Entomologia Experimentalis et Applicata **87:** 295–300, 1998.

Selection of biting sites on a human host by *Anopheles gambiae s.s.*, *An. arabiensis* and *An. quadriannulatus*

Teun Dekker[1], Willem Takken[1], Bart G. J. Knols[2], Edwin Bouman[3], Stephan van de Laak[1], Arjan de Bever[1] & Piet W. T. Huisman[1]

[1]*Laboratory of Entomology, Wageningen Agricultural University, PO Box 8031, 6700 EH, The Netherlands;* [2]*Present address: International Centre of Insect Physiology and Ecology, Nairobi, Kenya;* [3]*Present address: Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic*

Accepted: March 24, 1998

Key words: Anopheles gambiae sensu stricto, An. arabiensis, *An. quadriannulatus*, mosquito, biting behaviour, human, convection current, host odour

Abstract

The selection of biting sites on a human host of three closely related mosquito species belonging to the African *Anopheles gambiae* complex (Diptera: Culicidae), *Anopheles gambiae* Giles *s.s.*, *An. arabiensis* Patton and *An. quadriannulatus* Theobald, was investigated under controlled laboratory conditions. Although these species differ in host preference, they all showed a significant preference to bite the legs and feet, suggesting that a mutual not specifically human factor was involved in the selection of biting sites. In subsequent experiments with *An. gambiae s.s.* this factor was revealed by altering the position of the test person. In experiments with the test person sitting on a stool, the legs and feet were significantly preferred as biting sites, whereas lying on the ground, with the legs and feet sticking up, the latter body parts were bitten significantly less than the body parts closest to the ground (head, trunk and arms). The results indicate that mainly convection currents along the host are used by members of the *An. gambiae* complex in selecting a biting site. In contrast to *An. gambiae s.s.* and *An. arabiensis*, large numbers of bites by *An. quadriannulatus* also occurred on the head, possibly in response to exhaled breath. It is concluded that the selection of biting sites of members of the *Anopheles gambiae* complex is guided by convection currents and partially mediated by host odours.

Introduction

Many studies have underlined the importance of physical host-derived stimuli, such as heat and moisture, on the behaviour of mosquitoes (Gillies, 1980; Hocking, 1971; Wright & Kellogg, 1962). Until recently, it was assumed that these cues dominate behavioural responses in the close vicinity of a host, whilst chemical cues would only act at medium and long distances. However, Knols et al. (1994) and de Jong & Knols (1995a, 1996) showed that mosquitoes are also guided by odours when selecting a biting site on a human host and that the odours used in this process differ between species. In their studies *Anopheles atroparvus* van Thiel and *An. albimanus* Wiedemann preferred to bite around the nose of an human seated upright, whereas *An. gambiae* Giles *sensu stricto* showed a strong preference for biting the feet and ankles. Removal of exhaled breath and washing of the feet, respectively, resulted in a significant change in the distribution of biting sites on the body of the experimental person. The results were ascribed to known differences in host preference and responses to odours from the preferred biting sites. *An. atroparvus* and *An. albimanus* are opportunistic/zoophilic species and responded to breath, of which carbon dioxide has been shown to attract these species (Laarman, 1955; Wilton, 1975). *An. gambiae s.s.* is highly anthropophilic and responds poorly to CO_2 (Mboera & Takken, 1997; Takken et al., 1997). Its preference for biting the feet was thought to be the result of odours derived from this body region.

Further studies on the behaviour of *An. gambiae s.s.* (de Jong & Knols, 1995b; Knols et al., 1997) revealed that its attraction to the feet might be caused by the fatty acids produced by *Brevibacterium* spp. living on the human skin. This was reported to be unique to humans and therefore these compounds, as volatiles, may present a reliable cue for host seeking anthropophilic *An. gambiae s.s.* (Knols, 1996).

The six mosquito species tested in the biting experiments of Knols et al. (1994) and de Jong & Knols (1995a, 1996) belong to four different genera: *Aedes*, *Anopheles*, *Culex* and *Mansonia*. Besides host-preference, different species also differ in other behavioural traits, as has been shown by Takken et al. (1997) in their comparison between *An. gambiae s.s.* and *An. stephensi*. These two factors make it difficult to attribute the differences in the selection of biting sites to the innate host-preferences of these species. We therefore decided to study the selection of biting sites of three closely related species, belonging to the *An. gambiae* complex, of which the individual members display a large variety in host preference (White, 1974). Experiments were done with *An. gambiae s.s.*, a highly anthropophilic species, *An. quadriannulatus*, a highly zoophilic species and *An. arabiensis*, an opportunistic species. The responses of *An. gambiae s.s.* to five different adult men were studied. In addition we tested the effect of various body positions of the test person on the selection of biting sites by *An. gambiae s.s.*. Finally, since the geographical origin of a certain species and the way a colony is maintained may affect its behaviour, we also compared the biting behaviour of two West African strains of *An. gambiae s.s.*, which were maintained differently, and one East African strain.

Materials and methods

Mosquitoes. Three strains of *An. gambiae s.s.* were used. They originated from Moshi, Tanzania (KIL-strain), from Suakoko, Liberia (SUA-strain) and from The Gambiae (G3). The *An. arabiensis* strain originated from the Zambezi Valley, Zimbabwe, while the *An. quadriannulatus* strain originated from Skukuza, South Africa. For colony rearing, all strains were routinely offered a human arm to feed on, except for *An. gambiae s.s.* strain G3, which was fed on guinea pig. Oviposition took place on wet filter paper and larvae were fed with Tetramin® fish food. Pupae were collected from the larval trays and the emerging adults were kept in 30 cm cubic cages with a supply of 6% glucose in water. Experimental mosquitoes were 5–10 days old and had not been given the opportunity to feed on blood. They were put into glass vials individually 12–18 hour before the experiments started and had access to water on moist cotton wool plugs.

Experimental procedures. Experiments were carried out during the last 4 hours of the scotophase in climate-controlled chambers, at 27–28 °C and a r.h. of 65–75%, and illuminated below 20 Lux. Tests were conducted with five different volunteer male Caucasians: 23 years old (P1, P2 and P4); 51 years old (P3); 27 years old (P5)). The test person, who had not bathed for at least 9 hours, was sitting or lying under a bed net of 1.90 × 1.90 × 1.90 m. He was wearing tight underwear only. Mosquitoes were released individually through a small slit in the bed net (95 cm from the side and 27 cm from the top) and given a chance to bite within 5 min. The bite was located and the mosquito was removed. Mosquitoes which did not respond within 5 min were discarded.

Test series. In the test series in which differences between the three mosquito species, between different mosquito strains and between different human individuals, were studied, the test person was seated upright on a stool of 50 cm high, with his hands on his knees and facing the release site. The work on *An. gambiae s.s.* (G3) and *An. quadriannulatus* was done at the South African Institute for Medical Research, Johannesburg, South Africa, while all other experiments were carried out at the Laboratory of Entomology, Wageningen Agricultural University, The Netherlands. The *An. gambiae s.s.* (SUA) strain was used for testing biting patterns on subjects P1–P4 seated in an upright position. To see whether we could alter the preference of *An. gambiae s.s.* to bite the feet, we thoroughly washed the feet of three test persons with Unicura® non-perfumed medical soap, which contains the anti-bacterial compound 2,4,4′-trichloro-2′hydroxyphenyl ether (1%), at hourly intervals, similar to the procedures of de Jong & Knols (1995a). In addition, the effect of the position of the test person on the distribution of biting sites was studied. In these series the position of the body of the test person was changed from sitting on a stool to sitting on the ground with the legs stretched or lying on the ground, either with the legs stretched or with the legs kept upright.

Data analysis. The distribution of biting sites found in the different test series was compared with the expected number according to the relative skin surface of various body parts (Clark & Edholm, 1985) using a G-test (Sokal & Rohlf, 1981). Differences between the test series were tested with the G-test as well.

Results

The distribution of the bites of the different species and strains of the *An. gambiae* complex on the human body is shown in Table 1. The three different *An. gambiae s.s.* strains all showed a significant preference for biting the feet ($P<0.001$). The biting patterns were similar for the West (SUA) and East African (KIL) *An. gambiae s.s.* strains, but both differed significantly from the G3 strain. With the latter strain a large number of bites was located on the legs, reducing the number of bites on the feet. *An. arabiensis* also preferred to bite the legs and feet. *An. quadriannulatus*, however, bit these regions significantly less than the other two species ($P<0.001$). Instead a significant preference ($P<0.01$) to bite the head region was found for this species. Most bites of the three species were located on edges of the feet and around the hips.

Table 2 shows the distribution of biting sites on four different persons. The preference to bite the legs and feet was significant in all four cases. Although significant differences appeared between the test persons ($P<0.01$), these disappeared when the number of bites on the legs and feet are summed.

Table 3 shows the effect of washing the feet with medical soap for three test persons. No change in the preference of *An. gambiae s.s.* to bite the feet was observed after the feet of the test persons were washed with Unicura® . However, the position of the body of the test persons significantly altered this preference (Table 4). When the test person was sitting on the ground with the legs stretched, the bites were evenly distributed over the legs and feet. When lying on the ground the distribution of biting sites was in concordance with the relative size of the body parts. A dramatic change in the location of bites was found when the subject was lying on the ground with the legs up at an angle of 90 ° (Table 4). In this position biting occurred significantly in favour of the body parts closest to the ground and were mainly located on the edges of the body. On the ground, in horizontal position with the arms alongside the body, this resulted in high biting indices on the arms and subsequently lower biting indices on the trunk.

Discussion

The results show that different strains of *An. gambiae s.s.*, irrespective of their geographical origin, like the Suakoko and the Kilimanjaro strain, prefer to bite the legs and feet of humans and behaved nearly identical with respect to the selection of biting sites. In this behaviour *An. gambiae s.s.* differs from *Ae. aegypti* (Linnaeus), *Ae. simpsoni* (Theobald), *An. albimanus* Wiedemann, *An. atroparvus* van Thiel and *Culex quinquefasciatus* Say which prefer to bite the head region or bite randomly (reviewed by de Jong & Knols, 1996). Haddow (1956) found that *Eretmapodites chrysogaster* Graham also bit humans below the knee, although in this case this behaviour was thought to be vision-oriented. Furthermore, we assumed that feeding a highly anthropophilic species, like *An. gambiae s.s.*, on guinea pigs puts the colony through a high selection pressure with respect to host preference and its behaviour toward its originally preferred host. Indeed, Laarman (1958) reported that a laboratory strain of *An. maculipennis atroparvus* had lost its preference for human odour after a few generations of feeding on a rabbit. According to Laarman this effect was caused by selective pressure as a result of the feeding of the mosquitoes on the rabbit. In the present study the distribution of biting sites of *An. gambiae s.s.* G3, which had been raised on guinea pigs, differed significantly from that of the SUA and KIL strain which were fed on humans. However, a difference in the distribution of biting sites was also found within the same strain of *An. gambiae s.s.*, when different test persons were used (Table 2). There is also a large difference between our results and those of de Jong & Knols (1995a) (biting index on feet: 9.6 *vs* 5.6; biting index on legs: 0.39 vs 1.1) where both studies had used the same mosquito strain (*An. gambiae s.s.* var. Kilimanjaro). These results suggest firstly that the differences found between the G3 strain and the SUA and KIL strain are attributable to differences between the test persons and not necessarily to the type of blood host, and secondly that yet unknown differences between test persons, for instance variations in odour composition, might exist.

In line with the field experiences of Braack et al. (1994), *An. arabiensis* bit primarily on the legs and feet. This was also seen in *An. quadriannulatus*,

Table 1. Distribution of biting sites of *An. gambiae s.s.*, *An. arabiensis* and *An. quadriannulatus* on various body parts of a human host

	N[b]	Biting index[a]					G-tests	
		Head[c]	Trunk	Arms	Legs	Feet	1[d]	2[e]
An. gambiae s.s. Sua[P1]	218	0.11	0.22	0.63	0.34	9.6	P<0.001	a
An. gambiae s.s. Kil[P1]	244	0.33	0.28	0.37	0.42	9.7	P<0.001	a
An. gambiae s.s. G3[P5]	100	0.78	0.19	0.26	0.94	7.1	P<0.001	b
An. arabiensis[P1]	48	0	0	0	0.33	13	P<0.001	c
An. quadriannulatus[P5]	52	2.6	0.25	0.42	1.2	3.3	P<0.001	d
Skin surface (as % of total)		9	32	19	33	7		

[a] Biting index = % bites on a certain body part divided by % skin surface of that body part.
[b] Total number of bites recorded. [c] Head includes neck region. [d] Statistical comparison between distribution found and expected, based on relative skin surface. [e] Statistical comparison between treatments, treatments not followed by the same letter are different at P<0.01. [P1] Test person 1; [P5] Test person 5.

Table 2. Distribution of biting sites of *An. gambiae s.s.* 'Suakoko' on different human hosts

Test person	N[b]	Biting index[a]					G-tests	
		Head[c]	Trunk	Arms	Legs	Feet	1[d]	2[e]
P1[f]	218	0.11	0.22	0.63	0.39	9.6	P<0.001	a
P2	68	0	0.13	0.21	0.36	11	P<0.001	b
P3	40	0	0.09	0	0.85	10	P<0.001	b,c
P4	44	0	0	0.26	0.70	10	P<0.001	c

[a] Biting index as in Table 1. [b] Total number of bites recorded. [c] Head includes neck region. [d] G-tests as in Table 1. [e] Treatments not followed by the same letter are different at P<0.01. [f] Same data as in Table 1. P1 – Test person 1; P2– Test person 2, etc.

although the latter species had a high biting index on the head as well, in which it differed from its siblings (Table 1). Since both species are not anthropophilic (White, 1974), other factors than human-specific odours must have guided these two species to the lower parts of the body. Detailed experiments on the selection of biting sites by *An. gambiae s.s.* provided information on these factors. If the position of the test person was changed from sitting on a stool to sitting on the ground, the feet were no longer preferred above the legs and when the test person was lying on the ground, *An. gambiae s.s.* no longer selected a specific body part, but distributed its bites evenly over the body (Table 4). Moreover, when the feet and legs were kept in an upright position, the feet and legs were even less attractive than other body parts. Finally, in contrast with de Jong & Knols (1995a) in identical experiments, we did not find a change in the preference for a biting site after the feet were washed with Unicura® soap. From these results we conclude that in our study the preference to bite the feet was primarily caused by the fact that they were the body parts closest to the ground. The results suggest that the near-host orientation of *An. gambiae s.s.* and its siblings *An. arabiensis* and *An. quadriannulatus* took place merely on the basis of convection currents along the host (Lewis et al., 1969; Clark & Toy, 1975). In selecting a biting site, females of the *An. gambiae* complex gradually descend with the convection currents created by the body heat of the host, and bite the lowest parts encountered. The fact that with the test person sitting on the floor, the bites on the legs were merely located around the hips supports this idea, as a mosquito gradually descending along with the convection current will end up around the hips or feet.

It is difficult to find a suitable explanation for the incongruity between this study and that of de Jong & Knols (1995a) whose authors reported a change in behaviour after washing of the feet. In a recent study Jeganathan (1997) also found that *An. gambiae s.s.* changed its biting behaviour from primarily on the feet to randomly across the body, after washing the feet with Unicura® soap, thus confirming de Jong & Knols' observations. When the results of these three studies are summarized, *An. gambiae s.s.* was exposed to seven naked persons and had a significant

Table 3. Distribution of biting sites by *An. gambiae s.s.* 'Suakoko' on a human body after washing of the feet

Test person	N[b]	Biting index[a]					G-tests[d]
		Head[c]	Trunk	Arms	Legs	Feet	
P 1	165	0.11	0.34	0.37	0.39	9.6	P<0.001
P 2	21	0	0	1.7	0	9.6	P<0.001
P 3	30	0	0	0	1.0	9.6	P<0.001

[a] Biting index as in Table 1. [b] Total number of bites recorded. [c] Head includes neck region. [d] G-tests as in Table 1.

Table 4. Distribution of biting sites by *An. gambiae s.s.* 'Suakoko' on a human body with the body in different positions

Position of the body		N[b]	Biting index[a]					G-tests	
			Head[c]	Trunk	Arms	Legs	Feet	1[d]	2[e]
Sitting on a stool[P1,f]		218	0.11	0.22	0.63	0.39	9.6	P<0.001	a
Sitting on a stool[P2,f]		68	0	0.13	0.21	0.36	11	P<0.001	b
Sitting on the ground[P2]		38	0	0.33	0.55	1.8	3.0	P<0.001	c
Lying on the ground[P2]		56	0.60	0.45	1.6	1.2	1.3	n.s.	d
Lying on the ground, legs up[P1]		84	0.13	0.86	2.5	0.61	0.34	P<0.001	e

[a] Biting index as in Table 1. [b] Total number of bites recorded. [c] Head includes neck region. [d] G-tests as in Table 1. [e] Treatments not followed by the same letter are different at P<0.01. [f] Same data as Table 1, *An. gambiae s.s.* 'Suakoko'. [P1] Test person 1. [P2] Test person 2.

preference to bite the feet. Washing of the feet had a significant effect on the biting behaviour of this mosquito in two persons and in five persons no change was seen. There is a number of factors that is influenced by washing of the skin, such as temperature gradients, humidity levels and spatial odour composition. It is likely that these factors vary between persons, and that washing of the feet subsequently influences these factors differently. The combined results indicate that within the *An. gambiae* complex odours mediate the process of selection of biting sites.

There is another reason that we cannot exclude odours in the selection of biting sites by *An. gambiae s.l.*, because *An. quadriannulatus* preferred to bite the head region significantly more than what was expected on the basis of the relative skin surface of the head. This difference with *An. gambiae s.s.* and *An. arabiensis* is in accordance with Dekker & Takken (1998), who found that *An. quadriannulatus* was attracted to CO_2 in the field, while no such response was found for *An. gambiae s.s.* and *An. arabiensis* (Mboera et al., 1997). This suggests that in selecting a biting site, *An. quadriannulatus* might have responded partially to breath, as was previously seen for *An. atroparvus* (de Jong & Knols, 1995a) and *An. albimanus*, leading it to the legs and feet of the human body. In the field, *An. quadriannulatus* has also been observed to bite primarily the legs and feet of cattle (Dr. R. H. Hunt, pers. comm.). This may be correlated with the short hair depth and density around the feet, as has been found for Tabanidae (Mullens & Gerhardt, 1979). These authors found a positive correlation between mean hair depth at the point of landing on cattle and the mean length of the mouthparts of more than twenty tabanid species. More interestingly, closely related species appeared to select the same biting sites, whereas large differences were observed with other tabanid species of the same size. One may therefore cautiously speculate on the possibility that the distribution of biting sites of the relatively small sized *An. gambiae s.l.* is genetically determined and a result of the zoophilic ancestor species, which, presumably because of its size, went for the legs.

Acknowledgements

The authors thank Leo Koopman, Frans van Aggelen and André Gidding for maintaining the mosquito colonies used in this study. Dr. Maureen Coetzee and Dr. Richard Hunt are thanked for making provisions for a working visit by T.D. Part of this work was funded by the Netherlands Fund for the Prevention of Diseases.

References

Braack, L. E. O., M. Coetzee, R. H. Hunt, H. Biggs, A. Cornel & A. Gericke, 1994. Biting behavior and host-seeking behavior of *An. arabiensis* (Diptera: Culicidae) in Northeast South Africa. Journal of Medical Entomology 31: 333–339.

Clark, R. P. & O. G. Edholm, 1985. Man and his Thermal Environment. Edward Arnold, London.

Clark, R. P. & N. J. Toy, 1975. Natural convection around the human head. Journal of Physiology 244: 283–293.

Dekker T. & W. Takken, 1998. Field studies on the responses of *Anopheles arabiensis* Patton and *An. quadriannulatus* Theobald to CO_2, man and calf. Medical and Veterinary Entomology, in press.

Gillies, M. T., 1980. The role of carbon dioxide in host-finding by mosquitoes. Bulletin of Entomological Research 70: 525–532.

Haddow, A. J. 1956. Observations on the biting habits of African mosquitoes in the genus *Eretmapodites* Theobald. Bulletin of Entomological Research 46: 761–772.

Hocking, B., 1971. Blood-sucking behavior of terrestrial arthropods. Annual Review of Entomology 16: 1–26.

Jeganathan, N., 1997. Selection of biting sites by *Anopheles gambiae* and *An. atroparvus*. MSc thesis, University of London.

Jong, R. de & B. G. J. Knols, 1995a. Selection of biting sites on man by two malaria mosquito species. Experientia 51: 80–84.

Jong, R. de & B. G. J. Knols, 1995b. Olfactory responses of host-seeking *Anopheles gambiae s.s.* Giles (Diptera: Culicidae). Acta Tropica 59: 333–335.

Jong, R. de & B. G. J. Knols, 1996. Selection of biting sites by mosquitoes. In: Cardew, G. (ed.), Proceedings of CIBA foundation No. 200: Olfaction in Mosquito-Host Interactions. Wiley, Chichester, pp. 89–99.

Knols, B. G. J., 1996. Odour-mediated host-seeking behaviour of the Afro-tropical malaria vector *Anopheles gambiae* Giles, 213 pp. Ph.D. Thesis, Agricultural University Wageningen.

Knols, B. G. J., W. Takken & R. de Jong, 1994. Influence of human breath on the selection of biting sites by *Anopheles albimanus*. Journal of the American Mosquito Control Association 10: 423–426.

Knols, B. G. J., J. J. A. van Loon, A. Cork, R. D. Robinson, W. Adam, J. Meijerink, R. de Jong & W. Takken, 1997. Behavioural and electrophysiological responses of the female malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) to Limburger cheese volatiles. Bulletin of Entomological Research 87: 151–159.

Laarman, J. J., 1955. The host-seeking behaviour of the malaria mosquito *Anopheles atroparvus*. Acta Leidensia 25: 1–144.

Laarman, J. J., 1958. The host-seeking behaviour of anopheline mosquitoes. Tropical and Geographical Medicine 10: 293–305.

Lewis, H. E., A. R. Foster, B. J. Mullan, R. N. Cox & R. P. Clark, 1969. Aerodynamics of the human microenvironment. Lancet I: 1273–1277.

Mboera, L. E. G. & W. Takken, 1997. Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. Review of Medical and Veterinary Entomology 85: 355–368.

Mboera, L. E. G., B. G. J. Knols, A. Della Torre & W. Takken, 1997. The response of *Anopheles gambiae s.l.* and *An. funestus* (Diptera: Culicidae) to tents baited with human odour or carbon dioxide in Tanzania. Bulletin of Entomological Research 87: 173–178.

Mullens, B. A. & R. R. Gerhardt, 1979. Feeding behaviour of some Tennessee Tabanidae. Environmental Entomology 8: 1047–1051.

Sokal, R. R. & F. J. Rohlf, 1981. Biometry. W. H. Freeman and Co., New York. pp. 704–709.

Takken, W., T. Dekker & Y. Wijnholds, 1997. Odor-mediated behavior of *Anopheles gambiae s.s.* Giles and *An. stephensi* Liston in response to CO_2, acetone and 1-octen-3-ol. Journal of Insect Behavior 10: 395–407.

White, G. B., 1974. *An. gambiae* complex and disease transmission in Africa. Transactions of the Royal Society for Tropical Medicine and Hygiene 68: 278–299.

Wilton, D. P., 1975. Mosquito collections in El Salvador with ultraviolet and CDC miniature light traps with and without dry ice. Mosquito News 35: 522–525.

Wright, R. H. & F. E. Kellogg, 1962. Responses of *Aedes aegypti* to moist convection currents. Nature 194: 402–403.