8. Host-seeking behaviour of Afrotropical anophelines: field and semi-field studies

Wolfgang R. Mukabana, Evelyn A. Olanga and Bart G.J. Knols

Abstract

The search for a synthetic mosquito attractant based on one or more human-derived kairomones has been the goal of many laboratory studies. Besides alleviating the occupational risk to which volunteers participating in vector surveillance are subjected whilst performing landing catches, discovery of potent attractants also underpins the development and deployment of mass trapping devices for controlling transmission of mosquito-borne diseases. Whereas a few potential synthetic attractants have recently been developed and tested, not much has been done under open field conditions. Odour delivery methodologies are still needed and the potency of available attractants is too small at present to warrant full-scale application of this technology for malaria vector surveillance and control. Cheap traps and trapping devices that operate energy-free or utilise an affordable or renewable source of energy to power them, a cheap source of carbon dioxide, a cheap and easy method for attractant delivery, and discovery of generic attractants that can trap multiple vectors need to be developed. Only then shall the potential impact of this technology on incidence and prevalence of various vector-borne diseases come to fruition.

Keywords: *Anopheles*, malaria, kairomones, host-seeking behaviour, Africa

Introduction

About one million deaths and close to five hundred million clinical episodes of malaria occur throughout the world each year (Breman and Holloway 2007). Ninety percent of malaria-related deaths occur in sub-Saharan Africa (Boutin *et al*. 2005), mainly among pregnant women and in children below five years of age (Philips 2001). Malaria hampers socioeconomic development by interfering with savings and investment, lowering workforce productivity, promoting absenteeism, causing premature mortality and increasing medical costs (Sachs and Malaney 2002). This makes malaria-endemic countries not only poor but also associates them with lower and slower rates of economic growth. The global distribution of per-capita gross domestic product (GDP) shows a distinct correlation between malaria and poverty. The reverse is true for countries where malaria has been eradicated (Gallup and Sachs 2001).

Human malarias are transmitted by *Anopheles* mosquitoes and are caused by single or multiple infections of four species of *Plasmodium* parasites. These include *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. Recently, *P. knowlesi* has been reported to cause malaria in Southeast Asia (Cox-Singh *et al*. 2007, Kim-Sung *et al*. 2009). While *P. vivax* is most widely distributed, *P. falciparum*, which is the most widespread *Plasmodium* species in sub-Saharan Africa, causes the most severe complications. The main vectors of malaria in Africa are mosquitoes belonging to the *Anopheles gambiae* and *An. funestus* complexes.

The *An. gambiae* complex consists of seven morphologically indistinguishable sibling species namely *An. gambiae s.s.*, *An. arabiensis*, *An. bwambae*, *An. merus*, *An. melas*, *An. quadriannulatus* species A (Coetzee *et al*. 2000, White 1974) and *An. quadriannulatus* species B (Hunt *et al*. 1998). *Anopheles gambiae*, the nominal taxon, and *An. arabiensis* are the most important vector species

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in the complex (Coetzee *et al*. 2000, Gillies and De Meillon 1968, Gillies and Coetzee 1987, White 1974). The occurrence of 80% of the world's malaria in tropical Africa (WHO 1993) is due to the strong human biting habits (anthropophily) and relatively long lifespan of these two species (Collins and Besansky1994), besides *An. funestus* which has similar biological characteristics. *Anopheles gambiae* is a more efficient vector because of its endophilic and anthropophilic characteristics (Costantini *et al*. 1999). The vectorial capacity of *An. arabiensis* is slightly lower than that of *Anopheles gambiae* because of its ability to feed on other animals when human hosts are not available (Gillies and Coetzee 1987). Distinct chromosomal 'forms' of *An. gambiae*, which are strongly associated with specific habitats, exist in West Africa. Three of these so-called ecophenotypes (i.e. BAMAKO, MOPTI, and SAVANNAH) have been found to occur in sympatry at numerous sites (Coluzzi *et al*. 1979). Two other members of the complex, namely *An. melas* in West Africa and *An. merus* in East Africa, are localised vectors depending on their levels of contact with people. Of the other sibling species, *An. bwambae* is responsible for localised malaria transmission among the Bambute pygmies of Bwamba in Uganda whereas *An. quadriannulatus* is not considered a vector, in spite of its competence to transmit *P. falciparum* (Takken and Knols 1999). The Ethiopian population of *An. quadriannulatus* was recently recognised as being distinct (from its South African counterpart) and is designated *An. quadriannulatus* species B (Hunt *et al*. 1998).

The *An. funestus* complex consists of a group of nine species including *An. funestus*, *An. rivulorum*, *An. parensis*, *An. vaneedeni*, *An. leesoni*, *An. fuscivenosus*, *An. aruni*, *An. brucei* and *An. confusus* (Cohuet *et al.* 2004). *Anopheles funestus* is the main species within the *An. funestus* group that transmits malaria (Cohuet *et al*. 2004, Gillies and De Meillon 1968, Wilkes *et al*. 1996). It is endophilic and anthropophilic and is considered as one of the major vectors of malaria in Africa (Cohuet *et al*. 2004). Whereas *An. funestus, An. rivulorum,* and *An. leesoni* are widely distributed throughout sub-Saharan Africa, the other members of the group are more locally distributed: *An. parensis* and *An. confusus* are found in Eastern Africa, *An. vaneedeni* in the northern areas of South Africa, *An. aruni* in Zanzibar, *An. fuscivenosus* in Zimbabwe, and *An. brucei* in Nigeria. The distribution, ecological and behavioural aspects of the main vectors of malaria in Africa are presented in Table 1.

African malaria mosquitoes locate their blood meal hosts largely based on olfactory cues (Takken and Knols 1999). Physical cues, encompassing heat and moisture, also play a role that is hitherto not well understood (e.g. Mukabana *et al*. 2004, Olanga *et al*. 2010, Takken *et al*. 1997). Dissecting and analysing the broad spectrum of human emanations (Costantini *et al*. 1993) can provide an important basis for developing synthetic compounds or blends with desirable attractant (Logan and Birkett 2007), repellent or attractant 'masking' properties (Logan *et al*. 2008). Several compounds identified from human emanations have been demonstrated to exhibit attractant properties under varying experimental conditions. In this chapter we explore the extent to which odour baits have been employed to assess the host-seeking behaviour of African malaria mosquitoes under semi-field and field conditions. Emphasis is placed on studies conducted in the last decade, as earlier work is reviewed by Takken and Knols (1999).

Odour-mediated host-seeking behaviour of African malaria vectors in the field

Haddow (1942) was among the first to recognise the importance of host odours in the behaviour of African malaria vectors. He demonstrated that human body odour attracted anophelines to a house, and that the attraction was proportional to body mass. Since then numerous studies demonstrated the role of human odour in the attraction of malaria vectors (reviewed in Takken and Knols 1999). Few studies attempted to unravel the role of individual chemicals constituting

Table 1. Distribution, ecological and behavioural characteristics of the main vectors of malaria in Africa.

Species and distribution	Ecology and behaviour	Comments	References
An. arabiensis and An. gambiae Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo, Cote d'Ivoire, Democratic Republic of Congo, Djibouti, Eritrea, Guinea, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea Bissau, Kenya, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe	*Adults: An. arabiensis* is anthropophilic but rather opportunistic, sometimes preferring to feed on domestic animals rather than man; rests outdoors frequently, indoors more frequently; feeds outdoors and indoors frequently. *An. gambiae* is highly anthropophilic; mainly feeding and resting indoors. *Larvae:* breed in temporary pools of water that are typically small, shallow, open and sunlit; the water is normally warm, could be turbid, and could contain algae but no surface film and/or aquatic vegetation. The diversity of habitats could include burrow pits, drains, brick-pits, ruts, car tracks, foot and hoof prints, water holes, river overflows, pools left by receding rivers, backwaters, rice fields, etc.	The stamp of human activity, direct or indirect, is implicit in most breeding sites. *An. gambiae* is common in the wet season and in high rainfall areas. In Madagascar both species are predominantly zoophilic.	Gillies and DeMeillon 1968, Gillies and Coetzee 1987, Costantini *et al.* 1998, 1999, Van den Broek and Den Otter 1999, Coetzee *et al.* 2000, Duchemin *et al.* 2001, Gimnig *et al.* 2001, Dia *et al.* 2003, Pock Tsy *et al.* 2003, Moffett *et al.* 2007
An. melas Angola, Congo, Cote d'Ivoire, Democratic Republic of Congo Equatorial Guinea, Gambia, Guinea, Gabon, Gambia, Ghana, Liberia, Nigeria, Senegal, Sierra Leone	*Adults:* feed readily indoors and outdoors; highly anthropophilic but with considerable levels of zoophily; endophily and exophily almost balance; outdoor resting sites are mainly natural – shaded earth banks, tree bases / buttresses, termite mounds, sides of crab holes, and underneath vegetation. *Larvae:* typically breed in salt- or brackish water habitats – pools, ponds, lagoons, marshes and swamps.	High organic pollution is typical of the larval habitats. The species is confined to the west coast of Africa.	Gillies and DeMeillon 1968, White 1974, Bryan *et al.* 1987, Gillies and Coetzee 1987, Coetzee *et al.* 2000, Bogh *et al.* 2001, Moffett *et al.* 2007

Table 1. Continued.

Species and distribution	Ecology and behaviour	Comments	References
An. merus			
Madagascar, Kenya, Mauritius, Mozambique, Somalia, Seychelles, South Africa, Swaziland, Tanzania	*Adults*: can be caught resting indoors by day but are mainly exophilic; in the absence of domestic animals they bite man readily both indoors and outdoors, but have very high zoophilic tendencies. *Larvae*: most commonly found in brackish lagoons, ponds, swamps, pools and puddles.	Water in pools favoured by *An. merus* is sometimes black, showing a high degree of organic (vegetable) pollution. *An. merus* is confined to the east coast of Africa.	Gillies and DeMeillon 1968, Mosha and Subra 1982, Mosha and Petrarca 1983, Paskewitz *et al.* 1983, Sharp *et al.* 1984, Mnzava and Kilama 1986, Gillies and Coetzee 1987, Coetzee 1989, La Grange 1995, Van Rensburg *et al.* 1996, Coetzee *et al.* 2000, Pock Tsy *et al.* 2003, Moffett *et al.* 2007
An. quadriannulatus			
Ethiopia, Malawi, Mozambique, South Africa, Tanzania (Zanzibar only), Zimbabwe	*Adults*: are characteristically zoophagic and exophilic. *Larvae*: same as for *An. arabiensis* and *An. gambiae*.	Incriminated as a competent vector in the laboratory but not (yet) in the field.	Mpofu 1985, Gillies and Coetzee 1987, Coetzee 1989, Coetzee *et al.* 1993, La Grange 1995, Van Rensburg *et al.* 1996, Hunt *et al.* 1998, Takken *et al.* 1999, Fettene *et al.* 2002, 2004, Pates *et al.* 2006, Mzilahowa *et al.* 2008, Moffett *et al.* 2007, Sharp *et al.* 2007
An. coustani			
Common in most parts of Sub-Saharan Africa down to the Cape Province in South Africa. Also present in Madagascar and Mauritius	*Adults*: readily attack humans outdoors penetrating indoors only to a limited extent; conversely they readily enter stables and animal-baited traps; females rest primarily in tall vegetation; biting activity is fairly evenly spread out over the whole night. *Larvae*: breeds in natural collections of clear water with aquatic and semi-aquatic vegetation such as swamps, ponds, backwaters of streams, springs, ditches and rice fields.	-	Gillies and DeMeillon 1968, Gillies and Coetzee 1987, Logan *et al.* 1991, Adugna and Petros 1996, Antonio-Nkondjio *et al.* 2002, 2005, Dia 2005, Muturi *et al.* 2006, Moffett *et al.* 2007

Table 1. Continued.

Species and distribution	Ecology and behaviour	Comments	References
An. funestus Angola, Botswana, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Cote d'Ivoire, Democratic Republic of Congo, Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Kenya, Liberia, Madagascar, Malawi, Mali, Mauritania, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe, Equatorial Guinea	*Adults*: highly prefer to feed and rest indoors; very highly anthropophilic. *Larvae*: breed typically in large, less turbid, semi-permanent water bodies [swamps (near edges if deep), weedy banks of streams, rivers, furrows or ditches, protected portions of lake shores, ponds, etc.] or in water such as seepages which are fed from underground permanent sources. Shade from trees or bush is not critically important.	Larval habitats typically contain emergent vegetation (short grass, tall reeds, trailing plants, etc.). Larvae difficult to find: they dive and remain under water for prolonged periods.	Watson 1953, Bruce-Chwatt and Gockel 1960, Gillies and DeMeillon 1968, Gillies and Coetzee 1987, Gimnig *et al.* 2001, Moffett *et al.* 2007, Jawara *et al.* 2009
An. moucheti Cameroon, Democratic Republic of Congo, Ecuatorial Guinea, Gabon, Kenya, Nigeria, Uganda	*Adults*: highly anthropophilic/domestic; feed on man indoors; rest to variable extents indoors. *Larvae*: are found along the borders of slow moving streams and large rivers, or in pools and ponds where the water movement may be imperceptible. Although restricted to forest areas the larvae are always found where the canopy is broken with some penetration of direct sunlight.	*An. moucheti* is essentially a forest species and an important vector of malaria in almost all areas where it occurs.	Gillies and Coetzee 1987, Adugna and Petros 1996, Elissa *et al.* 1999, Sylla *et al.* 2000, Antonio-Nkondjio *et al.* 2002, 2005, Cano *et al.* 2006, Muturi *et al.* 2006, Moffett *et al.* 2007
An. nili Benin, Burkina Faso, Botswana, Cameroon, Chad, Côte d'Ivore, Ethiopia, Gabon, Ghana, Gambia, Kenya, Liberia, Mali, Nigeria, Senegal, Sierra Leone, South Africa, Sudan, Tanzania, Uganda	*Adults*: are anthropophilic (readily bite man indoors and outdoors), frequently resting indoors by day in some areas; have little contact with man and are rarely found indoors in other areas. *Larvae*: breed within vegetation or dense shade along the edges of streams and large rivers.	-	Elissa *et al.* 1999, Antonio-Nkondjio *et al.* 2002, 2005, Kenge *et al.* 2003, Awone-Ambene *et al.* 2004, Dia *et al.* 2005, Moffett *et al.* 2007

Table 1. Continued.

Species and distribution	Ecology and behaviour	Comments	References
An. pharoensis Angola, Burkina Faso, Burundi, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Egypt, Ethiopia, Gabon, Gambia, Ghana, Israel, Kenya, Malawi, Mauritania, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, South Africa, Sudan, Syria, Tanzania, Togo, Uganda, Zambia, Zimbabwe	*Adults*: endophagic and exophagic; highly exophilic; feed on humans but are more zoophilic; mainly rest on vegetation; females often found between stems of rice plants and on stems of reeds. *Larvae*: normally confined to fresh water and breed primarily in large vegetated swamps. Other habitats: grass shores of lakes among floating plants, rice fields, stagnant desert water, edges of streams, ditches, overgrown wells and temporarily flooded areas.	Is a secondary vector of malaria.	Bruce-Chwatt and Gockel 1960, Gillies and DeMeillon 1968, Gillies and Coetzee 1987, Wernsdorfer and Wernsdorfer 2003, Moffett *et al.* 2007, Jawara *et al.* 2009

attractive odours. Carbon dioxide (CO_2) was recognised as a universal mosquito attractant, including African anophelines (Mboera and Takken 1997). Apart from the studies that focused on CO_2 only, the number of semi-field and field studies that have been undertaken between 1993 and 2010 to further our understanding of odour-mediated host-seeking behaviour of African malaria vectors are limited (Table 2). These largely centered on understanding mosquito behavioural responses to crude, unrefined odour samples, mostly whole human odour (Table 2). Other unrefined odour bait sources that have been investigated within this period include human foot odour (Jawara *et al*. 2009, Murphy *et al*. 2001, Njiru *et al*. 2006, Olanga *et al*. 2010, Okumu *et al*. 2010a, Schmied *et al*. 2008), human breath (Knols *et al*. 1998, Mukabana *et al*. 2004) and sheep, goat, pig (Mahande *et al*. 2007), cattle (Costantini *et al*. 1993, 1998, Dekker and Takken 1998, Duchemin *et al*. 2001, Kweka and Mahande 2009, Kweka *et al*. 2009, Mahande *et al*. 2007, Tirados *et al*. 2006) and monkey odours (Costantini and Diallo 2001). A handful of these studies have been carried out in West Africa where malaria vector species, characterised by certain peculiarities (Costantini *et al*. 1999), differ in some ways from those found in eastern Africa, including the islands of the Indian Ocean (Duchemin *et al*. 2001).

Specific objectives of the studies conducted are diverse but can be grouped into four. These included: (1) development of mosquito sampling and surveillance tools (Costantini *et al*. 1993, Dia *et al*. 2005, Govella *et al*. 2009, Jawara *et al*. 2009, Knols *et al*. 1998, Laganier *et al*. 2003, Mathenge *et al*. 2002, 2004, 2005), (2) investigation of the basis of differences of attractiveness of humans to mosquitoes (Brady *et al*. 1997, Knols *et al*. 1995, Lacroix *et al*. 2005, Lindsay *et al*. 1993, Mboera *et al*. 1997, Mukabana *et al*. 2002, 2004); (3) determination of the interspecific host preferences of different mosquitoes species (Costantini and Diallo 2001, Costantini *et al*. 1998, Dekker and Takken 1998, Duchemin *et al*. 2001, Kweka and Mahande 2009, Kweka *et al*. 2009, Mahande *et al*. 2007, Tirados *et al*. 2006, Torr *et al*. 2008) and (4) identification of chemical attractants for Afrotropical anophelines (Beavers *et al*. 1998, Costantini *et al*. 1996, 2001, Gibson *et al*. 1997, Murphy *et al*. 2001, Njiru *et al*. 2006, Okumu *et al*. 2010a, Qiu *et al*. 2007). To date, nothing has been reported on the actual deployment of chemical attractants for operational mosquito control or surveillance in Africa.

The species of African *Anopheles* mosquitoes mentioned in the published studies are diverse and include *Anopheles gambiae s.s.*, *An. arabiensis*, *An. coustani*, *An. funestus*, *An. nili*, *An. pharoensis*, *An. quadriannulatus*, *An. sergenti*, *An. squamosus* and *An. ziemanni*. In terms of host preference *An. gambiae s.s.* (Costantini and Diallo 2001, Costantini *et al*. 1993, 1998), *An. funestus* (Duchemin *et al*. 2001) and *An. pharoensis* (Costantini *et al*. 1998) preferred to enter human-baited over animal-odour baited traps in dual-choice assays. This was also the case for *An. arabiensis* (Kweka *et al*. 2009, Torr *et al*. 2008). However, in some cases for *An. gambiae*, *An. arabiensis* (Duchemin *et al*. 2001, Kweka and Mahande 2009) and *An. funestus* (Costantini *et al*. 1998) this was the reverse. Carbon dioxide was identified as a cause for differences in human attractiveness to mosquitoes (Brady *et al*. 1997) and the chemical compounds 1-octen-3-ol + CO_2 (Beavers *et al*. 1998), L-lactic acid + CO_2 (Murphy *et al*. 2001), 7-octenoic acid (Costantini *et al*. 2001), and a blend containing ammonia + L-lactic acid + CO_2 + 3-methylbutanoic acid (Qiu *et al*. 1997) attracted various African mosquito species in the field.

Table 2. Published field and semi-field studies on odour-mediated host-seeking behaviour of African Anopheles mosquitoes undertaken between 1993 and 2010. F= Field study, S= Semi-field study.

Odour baits, Reference	F/S	Objective(s)	Study site and procedures	Results
Carbon dioxide (CO$_2$), human odour, calf odour; Costantini *et al.* 1993	F	Develop an odour-baited trap for field studies of mosquito host-seeking behaviour.	Study site: Burkina Faso. An Odour Baited Entry Trap (OBET) with live bait as sources of host stimuli was developed. Volatiles or gases emitted by live baits concealed in tents were sucked and channelled into a mosquito trapping device.	*Anopheles gambiae* s.l., *An. funestus* and *Mansonia* spp. were the most abundant species caught when traps were baited with a human, calf or CO$_2$. Species composition varied with different baits. Caught mosquitoes were mainly non-fed, with 100% survival rate.
CO$_2$, body odour (including breath); Brady *et al.* 1997	F	Determine the basis of differences in attractiveness of humans to mosquitoes.	Study site: Burkina Faso. Attractiveness of humans to mosquitoes was investigated based on Biting Catch (HBC) and OBETs baited with odour of individuals performing the HBC. Catches using OBETs were repeated with the individuals' CO$_2$ outputs equalised.	In the HBC, the 'most attractive' catcher in each team collected twice as many mosquitoes as the 'least attractive' catcher. The differences did change and were independent of catchers' skills. OBET assays found equal significant rankings for *An. gambiae* s.l. but ranking was lost when the men's CO$_2$ outputs were artificially standardised.
CO$_2$, body odour (including breath); Costantini *et al.* 1996, Gibson *et al.* 1997	F	Investigate the influence of CO$_2$ relative to other host odours in the host seeking behaviour of *An. gambiae, An. arabiensis.*	Study site: Noungou village, Burkina Faso. Direct choice tests were done using two OBETs placed side by side or 20 m apart. One OBET contained CO$_2$ alone; the other emitted the same concentration of CO$_2$ plus human odour.	More *An. gambiae* s.l. chose the human OBET when traps lay side by side. When placed 20 m apart the CO$_2$ baited traps caught 50, 40, 65 and 200% of *An. gambiae* s.l., *An. funestus, Ma. uniformis* and *An. pharoensis* of the human odour. All species gave similar dose-response curves when amounts of CO$_2$ were varied but behaviours differed when catches were compared with human bait: *An. arabiensis* chose CO$_2$ OBETs with a higher probability than *An. gambiae* s.s.
CO$_2$, human body odour; Mboera *et al.* 1997	F	Determine (1) the relative attractiveness of human odour to mosquitoes in the presence or absence of a human; (2) mosquito responses to different levels of CO$_2$.	Study site: Kikulukutu village, Kilombero district, Tanzania. Odours from a male volunteer sitting in an underground pit were pumped into an untreated bed net present inside a PVC tent. The relative attractiveness of the volunteer's odour against an empty tent, a tent occupied by a second volunteer or a tent with CO$_2$ (released at 300 or 1,500 ml/min) was then evaluated.	Human odour attracted significantly more *An. gambiae* and *An. funestus* than 'odour' from an empty pit. Physical presence of a volunteer did not increase his attractiveness over odour, from a competing human subject, pumped from a pit. Tents baited with humans attracted a larger proportion of both species than tents baited with CO$_2$ (300 ml/min), which in turn caught more mosquitoes than unbaited tents. This result was unchanged for *An. gambiae* when the amount of CO$_2$ was increased (1,500 ml/min). Releasing CO$_2$ at 1,500 ml/min equalled catches of *An. funestus* to those by man.

Table 2. Continued.

Odour baits, Reference	F/S	Objective(s)	Study site and procedures	Results
CO_2, 1-octen-3-ol; Beavers et al. 1998	F	Evaluate CO_2 and 1-octen-3-ol as mosquito attractants	Study site: Qara and Matruh Governorate, Egypt	Larger numbers of Ae. caspius and An. sergenti were collected in CO_2 and CO_2 + octenol traps than octenol alone. Traps baited with octenol + CO_2 resulted in greater capture rates but results were not significant when compared with CO_2 alone. At the release rate tested (0.5 mg/h at 21 °C), octenol was not an effective attractant.
Human, calf odour; Costantini et al. 1998	F	Investigate the role of whole host odours in the determination of mosquito preferences for a calf and a human.	Study site: Noungou village, Burkina Faso. Choice tests were performed using two OBETs placed side by side. Odours of a human and a calf (of similar mass) were drawn from two concealed tents and blown into the OBETs.	An. gambiae s.l. and An. pharoensis predominantly chose the human-baited trap; the calf-baited trap had high numbers of Cx. antennatus, An. rufipes, Cx. duttoni and Cx. nebulosus. An. funestus, Ma. africana, Ae. dalzieli and Ae. hirsutus were caught in low numbers but with a bias for the human-baited trap. Ma. uniformis was distributed randomly between the two traps.
Whole human odour, calf, CO_2; Dekker and Takken 1998	F	Investigate the response of two sibling species An. arabiensis and An. quadriannulatus to CO_2, a man or a calf.	Study site: South Africa. CO_2 was delivered as dry ice with average release rate of 800-900 ml/min. Lower rates of 250 and 180 ml/min were obtained from a CO_2 gas cylinder. Combinations of baits were compared in dual-choice tests using two mosquito nets placed 2.5 m apart and 10 cm off the ground. Mosquitoes entered the nets from below and were collected by a suction tube.	In the choice test between man and CO_2 (human equivalent of 250 ml/min), 81% of An. quadriannulatus and 20% of An. arabiensis were attracted to CO_2. High CO_2 release rates (cow equivalent of 800 ml/min) attracted significantly more An. quadriannulatus than CO_2 released at 250 ml/min. Up to 33% of the attraction of An. arabiensis to human emanations was attributed to CO_2. An. quadriannulatus was equally attracted to a calf and CO_2 (calf equivalent of 180 ml/min).
Human breath, CO_2; Knols et al. 1998	F	Test the feasibility of electric nets for sampling mosquitoes.	Study site: Namawala village, Tanzania. Mosquitoes were sampled using electric nets.	It was possible to sample An. squamosus, An. coustani and Mansonia africana using human breath-baited and CO_2 baited electric nets.
3-methyl-2-hexenoic acid, 7-octenoic acid, CO_2, whole human odour; Costantini et al. 2001	F	Synthesise and investigate role of the listed odourants as components of human-specific kairomones.	Study site: Burkina Faso. The acid(s) were released with either 'pure CO_2' (flow-rate 200 ml/min), or 'whole human odour' provided by an adult male sleeping inside a tent. These treatments were compared to 'pure CO_2' or 'whole human odour', which served as controls.	Only 7-octenoic acid significantly increased An. gambiae s.l. trap catches of CO_2. The treatment trap caught significantly fewer An. gambiae s.l. than the control trap, for both 'pure CO_2' and 'whole human odour' treatments, when 7-octenoic acid was offered in combination with 3-methyl-2-hexenoic acid.

Table 2. Continued.

Odour baits, Reference	F/S	Objective(s)	Study site and procedures	Results
Human and monkey odours; Costantini and Diallo 2001	F	Assess the tendency of An. gambiae and other malaria vectors to prefer human over monkey odour.	Study site: Senegal. Two adult Cercopithecus aethiops monkeys and a child of similar mass slept inside separate tents and their odours were drawn to paired OBETs so that approaching mosquitoes could experience both odour-laden streams before entering one of the two traps.	An. gambiae s.l, An. funestus, and An. nili clearly expressed a preference for human odour, with >90% of captured mosquitoes caught in the human baited trap. These results support the hypothesis that the strongly anthropophilic feeding preferences of An. gambiae did not evolve from an ancestral association with non-human primates.
Cow, human odour; Duchemin et al. 2001	F	Assess host preferences of An. gambiae, An. arabiensis and An. funestus.	Study site: Madagascar. Odours from a man and a calf of similar mass, concealed in different tents were drawn by fans to separate OBETs.	An. funestus 'preferred' human odour whereas An. gambiae and An. arabiensis preferred calf odour.
Limburger cheese volatiles, L-lactic acid, CO_2, hexanoic acid, foot odour, human odour; Murphy et al. 2001	F	Test if attractants derived from or related to human emanations elicit behavioural responses to An. gambiae and An. funestus.	Study site: Kenya. Hexanoic acid, L-lactic acid and cheese volatiles were offered in combination with CO_2 (dry ice). Emanations from a human or his worn socks were not combined with CO_2. Attractants were offered on 100% cotton 3.8 cm long dental wicks.	CDC miniature light traps baited with L-lactic acid plus CO_2 attracted significantly more mosquitoes than those baited only with CO_2 or the other attractants.
Whole human odour; Dia et al. 2005	F	Compare relative performance of OBETs and HBC.	Study site: Barkédji and Ngari villages, Senegal.	HBC was more effective indoors for surveying the anopheline fauna. Both methods were effective in sampling An. gambiae, An. arabiensis, An. funestus and An. nili, and mosquito age structure and infectivity rates did not differ between the methods.
Human foot odour, CO_2, ammonia, 1-octen-3-ol; Njiru et al. 2006	S	Assess reponses of An. gambiae to these odours alone or in combination	Study site: Kenya. Lab-reared mosquitoes were released in a 7×11 m screenhouse compartment with and given a choice of baited MM-X traps.	Increases in catches were observed as follows: 1-octen-3-ol > ammonia > foot odour > foot odour + ammonia > CO_2 > foot odour + ammonia + CO_2

Table 2. Continued.

Odour baits, Reference	F/S	Objective(s)	Study site and procedures	Results
Cow odour; whole human odour; Tirados et al. 2006	F	Monitor the blood feeding behaviour of An. arabiensis.	Study site: Ethiopia. Female An. arabiensis mosquitoes were collected by host-baited methods (light traps, human landing catches, cattle-baited traps) and from resting sites (huts and pit shelters). Anthropophily was gauged by comparing numbers of mosquitoes caught in OBETs baited with either human or cattle odour.	Where the ratio of cattle to humans was 0.6:1, 51% of outdoor resting mosquitoes and 66% of those collected indoors had fed on humans; human baits outdoors caught >2.5 times more mosquitoes than those indoors. Where humans slept outdoors close to their cattle, ~46% of resting mosquitoes collected outdoors had fed on humans despite the high cattle to human ratio of 17:1. In both sites, high proportions of blood meals were mixed cow + human: 22-25% at Fuchucha and 37% in the cattle camps. In the OBETs, the human-baited trap caught five times as many mosquitoes as the cattle-baited one.
Human, cattle, sheep, goat and pig odours; Mahande et al. 2007	F	Assess feeding behaviour of An. arabiensis.	Study site: Mabogini village, Moshi, Tanzania. Feeding behaviour of An. arabiensis was assessed using OBETs.	Cattle, human, sheep, goat and pig odours attracted 90.3%, 9.7%, 9.7%, 7.2%, and 7.3% of An. arabiensis mosquitoes, respectively (n = 506).
CO_2, L-lactic acid, ammonia, carboxylic fatty acids, ketones, alcohols, whole human odour; Qiu et al. 2007	F	Investigate effects of synthetic odour blends on mosquito catches with or without CO_2 as an additional stimulus.	Study site: Walikunda village, The Gambia. Attractants were placed in vials and placed in MM-X traps with or without CO_2. Human odour was piped to an MM-X trap by drawing air from a human-occupied nylon tent through lay-flat tubing. Sixteen odour blends with CO_2 and fourteen odour blends without CO_2 were tested. The control odour consisted of ammonia + L-lactic acid + CO_2.	Addition of CO_2 to synthetic odours increased the catch of all mosquito species in the MM-X traps. The blend of ammonia + L-lactic acid + CO_2 + 3-methylbutanoic acid attracted most mosquito species.
Foot odour, foot odour + CO_2, or foot odour + repellent (lemongrass); Schmied et al. 2008	S	Compare various odours against An. gambiae using two different trap designs (BGS or MMX-trap).	Study site: Ifakara, Tanzania. Female An. gambiae s.s. were released in an experimental flight arena that was placed inside a semi-field system.	The BGS trap caught about three times as many mosquitoes as the MM-X trap ($P=0.002$). Adding CO_2 (500 ml/min) to foot odour increased the number of mosquitoes caught by 268% for the MM-X ($P<0.001$) and 34% ($P=0.051$) for the BGS trap, compared to foot odour alone. When lemongrass leaves were added to foot odour, mosquito catches were reduced by 39% (BGS, $P<0.001$) and 38% (MM- X, $P=0.353$), respectively.

Table 2. Continued.

Odour baits, Reference	F/S	Objective(s)	Study site and procedures	Results
Cattle odour, human odour, CO_2, acetone, 1-octen-3-ol, 4-methylphenol, 3-n-propylphenol; Torr et al. 2008	F	Compare odour-oriented responses of *An. arabiensis* and *An. quadriannulatus* in the field.	Study site: Rekomitjie, Mana pools game reserve, Zimbabwe. Number of mosquitoes attracted and mosquito entry responses were estimated using electrocuting nets (E-nets) and OBETs, respectively. Mosquito landing responses were estimated by comparing catches from E-nets and cloth targets covered with an electrocuting grid.	Electric nets baited with odour from a single ox or a single man caught similar numbers of *An. arabiensis*; increasing the dose of human odour from one to three men increased the catch 4-fold. *An. quadriannulatus* catches from E-nets increased up to 6-fold in the progression: man, 3 men, ox and man plus ox. Entry responses of *An. arabiensis* were stronger with human odour (62%) than with ox odour (6%) or both (15%). *An. arabiensis* did not exhibit a strong entry response to carbon dioxide (0.2-2 l/min). Entry responses of *An. quadriannulatus* were low (<2%) with both cattle and human odour. Catches from an electrocuting target baited with either CO_2 or a blend of acetone, 1-octen-3-ol, 4-methylphenol and 3-n-propylphenol – components of natural ox odour – showed that virtually all mosquitoes arriving there alighted on it.
Cow, human subject; Kweka and Mahande 2009	F	Compare human landing catches, pit shelters, indoor resting collections and man/cow-baited entry traps for sampling mosquitoes at low density.	Study site: Mabogini village, Lower Moshi, Tanzania. The number of mosquitoes recovered indoors, from a pit shelter, from a cow-baited entry trap, from a human-baited entry trap and human landing catches were compared during a season of low mosquito density and malaria transmission.	Both *An. gambiae* s.l. and *Cx. quinquefasciatus* mosquitoes were collected. In general human landing catches recovered significantly fewer mosquitoes than the pit shelter and the cow-baited entry trap. The human biting catch collections did not differ significantly from the indoor resting collections. Though otherwise stated human landing catches were apparently not different from collections using the human-baited entry trap.
Cow body odour, cow urine, human subject; Kweka et al. 2009	F	Evaluate human landing catch collections and cow odour-baited resting boxes as sampling tools for *An. arabiensis*.	Study site: Mabogini village, Lower Moshi, Tanzania. Human landing catch collections and an unbaited resting box, a cow odour-baited resting box and a cow urine-baited resting box were evaluated as sampling tools for *An. arabiensis* mosquitoes.	Significantly more *An. arabiensis* mosquitoes were collected from urine baited resting boxes than by the human landing catches, an unbaited resting box and a resting box baited with cow odour only.

Table 2. Continued.

Odour baits, Reference	F/S	Objective(s)	Study site and procedures	Results
CO_2, foot odour, human subjects; Jawara et al. 2009	F	Determine if traps are best placed indoors or outdoors, the best height and distance away from a hut for collecting mosquitoes and the possibility of competition among outdoor traps and their influence on numbers of mosquitoes entering huts.	Study site: Walikunda village, The Gambia. MM-X traps were either placed indoors or outdoors in experimental huts, each occupied by one human subject. The traps were baited with CO_2 and human foot odour (socks worn for 12 hours). Traps were operated from 09:00 pm to 07:00 am the next morning. Hut windows were slightly opened at 6:00 pm to leave a gap of about 10 cm, simulating a poorly fitting window as observed in village huts, and they remained in this position until 6:30 am, while the doors remained closed.	The mosquito species collected included An. gambiae s.l. (9.1%), An. pharoensis (2.3%), An. ziemanni (1.4%), other anophelines (0.3%), Culex species (5.3%), Aedes (0.4%), and Mansonia species (81.2%). The effect of trap position (indoors or outdoors) and the presence or absence of a sleeper did not have any significant effect on the number of An. gambiae s.l. collected. The optimal placement of human odour-baited MM-X traps is at a height of 15 cm immediately outside huts. This maximizes capture of wild An. gambiae s.l. mosquitoes. The number of An. gambiae s.l. collected within the huts at the end of each night was not significantly affected by the presence of single or multiple traps immediately outside, compared to huts that had no trap outside.
CO_2, ammonia, 7 carboxylic acids, foot odour, human subjects; Okumu et al. 2010	F,S	Develop and evaluate under field conditions a super-attractive synthetic odor blend consisting of known mosquito attractants.	Study site: Tanzania. Binary choice assays were conducted inside a semi-field enclosure using baited traps placed 20 m apart to find optimum concentrations at which individual candidate attractants need to be added to constitute a blend maximally attractive to lab-reared An. gambiae mosquitoes. Field experiments were conducted inside experimental huts, where the blend was compared with adult male humans, directly and indirectly.	The blend consisting of carbon dioxide, ammonia, L-lactic acid and seven aliphatic carboxylic acids attracted three to five times more An. gambiae s.l. (mostly An. arabiensis) and An. funestus than humans when the two baits were in different experimental huts (10-100 m apart), but was equally or less attractive than humans when compared side by side within the same hut.

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Synthetic odour baits attractive to African anophelines under semi-field and field conditions

The discovery of synthetic odour baits that are capable of attracting mosquitoes much the same as a human being (Brady *et al*. 1997) can enhance the development of powerful tools for vector surveillance (Takken and Knols 1999) and control (Day and Sjogren 1994, Kline 2007, Logan and Birkett 2007, Takken and Knols 2009). Individual chemical compounds and blends thereof have been shown to attract African *Anopheles* mosquitoes under semi-field and field environments. The chemicals, which comprise of commonly known kairomones like CO_2, carboxylic acids, ketones, phenols, L-lactic acid, and ammonia, are described in subsequent paragraphs. This chapter is restricted to semi-field and field studies on African anophelines where CO_2 was tested in combination with crude odourants or at least one synthetic attractant (Table 2). The selection of candidate attractants for these studies was in many cases informed by laboratory studies, described in Chapter 7.

Carbon dioxide, a major constituent of human exhaled air (300-500 ml/min.), has been identified as an attractant for many mosquito species including the main vectors of malaria in Africa (Gillies 1980, Mboera and Takken 1997, Mboera *et al*. 1997, 2000, Takken 1991, Takken and Knols 1999). Gillies (1980) suggested that this compound acts as an activator, initiating flight responses, as well as being an attractant. There is strong evidence that CO_2 acts synergistically with other chemical compounds to attract host-seeking mosquitoes (Dekker *et al*. 2002, Kline *et al*. 1990, Murphy *et al*. 2001, Njiru *et al*. 2006, Takken and Kline 1989). Indeed, addition of CO_2 to traps baited with various synthetic compounds significantly increased catches of African anophelines including *An. gambiae s.s.* (Costantini *et al.* 2001, Murphy *et al*. 2001), *An. arabiensis* (Costantini *et al.* 2001, Murphy *et al*. 2001, Torr *et al*. 2008), *An. quadriannulatus* (Torr *et al*. 2008), *An. funestus* (Murphy *et al*. 2001), *An. sergenti* (Beavers *et al*. 1998), *An. pharoensis* (Qiu *et al*. 2007) and *An. ziemanni* (Qiu *et al*. 2007). In a field study in Burkina Faso, CO_2 added to 7-octenoic acid significantly increased the number of *An. gambiae s.s.* and *An. arabiensis* attracted to odour-baited entry traps. In The Gambia, MM-X traps baited with a synthetic odour blend consisting of ammonia, lactic acid, CO_2 and 3-methylbutanoic acid attracted large numbers of mosquitoes belonging to the genera *Mansonia*, *Anopheles* and *Culex* (Qiu *et al*. 2007). Field studies in Egypt demonstrated that CO_2 plus 1-octen-3-ol attracted similar numbers of *An. sergenti* as CO_2 alone (Beavers *et al*. 1998).

Limburger cheese, the smell of which is reminiscent of human foot odour, is known to attract African anopheline mosquitoes under laboratory conditions (De Jong and Knols 1995, Knols and De Jong 1996). One field study carried out in western Kenya, using Limburger cheese in its original form, also demonstrated this (Owino 2006) while another, carried out in the same region using a synthetic analogue of the cheese, did not (Murphy *et al*. 2001). In these studies Limburger cheese odours were delivered using either MM-X counter flow geometry traps (Owino 2006) or CDC miniature light traps (Murphy *et al*. 2001). Species of African malaria mosquitoes that were successfully trapped using Limburger cheese in its original form included *An. gambiae s.s.*, *An. arabiensis* and *An. funestus* (Owino 2006). The difference in results may be attributed to usage of synthetic versus authentic forms of the cheese and differences in odour delivery methodologies. In the case of Murphy *et al*. (2001), a combination of lactic acid and CO_2 was the only treatment that significantly attracted more *An. gambiae* s.l. and *An. funestus* mosquitoes, although numbers caught were very low. This may be attributed to the difficulty of baiting CDC light traps, the fan of which disperses odours widely.

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Synthetic blends consisting of acetone, 1-octen-3-ol, 4-methylphenol and 3-n-propylphenol, which are components of cattle odour, have recently been used to bait mosquito electrocuting nets (Torr *et al*. 2008). *Anopheles arabiensis* did not exhibit a strong entry response to CO_2 (0.2-2 l/min) baited entry traps. Catches from an electrocuting target baited with either CO_2 or a blend of acetone, 1-octen-3-ol, 4-methylphenol and 3-n-propylphenol induced overwhelmingly high *An. gambiae* s.l. landing rates (Torr *et al*. 2008). In a recent study, an odour blend consisting of known mosquito attractants namely CO_2, ammonia and carboxylic acids was evaluated (Okumu *et al*. 2010a; see Table 2 for compound names). Field experiments conducted inside experimental huts found the blend to attract three to five times more *An. arabiensis* than humans when the two baits were in different experimental huts (10-100 meters apart). However, the blend was equally or less attractive than humans when compared side by side within the same huts (Okumu *et al*. 2010a).

From the foregoing it is clear that one or more synthetic analogues of human sweat combined with CO_2 (Costantini *et al*. 2001, Qiu *et al*. 2007) or CO_2 combined with various aliphatic carboxylic acids (Okumu *et al*. 2010a) are currently the best attractants for host-seeking Afrotropical malaria mosquitoes under field conditions. Further research on the development of potent attractants needs to be carried out with the aim of (1) identifying additional candidate human specific odours (2) determine their optimum concentrations to attract host-seeking mosquitoes and (3) optimising existing synthetic odour blends.

Semi-field research on odour bait development

Historically, semi-field systems, which in essence are large outdoor screened cages, have been used for mosquito research in several countries and for several mosquito species (reviewed by Ferguson *et al*. 2008). Only since the late 1990s have such systems become a research tool to identify important kairomones for African anophelines. A major advantage of such systems is the fact that a fixed and controllable number of mosquitoes can be introduced in them, which delivers constant and comparable results. Moreover, by placing such systems directly in areas where malaria vectors occur in nature it is possible to rear offspring of field-collected gravid females, thereby ensuring that the genetic background of test mosquitoes is similar to that of the field population. Availability of efficient traps that can easily be baited with CO_2 and other attractants, such as the MM-X trap (see Njiru *et al*. 2006) and the BGS trap (see Schmied *et al*. 2008) has further strengthened semi-field studies on anopheline host-seeking behaviour. When using two such traps in simple cross-over designs to account for positional effects, rapid progress can be made to improve baits. For instance, the recent step-wise and incremental improvement of a powerful blend to attract *An. gambiae s.s.* by Okumu *et al*. (2010a) started with a basic blend of CO_2 and ammonia, and was then augmented with various concentrations of L-lactic acid, until an optimal concentration was found. Thereafter, each of seven aliphatic carboxylic acids was added to this base blend, at varying concentrations, until again the optimal increase in catch was reached. This approach culminated in the development of a blend that attracted 3-5 times as many mosquitoes than a human volunteer, when tested in experimental huts in a field setting. These developments clearly demonstrate the power of semi-field research, and we advocate the use of such systems not only for further attractant development, but also for the development of low-cost trapping devices, an aspect of bait-trap technology that has not received enough attention to date.

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The promise of using odour baits for malaria vector surveillance and control in Africa

Although the goals of developing potent insect attractants are diverse, the central goal lies in vector control and surveillance (Kline 2007, Logan and Birkett 2007). With respect to mosquitoes, impact on target populations can be achieved through mass trapping (Anonymous 2005, Kline and Lemire 1998), lure and kill technology (Day and Sjogren 1994, Kline 2007) or lure and contaminate technology (e.g. by using biopesticides like entomopathogenic fungi). The efficacy of mass trapping, despite its underlying conceptual, technical, logistical and financial limitations, has been successfully demonstrated for mosquito population reduction in the USA (Anonymous 2005, Kline 2007, Kline and Lemire 1998). However, this strategy is still being developed, with current efforts being centered on searching for new attractants and attractant formulations (Healy and Copland 2000, Healy *et al.* 2002, Okumu *et al.* 2010a, Qui *et al.* 2007), improving on existing ones (Smallegange *et al.* 2005), and developing trapping devices (Kline 2006, Kröckel *et al.* 2006). Efficacy trials of candidate synthetic attractants under field (Qiu *et al.* 2007) and semi-field conditions (Njiru *et al.* 2006, Okumu *et al.* 2010a, Olanga *et al.* 2010) are also underway.

In terms of surveillance the need of replacing the risk prone human landing catch for measuring the degree of contact between humans and mosquitoes (Service 1993) is urgent. The key challenge is to find synthetic attractants, which by acting as human surrogates can be used in sampling devices for malaria vectors (Brady *et al.* 1997). Whereas identification of a potent synthetic attractant will go a long way in helping to develop malaria vector surveillance tools and mass trapping devices, other challenges relate to suitable ways and means of delivering attractant odours. In the studies carried out thus far delivery methods of synthetic attractants included use of wicks (Murphy *et al.* 2001), glass vials (Costantini *et al.* 2001, Qiu *et al.* 2007), sealed polythene sachets (Torr *et al.* 2008), and nylon strips (Okumu *et al.* 2010b). The use of wicks, glass vials and nylon strips has the major disadvantage that whereas release rates of synthetic odours can be measured by weighing before and after placement in the field, measurement may not be as precise, especially for compounds which tend to absorb water such as octenol. This is in contrast to sealed polythene sachets, which guarantee measurable constant release rates of synthetic attractants. However, the use of pressurised cylinders (Costantini *et al.* 2001, Qiu *et al.* 2007, Torr *et al.* 2008) or dry ice (Murphy *et al.* 2001) as delivery methods of CO_2 is disadvantageous as the methods are not logistically sound. They are expensive and laborious for use in the field. Handy sources and more effective methods of delivering CO_2 and other gaseous kairomones should be sought.

Besides the disadvantages associated with odour delivery technologies, the physical gadgets useful for handling and dispensing attractant-impregnated materials are limiting. Field experiments have so far relied on using odour-baited entry traps (Brady *et al.* 1997, Costantini at Diallo 2001, Costantini *et al.* 1993, 1996, 1998, Dia *et al.* 2005, Gibson *et al.* 1997, Kweka and Mahande 2009, Kweka *et al.* 2009, Mahande *et al.* 2007, Tirados *et al.* 2006, Torr *et al.* 2008); mosquito magnet model X (MM-X) traps (Jawara *et al.* 2009, Okumu *et al.* 2010a, Qiu *et al.* 2007), CDC miniature light traps (Murphy *et al.* 2001), BGS traps (Schmied *et al.* 2008), electric nets (Knols *et al.* 1998, Torr *et al.* 2008) and resting boxes (Kweka *et al.* 2009). Apart from the MM-X and BGS trap none of the other gadgets is well suited at present for delivering attractants in a desirable manner.

In general the bottlenecks that might impede the use of synthetic attractants for malaria vector surveillance and control in Africa include (1) lack of cheap traps and trapping devices, (2) lack of affordable sources of energy to power traps, (3) lack of cheaper sources of CO_2, this being a key kairomone for many malaria vectors and may be a chemical that will remain an integral

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component of attractive blends, (4) lack of cheap and easy methods for delivering mosquito synthetic attractants, (5) low diversity of synthetic attractants, and (6) lack of a generic attractant that can trap multiple vectors.

Concluding remarks

This chapter has focused on odour baits that have been employed to assess the host-seeking behaviour of African *Anopheles* mosquitoes under field and semi-field conditions. The reported findings on mosquito responses to synthetic attractants provide optimism that the search for a surrogate human is possible. A potent synthetic human odour will enhance the development of powerful mosquito trapping devices that can be exploited to increase the success of control programmes through forecasting epidemics accurately and for formulating, planning and rolling out control activities.

References

Adugna N and Petros B (1996) Determination of the human blood index of some anopheline mosquitoes by using ELISA. Ethiop Med J 34: 1-10.

Anonymous, 2005. Collier Mosquito Control District - completed projects. Stevens' Landing. Available at: http://www.collier-mosquito.org/stevens_landing.php (accessed November 30, 2009).

Antonio-Nkondjio C, Awono-Ambene P, Toto JC, Meunier JY, Zebaze-Kemleu S, Nyambam R, Wondji CS, Tchuinkam T and Fontenille D (2002) High malaria transmission intensity in a village close to Yaounde the capital city of Cameroon. J Med Entomol 39: 350-355.

Antonio-Nkondjio C, Simard F, Awono-Ambene P, Ngassam P, Toto J, Tchuinkam T and Fontenille D (2005) Malaria vectors and urbanization in the equatorial forest region of south Cameroon. Trans R Soc Trop Med Hyg 99: 347-354.

Awone-Ambene HP, Kengne P, Simard F, Antonio-Nkondjio C and Fontenille D (2004) Description and bionomics of *Anopheles* (Cellia) *ovengensis* (Diptera: Culicidae) a new malaria vector species of the *Anopheles nili* group from South Cameroon. J Med Entomology 41: 561-568.

Beavers GM, Hanifa HA and Tetreault GE (1998) Response of mosquitoes (Diptera: Culicidae) to carbon dioxide and octenol in Egypt. J Egypt Soc Parasitol 28(2): 303-312.

Bøgh C, Clarke SE, Pinder M, Sanyang F and Lindsay SW (2001) Effect of passive zooprophylaxis on malaria transmission in The Gambia. J Med Entomol 38: 822-828.

Boutin JP, Pradines B, Pages F, Legros F, Rogier C and Migliani R (2005) Epidemiology of Malaria. Rev Prat 55(8): 833-840.

Brady J, Costantini C, Sagnon N, Gibson G and Coluzzi M (1997) The role of body odours in the relative attractiveness of different men to malarial vectors in Burkina Faso. Annal of Trop Med Parasitol 91: 121-122.

Breman JG and Holloway CN (2007) Malaria Surveillance Counts. Am J Trop Med Hyg, 77 (Suppl 6): 36-47.

Bruce-Chwatt LJ and Göckel CW (1960) A study of the blood-feeding patterns of *Anopheles* mosquitoes through precipitin tests. Bull WHO 22: 685-720.

Bryan, JH, Petrarca V, Di Deco MA and Coluzzi M (1987) Adult behaviour of members of the *Anopheles gambiae* complex in the Gambia with special reference to *An. melas* and its chromosomal variants. Parasitologia 29: 221-249.

Cano J, Angel Desalzo M, Moreno M, Chen Z, Nzambo S, Bobuakasi L, Buatiche JN, Ondo M, Micha F and Benito A (2006) Spatial variability in the density distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). Malar J 5: 21.

Coetzee M (1989) Comparative morphology and multivariate analysis for the discrimination of four members of the *Anopheles gambiae* group in Southern Africa. Mosq Sys 21: 100-116.

Coetzee M (2004) Distribution of the African malaria vectors of the *Anopheles gambiae* complex. Am J Trop Med Hyg 70: 103-104.

Coetzee M, Craig M and Le Sueur D (2000) Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. Parasitology Today 16: 74-77.

Coetzee M, Hunt RH, Baack L and Davidson G (1993) Distribution of mosquitoes belonging to the *Anopheles gambiae* complex, including malaria vectors, south of latitude 15uS. S Afr J Sci 89: 227-231.

Cohuet A, Simard F, Wondji CS, Antonio-Nkondjio C, Awono-Ambene P and Fontenille D (2004) High malaria transmission intensity due to *Anopheles funestus* (Diptera: culicidae) in a village of Savannah-forest transition area in Cameroon. J Med entomol 41: 901-905.

Collins FH and Besansky NJ (1994) Vector biology and the control of malaria in Africa. Science 264: 1874-1875.

Coluzzi M, Sabatini A, Petrarca V and di Deco MA (1979) Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. Trans R Soc Trop Med Hyg, 73: 483-497.

Costantini C and Diallo M (2001) Preliminary lack of evidence for simian odour preferences of savanna populations of *Anopheles gambiae* and other malaria vectors. Parassitologia 43: 179-182.

Costantini C, Birkett MA, Gibson G, Ziesmann J, Sagnon NF, Mohammed HA, Coluzzi M and Pickett JA (2001) Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. Med Vet Entomol 15: 259-266.

Costantini C, Gibson G, Brady J, Merzagora L and Coluzzi M (1993) A new odour-baited trap to collect host-seeking mosquitoes. Parassitologia 35: 5-9.

Costantini C, Gibson G, Sagnon N, della Torre A, Brady J and Coluzzi M (1996) Mosquito responses to carbon dioxide in a West African Sudan savanna village. Med Vet Entomol 10: 220-227.

Costantini C, Sagnon N, Della TA, Diallo M, Brady J, Gibson G and Coluzzi M (1998) Odour-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. Am J Trop Med Hyg 58: 56-63.

Costantini C, Sagnon NF, Torre AD and Coluzzi M (1999) Mosquito behavioural aspects of vector-human interactions in the *Anopheles gambiae* complex. Parassitologia 41: 209-217.

Cox-Singh J, Davis TME, Lee K, Shamsul SG, Matusop A, Ratnam S, Rahman HA, Conway DJ and Singh B (2007) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clinical infectious Diseases 46: 16-171.

Day JF and Sjogren RD (1994) Vector control by removal trapping. Am J Trop Med Hyg 50: 126-133.

De Jong R and Knols BGJ (1995) Olfactory responses of host-seeking *Anopheles gambiae s.s.* Giles (Diptera: Culicidae). *Acta Tropica*, 59: 333-335.

Dekker T and Takken W (1998) Differential responses of mosquito sibling species *Anopheles arabiensis* and *An. quadriannulatus* to carbon dioxide, a man or a calf. Med Vet Entomol 12: 136-140.

Dekker T, Steib B, Cardé RT and Geier M (2002) L-lactic acid: a human-signifying host cue for the anthropophilic mosquito *Anopheles gambiae*. Med Vet Entomol 16: 91-98.

Dia I, Diallo D, Duchemin J, Ba Y, Konate L, Costantini C and Diallo M (2005) Comparisons of human-landing catches and odour-baited entry traps for sampling malaria vectors in Senegal. J Med Entomol 42(2): 104-109.

Duchemin JB, Pocktsy JM, Rabarison P, Roux J, Coluzzi M and Costantini C (2001) Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odour-baited entry traps. Med Vet Entomol 15: 50-57.

Elissa N, Karch S, Bureau P, Ollomo B, Lawoko M, Yangari P, Ebang B and Georges AJ (1999) Malaria transmission in a region of savanna-forest mosaic Haut-Ogooue Gabon. J Am Mosq Control Assoc 15: 15-23.

Fettene M, Hunt RH, Coetzee M and Tessema F (2004) Behaviour of *Anopheles arabiensis* and *Anopheles quadriannulatus* sp. B mosquitoes and malaria transmission in Southwestern Ethiopia. Afr Entomol 12: 83-87.

Fettene M, Koekemoer LL, Hunt RH and Coetzee M (2002) PCR assay for identification of *Anopheles quadriannulatus* species B from Ethiopia and other sibling species of the *Anopheles gambiae* complex. Med Vet Entomol 16: 214-217.

Gallup JL and Sachs JD (2001) The economic burden of malaria. Am J Trop Med Hyg 64: 85-96.

Gibson G, Constantini C, Sagnon F, Torre A and Coluzzi M (1997) The responses of *Anopheles gambiae* and other mosquitoes in Burkina Faso to CO_2-the start of a search for synthetic human odor. Annal Trop Med Parasitol 91: 123-124.

Gillies MT (1980) The role of carbon dioxide in host finding by mosquitoes (Diptera: Culicidae): a review. Bull Entomol Res 70: 525-532.

Gillies MT and Coetzee M (1987) A supplement to the *Anophelinae* of African South of the Sahara. South African Institute of Medical Research, Johannesburg, South Africa.

Gillies MT and De Meillon B (1968) The *Anophelinae* of Africa South of the Sahara. South African Institute of Medical Research, Johannesburg, South Africa.

Gimnig JE, Ombok M, Kamau L and Hawley WA (2001) Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. J Med Entomol 38: 282-288.

Govella NJ, Chaki PP, Geissbuehler Y, Kannady K, Okumu F, Charlwood JD, Anderson RA and Killeen GF (2009) A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex. Malar J 8: 157.

Haddow AJ (1942) The mosquito fauna and climate of native huts at Kisumu, Kenya. Bull Entomol Res 33: 91-142.

Healy TP and Copland MJW (2000) Human sweat and 2-oxopentanoic acid elicit a landing response from *Anopheles gambiae*. Med Vet Entomol 14: 195-200.

Healy TP, Copland MJW, Cork A, Przyborowska A and Halket JM (2002) Landing responses of *Anopheles gambiae* elicited by oxocarboxylic acids. Med Vet Entomol 16: 126-132.

Hunt RH, Coetzee M and Fettene M (1998) The *Anopheles gambiae* complex: a new species from Ethiopia. Trans R Soc Trop Med Hyg 92: 231-235.

Jawara M, Smallegange RC, Jeffries D, Nwakanma DC, Awolola TS, Knols BGJ, Takken W and Conway DJ (2009) Optimizing odor-baited trap methods for collecting mosquitoes during the malaria season in The Gambia. PLoS One 4: e8167.

Kenge P, Awono-Ambene P, Antonio-Nkondjio C, Simard F and Fontenille D (2003) Molecular identification of the *Anopheles nili* group of African malaria vectors. Med Vet Entomol 17: 67-74.

Kim-Sung L, Cox-Singh J, Brooke G, Matusop A and Singh B (2009) *Plasmodium knowlesi* from archival blood films: further evidence that human infections are widely distributed and not newly emergent in Malaysian Borneo. Int J Parasitol. 2009 August; 39(10): 1125-1128.

Kline L (2006) Traps and trapping techniques for adult mosquito control. J Am Mosq Control Assoc 22(3): 490-496.

Kline DL (2007) Semiochemicals, traps/targets and mass trapping technology for mosquito management. J Am Mosq Control Assoc 23: 241-251.

Kline DL and Lemire GF (1998) Evaluation of attractant baited traps/targets for mosquito management on Key Island, Florida, USA. J Vect Ecol 23: 171-185.

Kline DL, Takken W, Wood JR and Carlson D (1990) Field studies on the potential of butanone, carbon dioxide, honey extract, l-octen-3-ol, lactic acid, and phenols as extracts for mosquitoes. Med Vet Entomol 4: 383-391.

Knols BGJ and De Jong R (1996) Limburger cheese as an attractant for the malaria mosquito *Anopheles gambiae s.s.* Parasitol Today, 12: 159-160.

Knols BGJ, De Jong R, Takken W (1995) Differential attractiveness of isolated humans to mosquitoes in Tanzania. Trans Roy Soc Trop Med Hyg 89: 604-606.

Knols BGJ, Mboera LEG and Takken W (1998) Electric nets for studying odour-mediated host seeking behavior of mosquitoes. Med Vet Entomol 12: 116-120.

Kröckel U, Rose A, Eiras A and Geier M (2006) New tools for surveillance of adult *Aedes aegypti*: comparison of trap catches with human landing collections in an urban environment. J Am Mosq Control Assoc 22: 229-238.

Kweka EJ and Mahande AM (2009) Comparative evaluation of four mosquitoes sampling methods in rice irrigation schemes of lower Moshi, northern Tanzania. Malar J 8: 149

Kweka EJ, Mwang'onde BJ, Kimaro E, Msangi S, Massenga CP and Mahande AM (2009) A resting box for outdoor sampling of adult *Anopheles arabiensis* in rice irrigation schemes of lower Moshi, northern Tanzania. Malar J 8: 82

La Grange JJP (1995) Survey of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of Swaziland. Afr Entomol 3: 217-219.

Laganier R, Randimby FM, Rajaonarivelo V and Robert V (2003) Is the Mbita trap a reliable tool for evaluating the density of anopheline vectors in the highlands of Madagascar? Malar J 2: 42.

Lacroix R, Mukabana WR, Gouagna LC and Koella JC (2005) Malaria infection increases attractiveness of humans to mosquitoes. PLoS Biol 3: 1590-1593.

Lindsay SW, Adiamah JH, Miller JE, Pleass RJ and Armstrong JRM (1993) Variation in attractiveness of human subjects malaria mosquitoes (Diptera: Culicidae) in The Gambia. J Med Entomol 30: 368-373.

Logan JG and Birkett MA (2007) Semiochemicals for biting fly control: their identification and exploitation. Pest Manag Sci, 63: 647-657.

Logan JG, Birkett MA, Clark SJ, Powers S, Seal NJ, Wadhams LJ, Mordue AJ and Pickett JA (2008) Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. J Chem Ecol 34: 308-322.

Logan TM, Linthicum KJ, Thande PC, Wagateh JN and Roberts CR (1991) Mosquito species collected from a marsh in western Kenya during the long rains. J Am Mosq Control Assoc 7: 395–399

Mahande A, Mosha F, Mahande J and Kweka E (2007) Feeding and resting behaviour of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. Malaria J 6: 100.

Mathenge EM, Killeen GF, Oulo DO, Irungu LW, Ndegwa PN and Knols BGJ (2002) Development of an exposure-free bednet trap for sampling Afrotropical malaria vectors. *Med Vet Entomol* 16: 67-74.

Mathenge EM, Misiani GO, Oulo DO, Irungu LW, Ndegwa PN, Smith TA, Killeen GF and Knols BG (2005) Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of Anopheles arabiensis, An. funestus and culicine species in a rice irrigation in western Kenya. Malar J 4: 7.

Mathenge EM, Omweri GO, Irungu LW, Ndegwa PN, Walczak E, Smith TA, Killeen GF and Knols BG (2004) Comparative field evaluation of the Mbita trap, the Centers for Disease Control light trap, and the human landing catch for sampling of malaria vectors in western Kenya. Am J Trop Med Hyg 70: 33-37.

Mboera LEG and Takken W (1997) Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. Annu Rev Entomol 85: 355-368.

Mboera LEG, Knols BGJ, Braks MAH and Takken W (2000) Comparison of carbon dioxide-baited trapping systems for sampling outdoor mosquito populations in Tanzania. Med Vet Entomol 14: 257-263.

Mboera LEG, Knols BGJ, Takken W and Della Torre A (1997) The response of *Anopheles gambiae* s.l. and *An. funestus* (Diptera: Culicidae) to tents baited with human odour or carbon dioxide in Tanzania. Bull Entomol Res 87: 173-178.

Mnzava AE and Kilama WL (1986) Observations on the distribution of the *Anopheles gambiae* complex in Tanzania. Acta Trop 43: 277-282.

Moffett A, Shackelford N and Sarkar S (2007) Malaria in Africa: vector species' niche models and relative risk maps. PLoS One 2: e824.

Mosha FW and Petrarca V (1983) Ecological studies on *Anopheles gambiae* complex sibling species on the Kenya coast. Trans R Soc Trop Med Hyg 77: 344-345.

Mosha FW and Subra R (1982) Ecological studies on *Anopheles gambiae* complex sibling species in Kenya 1 preliminary observations on their geographical distribution and chromosomal polymorphic inversions. Document WHO/VBC/82867. World Health Organization, Geneva, Switzerland.

Mpofu SM (1985) Seasonal vector density and disease incidence patterns of malaria in an area of Zimbabwe. Trans R Soc Trop Med Hyg 79: 169-175.

Mukabana WR, Takken W, Coe R and Knols BGJ (2002) Host-specific cues cause differential attractiveness of Kenyan men to the malaria mosquito *Anopheles gambiae*. Malar J 1: 17.

Mukabana WR, Takken W, Killeen GF and Knols BGJ (2004) Allomonal effect of breath contributes to differential attractiveness of humans to the African malaria vector *Anopheles gambiae*. Malar J 3: 1.

Murphy MW, Dunton RF, Perich MJ and Rowley WA (2001) Attraction of *Anopheles* (Diptera: Culicidae) to volatile chemicals in Western Kenya. J Med Entomol 38: 242-244.

Muturi E, Shililu J, Jacob B, Gu W, Githure J and Novak R (2006) Mosquito species diversity and abundance in relation to land use in a riceland agroecosystem in Mwea, Kenya. J Vector Ecol 31: 129-137.

Mzilahowa T, Ball AJ, Bass C, Morgan JC, Nyoni B, Steen K, Donnelly MJ and Wilding CS (2008) Reduced susceptibility to DDT in field populations of *Anopheles quadriannulatus* and *Anopheles arabiensis* in Malawi: evidence for larval selection. Med Vet Entomol 22: 258-263

Njiru BN, Mukabana WR, Takken W and Knols BGJ (2006) Trapping of the malaria vector *Anopheles gambiae* with odour-baited MM-X traps in semi-field conditions in western Kenya. Malaria J 5: 39.

Okumu FO, Killeen GF, Ogoma S, Biswaro L, Smallegange RC, Mbeyela E, Titus E, Munk C, Ngonyani H, Takken W, Mshinda H, Mukabana WR and Moore SJ (2010a) Development and field evaluation of a synthetic mosquito lure that is more attractive than humans. PLoS One 5: e8951.

Okumu FO, Biswaro L, Mbeyela E, Killeen GF, Mukabana WR and Moore SJ (2010b) Using nylon strips to dispense mosquito attractants for sampling the malaria vector *Anopheles gambiae s.s.* J Med Entomol *(*in press).

Olanga EA, Okal M, Mbadi PA, Kokwaro E and Mukabana WR (2010) Attraction of *An. gambiae* to odour baits augmented with heat and moisture. Malar J 9: 6.

Owino EA (2006) Field evaluation of Limburger cheese as an odour bait source for sampling afrotropical malaria vectors. MSc Thesis, University of Nairobi, Nairobi, Kenya.

Paskewitz SM, Ng K, Coetzee M and Hunt RH (1993) Evaluation of the polymerase chain reaction method for identifying members of the *Anopheles gambiae* (Diptera: Culicidae) complex in southern Africa. J Med Entomol 30: 953-957.

Pates HV, Takken W, Curtis CF and Jamet H (2006) Zoophilic *Anopheles quadriannulatus* species B found in a human habitation in Ethiopia. Ann Trop Med Parasitol 100(2): 177-179.

Phillips RS (2001) Current status of malaria and potential for control. Clin Microbiol Rev 14: 208-226.

Pock Tsy JM, Duchemin JB, Marrama L, Rabarison P, Le Goff G, Rajaonarivelo V and Robert V (2003) Distribution of the species of the *Anopheles gambiae* complex and first evidence of *Anopheles merus* as a malaria vector in Madagascar. Malar J 8: 33.

Qiu YT, Smallegange RC, Ter BC, Spitzen J, Van Loon JJ, Jawara M, Milligan P, Galimard AM, Van Beek TA, Knols BG and Takken W (2007) Attractiveness of MM-X traps baited with human or synthetic odor to mosquitoes (Diptera: Culicidae) in The Gambia. J Med Entomol 44: 970-983.

Sachs J and Malaney P (2002) The economic and social burden of malaria. Nature 11: 681–685.

Schmied WH, Takken W, Killeen GF, Knols BG and Smallegange RC (2008) Evaluation of two counterflow traps for testing behaviour-mediating compounds for the malaria vector *Anopheles gambiae s.s.* under semi-field conditions in Tanzania. Malar J 7: 230.

Service MW (1993). Mosquito ecology, field sampling methods, 2nd ed. Elsevier Applied Science, London, UK.

Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI, Durrheim DN, Ridl FC, Morris N, Seocharan I, Kunene S, La Grange JJP, Mthembu JD, Maartens F, Martin CL and Barreto A (2007) Seven years of regional malaria control collaboration – Mozambique, South Africa, and Swaziland. Am. J. Trop. Med. Hyg. 76: 42-47.

Smallegange RC, Qiu YT, van Loon JJ and Takken W (2005) Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito *Anopheles gambiae sensu stricto* (Diptera: Culicidae). Chem Senses 30: 145-152.

Sylla EHK, Kun JFJ and Kremsner PG (2000) Mosquito distribution and entomological inoculation rates in three malaria-endemic areas in Gabon. Trans R Soc Trop Med Hyg 94: 652-656.

Takken W (1991) The role of olfaction in host-seeking of mosquitoes: a review. Insect Sci Appl 12: 287-295.

Takken W and Kline DL (1989) Carbon dioxide and l-octen-3-ol as mosquito attractants. J Am Mosq Control Assoc 5: 311-316.

Takken W and Knols BGJ (1999) Odor-mediated behaviour of Afrotropical malaria mosquitoes. Annu Rev Entomol 44: 131-157.

Takken W and Knols BGJ (2009) Malaria vector control: current and future strategies. Trends Parasitol 25: 101-104.

Takken W, Knols BGJ and Otten H (1997) Interactions between physical and olfactory cues in the host-seeking behaviour of mosquitoes: the role of relative humidity. Ann Trop Med Parasitol 91: 119-120.

Tirados I, Costantini C, Gibson G and Torr SJ (2006) Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. Med Vet Entomol 20: 425-437.

Torr S, Della Torre A, Calzetta M, Costantini C and Vale GA (2008) Towards a fuller understanding of mosquito behaviour: use of electrocuting grids to compare the odour-orientated responses of *Anopheles arabiensis* and *An. quadriannulatus* in the field. Med Vet Entomol 22: 93-108.

Van den Broek IVF and Den Otter CJ (1999) Olfactory sensitivities of mosquitoes with different host preferences (*Anopheles gambiae s.s.*, *An. arabiensis*, *An. quadriannulatus*, *An. m. atroparvus*) to synthetic host odours. J Insect Physiol 45: 1001-1010.

Van Rensburg AJ, Hunt RH, Koekemoer LL, Coetzee M, Shiff CJ and Minjas J (1996) The polymerase chain reaction method as a tool for identifying members of the *Anopheles gambiae* complex (Diptera: Culicidae) in northeastern Tanzania. J Am Mosq Control Assoc 12: 271-274.

Watson M (1953) African highway. John Murray, London, UK.

Wernsdorfer G and Wernsdorfer WH (2003) Malaria at the turn from the 2nd to the 3rd millenium. Wien klin Wochenschr 115 Suppl 3: 2-9.

Wilkes TJ, Matola YG and Charlwood JD (1996) *Anopheles rivulorum*, a vector of human malaria in Africa. Med Vet Entomol 10: 108-110.

White GB (1974) *Anopheles gambiae* complex and disease transmission in Africa. Trans R Soc Trop Med Hyg 68: 278-301.

World Health Organization (1993) World malaria situation in 1991. Part I. Weekly Epidemiol Rec 34: 246.