Why humans are attractive to malaria mosquitoes

Malaria poses serious problems to human health, especially in sub-Saharan Africa. Blood-feeding female mosquitoes are the vectors of the malaria parasites. The mosquitoes use host odours to find their blood sources, but we know little about the semiochemicals that mediate this behaviour. A combined study including behavioural experiments, electrophysiological techniques and chemical analysis is undertaken to identify the volatile human-specific compounds that are used in the host-seeking behaviour of the females of the malaria mosquito *Anopheles gambiae* sensu stricto. Traps baited with these chemicals may assist in monitoring populations and reduce contact between the vector and human host.

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Malaria

Worldwide between 300 and 500 million people suffer from malaria and over one million people, especially children under the age of five and pregnant women, die as a result of this disease each year. Most of the malaria cases occur in sub-Saharan Africa and these result in about 90% of global mortality due to malaria. In this region, the females of the mosquito *Anopheles gambiae* Giles sensu stricto (Diptera: Culicidae; figure 1) are the principle vectors of the parasite *Plasmodium falciparum*, which is the main cause of severe clinical malaria and death. Treatment and control of the disease have become difficult due to the spread of drug-resistant strains of the parasites and of strains of mosquitoes that became resistant to insecticides or changed their biting behaviour. Social and economical factors also play an important role. New, more effective and inexpensive control measures are needed (Greenwood & Mutabingwa 2002).

Host odours

Only female mosquitoes take blood meals; blood proteins are required for egg development. Blood is taken from a wide range of vertebrate hosts. Several studies showed that host odours play a major role in the orientation and host-finding of blood-seeking (nocturnal) female mosquitoes (Takken 1991, Takken & Knols 1999). In some species a definite host preference is present, which is determined genetically. Some species feed on one kind of host only. Since host-seeking is mediated by an olfactory stimulus, it is possible that the odour complex of the host contains host-specific chemicals to which only certain mosquito species respond. Specificity can also be due to quantitative differences in abundance of generally occurring host-derived chemicals.

In Africa the main vectors of human malaria are species of *A. gambiae* sensu lato. This is a group of seven mosquito species, including vectors and non-vectors. Most members are opportunistic in their feeding behaviour: they may bite humans and animals, depending on host availability. The females of *A. gambiae* s.s., however,are highly anthropopha-

Figure 1. A blood-feeding *Anopheles gambiae* s.s.female on a human arm. Photo: B. Mans
Een bloedzuigend vrouwtje van Anopheles gambiae *s.s.*

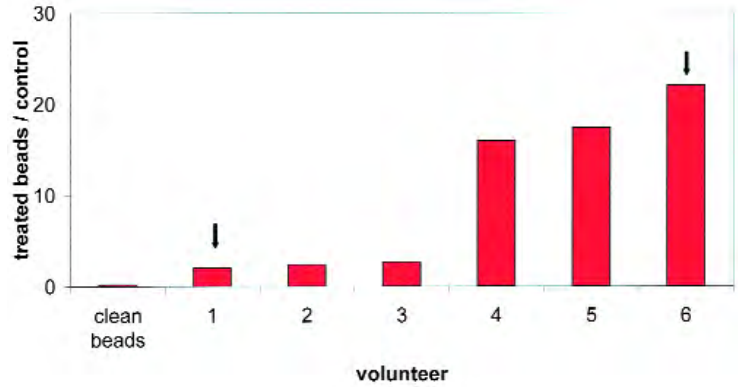

Figure 2. Attractiveness of glass beads handled by six volunteers to females of the malaria mosquito *A. gambiae* s.s., when tested individually in a dual-choice olfactometer (treated beads versus clean beads + ammonia). The attractiveness is given as the number of mosquitoes attracted to the treated beads over the number of mosquitoes attracted to the control stimulus. Beads of persons on the right attracted high numbers of mosquitoes compared to clean beads. Arrows show the individuals tested directly against each other (see figure 3).
Aantrekkelijkheid van knikkers die in contact zijn geweest met handen van zes vrijwilligers voor vrouwtjes van de malariamug A. gambiae *s.s., individueel getest in een tweekeuze-olfactometer ('reukmeter'; behandelde knikkers versus schone knikkers + ammoniak). De aantrekkelijkheid is weergegeven als het aantal tot de knikkers aangetrokken muggen gedeeld door het aantal tot de schone knikkers aangetrokken muggen. Knikkers van personen rechts in de grafiek trokken veel muggen aan in vergelijking tot schone knikkers. De pijlen geven de individuen aan die tevens als tweetal zijn getest (zie figuur 3).*

gic: they have a preference for human blood (White 1974, Pates *et al.* 2001a), which makes them the most important vectors of human malaria. Morphology, physiology and behaviour of the females are entirely adapted to feeding from their preferred host. Therefore, it is possible that they use human-specific odour blends to find their blood hosts. This knowledge may be used to improve the effectiveness of (odorous) mosquito traps that can be deployed to interrupt the contact between vector and human host.

It has been shown that anthropophilic mosquito females use chemical compounds present in the human breath or that evaporate from the human skin to locate their blood hosts from a distance. For example, in the field, females of *A. gambiae* s.s. are caught in traps baited with carbon dioxide (CO_2) (Gillies 1980, Costantini *et al.* 1996). However, CO_2 is exhaled by all warm-blooded vertebrates and it is unlikely that a mosquito species like *A. gambiae* s.s.,which has a selective host preference, will be guided to its host by this compound alone. CO_2 seems to be more important for opportunistic and zoophilic species (Mboera *et al.* 1997, Mboera & Takken 1997, Dekker & Takken 1998, Takken & Knols 1999). Research in laboratories showed that human sweat and human skin residues are highly attractive for *A. gambiae* s.s. (Braks *et al.* 1997; Braks & Takken 1999; Healy & Copland 2000). Also chemicals, like short-chain carboxylic acids, ammonia, and L-lactic acid, which are present in human sweat and/or in human skin emanations, were found to be attractive (*e.g.* Knols *et al.* 1997, Braks *et al.* 2001, Costantini *et al.* 2001, Dekker *et al.* 2002, Smallegange *et al.* 2002). To date however, it is still not clear which combination of volatiles the *A. gambiae* s.s.-females use to find humans. The (combination of) attractants identified thus far are less attractive than the total odour of the natural host. In our laboratory research is undertaken to unravel this mystery.

Interdisciplinary research

The majority of the compounds present in human sweat and skin emanations have been identified (Cork & Park 1996, Bernier *et al.* 2000, Meijerink *et al.* 2000). Theoretically, we can use this knowledge to test which compounds play a role in the host-seeking behaviour of the malaria mosquitoes by testing them one by one and in all possible combinations in behavioural tests. However, this is not feasible as it concerns more than 300 components! It will take years to test all these chemicals for their behavioural relevance. Therefore, at Wageningen University we are using another inter- disciplinary approach.

Some people are bitten by mosquitoes more often than others (Curtis 1986). We used this phenomenon in our research and compared the odours of highly attractive indivi-

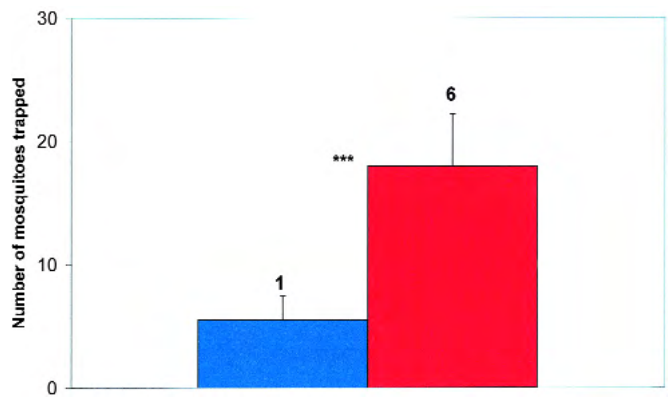

Figure 3. Attractiveness of handled glass beads of a highly and a less attractive volunteer (see figure 2) when tested simultaneously. The mean of four experiments is shown. In each experiment 30 female mosquitoes were released (Chi-square-test: *** P<0.0001; error bars of mean are shown).
*Aantrekkelijkheid van knikkers van een zeer aantrekkelijke en van een minder aantrekkelijke vrijwilliger (zie figuur 2) die rechtstreeks tegen elkaar zijn getest. De grafiek laat het gemiddelde van vier experimenten zien. In elk experiment werden 30 vrouwelijke muggen losgelaten (Chi-kwadraattoets: *** P<0,0001; standaardfout van het gemiddelde is eveneens weergegeven).*

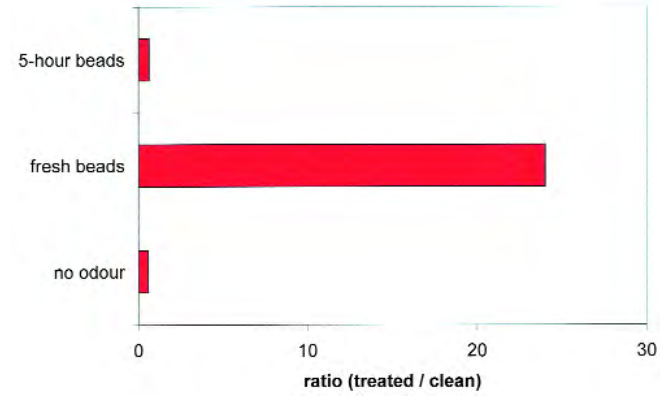

Figure 4. Attractiveness of handled beads of one person immediately and five hours after handling. The attractiveness is given as the number of mosquitoes attracted to the treated beads over the number of mosquitoes attracted to clean beads.
De aantrekkelijkheid van vastgehouden knikkers direct en vijf uur na behandeling. De aantrekkelijkheid is weergegeven als het aantal tot behandelde knikkers aangetrokken muggen gedeeld door het aantal aangetrokken tot schone knikkers.

duals with those of less attractive ones. A group of 28 volunteers was asked to rub glass beads in their hands during a few minutes. This method can be used to transfer attractive emanations from the human hand to the glass surface (Bernier *et al.* 1999). The handled glass beads of each person were tested six times for their attractiveness in behavioural tests with *A. gambiae* s.s.-females. During these experiments the beads of one person were tested against clean beads and a concentration of ammonia shown to be attractive as the standard control odour. Indeed, these bio-assays revealed that human individuals can be ranked based on their attractiveness to female mosquitoes (figure 2). To be certain, we tested beads handled by a highly attractive person versus beads handled by a less attractive person. These experiments confirmed our first results (figure 3).

Thermal desorption combined with gas chromatography (GC) and mass spectrometry (MS) (see Bernier *et al.* 1999) are used to find the differences in the odour profiles of most and least attractive volunteers, and to identify the compounds involved. These differences may be qualitative and/or quantitative. In addition, it is possible that not only attractive but also repellent compounds cause the differences in attractiveness between individuals.

The attractiveness of the handled beads was found to decrease quickly and disappeared within five hours after handling (figure 4). This suggests that at least some of the attractive compounds are very volatile. This knowledge can be used in our attempt to identify the chemicals responsible for the attractiveness of humans to female mosquitoes. To narrow down our search we are looking for human-specific compounds.

Based on the differences in human odour profiles we may be able to identify chemicals involved in the host-location by *A. gambiae* s.s.-females. A first possible step is to use electrophysiological methods: identified chemicals can be tested for their biological activity with a so-called ElectroAntennoGram (EAG) and Single Sensillum Recording techniques (see Meijerink *et al.* 2000). These techniques show whether the olfactory sensilla on the antennae of the female mosquito respond to these odorous components. Or in other words, whether the mosquito can 'smell' a certain single chemical or a combination of compounds. EAG in combination with GC and MS (EAG-GC-MS) can also be used as an intermediate step (Smid *et al.* 2002). It will show to which identified compounds in the human odour profile the olfactory sensilla of the mosquito are responding. These compounds will be worth studying.

In the end, it is necessary to return to the behavioural tests. A mosquito may be able to smell a specific odour, but this information does not tell us if the compound is attractive, repellent or neutral to the host-seeking females. In dual-choice olfactometers (Braks *et al.* 1997; figure 5) in the laboratory, single chemicals and combinations of chemicals can be screened for their attractiveness. Finally, field experiments in Africa with wild *A. gambiae* s.s.-mosquitoes have to reveal the usefulness of traps baited with the promising chemicals under natural circumstances when they have to compete with human baits.

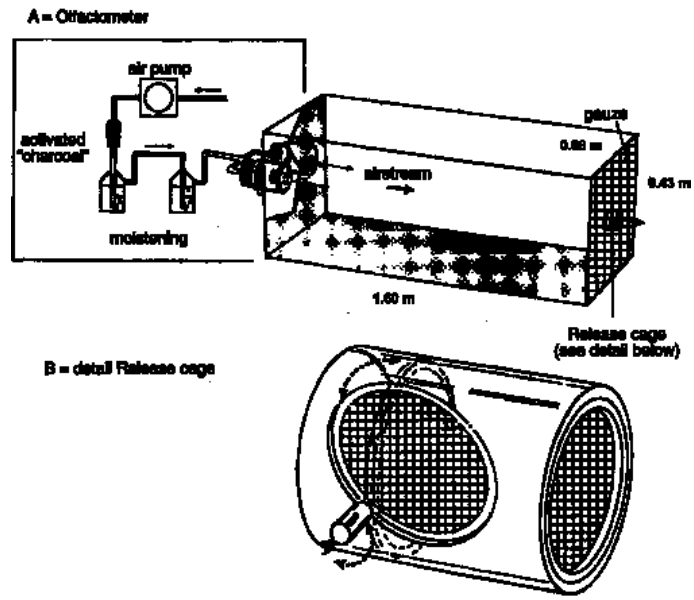

Figure 5. Diagram of the dual-port olfactometer (A) and a mosquito release cage (B) (after Pates *et al.* 2001b).
Schematische weergave van een tweekeuze-olfactometer (A) en een loslaatkooitje (B) (naar Pates et al. *2001b).*

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Samenvatting

Waarom mensen aantrekkelijk zijn voor malariamuggen

Elk jaar raken 300 tot 500 miljoen mensen besmet met malariaparasieten. Met name in Afrika ten zuiden van de Sahara vormt de ziekte malaria een groot probleem. Het is daar doodsoorzaak nummer één onder kinderen tot vijf jaar oud. De ziekte wordt overgebracht door bloedzuigende vrouwelijke muggen. In Afrika zijn de vrouwtjes van de muggensoort *Anopheles gambiae* sensu stricto de belangrijkste vector van de malariaparasiet *Plasmodium falciparum*. De bestrijding van malaria wordt onder andere bemoeilijkt door de ontwikkeling van resistentie van de parasiet tegen medicijnen en van de mug tegen insecticiden. Nieuwe, effectievere en goedkopere bestrijdingsmethoden zullen ontwikkeld moeten worden.

Onderzoek heeft uitgewezen dat muggenvrouwtjes geuren gebruiken om hun bloeddonoren te vinden. Aangezien de vrouwtjes van *A. gambiae* s.s. hoofdzakelijk mensen steken is het mogelijk dat een aantal van deze geuren mensspecifiek zijn. Door gebruik te maken van de combinatie van gedragsonderzoek, elektrofysiologische en analytisch-chemische technieken proberen we te achterhalen welke geurstoffen dat zijn. Een vangsysteem gecombineerd met deze lokstoffen kan gebruikt worden om aantalsfluctuaties in muggenpopulaties te volgen en om het contact tussen mug en mens te onderbreken om zo verdere overdracht van malariaparasieten te voorkomen.