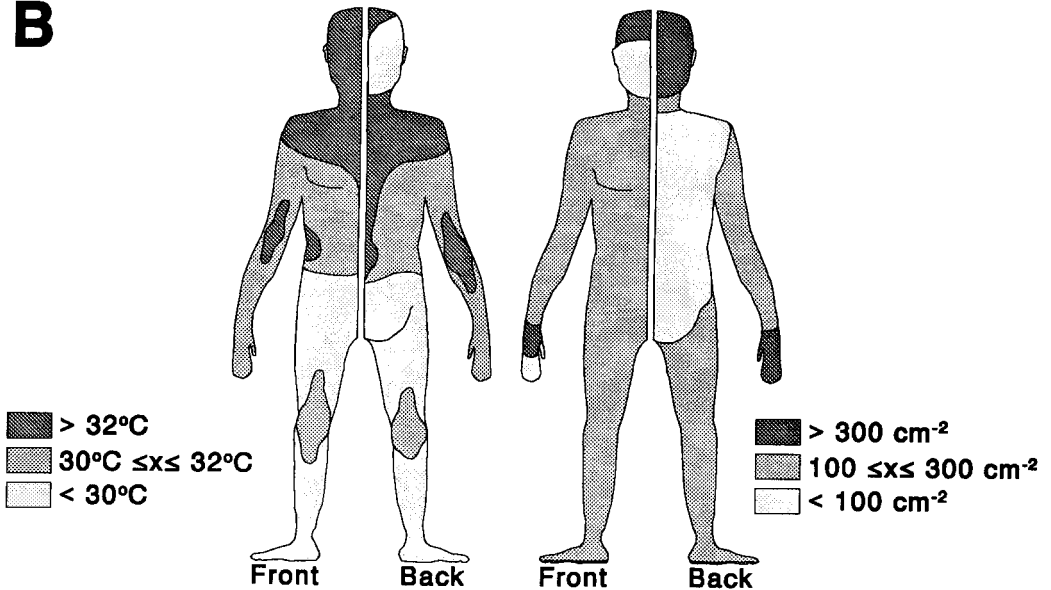
A**B**

Fig. 1. (A) Distribution of *Anopheles albimanus* bites on a human host before (left, $n = 100$) and after (right, $n = 100$) breath removal. All bites on front and back are superimposed on half silhouettes. (B) Categorized skin temperatures (left, after Clarke and Edholm 1985) and eccrine sweat gland densities (right, after Marples 1969).

Both treatments were completed in 3 test days. The distribution of biting sites was analyzed by comparing relative skin surface areas of various body parts (Clarke and Edholm 1985) with received and expected number of bites using a G-test (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

After release, the mosquitoes responded rapidly to the presence of the host and all but 7% bit within the 3-min experimental period. It was clearly visible that the mosquitoes reduced their

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

(A)										
Host	Body part				G-test ²					
	Head ¹	Trunk	Arms	Legs						
Normal	49	14	28	9	a					
Modified (breath removed)	20	33	31	16	b					
Expected ³	9	32	19	40	c					

(B)										
Host	Skin temperature ⁴									G-test
	Low			Medium			High			
	Eccrine sweat gland density ⁵									
	Low	Medi-um	High	Low	Medi-um	High	Low	Medi-um	High	
Normal	0	9	5	4	20	6	18	26	12	a
Modified (breath removed)	0	11	0	4	29	7	13	32	4	b
Expected	5	28	4	9	33	3	6	7	5	c

(C)										
Host	Skin temperature ⁴				Eccrine sweat gland density ⁵					
	Low	Medi-um	High	G-test	Low	Medi-um	High	G-test		
	Normal	14	30		56	a	22		58	20
Modified (breath removed)	11	40	49	a	17	72	11	b		
Expected	37	45	18	b	20	68	12	b		

¹ Head includes neck region.
² G-test of goodness of fit (Sokal and Rohlf 1981). Treatments not followed by the same letter are different at $P < 0.01$.
³ Relative skin surface (after Clarke and Edholm 1985) expressed as expected number of bites.
⁴ Categorized (after Clarke and Edholm 1985). Low, $< 30^{\circ}\text{C}$; Medium, $30^{\circ} \leq x \leq 32^{\circ}\text{C}$; High, $> 32^{\circ}\text{C}$.
⁵ Categorized (after Marples 1969). Low, < 100 glands cm^{-2} ; Medium, $100 \leq x \leq 300$ glands cm^{-2} ; High, > 300 glands cm^{-2} .

flight speed when approaching the frontal part of the face, most likely upon entering the cone of exhaled breath. This could have resulted in the nonrandom biting site distribution as shown in Fig. 1A and Table 1A. Up to 5.4 times the expected number of bites were recorded on the head, predominantly on the face. However, the influence of other factors such as vision, skin temperature, and skin humidity cannot be excluded. In order to determine whether correlations between biting-site selection and these skin properties exist, we projected the bites on categorized skin temperature and/or eccrine sweat gland density patterns of the body (Fig. 1B). Because the main discharge of water by the skin is affected by eccrine sweat glands, we used their apparent densities (Fig. 1B) to assess humidity levels above the skin. As shown in Table 1B and C correlations indeed exist, especially with the warmer parts of the body and to a lesser extent

with skin surfaces excreting relatively large amounts of water.

In previous work (De Jong and Knols 1994) we found similar patterns for selection of biting sites by *An. atroparvus*, which also occurred on the head and correlated with elevated skin temperature. However, removal of exhaled breath drastically changed the biting-site selection of this species, with the percent of bites on the head dropping from 50 to 19% ($P < 0.001$). Furthermore, biting no longer correlated with skin temperature and densities of eccrine sweat glands as before. On this basis it was concluded that breath dominates biting-site selection by *An. atroparvus*. In olfactometer experiments, Laarman (1955) previously showed the attractiveness of breath, and in particular its CO_2 content. In order to verify our hypothesis that biting by *An. albimanus* is also influenced by breath, we conducted a similar experiment where breath was removed

from the bednet. The results are shown in Table 1A and Fig. 1A. A significant reduction ($P < 0.001$) in the number of bites on the head was found. Mosquitoes still preferred to bite the warmer parts of the body, but the correlation with eccrine sweat gland densities disappeared. This shows that in these tests, breath influenced selection of biting sites, thus confirming its role in the host location of this mosquito species.

We also observed some interesting changes in orientation behavior. Mosquitoes generally flew closer to the ground and were sometimes seen flying very close to the body without immediately landing, resulting in delayed biting. Another indication of the importance of breath is that a few mosquitoes that escaped during the experiments were seen hovering outside the bednet near the exhaust of the plastic tube used for removing breath. The apparent difficulty in locating the host when breath was removed was also expressed in the slightly higher percentage of non-responding mosquitoes (12%).

Our theory that breath, and probably its CO_2 content, influences biting-site selection by generalistic blood feeders, such as *An. albimanus*, is supported by these observations. However, we cannot exclude other chemicals commonly found in animals, for example, acetone (Krotozynski et al. 1977), also present in human breath, as responsible for guiding this species to its hosts.

ACKNOWLEDGMENTS

We sincerely thank D. L. Kline, U.S. Department of Agriculture Medical and Veterinary Entomology Research Laboratory, Gainesville, FL, for providing experimental facilities. The present work was partially funded by the European Community as part of the project Behavioural Studies on Malaria Vectors under project numbers TS3-CT92-0101 and TS3-CT91-0032. The provision of travel funds by the Dr. J. L. Dobberke foundation is gratefully acknowledged.

REFERENCES CITED

- Breeland, S. G. 1972. Studies on the ecology of *Anopheles albimanus*. Am. J. Trop. Med. Hyg. 21: 751-754.
- Burgess, L. 1959. Probing behaviour of *Aedes aegypti* in response to heat and moisture. Nature 184:1968-1969.
- Clarke, R. P. and O. G. Edholm. 1985. Man and his thermal environment. Edward Arnold, London.
- Clements, A. N. 1963. The physiology of mosquitoes. Pergamon Press, Oxford.
- De Jong, R. and B. G. J. Knols. 1994. Odour-mediated selection of biting sites on man by two malaria species. Experientia (in press).
- Friend, W. F. and J. J. B. Smith. 1977. Factors affecting feeding by bloodsucking insects. Annu. Rev. Entomol. 22:309-331.
- Gillies, M. T. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bull. Entomol. Res. 70:525-532.
- Gillies, M. T. and T. J. Wilkes. 1972. The range of attraction of animal baits and carbon dioxide for mosquitoes. Bull. Entomol. Res. 61:389-404.
- Krotozynski, B., G. Gabriel and H. O'Neill. 1977. Characterization of human expired air: a promising investigative and diagnostic technique. J. Chromatogr. Sci. 15:239-244.
- Laarman, J. J. 1955. The host-seeking behaviour of the malaria mosquito *Anopheles atroparvus*. Acta Leiden. 25:1-144.
- Loyola, E. G., L. González-Cerón, M. H. Rodríguez, J. I. Arredondo-Jiménez, S. Bennett and D. N. Bown. 1993. *Anopheles albimanus* (Diptera: Culicidae) host selection patterns in three ecological areas of the coastal plains of Chiapas, southern Mexico. J. Med. Entomol. 30:518-523.
- Marples, M. J. 1969. Life on the human skin. Sci. Am. 220:108-115.
- Posey, K. H. and C. E. Schreck. 1981. An airflow apparatus for selecting female mosquitoes for use in repellent and attraction studies. Mosq. News 41:566-568.
- Ramsey, J. M. D., D. N. Bown, J. Aron and J. F. Méndez. 1986. Field tests in Chiapas, Mexico, of a rapid detection method for malaria in anopheline vectors with low infection rates. Am. J. Trop. Med. Hyg. 35:234-238.
- Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Co., New York.
- Swellengrebel, N. H. and A. De Buck. 1938. Malaria in the Netherlands. Scheltema and Holkema Ltd., Amsterdam.
- Takken, W. 1991. The role of olfaction in hostseeking of mosquitoes: a review. Insect Sci. Appl. 12:287-295.
- Van Thiel, P. H. and C. Weurman. 1947. L'attraction exercée sur *Anopheles maculipennis atroparvus* par l'acide carbonique dans l'appareil de choix II. Acta Trop. 4:1-9.
- Wilton, D. P. 1975. Mosquito collections in El Salvador with ultraviolet and CDC miniature light traps with and without dry ice. Mosq. News 35:522-525.