7. Host-seeking behaviour of mosquitoes: responses to olfactory stimuli in the laboratory

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Abstract

This chapter reviews the current understanding of odour-mediated host-seeking behaviour in mosquitoes based on laboratory (indoor) studies. Most recent studies have focused on *Aedes aegypti* L., *Anopheles gambiae sensu stricto* Giles and *Culex quinquefasciatus* Say, as these species are all strongly associated with human biting and disease transmission. Where relevant, reference to other mosquito species is provided. Host seeking in mosquitoes is mediated primarily by chemical cues, and therefore most studies focus on the response to host odours and identification of specific compounds or odour blends. Laboratory tools for the study of odour-mediated behaviour include Y-tube and dual-choice (or dual-port) olfactometers, wind tunnels and room-size arenas in which mosquitoes can fly freely. Mosquitoes are observed individually or in groups, usually at a specified age or range of ages, at a pre-determined time of the day when they are considered to be naturally responsive to host cues. *Aedes aegypti* and *An. gambiae* respond strongly to natural human skin emanations, whereas *Cx. quinquefasciatus* shows variation in this behaviour dependent on its geographic origin. Carbon dioxide acts as a principal stimulus for each of these mosquito species although with a variable species-specific effect. Several host-derived compounds have been identified that cause behavioural responses in these mosquito species, including ammonia, L(+)-lactic acid and aliphatic carboxylic acids. In addition, acetone and dimethyl disulfide enhance the attraction of blends of these compounds to *Ae. aegypti*. Some other ketones, such as 6-methyl-5-hepten-2-one and geranyl acetone disrupt the host-seeking process of *Ae. aegypti* and *An. gambiae*. Several aldehydes, such as octanal and nonanal, are attractive to *Cx. quinquefasciatus*, and reduce upwind flight and the total number of landings in *Ae. aegypti*. Based on these data odour blends have been identified that are attractive to these mosquito species, albeit less so than natural human odour, indicating that additional, hitherto unidentified, semiochemicals are involved in host-seeking behaviour that are likely to improve the efficacy of blends. Behavioural research combined with studies on molecular olfaction is ongoing to discover compounds that affect the host-seeking process, and further research under semi-field and field conditions is required to explore the effectiveness of putative attractants and repellents in natural circumstances.

Keywords: bioassay, host-odours, kairomone, odour baits, olfaction, repellent, synthetic blend, vector-borne disease

Introduction

Some species of mosquitoes are vectors of pathogens that give rise to several important infectious diseases of humans and animals. Several of these diseases thrive in tropical climates, where the abundance of vectors, aided by favourable environmental conditions, allows for high levels of transmission. Approximately half of the world's population, especially in less developed countries, are at risk (Jones *et al.* 2008, WHO 2008, 2009). Although new progress has recently been made in the prevention and control of vector-borne diseases, current methods appear insufficient to prevent many cases of illnesses and deaths due to various factors (e.g. societal, economical, political, mosquito and parasite resistance) (Greenwood *et al.* 2008, Morrison *et al.* 2008, Takken

and Knols 2009). In addition, the recent expansion of vector-borne diseases into more temperate, industrialised countries, such as the occurrence of West Nile virus and Chikungunya in the USA and Europe, respectively (Gould 2003, Gould and Fikrig 2004, Takken and Knols 2007, Vazeille *et al.* 2008), demonstrate the need for further development and implementation of measures to protect people at risk (e.g. with bednets, vaccines) and to control mosquito vectors (e.g. with insecticides, biological control agents, treated surface and materials, the sterile insect technique, monitoring tools).

In tropical areas, *An. gambiae sensu stricto* (hereafter termed *An. gambiae*), *Ae. aegypti* and *Cx. quinquefasciatus* are medically important mosquito vectors of infectious pathogens such as malaria, dengue and bancroftian filariasis, respectively (Cook 1996). The females of these mosquito species are considered to be anthropophilic (Takken and Knols 1999) and often live in close association with humans. Since host seeking by mosquitoes is mainly mediated by olfactory stimuli (Takken 1991, Takken and Knols 1999), it is likely that the odour complex of the blood-host contains host-specific and non-host-specific chemicals to which these mosquito species respond (Gillies 1988). It is important to note that the semiochemicals involved in the attraction of mosquitoes to hosts may include both repellents and attractants, and the effects of some chemicals are likely to be dose-dependent. Once these semiochemicals have been identified, it is expected that they can be incorporated into technologies that can be used to trap, kill or repel mosquitoes (Cook *et al.* 2007, Day and Sjogren 1994, Hassanali *et al.* 2008, Kline 2006, 2007, Logan and Birkett 2007, Takken and Knols 2009) as has been successfully demonstrated for tsetse flies (see Chapter 12, this volume; also see Chapter 17, this volume). Ultimately, some of these products may improve or even replace current control technologies or sampling tools in epidemiological studies of mosquito-borne diseases. Improvement of present methods could be achieved by adding an odour bait to an existing mosquito trap, by replacing a bait with a better performing one, or by combining a newly developed odour trap with current mosquito control tools.

Crumb (1922) was the first to describe the role of host-derived semiochemicals in mosquito behaviour. In addition, Rudolfs (1922) and Van Thiel (1937) discovered the unique and important role of carbon dioxide (CO_2) (see review of Mboera and Takken 1997). Since then many mosquito attractants have been discovered, but there is little evidence to explain how these compounds act as olfactory mediators of behaviour and none are as attractive as natural odour blends (Bernier *et al.* 2007, Bosch *et al.* 2000, Bowen 1991, Okumu *et al.* 2010, Spitzen *et al.* 2008, Smallegange *et al.* in press, Takken and Knols 1999). Only a few odorants are used as baits for mosquito surveillance or control, in contrast to the extensive use of host-derived baits for other insects of medical or veterinary importance (e.g. tsetse flies, screwworm, biting midges, triatomine bugs) (Cork and Hall 2007, Cruz-Lopez *et al.* 2001, Logan and Birkett 2007, Qiu *et al.* 2007b, Vale 1993, see also Chapters 10 and 12 of this volume).

Since the review of Takken and Knols (1999), which summarised our knowledge on the role of semiochemicals in the life history of African malaria mosquitoes at the close of the last century, new techniques and relevant information have become available. In this chapter, we describe recent results of laboratory studies on the identification of semiochemicals that play a role in the host-seeking behaviour of mosquitoes, with emphasis on *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, the species that have been studied in most detail over the last decade. It is not the purpose of this chapter to go into details of each step involved in the host-seeking process (Takken 1996, see also Chapter 1 of this volume) or of the strategies employed by mosquitoes to find distant odour sources (the latter has recently been reviewed by Cardé and Willis (2008) for insects in general). Instead, we will provide an overview of the individual chemicals and

synthetic odour blends that have been tested for their possible effect on the behaviour of host-seeking mosquitoes. We also briefly describe methods and bioassays deployed to identify candidate semiochemicals. Subsequently, we will discuss problems and knowledge gaps that are encountered in this research area. Studies on anopheline host-seeking behaviour performed in semi-field and field situations are discussed in Chapter 8 (this volume).

Identification of candidate semiochemicals

Anopheles gambiae and *Ae. aegypti* are considered to be highly anthropophilic mosquito species, i.e. the females express a preference for biting humans to get their blood meal (Gillies 1964, Harrington 2001, Takken and Knols 1999, White 1974). Several hundred volatile compounds emitted by humans have been identified, especially in the last twenty years (Bernier *et al.* 1999, 2000, 2002, Cork and Park 1996, Curran *et al.* 2005, 2007, Deng *et al.* 2004, Ellin *et al.* 1974, Gallagher *et al.* 2008, Hasegawa *et al.* 2004, Haze *et al.* 2001, Healy and Copland 2000, Healy *et al.* 2002, Krotoszynski *et al.* 1997, Meijerink *et al.* 2000, Natsch *et al.* 2006, Penn *et al.* 2007, Perry *et al.* 1970, Philips 1997, Zeng *et al.* 1991, 1996). It is assumed that the host-seeking behaviour of mosquitoes is mediated by a blend of specific compounds, and not by the complete assemblage of compounds present in host odour (Zwiebel and Takken 2004). Theoretically, knowledge about the compounds present in the human odour profile can be used to test which compounds play a role in the host-seeking behaviour of anthropophilic mosquito species. However, testing the behavioural effect of each compound in different concentrations and in all possible combinations is not practical because of the large number of compounds present in human emanations (Bernier *et al.* 2000, Curran *et al.* 2005, Penn *et al.* 2007, Philips 1997). Here we outline some techniques that can be used to narrow down the selection of candidate compounds (Logan and Birkett 2007, Smallegange *et al.* 2003, Van der Goes van Naters and Carlson 2006).

It has been shown that some persons are bitten by mosquitoes more often than others (Curtis 1986, Lindsay *et al.* 1993, Scott *et al.* 2006). This may be the result of a differential attractiveness of the body odours of human individuals to mosquitoes (Bernier *et al.* 2002, Knols *et al.* 1995, Logan *et al.* 2008, Lindsay *et al.* 1993, Mukabana *et al.* 2002, Qiu *et al.* 2004a, 2006a, Schreck *et al.* 1990). Comparing the odour profiles of highly and poorly attractive individuals can reveal compounds that play a role in host selection. Gas-chromatography (GC) and mass-spectrometry (MS) are used to study the differences in the odour profiles of individuals and to identify the compounds involved (Bernier *et al.* 1999, 2000, 2002, Logan *et al.* 2008). These differences may be qualitative and/or quantitative, which makes it difficult to identify the compounds involved in host selection. The discovery that human skin emanations collected on glass beads lose their attractiveness after a certain period of time suggests that volatile compounds decreasing in relative abundance during this period play a role in the attractiveness of the emanations (Bernier *et al.* 2000, 2002, Qiu *et al.* 2004a, Schreck *et al.* 1981, Smallegange *et al.* 2003). Comparing odour profiles of differential attractive individuals resulted in weak attractants and putative repellents for *Ae. aegypti* (Bernier *et al.* 2002, Logan *et al.* 2008).

Skin microbiota plays a role in the production of human body odour (Leyden *et al.* 1981, Shelley *et al.* 1953). Blood agar incubated with microbiota from human feet or *Staphylococcus epidermidis*, a bacteria species commonly found on human skin, is attractive to *An. gambiae*. Identifying volatile compounds produced by microorganisms present on human skin revealed 10 putative attractants for this mosquito species (Verhulst *et al.* 2009).

Although not a new technology we should add here that, in addition to identifying volatile organic compounds emanating from the human skin and comparing odour profiles of individuals, electrophysiological methods, such as electroantennography (EAG) and single sensillum recording (SSR) techniques, either without or in combination with GC and MS, can be applied to identify which compounds in the human odour profile stimulate the olfactory sensilla on mosquito antennae or maxillary palps (see Chapter 3 in this volume for more details, as well as Logan *et al.* 2008, Lu *et al.* 2007, Qiu *et al.* 2004b). Ultimately, the effects of odours on mosquitoes must be investigated directly, with behavioural assays. The sensory systems of a mosquito may be able to detect a volatile compound, but this does not necessarily mean that a motor response is triggered, or that the response is necessarily related to the behaviour under investigation. Behavioural observations are required to establish the mechanisms by which odorants guide mosquitoes to a host, e.g. they may act as attractants, repellents, activators or arrestants (Costantini *et al.* 2001, Logan *et al.* 2008, Mukabana *et al.* 2004).

The publication of the full gene sequence of *An. gambiae* (Holt *et al.* 2002) and *Ae. aegypti* (Nene *et al.* 2007) has enabled rapid progress in molecular genetics. The sequencing of the *Cx. quinquefasciatus* genome has almost been completed (Pelletier and Leal 2009, Waterhouse *et al.* 2008). A large family of olfactory receptor genes (ORs) and gustatory receptor genes (GRs) has been identified (Van der Goes van Naters and Carlson 2006). These genes play a crucial role in the binding of olfactory molecules to neural membranes (see Chapter 2, this volume). The molecular regulation of olfaction appears highly conserved in the animal kingdom, although the OR and GR families are highly specific to species (Robertson and Wanner 2006). This would explain why some insects express a highly selective host preference, as found in *An. gambiae* and *Ae. aegypti*. Work on the molecular regulation of olfaction in *Drosophila* and *Xenopus* has elucidated the processes by which small molecules control physiological activity in insects (Hallem *et al.* 2006, Rutzler and Zwiebel 2005). This knowledge is currently being used for the identification of semiochemicals to which mosquitoes respond (Carey *et al.* 2010, Hallem *et al.* 2004, Justice *et al.* 2003, Leal *et al.* 2008, Wang *et al.* in press and Chapter 2, this volume).

Combined with powerful electrophysiological techniques (Chapter 3, this volume), molecular genetics can provide new insights into the olfactory behaviour of mosquitoes and can be used as a tool for the rapid identification of candidate semiochemicals. Behavioural studies, however, remain essential for the final assessment of the role of these chemicals in the insect's life-history. The olfactory receptor neurons may be able to detect certain chemical stimuli, the brain will associate them with a specific behavioural response (Klowden 1995, see also De Bruyne and Baker 2008).

Similar strategies are used to discover olfactory cues that affect mosquitoes with more variable host preferences, such as *Cx. quinquefasciatus*. The host-preference of *Cx. quinquefasciatus* appears to be region-dependent, as the following examples illustrate. Field experiments in Tanzania showed that more *Cx. quinquefasciatus* were caught in human-odour baited traps than in traps baited with goat or calf odour (Mboera and Takken 1999). By contrast, blood meal analyses have shown that in Kenya this species has a preference for livestock over human hosts (Muturi *et al.* 2008). In a study in Tucson, USA, blood meal analysis revealed that 50% of *Cx. quinquefasciatus* females fed on humans and 32% on birds, respectively (and therefore this species is considered to be a potential West Nile virus vector; Zinser *et al.* 2004), whereas in Tenessee, USA, and Mexico they were found to feed less on humans than on birds (Eilizondo-Quiroga *et al.* 2006, Savage *et al.* 2007). In addition, a greater percentage of *Cx. quinquefasciatus* females, from a colony originating in Florida, USA, responded to chicken than to human odour in an olfactometer study (Allan *et al.*

2006b). These latter results indicate that in the USA *Cx. quinquefasciatus* is less anthropophilic and more ornithophilic than in Tanzania. Similarly, in Senegal and Madagascar *An. gambiae* populations were found to feed predominantly on cattle (Diatta *et al.* 1998, Duchemin *et al.* 2001). In addition, *Ae. aegypti* responds not only to human odour, but also to mouse and chicken odour (Allan *et al.* 2006b, McCall *et al.* 1996, McIver 1968), suggesting that this species is actually an opportunistic feeder. The reliability of results obtained in host preference studies, however, depends on the method used (field and laboratory), the geographic area covered (field) or geographic origin of the mosquitoes (laboratory) and the availability of host species (field and laboratory), which demands for caution in using labels such as 'anthropophilic' and to confine research to analysing only human odours (Kilpatrick *et al.* 2006, Lefèvre *et al.* 2009, Torr *et al.* 2008).

A wider host-range broadens the search for volatile organic compounds mediating host-seeking behaviour. Overlap in odour profiles of different blood-host species of a mosquito species, such as human and chicken, may reveal attractive compounds such as nonanal (Syed and Leal 2009). However, differences in these odour profiles may explain differences in relative attraction to these hosts due to allomones or additional kairomones (Dicke and Sabelis 1988) emitted by the host.

Compounds present in bovine emanations have been thoroughly examined in relation to the development of surveillance and control tools for tsetse, cattle and stable flies using the techniques described above and have been published (e.g. Birkett *et al.* 2004, Bursell *et al.* 1988, Schofield and Brady 1997, Schofield *et al.* 1995, 1997, Spinhirne *et al.* 2003, 2004, Torr *et al.* 1995, Vale 1980, Vale and Hall 1985a,b, Vale *et al.* 1988 and Chapter 12, this volume). Also, several compounds emitted by chickens have been identified, for example by solvent extraction and volatile trapping of faeces, feathers, feet or skin, and include alcohols, aldehydes, carboxylic acids, diones and ketones (Bernier *et al.* 2008, Cooperband *et al.* 2008, Haathi and Fales 1967, Williams *et al.* 2003).

Although in nature mosquitoes do not feed on blood directly, females of both *Cx. quinquefasciatus* and *Ae. aegypti* were found to be attracted to the odour of bovine and avian blood. *Culex* females landed more on membranes filled with avian than with bovine blood, whereas *Aedes* mosquitoes showed no landing preference (Allan *et al.* 2006a). The volatile chemicals emanating from blood have been identified (Ashley *et al.* 1992, Issachar *et al.* 1982, Sastry *et al.* 1980) but few of these have been tested for their effects on mosquitoes in the laboratory, although components found in exhaled breath and skin emanations may be similar to those present in blood (Sastry *et al.* 1980).

An initial screening of compounds for their effect on the host-seeking behaviour of mosquitoes in a laboratory before testing them under field conditions has the advantage that the environmental circumstances during the bioassays and physiological state (e.g. age, time since sugar or bloodmeal, stage in gonotrophic cycle) of the mosquitoes can be controlled during the experiments. Before the results of a bioassay are put into practical use, they should first be validated in semi-field and/or field experiments (Knols *et al.* 2002, see also Chapter 8 in this volume). (Semi-)field studies provide information about the effects of natural temperature, humidity, wind, the presence of competitive odours, etc. on the responses to odours, and can be used to test the effectiveness of an odour bait over larger distances than can be tested in the laboratory (Njiru *et al.* 2006, Okumu *et al.* 2010, Williams *et al.* 2006a). Field evaluations can also reveal simultaneously whether other vectors are attracted or repelled by the odour compounds tested (Jawara *et al.* 2009, Qiu *et al.* 2007a).

Bioassay techniques in the laboratory

In the laboratory, host-seeking behaviour in response to odour can be evaluated by observing individual insects. This can be done by direct observation (human landing catches) in a cage (De Jong and Knols 1995, Dekker *et al.* 1998, Knols *et al.* 1994a) or wind tunnel (Takken *et al.* 1997) or by video recording (Beeuwkes *et al.* 2008, Cooperband and Cardé 2006, Dekker *et al.* 2005, Healy and Copland 1995, Young *et al.* 1993).

Y-tube and dual-port (or dual-choice) olfactometers are used to assess the response of mosquitoes to a choice between an odour and clean air or two different odours (Geier and Boeckh 1999, Knols *et al.* 1994b, Posey *et al.* 1998). Y-tube olfactometers (Figure 1A) are conveniently small devices for rapid screening of odours. *Aedes aegypti* in particular responds well in Y-tubes (Geier and Boeckh 1999, Logan *et al.* 2008). Dual-port olfactometers (Figure 1B) are more suitable for mosquito species that perform better in larger arenas, such as *An. gambiae* (Knols *et al.* 1994b).

Mosquito responses to odours can also be studied in large indoor cages, for instance inside a bednet or a closed room. These tools have been used for studies on the selection of biting sites on volunteers (De Jong and Knols 1995, Dekker *et al.* 1998, Knols *et al.* 1994a) and for studies with insect repellents (Curtis and Hill 1988). By placing mosquito traps in indoor cages (Figure 1C), the effect of different odorants on trapping efficacy can be investigated (Silva *et al.* 2005, Smallegange *et al.* 2009).

Several methods have been developed for testing putative repellents. The most commonly used methods to test putative repellents for employment directly on the human skin have recently been reviewed by Barnard *et al.* (2007). Various types of olfactometers have been used to test compounds for their efficacy as spatial repellents (Bernier *et al.* 2005, Dogan and Rossignol 1999, Grieco *et al.* 2005, Kline *et al.* 2003, Logan *et al.* 2008, Waka *et al.* 2006).

Laboratory-based studies have been done with individual mosquitoes or with groups of up to 50 mosquitoes per test. In wind tunnels, experiments are usually done with one insect at a time (Healy and Copland 1995, Takken *et al.* 1997), whereas in olfactometers most studies are based on the results of groups of 15 to 50 insects. Because of the possibility of learning (Alonso and Schuck-Paim 2006, McCall and Eaton 2001, Tomberlin *et al.* 2006), individual mosquitoes should not be tested more than once, and each test should be performed with naïve insects.

Effects of physiology on behaviour

It should be taken into account that the host-seeking process of mosquitoes includes different phases. After an inactive period during which reproductive maturation (Lehane 1991) and development of the antennal sensitivity takes place (Davis 1984), female activity increases (influenced by circadian rhythm; see Klowden 1996). This increase makes it more likely for the females to encounter host-derived stimuli. Activation takes place as soon as these stimuli have been detected. This phase is followed by the orientation process that will bring the mosquitoes in the immediate vicinity of the host. As soon as the host has been located, the female will land and start blood feeding (Takken 1996). The mosquitoes will respond to different stimuli, among which olfactory, within each phase. Therefore, the choice for a certain bioassay may influence the discovery of semiochemicals that play a role in a specific phase of the mosquito host-seeking process.

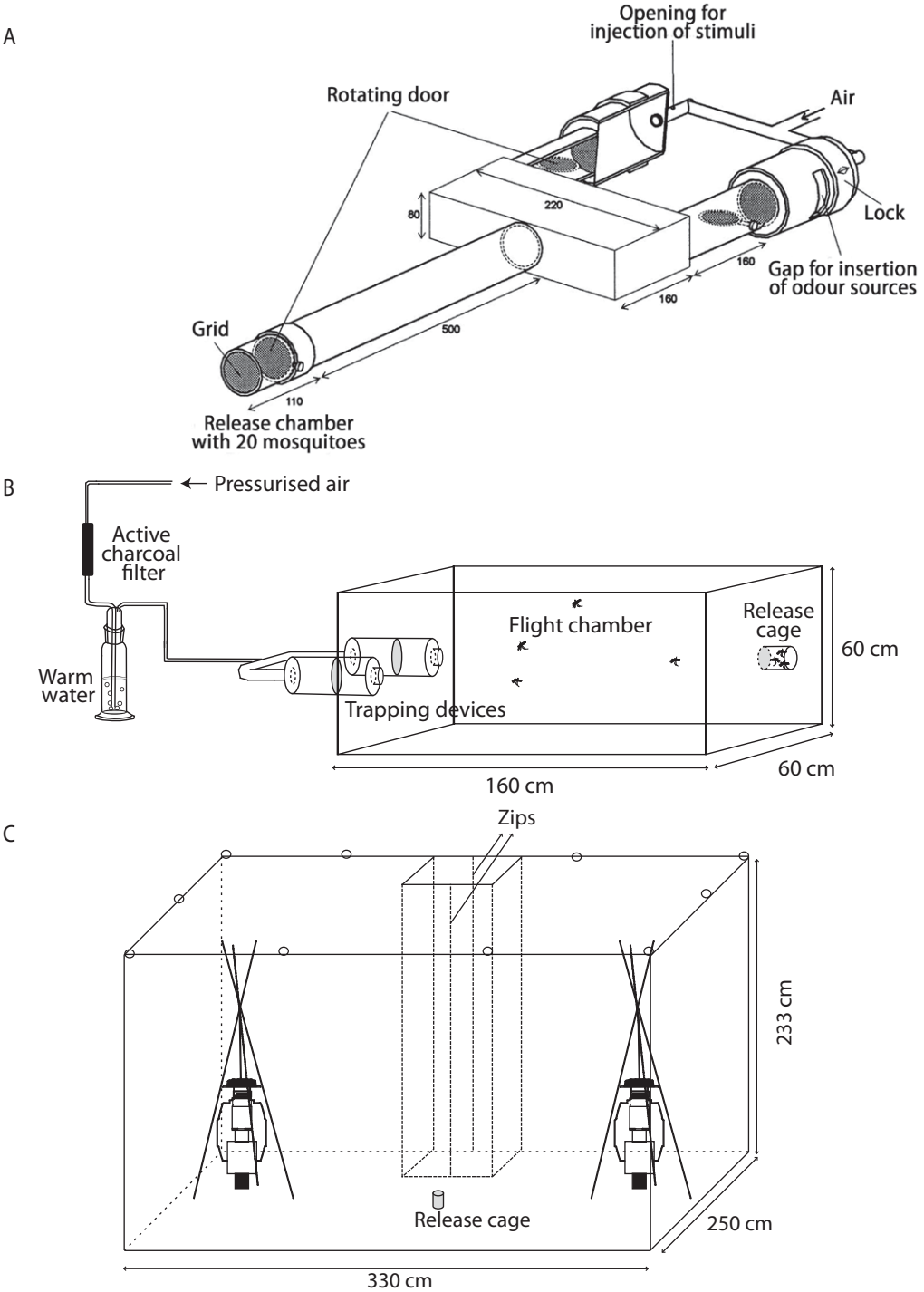

Figure 1. Schematic drawings of (A) an Y-tube olfactometer (from Geier et al. *1999a), (B) a dual-port olfactometer (modified after Spitzen* et al. *2008) and (C) two MM-X traps in an indoor netted cage.*

Olfactory cues are important external factors affecting host-seeking behaviour from a distance, whereas visual and physical cues, such as heat and moisture, play a role in close vicinity of the blood-host (Takken and Knols 1999). However, even when host-stimuli are present, host-seeking behaviour may not be expressed as a result of endogenous, physiological factors. These physiological factors include the circadian rhythm, age, nutritional state, mating condition, and presence of eggs, and should be considered in any study on insect behaviour (Klowden 1995, 1996).

Aedes aegypti is a diurnal mosquito and its host-seeking behaviour should therefore be studied during daytime under illuminated conditions (Geier and Boeckh 1999, Klowden 1995). Both *An. gambiae* and *Cx. quinquefasciatus* are nocturnal. Biting activity of the latter species was observed throughout the night (Chandra 1995), allowing olfactometer experiments during the scotophase (Mboera *et al.* 1998). Puri *et al.* (2006), however, observed that the mosquitoes were most active during a period of one hour after dusk, and therefore performed experiments in dim light during this short period of time only. Experiments examining the host-seeking behaviour of *An. gambiae* are usually performed, in the dark or under moonlight conditions (Knols *et al.* 1994b), during the last 4 hours of the scotophase, when this species has frequently been reported to be naturally active (Haddow and Ssenkubuge 1973, Killeen *et al.* 2006, Maxwell *et al.* 1998). Shifts in timing of host-seeking activity, however, have occasionally been observed, chiefly because of vector-control interventions (Braimah *et al.* 2005).

Host-seeking behaviour of *Ae. aegypti* increases from no response to host cues directly after adult emergence to more than 90% of the females responding to host-odours 102 hours post emergence (Davis 1984). Therefore, the best results can be obtained with females of 4-days-old or older. Generally, adults are tested within a specified range of ages, which varies overall from 3-10 days, to control for the effects of aging.

A blood meal changes the mosquito's physiology and behaviour, by affecting the sensitivity of the olfactory receptor organs (Davis 1984, Qiu *et al.* 2006b, see also Chapter 3 in this volume). After a full blood meal, *An. gambiae* is no longer attracted to host stimuli for 24 hours, with host-seeking behaviour returning to pre-blood meal levels 72 hours post feeding (Takken *et al.* 2001). In mated *Ae. aegypti*, the inhibition begins 30 hours after ingestion of the blood and reaches its maximum between 36 and 72 hours post feeding. The inhibition is less pronounced in unmated *Ae. aegypti* females (Klowden and Lea 1979). Nutritional status also affects the intensity of the inhibition (Klowden 1986). Test insects may be starved for up to 18 hours prior to testing. This can be done by replacing the carbohydrate food source with water before the experiment. Infection with pathogens/parasites may alter the host-seeking behaviour as well (Hurd 2003, Schaub 2006).

Bioassay results with semiochemicals in laboratory studies

In this section an overview is given of semiochemicals identified to play a role in the host-seeking process of *Ae. aegypti*, *An. gambiae*, *Cx. quinquefasciatus*, and some other mosquito species. The compounds, the bioassay in which they were tested and the effect that has been found are summarised in Tables 1-3. As some bioassays cannot differentiate between an inhibitory and a repellent effect, both terms are used interchangeably. Although, as Kennedy (1978) pointed out, this term does not reveal the many processes and underlying mechanisms involved, we use the term 'attractant' to refer to a chemical or blend that has been found to induce mosquitoes to orientate towards the odour source.

Aedes aegypti

A human hand as the odour source stimulates *Ae. aegypti* females to fly upwind in a Y-tube olfactometer. A similar result was achieved by using high doses of heated or unheated ethanol skin extracts (Geier and Boeckh 1999, Geier *et al.* 1996). Liquid chromatography of the skin extract revealed active fractions of which one contained lactic acid and another probably ammonia (Geier *et al.* 1996, 1999a).

In humans, L-(+)-lactic acid (hereafter lactic acid) is present in breath and on the skin. Human skin rubbing extracts contain lactic acid in significantly higher quantities than skin extracts of 12 other mammals and chickens (Dekker *et al.* 2002) and have, presumably for that reason, received specific attention as a kairomone for anthropophilic mosquitoes. More interestingly, lactic acid was found in higher amounts on glass beads that were handled by individuals that were more attractive to *Ae. aegypti* than individuals with lower amounts of the compound in their skin emanations. From numerous studies it was shown that lactic acid plays a major role in the host-seeking behaviour of *Ae. aegypti* (Table 1) (Acree *et al.* 1968, Bernier *et al.* 2002, Eiras and Jepson 1991, Geier *et al.* 1996, Smith *et al.* 1970). Lactic acid is a weak attractant on its own but acts synergistically with carbon dioxide (CO_2), a major component of exhaled breath, and other volatiles emanating from human skin (Acree *et al.* 1968, Allan *et al.* 2006a, Bernier *et al.* 2002, Bosch *et al.* 2000, Eiras and Jepson 1991, Geier and Boeckh 1999, Geier *et al.* 1996, 1999a,b, Smith *et al.* 1970, Williams *et al.* 2006a). An ethanol extract of human skin was no longer attractive to *Ae. aegypti* females, presumably because the lactic acid had been removed from the extract (Geier *et al.* 1996).

Carbon dioxide on its own is an activator and weak attractant for *Ae. aegypti*, increasing take-off, flight velocity and flight duration, although homogeneous CO_2 plumes decrease upwind flight and trap catches (Allan *et al.* 2006b, Bernier *et al.* 2007, Bosch *et al.* 2000, Dekker *et al.* 2001b, 2005, Eiras and Jepson 1991, Geier and Boeckh 1999, Geier *et al.* 1999a,b, Kline *et al.* 2006). An encounter with a single CO_2 filament increases the responsiveness to human skin odours (Dekker *et al.* 2005).

The presence of carboxylic acids on the skin is unique to humans compared to other mammals, birds and rodents (Nicolaides *et al.* 1968). Saturated aliphatic carboxylic acids with chain length C1-C3, C5-C8, and C13-C18 were found to increase the attractiveness of lactic acid at certain doses to *Ae. aegypti* females. The attractiveness increased even further when a second carboxylic acid was added, as long as one is a short chain (C1-3) and the other a medium length (C5-8) carboxylic acid. Nonanoic and undecanoic acid reduced the effect of lactic acid, whereas butanoic, decanoic and dodecanoic acid had no effect (Bosch *et al.* 2000). Acetic acid on its own was found to be attractive to host-seeking *Ae. aegypti* females and to elicit a landing response. Several other carboxylic acids, including two aromatics, induced landing responses (Table 1) (Allan *et al.* 2006a). In contrast, hexanoic acid reduced the number of landings (Douglas *et al.* 2005), probably due to the high doses used. Propanoic, butanoic, 3-methylbutanoic, pentanoic, heptanoic, decanoic, dodecanoic, tetradecanoic, pentadecanoic, hexadecanoic, heptadecanoic, and octadecanoic acid did not attract *Ae. aegypti* females, even though some of these acids were present at higher levels in the skin emanations of highly attractive persons (Allan *et al.* 2006a, Bernier *et al.* 2002, Bosch *et al.* 2000). Propanoic and pentanoic acid did not influence the attractiveness of CO_2 (Bosch *et al.* 2000).

Ammonia was also found to enhance the attractiveness of lactic acid, although it is not attractive on its own or in combination with CO_2 (Geier *et al.* 1999a). Propanoic and pentanoic acid enhanced the effect of a lactic acid and ammonia blend. A blend of ammonia, lactic acid, and two carboxylic

Table 1. Overview of chemical compounds that have been tested for their impact on the host-seeking behaviour of Aedes aegypti *using behavioural assays in the laboratory. (Details about concentrations of compounds are omitted).*

Compound	Response	Behavioural assay	Reference
carbon dioxide (CO_2)	(Weakly) attractive on its own;	Wind tunnel	Eiras and Jepson (1991)
	flight activating effect; synergism	Wind tunnel tube	Geier *et al.* (1999a)
	with lactic acid but not ammonia	Y-tube	Geier *et al.* (1999b)
		Y-tube	Geier and Boeckh (1999)
		Y-tube	Bosch *et al.* (2000)
		Dual-port olfactometer	Allan *et al.* (2006b)
		Dual-port olfactometer	Kline *et al.* (2006)
		Dual-port olfactometer	Bernier *et al.* (2007)
	Homogeneous CO_2 reduced trap catch	Dual-port olfactometer	Dekker *et al.* (2001b)
	Sensitizes to skin odours	Wind tunnel (3D)	Dekker *et al.* (2005)
ammonia (NH_3)	Not attractive on its own	Y-tube	Geier *et al.* (1999b)
	Enhanced the effect of lactic acid	Y-tube	Williams *et al.* (2006a)
	NH_3+hexanoic acid improved blend of lactic acid + acetone + dimethyl disulfide		
Alcohols			
4-hexen-1-ol	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
1-hepten-3-ol	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
glycerol	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
Aldehydes			
hexanal	Reduced the number of landings	Landing assay	Douglas *et al.* (2005)
heptanal	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
octanal	Reduced the number of landings	Landing assay	Douglas *et al.* (2005)
	Reduced upwind flight	Y-tube	Logan *et al.* (2008)
nonanal	Not attractive	Dual-port olfactometer	Bernier *et al.* (2002)
	Reduction of upwind flight	Y-tube	Logan *et al.* (2008)
decanal	Reduced upwind flight	Y-tube	Logan *et al.* (2008)
z-4-decanal	Reduced the number of landings	Landing assay	Douglas *et al.* (2005)
Aliphatic carboxylic acids			
lactic acid (LA)	Weakly attractive on its own;	Dual-port olfactometer	Acree *et al.* (1968)
	Synergism with CO_2 and other skin	Dual-port olfactometer	Smith *et al.* (1970)
	components	Wind tunnel	Eiras and Jepson (1991)
		Y-tube	Geier *et al.* (1996)
		Y-tube	Geier and Boeck (1999)
		Wind tunnel tube	Geier *et al.* (1999a)
		Y-tube	Geier *et al.* (1999b)
		Y-tube	Bosch *et al.* (2000)
		Dual-port olfactometer	Bernier *et al.* (2002)
		Y-tube	Williams *et al.* (2006a)
		Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
methanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)

Table 1. Continued

Compound	Response	Behavioural assay	Reference
acetic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
propanoic acid	Not attractive on its own	Y-tube	Bosch *et al.* (2000)
		Dual-port olfactometer	Allan *et al.* (2006a)
	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Enhanced the effect of LA + NH_3	Y-tube	Bosch *et al.* (2000)
	No landing response	Landing assay	Allan *et al.* (2006a)
butanoic acid	No effect on attractiveness of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
3-methyl butanoic acid	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
pentanoic acid	Not attractive on its own	Y-tube	Bosch *et al.* (2000)
	Enhanced the effect of LA		
	Enhanced the effect of LA + NH_3		
hexanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Attractive when combined with LA + NH_3	Y-tube	Williams *et al.* (2006a)
	Hexanoic acid + NH_3 improved blend of lactic acid + acetone + dimethyl disulfide	Y-tube	Williams *et al.* (2006a)
	Reduced number of landings	Landing assay	Douglas *et al.* (2005)
heptanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
octanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
nonanoic acid	Reduced the attractive effect of LA	Y-tube	Bosch *et al.* (2000)
decanoic acid	No effect on the attractiveness of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
undecanoic acid	Reduced the attractive effect of LA	Y-tube	Bosch *et al.* (2000)
dodecanoic acid	No effect on the attractiveness of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
tridecanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
tetradecanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
pentadecanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
hexadecanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
		Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)

Table 1. Continued

Compound	Response	Behavioural assay	Reference
heptadecanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
octadecanoic acid	Enhanced the effect of LA	Y-tube	Bosch *et al.* (2000)
	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
		Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
Blend of lactic acid, ammonia and two carboxylic acids (C1-C3 and C5-C8)	Attractive	Y-tube	Bosch *et al.* (2000)
Blend of octanal, hexanal, Z-4 decenal, decanal, hexanoic and octanoic acid	Reduced number of landings	Landing assay	Douglas *et al.* (2005)
Aromatic carboxylic acids			
benzoic acid	Induced landing response	Landing assay	Allan *et al.* (2006a)
2-hydroxybenzoic acid	Induced landing response	Landing assay	Allan *et al.* (2006a)
Ketones			
acetone	Attractive on its own; Enhanced the effect of LA	Dual-port olfactometer	Bernier *et al.* (2003)
		Dual-port olfactometer	Bernier *et al.* (2007)
	Improved blend of LA + NH_3 + hexanoic acid	Y-tube	Williams *et al.* (2006a)
butanone	Weakly attractive	Dual-port olfactometer	Bernier *et al.* (2002)
2-pentanone	Weakly attractive	Dual-port olfactometer	Bernier *et al.* (2002)
3-pentanone	Weakly attractive	Dual-port olfactometer	Bernier *et al.* (2002)
6-methyl-5-hepten-2-one	Weakly attractive	Dual-port olfactometer	Bernier *et al.* (2002)
	Reduced upwind flight	Y-tube olfactometer	Logan *et al.* (2008)
2-decanone	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
geranyl acetone	Reduced upwind flight	Y-tube olfactometer	Logan *et al.* (2008)
Sulfides			
methyl sulfide	Attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
carbon disulfide	Attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
dimethyl disulfide	Attractive on its own	Dual-port olfactometer	Bernier *et al.* (2003, 2007)
		Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
	Enhanced the effect of LA	Dual-port olfactometer	Bernier *et al.* (2003, 2007)
	Enhanced the effect of lactic acid + acetone	Lab. trapping exp's	Silva *et al.* (2005)
		Y-tube	Williams *et al.* (2006a)
		Dual-port olfactometer	Bernier *et al.* (2007)
ethyl disulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)

Table 1. Continued

Compound	Response	Behavioural assay	Reference
methyl propyl disulfide	Not attractive	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
dimethyl trisulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
Miscellaneous			
dichloromethane	Attractive on its own Enhanced the effect of LA	Dual-port olfactometer	Bernier *et al.* (2003)
pyridine	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
4-pyridinamine	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
benzene	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
toluene	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
heptane	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
1*H*-indole	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
2-octene	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
styrene	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
2-nonene	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
1,3-butanediamine	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
pentacosane	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
cholesterol	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)
squalene	Not attractive on its own	Dual-port olfactometer	Bernier *et al.* (2002)

acids from the C1-C3 and C5-C8 groups was almost as attractive as an extract of human skin residues (attracting 68.1% and 77.9% of the tested mosquitoes, respectively). The difference in attractiveness may have been caused by 'missing' compounds in the synthetic blend or because the concentrations and proportions of the compounds in the blend were not optimal (Bosch *et al.* 2000).

Another promising attractive blend is the combination of lactic acid, acetone, and dimethyl disulfide (Bernier *et al.* 2007, Silva *et al.* 2005, Williams *et al.* 2006a). The latter two compounds have been detected in human breath and in human skin emanations (Bernier *et al.* 2000, Philips 1997). MM-X traps (Woodstream, Lititz, PA, USA; obtained originally from American Biophysics, East Greenwich, RI, USA) baited with this synthetic blend were attractive to *Ae. aegypti* females under controlled laboratory conditions (Silva *et al.* 2005). However, natural human odour was significantly more attractive than the triple blend in two-choice olfactometer experiments (Bernier *et al.* 2007). The addition of ammonia and hexanoic acid improved the attractiveness of the blend, and the addition of acetone to a blend of lactic acid, ammonia and hexanoic acid increased attraction to the latter blend (Williams *et al.* 2006a). BG-Sentinel traps (BioGents AG, Germany) baited with the four blends mentioned above (i.e. combinations of ammonia, hexanoic acid, lactic acid, acetone and dimethyl disulfide) appeared to be highly effective and *Ae. aegypti*-specific in field experiments in Australia (Williams *et al.* 2006a,b). However, this seemed to be due to visual more than olfactory cues (Williams *et al.* 2006a).

Acetone, dimethyl disulfide and dichloromethane, attractants on their own, were found to act synergistically when combined in a binary blend with lactic acid (Allan *et al.* 2006a, Bernier *et al.* 2003, 2007). The binary blends were less attractive than the triple blend mentioned above (Bernier *et al.* 2007). *Aedes aegypti* females also responded to other sulfides that are found on human skin, in human breath or may be present in human blood (Ashley *et al.* 1992, Bernier *et al.* 2000, Krotoszynski *et al.* 1977, Philips 1997); carbon disulfide, methyl propyl disulfide and methyl sulfide, either in a dual-choice olfactometer and/or in landing assays were found to be attractive, but dimethyl trisulfide and ethyl disulfide were not (Allan *et al.* 2006a) (Table 1).

Several ketones, which were found to be present in higher amounts on glass beads handled by a person that was more attractive to *Ae. aegypti*, proved to be weak attractants in a dual-port olfactometer (Table 1). Two of these compounds, butanone and 2-pentanone, decreased after the skin sample had aged for 8 hours. In contrast, one of the ketones that was found to be a weak attractant, 6-methyl-5-heptene-2-one, was also found in lower quantities on a day when a person was more attractive (Bernier *et al.* 2002). Similarly, Logan *et al.* (2008) showed that 6-methyl-5-heptene-2-one and especially geranyl acetone may be responsible for the decreased attraction of certain individuals. The former inhibited flight activation and probing activity but not relative attraction, whereas the latter reduced flight activity over all doses tested as well as relative attraction and probing activity.

The aldehydes octanal, nonanal and decanal, which are present at high levels in the skin emanations of less attractive individuals, caused a significant reduction in upwind flight of *Ae. aegypti* females and reduced attraction to a human hand at certain concentrations (Bernier *et al.* 2002, Logan *et al.* 2008). This indicates that compounds that are present on human skin may act as repellents or 'mask' the attractive effects of other skin compounds. The abundance of these compounds may explain the relative attractiveness of different individuals to this mosquito species.

Hexanal, octanal and z-4-decanal, all aldehydes produced by the seabird crested auklet (*Aethia cristatella* Pallas), were shown to decrease the number of landings on a human hand by *Ae. aegypti* mosquitoes at doses similar to concentrations produced by this bird species (Douglas *et al.* 2001, 2005). Octanal (2.5%) was as repellent as a mixture of four aldehydes, including octanal and two carboxylic acids mimicking the auklet odorant in its natural composition. Both 2% hexanal and 2% hexanoic acid reduced the number of mosquito landings to the same extent (Douglas *et al.* 2005).

Several other compounds, which were interesting because they were more abundant in the skin emanations of a less attractive individual or were found in higher or lesser amounts when the skin emanations of a certain person were more attractive on some days than on other days, have been screened (Bernier *et al.* 2002). These compounds, which include two alcohols, 4-hexen-1-ol and 1-hepten-3-ol, with structural similarity to 1-octen-3-ol, an attractant for some mosquito and haematophagous fly species (e.g. Takken and Kline 1989, Schofield *et al.* 1997, Vale and Hall 1985a,b), were not attractive when tested individually (Table 1).

Aedes albopictus (Skuse), a competent vector for many arboviruses, which exhibits an opportunistic host-feeding pattern and is extending its range around the world (Gratz 2004, Richards *et al.* 2006, Turell *et al.* 2005) has been shown to be activated (in a Y-tube olfactometer) to a greater degree by hexanoic acid, ethyl butyrate and dimethyl disulfide than by lactic acid. These compounds were also shown to be attractive to this mosquito species (Wang *et al.* 2006). In contrast, Shirai *et al.* (2001) found a repellent, or inhibitory, effect of lactic acid for this mosquito species when applied on normally attractive human or mouse skin.

Anopheles gambiae s.s.

Experiments in an African village conducted by Haddow (1942) indicated that the host-seeking behaviour of *An. gambiae* females is mediated primarily by human odour. This has been proven by many studies since (reviewed by Takken and Knols 1999). In the laboratory, *An. gambiae* is strongly attracted to human emanations, in particular skin emanations. A single finger (Dekker *et al.* 2001b, Smallegange *et al.* 2002), ethanol washings of human hands and feet (Braks 1999), human sweat (Braks and Takken 1999, Meijerink *et al.* 2000, Smallegange *et al.* in press), and skin emanations of human hands or feet transferred to fabric (Dekker *et al.* 2001a, Pates *et al.* 2001b, Qiu *et al.* 2004b, Smallegange *et al.* in press, Spitzen *et al.* 2008) or glass beads (Qiu *et al.* 2004a, 2006a) are highly attractive when tested in wind tunnels, Y-tube or dual-choice olfactometers or indoor experiments with mosquito traps.

Several compounds present in human sweat or on human skin have been tested individually or in blends under laboratory conditions to identify their effect on *An. gambiae* females (Table 2). Ammonia is an important kairomone for this mosquito species; it is attractive over a wide range of concentrations and suppresses the inhibitory or repellent effect of a carboxylic acid mixture (Braks *et al.* 2001, Smallegange *et al.* 2005). However, at high doses ammonia has a repellent, or inhibitory, effect (Smallegange *et al.* 2005).

The role of lactic acid in the host-orientation phase seems to be different in *An. gambiae* compared to *Ae. aegypti*; on its own it is either not an attractant, or only a weak attractant (Braks *et al.* 2001, Dekker *et al.* 2002, Healy and Copland 2000, Smallegange *et al.* 2002, 2005). However, it has been found to augment the attractiveness of natural human skin odour, carbon dioxide, and synthetic odour blends, indicating that lactic acid is an essential compound in the orientation of *An. gambiae* to human hosts (Dekker *et al.* 2002, Qiu 2005, Smallegange *et al.* 2005, 2009).

Variable results have been obtained in field studies with carbon dioxide (Costantini *et al.* 1996, Knols *et al.* 1998, Mboera and Takken 1997, Qiu *et al.* 2007a). For this species the compound is considered to indicate the presence of a potential host while host-selection is accomplished by host-specific cues (Takken and Knols 1999). Dual-port olfactometer studies have shown that CO_2 is a poor kairomone for *An. gambiae* that can even have a repellent, or inhibitory, effect depending on the structure of the plume and the positioning of the release point (Dekker *et al.* 2001b, Spitzen *et al.* 2008). The compound, however, activates *An. gambiae*, guiding them towards the release point of CO_2 after which human skin emanations attract the mosquitoes into a trapping device (Dekker *et al.* 2001b, Healy and Copland 1995, Knols *et al.* 1994b, Spitzen *et al.* 2008). This was demonstrated by the fact that CO_2 released in a turbulent plume in front of the trap entrance enhanced the attractiveness of skin emanations. This combination was more attractive than the synthetic blend of ammonia, lactic acid and CO_2 (although the former two compounds increased the effect of CO_2), indicating that additional compounds are involved in the host-seeking behaviour of this mosquito species (Spitzen *et al.* 2008). Recently, (semi-)field studies confirmed that the addition of CO_2 to human emanations increases the number of *An. gambiae* caught in odour baited mosquito traps (Jawara *et al.* 2009, Njiru *et al.* 2006, Schmied *et al.* 2008).

Human sweat has been shown to stimulate *An. gambiae* females to land at the odour source, whereas a mixture of 22 aliphatic carboxylic acids, which have been identified from human sweat, did not elicit a landing response (Healy and Copland 2000). However, a blend of 12 aliphatic carboxylic acids was found to be attractive when highly diluted (Knols *et al.* 1997). A similar blend, although repellent or inhibitory on its own, was attractive when combined with ammonia and

Table 2. Overview of chemical compounds that have been tested for their impact on the host-seeking behaviour of Anopheles gambiae s.s. *using behavioural assays in the laboratory. (Details about concentrations of compounds are omitted).*

Compound	Response	Behavioural assay	Reference
carbon dioxide (CO_2)	Activation and upwind flight	Wind tunnel	Healy and Copland (1995)
	(Weakly) attractive on its own	Dual-port olfactometer	Knols *et al.* (1994b)
	Homogeneous CO_2 reduced trap	Dual-port olfactometer	Dekker *et al.* (2001b)
	catch; Turbulent CO_2 caused activation and attractive synergism with skin emanations	Dual-port olfactometer	Spitzen *et al.* (2008)
ammonia (NH_3)	Attractive or repellent / inhibitory effect depending on doses	Dual-port olfactometer	Braks *et al.* (2001)
		Dual-port olfactometer	Smallegange *et al.* (2005)
		Y-tube	Smallegange *et al.* (2002)
Alcohols			
3-methyl-1-butanol	Repellent / inhibitory effect when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
4-ethylphenol	Repellent / inhibitory effect when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
1-dodecanol	No effect when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
Aliphatic carboxylic acids			
lactic acid (LA)	No landing response	Wind tunnel	Healy and Copland (2000)
	Not or weakly attractive on its own	Dual-port olfactometer	Braks *et al.* (2001)
		Y-tube	Smallegange *et al.* (2002)
	Augmented attractiveness of CO_2 and human odour	Y-tube	Dekker *et al.* (2002)
	Synergism when combined with NH_3 and carboxylic acids	Dual-port olfactometer	Smallegange *et al.* (2005)
		Dual-port olfactometer	Smallegange *et al.* (2009)
acetic acid	No effect in combination with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
propanoic acid	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
2-methylpropanoic acid	No effect in combination with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
butanoic acid	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
3-methyl butanoic acid	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
pentanoic acid	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
hexanoic acid	No effect on its own	Y-tube	Smallegange *et al.* (2002)
	Synergism when combined with NH_3 + LA or repellent / inhibitory effect depending on doses	Dual-port olfactometer	Smallegange *et al.* (2009)

Table 2. Continued.

Compound	Response	Behavioural assay	Reference
heptanoic acid	Synergism when combined with NH_3 + LA or repellent / inhibitory effect depending on doses	Dual-port olfactometer	Smallegange *et al.* (2009)
octanoic acid	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
nonanoic acid	No effect when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
decanoic acid	No effect when combined with NH_3 and LA	Dual-port olfactometer	Smallegange *et al.* (2009)
dodecanoic acid	No effect when combined with + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
tridecanoic acid	No effect when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
tetradecanoic acid	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
	Attractive when combined with NH_3 + LA	Indoor trapping experiment	Smallegange *et al.* (2009)
hexadecanoic acid	No effect in combination with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2009)
blend of 12 carboxylic acids	Attractive	Dual-port olfactometer	Knols *et al.* (1997)
	Repellent / inhibitory effect	Dual-port olfactometer	Smallegange *et al.* (2005)
	Synergism when combined with NH_3 + LA	Dual-port olfactometer	Smallegange *et al.* (2005)
blend of 22 carboxylic acids	No landing response	Wind tunnel	Healy and Copland (2000)
blend of ammonia, lactic acid and 7 carboxylic acids	Attractive	Dual-port olfactometer	Smallegange *et al.* (2009)
		Indoor trapping experiment	Smallegange *et al.* (2009)
Unsaturated carboxylic acids			
7-octenoic acid	Attractive when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
combination of (E/Z)-3-methyl-2-hexenoic acid isomer mixture and 7-octenoic acid	Reduced response to CO_2	Dual-port olfactometer	Costantini *et al.* (2001)
Oxocarboxylic acids			
2-oxopropanoic acid	No landing response	Wind tunnel	Healy *et al.* (2002)
2-oxobutanoic acid	Induced landing response	Wind tunnel	Healy *et al.* (2002)
2-oxo-3-methylbutanoic acid	Induced landing response	Wind tunnel	Healy *et al.* (2002)
2-oxopentanoic acid	Induced landing response	Wind tunnel	Healy and Copland (2000)
		Wind tunnel	Healy *et al.* (2002)

Table 2. Continued.

Compound	Response	Behavioural assay	Reference
2-oxo-3-methylpentanoic acid	Induced landing response	Wind tunnel	Healy *et al.* (2002)
2-oxo-4-methylpentanoic acid	Induced landing response	Wind tunnel	Healy *et al.* (2002)
2-oxohexanoic acid	Induced landing response	Wind tunnel	Healy *et al.* (2002)
2-oxooctanoic acid	No landing response	Wind tunnel	Healy *et al.* (2002)
2-hydroxypentanoic acid	No landing response	Wind tunnel	Healy *et al.* (2002)
Ketones			
acetone	Activating effect when combined with CO_2	Wind tunnel	Takken *et al.* (1997)
	Enhanced the effect of LA, decreased effect of NH_3	Dual-port olfactometer	Qiu (2005)
	No aditional attractiviness when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
6-methyl-5-hepten-2-one	Repellent / inhibitory effect when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
geranyl acetone	Repellent / inhibitory effect when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
Miscellaneous			
benzothiazole	Repellent / inhibitory effect	Dual-port olfactometer	Qiu *et al.* (2004a)
indole	Repellent / inhibitory effect when tested alone and when combined with NH_3 + LA	Dual-port olfactometer	Qiu (2005)
blend of 1-butanol, 2,3-butanedione, 2-methyl-1-butanol, 2-methylbutanal, 2-methylbutanoic acid, 3-hydroxy-2-butanone, 3-methyl-1-butanol, 3-methylbutanal, 3-methylbutanoic acid, benzeneethanol	Attractive	Indoor trapping experiment	Verhulst *et al.* (2009)

lactic acid (Smallegange *et al.* 2005). In dual-choice olfactometer experiments, this blend was less attractive than a worn sock, suggesting that more volatiles play a role in the host-orientation of *An. gambiae* (Smallegange *et al.*, in press).

In addition, seven individual saturated aliphatic carboxylic acids (propanoic, butanoic, 3-methyl-butanoic, pentanoic, heptanoic, octanoic and tetradecanoic acid) and an unsaturated carboxylic

acid (7-octenoic acid) increased the attractiveness of ammonia when combined in a tripartite blend together with lactic acid (Qiu 2005, Smallegange *et al.* 2009). A blend consisting of ammonia, lactic acid and the seven saturated carboxylic acids was attractive both in a dual-choice olfactometer and in indoor trapping experiments in a netted cage (Smallegange *et al.* 2009). Moreover, this synthetic blend, combined with CO_2, attracted more mosquitoes than humans when present in different experimental huts, whereas it was equally or less attractive than humans when present within the same hut (Okumu *et al.* 2010).

Hexanoic and heptanoic acid were found to have inhibitory/repellent effects at certain doses (Smallegange *et al.* 2002, 2009). A range of other carboxylic acids (acetic, 2-methylpropanoic, nonanoic, decanoic, dodecanoic, tridecanoic and hexadecanoic acid) had no effect on the attractiveness of ammonia combined with lactic acid (Smallegange *et al.* 2009). The combination of 7-octenoic acid and the isomer mixture of (E/Z)-3-methyl-2-hexenoic acid reduced the response to CO_2 in a dual-choice olfactometer (Costantini *et al.* 2001). The latter is a major component in human axillary odour, the former a minor component (Zeng *et al.* 1991, 1996).

Six oxocarboxylic acids (2-oxobutanoic, 2-oxo-3-methylbutanoic, 2-oxopentanoic, 2-oxo-3-methylpentanoic, 2-oxo-4-methylpentanoic and 2-oxohexanoic acid), three of which have been identified in human sweat, caused a landing response in *An. gambiae* females (Table 2). More mosquitoes landed in response to 2-oxopentanoic acid than to the other oxocarboxylic acids. The landing response to this compound was found to be temperature dependent and decreased to control levels within 10 minutes after application (Healy and Copland 2000, Healy *et al.* 2002).

Three alcohols (1-dodecanol, 4-ethyl phenol, 3-methyl-butanol) individually added to ammonia and lactic acid had no effect, inhibited or repelled *An. gambiae* females (Qiu 2005), although two of them (1-dodecanol, 3-methyl-butanol) were found at high levels in incubated sweat, which was found to be more attractive than fresh sweat (Braks and Takken 1999, Meijerink *et al.* 2000).

Mosquitoes preferred ammonia over a blend of ammonia, lactic acid and either geranyl acetone or 6-methyl-5-hepten-2-one, depending on the doses of the ketones (Qiu 2005). These two ketones were found in equal quantities in fresh and incubated sweat (Meijerink *et al.* 2000).

Acetone is well known as an attractive kairomone for tsetse flies. This compound has been found in blood, milk, urine and breath of cattle, which is the host of these vectors of trypanosomiasis (Jordan 1986, Torr *et al.* 1995, Vale 1980, Vale and Hall 1985a,b). It is also relatively abundant in human breath (Philips 1997) and was found in fresh but not in incubated sweat (Meijerink *et al.* 2000). A human-equivalent physiological concentration of acetone had an activating effect on *An. gambiae* when added to CO_2 (Takken *et al.* 1997). It was also found to increase the attractiveness of lactic acid, but decreased the attractiveness of ammonia and was not attractive on its own. Although the triple blend of ammonia, lactic acid and acetone was attractive, it was as attractive as ammonia alone (Qiu 2005).

When tested alone, indole, which is abundantly present in incubated sweat (Meijerink *et al.* 2000), caused either no effect, inhibition or repelled, depending on the concentration tested. A blend of ammonia, lactic acid and indole was significantly less attractive than ammonia alone (Qiu 2005). A compound found in the headspace of worn nylon stockings but suspected to be not specifically human, benzothiazole, repelled *An. gambiae* females at close range in a dual-port olfactometer (Qiu *et al.* 2004b).

An. gambiae is attracted to volatiles produced by human skin microbiota both in dual-choice olfactometer and in indoor trapping experiments. Moreover, MM-X traps baited with a blend of 10 compounds present in the headspace of human feet microbiota (1-butanol, 2,3-butanedione, 2-methyl-1-butanol, 2-methylbutanal, 2-methylbutanoic acid, 3-hydroxy-2-butanone, 3-methyl-1-butanol, 3-methylbutanal, 3-methylbutanoic acid, benzeneethanol) caught significantly more *An. gambiae* during indoor trapping experiments than unbaited traps (Verhulst *et al.* 2009).

Another member of the *An. gambiae* complex, *An. quadriannulatus* (Theobald), has long been considered to be zoophilic and therefore of no medical importance (Takken *et al.* 1999). Hence its host-seeking behaviour has rarely been studied. Pates *et al.* (2001a), however, showed that this species has an equal preference for humans and calves. In addition, *An. quadriannulatus* preferred human odour over a synergistic combination of cow odour with CO_2 in a dual-port olfactometer. Carbon dioxide alone was not attractive, whereas CO_2 with 1-octen-3-ol had a inhibitory or repellent effect. The effect of acetone was not clear (Pates *et al.* 2005). A recent field study with *An. quadriannulatus* confirmed the finding that this species is attracted to human and cattle odours (Torr *et al.* 2008).

Culex quinquefasciatus

Almost 60% of the *Cx. quinquefasciatus* females from a colony that originated from Sri Lanka that were released individually in a dual-port olfactometer were attracted within three minutes to polyamide stockings that had been worn by a human volunteer for the preceding 48-60 hours (Mboera *et al.* 1998), whereas, in a separate study, less than 25% of the mosquitoes from a colony in the USA released in groups of 50-70 females were attracted to a human hand (Allan *et al.* 2006b). Skin rubbings and ethanol washings of human hands and feet or the back were attractive to groups or individual female mosquitoes (Braks *et al.* 1999). These results demonstrate that *Cx. quinquefasciatus* from different parts of the world are attracted to sources of volatiles that are present on human skin.

In laboratory studies carbon dioxide has little or no attractive effect on this species. A high concentration was found to be repellent. Contrary to what has been found for other mosquito species, no synergistic effect of CO_2 in combination with skin emanations has been observed (Allan *et al.* 2006b, Braks *et al.* 1999, Mboera *et al.* 1998).

Culex quinquefasciatus was attracted to lactic acid in dual-port olfactometer studies (Allan *et al.* 2006a, Braks *et al.* 1999). Ethanol washings from human hands and feet were significantly more attractive than lactic acid alone, demonstrating that lactic acid is not the only attractive component on human skin (Braks *et al.* 1999). Eighteen other compounds present in human skin emanations were found to be attractive at certain doses to this species when tested individually in a Y-tube olfactometer (Table 3). Some of these attracted over 75% of the mosquitoes released: heptanal, nonanal, ethylene glycol, benzyl alcohol, and eleven carboxylic acids (Puri *et al.* 2006). Several of these compounds, however, were not found to have an effect on mosquitoes in a dual-port olfactometer study, possibly due to the concentrations at which these compounds were tested (Table 3) (Allan *et al.* 2006a,b). Other alcohols and carboxylic acids had either no effect or exhibited decreased behavioural responses compared to the control, depending on the doses applied in a Y-tube olfactometer (Table 3) (Puri *et al.* 2006).

Recent field experiments showed that not only CO_2 enhances trap catches, but also nonanal. The latter compound is dominantly present in the odour profile of pigeons, chickens and humans from

Table 3. Overview of chemical compounds that have been tested for their impact on the host-seeking behaviour of Culex quinquefasciatus *using behavioural assays in the laboratory. (Details about concentrations of compounds are omitted).*

Compound	Response	Behavioural assay	Reference
carbon dioxide (CO_2)	Not or weakly attractive on its own; repellent / inhibitory effect at high concentration; no synergism with host emanations	Dual-port olfactometer	Mboera *et al.* (1998)
		Dual-port olfactometer	Braks *et al.* (1999)
		Dual-port olfactometer	Allan *et al.* (2006b)
Alcohols			
ethylene glycol	Attractive	Y-tube	Puri *et al.* (2006)
glycerol	Repellent / inhibitory effect	Y-tube	Puri *et al.* (2006)
phenol	No response	Y-tube	Puri *et al.* (2006)
benzyl alcohol	Attractive	Y-tube	Puri *et al.* (2006)
decanol	Attractive or repellent / inhibitory effect depending on doses	Y-tube	Puri *et al.* (2006)
cholesterol	Attractive	Y-tube	Puri *et al.* (2006)
Aldehydes			
propanal	Attractive	Y-tube	Puri *et al.* (2006)
hexanal	No response	Dual-port olfactometer	Allan *et al.* (2006b)
heptanal	Attractive	Y-tube	Puri *et al.* (2006)
benzaldehyde	No response	Y-tube	Puri *et al.* (2006)
		Dual-port olfactometer	Allan *et al.* (2006b)
nonanal	Attractive	Y-tube	Puri *et al.* (2006)
	No response	Dual-port olfactometer	Allan *et al.* (2006b)
Aliphatic carboxylic acids			
lactic acid (LA)	Attractive	Dual-port olfactometer	Allan *et al.* (2006a)
		Dual-port olfactometer	Braks *et al.* (1999)
	No landing response	Landing assay	Allan *et al.* (2006a)
acetic acid	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
propanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
butanoic acid	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
3-methyl butanoic acid	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
hexanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
heptanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
octanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
nonanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
decanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
undecanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
dodecanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
tridecanoic acid	Attractive	Y-tube	Puri *et al.* (2006)

Table 3. Continued.

Compound	Response	Behavioural assay	Reference
tetradecanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
pentadecanoic acid	Repellent / inhibitory effect	Y-tube	Puri *et al.* (2006)
hexadecanoic acid	Repellent / inhibitory effect	Y-tube	Puri *et al.* (2006)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
heptadecanoic acid	Attractive	Y-tube	Puri *et al.* (2006)
octadecanoic acid	Repellent / inhibitory effect	Y-tube	Puri *et al.* (2006)
	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
Aromatic carboxylic acids			
benzoic acid	No landing response	Landing assay	Allan *et al.* (2006a)
2-hydroxybenzoic acid	No landing response	Landing assay	Allan *et al.* (2006a)
Sulfides			
methyl sulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
carbon disulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
dimethyl disulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
ethyl disulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	No landing response	Landing assay	Allan *et al.* (2006a)
methyl propyl	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
disulfide	Induced landing response	Landing assay	Allan *et al.* (2006a)
dimethyl trisulfide	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006a)
	Induced landing response	Landing assay	Allan *et al.* (2006a)
Miscellaneous			
meso-2,3-butanediol	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006b)
2,3-butanediol	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006b)
α-pinene	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006b)
β-myrcene	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006b)
2,3-docosanediol	Not attractive on its own	Dual-port olfactometer	Allan *et al.* (2006b)
blend of hexanal, α -pinene, benzaldehyde, β-myrcene, and nonanal	Not attractive	Dual-port olfactometer	Allan *et al.* (2006b)

various ethnic backgrounds. Traps baited with both nonanal and CO_2 caught the highest number of *Cx. quinquefasciatus* (Syed and Leal 2009).

Culex quinquefasciatus females assayed in a dual-port olfactometer responded to a different extent to odour sources of animal origin (bovine blood, avian blood, chickens, chicken feathers, chicken feathers combined with CO_2, cotton balls rubbed over chicken feathers and hexane extracts of chicken feathers) (Allan *et al.* 2006a,b). However, there was no response to five compounds identified from feathers (Williams *et al.* 2003) individually or as a blend (hexanal, benzaldehyde, nonanal, α-pinene, and β-myrcene), or to three individual compounds (meso-2,3-butanediol, 2,3-butanediol and 2,3-docosanediol) that have been identified in the preen gland of hens (Allan *et al.* 2006b, Haathi and Fales 1967; Table 3).

In a Y-tube olfactometer, fresh chicken faeces was attractive to *Cx. quinquefasciatus*. Eight aldehydes ((E)-2-decanal, undecanal, dodecanal, tetradecanal, pentadecanal, hexadecanal, heptadecanal and octadecanal) identified in the headspace of the faeces elicited electroantennogram responses, as well as eight unknown compounds (Cooperband *et al.* 2008), and should be tested for their effect on the host-seeking behaviour of *Cx. quinquefasciatus* females.

Volatile compounds associated with blood (aliphatic and aromatic carboxylic acids and sulphides) showed no attractiveness in olfactometer assays, with the exception of lactic acid (Table 3). However, in cage assays, significantly more landings were observed on collagen sausage casings treated with acetic, hexadecanoic, and octadecanoic acid, dimethyl trisulfide or methyl propyl disulfide than on casings treated with water (Allan *et al.* 2006a).

Female *Cx. nigripalpus* Theobald, another mosquito species associated with West Nile virus transmission in the USA (Godsey *et al.* 2005, Sardelis *et al.* 2001), were attracted to tetradecanoic acid, dimethyl disulfide and methyl propyl disulfide and landed on casings treated with lactic acid, octadecanoic acid, dimethyl trisulfide, ethyl disulfide or methyl propyl disulfide, although responses to bovine blood were low (Allan *et al.* 2006a). *Culex nigripalpus* and *Cx. tarsalis* Coquillett did not respond to human odour, whereas they were attracted to chicken odour in a dual-port olfactometer. *Culex tarsalis* was significantly more responsive to CO_2 than were *Ae. aegypti*, *Cx. quinquefasciatus* and *Cx. nigripalpus* (Allan *et al.* 2006b).

Discussion

In the last decade much progress has been made in the identification of kairomones and allomones of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*. However, in the laboratory assays these compounds and blends are less attractive than natural odours and work should continue to identify the 'missing' compounds. Recent advances in molecular olfaction and neurophysiology are expected to contribute to a rapid identification of essential odorants that are lacking from the current blends.

Robust behavioural assays are available for screening olfactory cues to which mosquitoes respond, although some devices are not suitable for all species. For example, *An. gambiae* behaviour is erratic in Y-tube assays (Smallegange *et al.* 2002), whereas *Ae. aegypti* and *Cx. quinquefasciatus* behaviour is normal and consistent in this device (Geier and Boeckh 1999, Puri *et al.* 2006).

The main bottleneck of high-throughput testing of candidate semiochemicals is the relatively time-consuming operation of the behavioural bioassays, in contrast to neurophysiological studies.

Three-layer olfactometers, in which three sets of mosquitoes can be tested simultaneously, as developed by the USDA research laboratory in Gainesville, Florida, USA (Posey *et al.* 1998), are a valuable means of increasing the rate at which mosquitoes can be tested. Nevertheless, each batch of novel odour blends needs to be tested in sufficient replicates, which slows down the bioassay evaluation. The development of tools that might accelerate the behavioural assays may be very useful so that new producers of semiochemicals will not be disappointed. These behavioural assays require large numbers of female mosquitoes, which can be a burden on the research budget as this requires the availability of climate-controlled insectaries and trained staff.

Progress in the development of digital recording techniques allows for the study of insect behaviour using 3D image analysers (see publications for details of products available). Patterns of flight behaviour in response to specific host-odours and blends of odours have been studied, revealing interesting behavioural changes during odour-mediated upwind anemotaxis (Beeuwkes *et al.* 2008, Braks *et al.* 2005, Cooperband and Cardé 2006, Chapter 6 in this volume). Thus, the specific role of a semiochemical in the mosquito behavioural repertoire can be established. This will facilitate the discovery of more effective odorant blends with which to modify or control mosquito behaviour.

Current techniques in semiochemistry allow for the rapid analysis of volatiles of human and animal origin (Bernier *et al.* 1999, Curran *et al.* 2007, Penn *et al.* 2007). This poses a potentially huge problem for the behavioural ecologist, as it is unlikely that all compounds present in host emanations are involved in host-seeking behaviour (Zwiebel and Takken 2004), and it is impractical to examine each compound in a behavioural assay. High-throughput testing of semiochemicals is possible at the neurophysiological level (Chapter 2 and 3, this volume), but encounters logistical obstructions at the behavioural level. Thus, chemical ecologists will probably rely on testing selected compounds and blends, based on results from neurophysiology and historical evidence. The exploration of chemical libraries for small molecules that might affect mosquito behaviour, as is currently in progress at different research facilities, will add a potentially large number of compounds that require behavioural testing (Justice *et al.* 2003, Zwiebel and Takken 2004).

At present, a novel and promising technique is under development by several research groups, in which differences in attractiveness to mosquitoes between human individuals is being exploited to study chemical profiles with the aim of identifying critical chemical groups or compounds that cause these differences (Bernier *et al.* 2002, Logan *et al.* 2008, Smallegange *et al.* 2003, Qiu *et al.* 2006a). Such compounds may be either kairomones or allomones (Dicke and Sabelis 1988). If the compounds that are significantly more abundant in highly attractive persons can be identified, it is likely to provide a rapid method for the development of attractive odour blends for mosquitoes, as it is expected that these compounds play a role in the attraction of these individuals. Alternatively, poorly attractive individuals may produce compounds that quench or even suppress the activity of other chemicals, so that the mosquito can no longer identify the source or, perhaps, is even repelled from it (Logan *et al.* 2008).

The relative concentrations of semiochemicals in odour blends is a fundamental factor in determining their attractiveness. For example, *Cx. quinquefasciatus* was not attracted to certain synthetic volatiles of chicken origin (Allan *et al.* 2006b), whereas it did respond to natural bird odours. This may indicate that either critical volatiles were missing or that the relative concentrations of components in the blend were not appropriate. This example illustrates some of the difficulties inherent in the development of effective synthetic odour baits. Knowledge of the naturally occurring ratios of compounds in host volatiles is not necessarily useful; artificial

baits for trapping and killing tsetse represent the most successful application of semiochemicals to control vectors in the world, and yet the blend of chemicals in the bait is only loosely related to the naturally occurring ratios and concentrations of compounds emitted by live hosts (Vale and Hall 1985a,b). It is also worth noting that compounds and blends of compounds that are attractive at a particular concentration, may be repellent or cause inhibition at higher concentrations (e.g. Knols *et al.* 1997, Smallegange *et al.* 2005, Vale and Hall 1985a). Therefore, single compounds and blends should be tested across a range of concentrations in a methodical way, similar to range testing doses of drugs or toxins. Differences in the concentrations of compounds tested may explain differences in the results of assays based on the same compounds (Allan *et al.* 2006a,b, Puri *et al.* 2006). Similarly, the type of assay used (Y-tube olfactometer, dual-port olfactometer, landing assay) may affect the results obtained.

A more directed approach may help to identify the most important compounds controlling host-seeking behaviour of particular species. For example, having found that hexane extracts of chicken feathers were attractive to *Cx. quinquefasciatus* while ether extracts were not (Allan *et al.* 2006b), Bernier *et al.* (2008) examined these extracts and found that the ether extracts contained aldehydes, which were also present in the hexane extracts, whereas the hexane extracts also contained alcohols, ketones and diones. Subsequent behavioural assays can focus now on compounds of the latter three groups or can be preceded by either on-line (coupled with GC) or off-line EAG recordings to determine whether components present in the hexane extract are detected by the mosquito olfactory structures as Cooperband *et al.* (2008) did with acidified chicken faeces. A similar approach could be used to identify more active compounds, in addition to lactic acid and ammonia, which are present in the fractions of skin extracts attracting *Ae. aegypti* (Geier *et al.* 1996, 1999a). Unidentified compounds may yet be found that increase the attractiveness of the best blends available so far (Williams *et al.* 2006a).

It has been shown repeatedly that a compound may be more attractive in mixtures than when applied singly (Geier *et al.* 1999a, Smallegange *et al.* 2005). *Aedes aegypti* females are more attracted to triple blends of lactic acid, acetone, dimethyl disulfide or dichloromethane than to binary blends, whereas binary blends are more attractive than single compounds (Bernier *et al.* 2007). A similar observation was found for tsetse flies: a mixture of three chemicals is more attractive than individual chemicals and more tsetse flies were trapped when the mixture was applied at higher doses for all components, except octenol, which reduces trap catches at higher doses, showing again the importance of controlling concentrations (Vale and Hall 1985b).

Compounds that have no clear effect on behaviour when used individually may, however, play an important role in a mixture, as was shown for lactic acid; although it is not or only weakly attractive to *An. gambiae* when applied alone, it has a synergistic effect when presented with ammonia and carboxylic acids (Qiu 2005, Smallegange *et al.* 2005, 2009). Similarly, *Ae. aegypti* is not attracted to ammonia alone, whereas this compound enhances the effect of lactic acid (Geier *et al.* 1999a). This phenomenon makes it even harder to 'predict' which mixture of compounds will produce an effective synthetic blend that is highly attractive to mosquitoes.

For *Ae. aegypti* and *An. gambiae* particular blends of kairomones attract a reasonably large number of mosquitoes in bioassays when tested on their own. However, the natural odour complex of the human host was significantly more attractive than any synthetic blend when compared directly against each other (Bernier *et al.* 2007, Spitzen *et al.* 2008, Smallegange *et al.* in press). It is therefore likely that essential components are lacking in these, otherwise, attractive blends.

Another complication in the identification of kairomones is that a chemical may be attractive when applied alone, but shows an inhibitory or a (spatial) repellent effect in combination with human odours. Such an effect has been found for the widely used mosquito repellent DEET (N,N-diethyl-3-methylbenzamide), which was found to be (weakly) attractive to *Ae. aegypti* when used on its own (Bernier *et al.* 2005, Dogan *et al.* 1999, Kline *et al.* 2003, Mehr *et al.* 1990), but caused (spatial) repellence or inhibited attraction when combined with natural human odours (Bernier *et al.* 2005, Curtis 1992, Dogan *et al.* 1999, Kline *et al.* 2003). Recently, however, Syed and Leal (2008) demonstrated true behavioural repellency of *Cx. quinquefasciatus* by DEET in the absence of other chemostimuli.

In addition, the method of testing putative repellents can conceal its possible effect. Based on the definition of a repellent as 'a chemical which causes insects to make oriented movements away from its source' (Dethier *et al.* 1960), it remains unclear from most Y-tube and dual-choice olfactometer assays whether compounds that attract significantly fewer mosquitoes than the control are truly repellents (Dogan and Rossignol 1999), because in these devices the insects cannot move away from the source. However, when the term repellent is used to designate products that intent to reduce the biting-rate of haematophagous insects (see White 2007), this term can be used for chemicals that have been shown to reduce the number of mosquitoes attracted to a natural or synthetic odour source when tested in a Y-tube or dual-choice olfactometer. Especially area or spatial repellents, which prevent mosquitoes from reaching their blood-host and are thus effective at a distance from the source of application (see Strickman 2007), may be discovered with these kind of bioassays. Several methods have been developed to test chemicals for their ability to be applied as mosquito repellents, either as a spatial or topical repellent. Each method has its advantages and disadvantages (e.g. Barnard *et al.* 2007, Chareonviriyaphap *et al.* 2002, Dogan and Rossignol 1999, Grieco *et al.* 2005, Hao *et al.* 2008, Kline *et al.* 2003, Klun *et al.* 2005, Rutledge and Gupta 2004). Which bioassay should be used may depend on the research question and possible future application method and may depend on the mode of action of the repellent. For these reasons we suggest to name chemicals that show a inhibitory or repellent effect in laboratory bioassays 'putative repellents' until their effectiveness has been proven in the field.

The previous paragraphs show that the bioassay used to test semiochemicals as behaviour-mediating compounds for mosquitoes may provide an indication that a certain compound plays a role in the host-seeking process. However, the result may not be conclusive of the exact role of this compound. For example, most Y-tube and dual-choice olfactometers cannot discriminate between an inhibitory and a repellent effect (Dogan and Rossignol 1999); additional, appropriate bioassays should be performed. Since the host-seeking process exists of different phases (Takken 1996), the choice for a certain type of bioassay determines which phase is examined. For example, the activating effect of a chemical may be missed in a landing assay or an olfactometer when it is not combined with a chemical that induces a landing response or that is an attractant, whereas it may be noticed when video recording in combination with a 3D image analyser is used. Therefore, when no response has been found in a specific bioassay care should be taken to conclude that a certain compound has no effect on the host-seeking behaviour of a certain mosquito species; the compound may play a role in another host-seeking phase. In an olfactometer, attraction may not be visible, even though the mosquitoes make oriented movements towards the source of a chemical (Dethier *et al.* 1960), when the insects do not fly into the trapping system with which the olfactometer is applied.

Although similarities have been found in the response to certain olfactory cues (e.g. ammonia, lactic acid, aliphatic carboxylic acids, some ketones and aldehydes in *Ae. aegypti*, *An. gambiae*

and *Cx. quinquefasciatus*), it is clear that different mosquito species use different odour cues to find their blood-host, and the role of the individual compounds varies between species, even for species that have the same host preference. Finally, much research is required to establish the range of variation in behaviour within wild populations of a given mosquito species across its geographical distribution, for variability within populations, and for the effects of colonisation on natural host-seeking behaviour, as indicated by some disparities between the results of studies based on different populations of all three species reviewed here (Lefèvre *et al.* 2009; Williams *et al.* 2003, 2006c). It is likely that different attractive blends, odour delivery systems and trap types have to be used to manage different mosquito populations (Kline 2007).

Conclusions

Several compounds found in human emanations have been shown to cause a behavioural response in the laboratory in at least one of the three mosquito species, *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*. Carbon dioxide is a universal kairomone, but with distinctly different effects on the three species. In the laboratory, *Aedes aegypti* is strongly activated by this compound, whereas for *An. gambiae* it is also a synergist and effective when released at a distance from the other kairomones. The behavioural response of *Cx. quinquefasciatus* to CO_2 is at best weak.

Composed odour blends are attractive to mosquitoes in the laboratory. These include a combination of ammonia, lactic acid, hexanoic acid, acetone and dimethyl disulfide for *Ae. aegypti*, and of ammonia, lactic acid and aliphatic carboxylic acids for *An. gambiae*. However, there is not yet a blend that is equally attractive or more so than natural human odours. Studies comparing (1) odours from poorly and highly attractive persons, (2) odours from a preferred host and a non-host and (3) blood-host preferences of mosquitoes from different geographic regions are likely to reveal critical compounds responsible for the strong attractiveness of humans to the three mosquito species reviewed here.

Six-methyl-5-hepten-2-one and geranyl acetone are candidate repellents, as these cause inhibition or repellence in *Ae. aegypti* (Logan *et al.* 2008) and *An. gambiae* (Qiu 2005). Interestingly, the release of these compounds from a human arm has been found to decrease when treated with DEET (Syed and Leal 2008).

Synthetic blends can be exploited to lure mosquitoes into odour-baited traps, manipulate them away from human houses or cause general disruption to normal host-seeking behaviour by mass release of human odorants (Day and Sjogren 1994, Kline 2007). Chapter 8 of this volume discusses how laboratory-based results may be transferred to the (semi-)field for further testing prior to their application.

It is expected that the studies in progress by several research consortia will lead to novel classes of odorant blends with which mosquito disease vectors can be monitored or controlled. Recent results from trapping with odour-baited MM-X or BG-Sentinel traps (Kline 2007, Kröckel *et al.* 2006, Mboera *et al.* 2000, Njiru *et al.* 2006, Qiu *et al.* 2007a, Schmied *et al.* 2008, Williams *et al.* 2006b) suggest that the application of such traps, provided the odorant stimulus is highly competitive with natural host odour, are likely to become reality in the near future. Novel synthetic odorant blends that disrupt the natural behaviour of mosquito vectors may be used in addition to current control methods (Greenwood 2008, Takken and Knols 2009) to achieve a greater effect than is presently possible.

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References

Acree F, Jr, Turner RB, Gouck HK, Beroza M and Smith N (1968) L-lactic acid: A mosquito attractant isolated from humans. Science 161: 1346-1347.

Allan SA, Bernier UR and Kline DL (2006a) Attraction of mosquitoes to volatiles associated with blood. J Vector Ecol 31: 71-78.

Allan SA, Bernier UR and Kline DL (2006b) Laboratory evaluation of avian odors for mosquito (Diptera: Culicidae) attraction. J Med Entomol 43: 225-231.

Alonso WJ and Schuck-Paim C (2006) The 'ghosts' that pester studies on learning in mosquitoes: guidelines to chase them off. Med Vet Entomol 20: 157-165.

Ashley DL, Bonin MA, Cardinali FL, McCraw JM, Holler JS, Needham LL and Patterson DG, Jr (1992) Determining volatile organic compounds in human blood from a large sample population by using purge and trap gas chromatography/ mass spectrometry. Analytical Chemistry 64: 1021-1029.

Barnard DR, Bernier UR, Xue R-D and Debboun M (2007) Standard methods for testing mosquito repellents. In: Debboun M, Frances SP and Strickman D (eds) Insect repellents. Principles, methods, and uses. CRC Press, Boca Raton, FL, USA, pp 103-110.

Beeuwkes J, Spitzen J, Spoor CW, Van Leeuwen JL and Takken W (2008) 3-D flight behaviour of the malaria mosquito *Anopheles gambiae s.s.* inside an odour plume. Proceedings of Experimental and Applied Entomology, N.E.V., Amsterdam 19: 137-146.

Bernier UR, Allan SA, Quinn BP, Kline DL, Barnard DR and Clark GG (2008) Volatile compounds from the integument of White Leghorn chickens (*Gallus gallus domesticus* L.): Candidate attractants of ornithophilic mosquito species. J Sep Sci 31: 1092-1099.

Bernier UR, Booth MM and Yost RA (1999) Analysis of human skin emanations by gas chromatography/mass spectrometry. 1.Thermal desorption of attractants for the yellow fever mosquito (*Aedes aegypti*) from handled glass beads. Anal Chem 71: 1-7.

Bernier UR, Furman KD, Kline DL, Allan SA and Barnard DR (2005) Comparison of contact and spatial repellency of catnip oil and N,N-diethyl-3-methylbenzamide (Deet) against mosquitoes. J Med Entomol 42:306-311.

Bernier UR, Kline DL, Allan SA and Barnard DR (2007) Laboratory comparison of *Aedes aegypti* attraction to human odors and to synthetic human odor compounds and blends. J Am Mosq Control Assoc 23: 288-293.

Bernier UR, Kline DL, Barnard DR, Schreck CE and Yost RA (2000) Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for yellow fever mosquito (*Aedes aegypti*). Anal Chem 72: 747-756.

Bernier UR, Kline DL, Posey KH, Booth MM, Yost RA and Barnard DR (2003) Synergistic attraction of *Aedes aegypti* (L.) to binary blends of L-lactic acid and acetone, dichloromethane, or dimethyl disulfide. J Med Entomol 40: 653-656.

Bernier UR, Kline DL, Schreck CE, Yost RA and Barnard DR (2002) Chemical analysis of human skin emanations: comparison of volatiles from humans that differ in attraction of *Aedes aegypti* (Diptera: Culicidae). J Am Mosq Control Assoc 18: 186-195.

Birkett MA, Agelopoulos N, Jensen K-MV, Jespersen JB, Pickett JA, Prijs HJ, Thomas G, Trapman JJ, Wadhams LJ and Woodcock CM (2004) The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. Med Vet Entomol 18: 313-322.

Bosch OJ, Geier M and Boeckh J (2000) Contribution of fatty acids to olfactory host finding of female *Aedes aegypti*. Chem Senses 25: 323-330.

Bowen MF (1991) The sensory physiology of host-seeking behavior in mosquitoes. Annu Rev Entomol 36: 139-158.

Braimah N, Drakeley C, Kweka E, Mosha F, Helinski M, Pates H, Maxwell C, Massawe T, Kenward MG, Curtis C (2005) Tests of bednet traps (Mbita traps) for monitoring mosquito populations and time of biting in Tanzania and possible impact of prolonged insecticide treated net use. Int J Trop Insect Sci 25: 208-213.

Braks MAH (1999) Human skin emanations in the host-seeking behaviour of the malaria mosquito *Anopheles gambiae*. PhD thesis Wageningen University, Wageningen, the Netherlands. 123 pp.

Braks MAH, Cooperband MF and Cardé RT (2005) 3-D flight track analyses of *Culex* mosquito orientation to oviposition-related odors. In: Noldus LP (ed) Proceedings Measuring Behavior 2005, Wageningen, the Netherlands, pp 209-210.

Braks MAH, Koenraadt CJM, Briet OJT and Takken W (1999) Behavioural responses of *Culex quinquefasciatus* (Diptera: Culicidae) to human odour, carbon dioxide and lactic acid. Proceedings of Experimental and Applied Entomology, N.E.V., Amsterdam 10: 91-97.

Braks MAH, Meijerink J and Takken W (2001) The response of the malaria mosquito, *Anopheles gambiae*, to two components of human sweat, ammonia and L-lactic acid, in an olfactometer. Physiol Entomol 26: 142-148.

Braks MAH and Takken W (1999) Incubated human sweat but not fresh sweat attracts the malaria mosquito *Anopheles gambiae sensu stricto*. Journal of Chemical Ecology 25: 663-672.

Bursell E, Gough AJE, Beevor PS, Cork A, Hall DR and Vale GA (1988) Identification of components of cattle urine attractive to tsetse flies, *Glossina* spp. (Diptera: Glossinidae). Bull Entomol Res 78: 281-291.

Cardé RT and Willis MA (2008) Navigational strategies used by insects to find distant, wind-borne sources of odor. J Chem Ecol 34: 854-866.

Carey AF, Wang G, Su C-Y, Zwiebel LJ and Carlson JR (2010) Odorant reception in the malaria mosquito *Anopheles gambiae*. Nature (in press) doi:10.1038/nature08834.

Chandra G (1995) Short report: peak period of filarial transmission. Am J Trop Med Hyg 53: 378-379.

Chareonviriyaphap T, Prabaripai A and Sungvornyothrin S (2002) An improved excito-repellency test chamber for mosquito behavioral tests. J Vector Ecol 27: 250-252.

Cook GC (1996) Manson's tropical diseases. 20th edn. WB Saunders Company Ltd, London, UK.

Cook SM, Khan ZR and Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52: 375-400.

Cooperband MF and Cardé RT (2006) Orientation of *Culex* mosquitoes to carbon dioxide-baited traps: flight manoeuvres and trapping efficiency. Med Vet Entomol 20: 11-26.

Cooperband MF, McElfresh JS, Millar JG, Cardé RT (2008) Attraction of female *Culex quinquefasciatus* Say (Diptera: Culicidae) to odors from chicken feces. J Insect Physiol 54: 1184–1192.

Cork A and Hall MJR (2007) Development of an odour-baited target for female New World screwworm, *Cochliomyia hominivorax*: studies with host baits and synthetic wound fluids. Med Vet Entomol 21: 85-92.

Cork A and Park KC (1996) Identification of electrophysiologically-active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat extracts. Med Vet Entomol 10: 269-276.

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, 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.

Crumb SE (1922) A mosquito attractant. Science 55: 446-447.

Cruz-Lopez L, Malo EA, Rojas JC and Morgan ED (2001) Chemical ecology of triatomine bugs: vectors of Chagas disease. Med Vet Entomol 15: 351-357.

Curran AM, Rabin SI, Prada PA and Furton KG (2005) Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. J Chem Ecol 31: 1607-1619.

Curran AM, Ramirez CF, Schoon AA and Furton KG (2007) The frequency of occurrence and discriminatory power of compounds found in human scent across a population determined by SPME-GC/MS. J Chromatogr B 846: 86-97.

Curtis CF (1986) Fact and fiction in mosquito attraction and repulsion. Parasitology Today 11: 316-318.

Curtis CF (1992) Personal protection methods against vectors of disease. Rev Med Vet Entomol 80: 543-553.

Curtis CF and Hill N (1988) Comparison of methods of repelling mosquitoes. Entomol Exp Appl 49: 175-179.

Davis EE (1984) Development of lactic acid-receptor sensitivity and host-seeking behaviour in newly emerged female Aedes aegypti mosquitoes. J Insect Physiol 30: 211-215.

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

De Bruyne M and Baker TC (2008) Odor detection in insects: volatile codes. J Chem Ecol 34: 882-897.

De Jong R and Knols BGJ (1995) Selection of biting sites on man by two malaria mosquito species. Cellular and Molecular Life Sciences (formerly Experientia) 51: 80-84.

De Jong R and Knols BGJ (1996) Selection of biting sites by mosquitoes: In: Bock GR and Cardew G (eds) Olfaction in mosquito host interactions. John Wiley and Sons Ltd, Chichester, UK, pp 89-103.

Dekker T, Geier M and Cardé RT (2005) Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. J Exp Biol 208: 2963-2972.

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.

Dekker T, Takken W and Braks MAH (2001a) Innate preference for host-odor blends modulates degree of anthropophagy of *Anopheles gambiae* sensu lato (Diptera: Culicidae). J Med Entomol 38: 868-871.

Dekker T, Takken W and Cardé RT (2001b) Structure of host-odour plumes influences catch of *Anopheles gambiae s.s.* and *Aedes aegypti* in a dual-choice olfactomer. Physiol Entomol 26: 124-134.

Dekker T, Takken W, Knols BGJ, Bouman E, Van de Laak S, De Bever A and Huisman PWT (1998) Selection of biting sites on a human host by *Anopheles gambiae s.s.*, *An. arabiensis* and *An. quadriannulatus*. Entomol Exp Appl 87: 295-300.

Deng C, Zhang X and Li N (2004) Investigation of volatile biomarkers in lung cancer blood using solid-phase microextraction and capillary gas chromatography–mass spectrometry. Journal of Chromatography B 808: 269-277.

Dethier VG, Browne LB and Smith CN (1960) The designation of chemicals in terms of the responses they elicit from insects. J Econ Entomol 53: 134-136.

Diatta M, Spiegel A, Lochouarn L, and Fontenille D (1998) Similar feeding preferences of *Anopheles gambiae* and *A. arabiensis* in Senegal. Trans R Soc Trop Med Hyg 92: 270-272.

Dicke M and Sabelis MW (1988) Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? Funct Ecol 2. 131-139

Dogan EB, Ayres JW and Rossignol PA (1999) Behavioural mode of action of deet: inhibition of lactic acid attraction. Med Vet Entomol 13: 97-100.

Dogan EB and Rossignol PA (1999) An olfactometer for discriminating between attraction, inhibition, and repellency in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 36: 788-793.

Douglas HD, Co JE, Jones TH and Conner WE (2001) Heteropteran chemical repellents identified in the citrus odor of a seabird (crested auklet: *Aethia cristatella*): evolutionary convergence in chemical ecology. Naturwissenschaften 88: 330-332.

Douglas HD, Co JE, Jones TH, Conner WE and Day JF (2005) Chemical odorant of colonial seabird repels mosquitoes. J Med Entomol 42: 647-651.

Duchemin J. B., Leong Pock Tsy J. M., 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.

Elizondo-Quiroga A, Flores-Suarez A, Elizondo-Quiroga D, Ponce-Garcia G, Blitvich BJ, Contreras-Cordero JF, Gonzalez-Rojas JI, Mercado-Hernandez R, Beaty BJ and Fernandez-Salas I (2006) Host-feeding preference of *Culex quinquefasciatus* in Monterrey, Northeastern Mexico. J Am Mosq Control Assoc 22: 654-661.

Eiras AE and Jepson PC (1991) Host location by *Aedes aegypti* (Diptera Culicidae): a wind tunnel study of chemical cues. Bull Entomol Res 81: 151-160.

Ellin RI, Farrand RL, Oberst FW, Crouse CL, Billups NB, Koon WS, Musselman NP and Sidell FR (1974) An apparatus for the detection and quantitation of volatile human effluents. J Chromatogr 100: 137-152.

Gallagher M, Wysocki CJ, Leyden JJ, Spielman AI, Sun X and Preti G (2008) Analyses of volatile organic compounds from human skin. Br J Dermatol 159:780-791.

Geier M and Boeckh J (1999) A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. Entomol Exp Appl 92: 9-19.

Geier M, Bosch OJ and Boeckh J (1999a) Ammonia as an attractive component of host odour for the Yellow fever mosquito *Aedes aegypti*. Chem. Senses 24: 647-653.

Geier M, Bosch OJ and Boeckh J (1999b) Influence of odour plume structure on upwind flight of mosquitoes towards hosts. J Exp Biol 202: 1639-1648.

Geier M, Sass H and Boeckh J (1996) A search for components in human body odour that attract female *Aedes aegypti*. In: Bock GR and Cardew G (eds) Olfaction in mosquito host interactions. John Wiley and Sons Ltd, Chichester, UK, pp 132-148.

Gillies MT (1964) Selection for host preference in *Anopheles gambiae*. Nature 203: 852-854.

Gillies MT (1988) Anopheline mosquitos: vector behaviour and bionomics. In: Wernsdorfer WH and McGregor I (eds) Malaria - principles and practice of malariology. Churchill Livingstone, Edinburgh, UK, pp 453-485.

Godsey MS, Jr., Blackmore MS, Panella NA, Burkhalter K, Gottfried K, Halsey LA, Rutledge R, Langevin SA, Gates R, Lamonte KM, Lambert A, Lanciotti RS, Blackmore CG, Loyless T, Stark L, Oliveri R, Conti L and Komar N (2005) West Nile virus epizootiology in the southeastern United States, 2001. Vector Borne Zoonotic Dis 5: 82-89.

Gould EA (2003) Implications for Northern Europe of the emergence of West Nile virus in the USA. Epidemiology and Infection 131: 583-589.

Gould LH and Fikrig E (2004) West Nile virus: a growing concern? J Clin Invest 113:1102-1107.

Gratz NG (2004) Critical review of the vector status of *Aedes albopictus*. Med Vet Entomol 18: 215-227.

Greenwood BM (2008) Control to elimination: implications for malaria research. Trends in Parasitology 24: 449-454.

Greenwood BM, Fidock DA, Kyle DE, Kappe SHI, Alonso PL, Collins FH and Duffy PE (2008) Malaria: progress, perils, and prospects for eradication. J Clin Invest 118: 1266-1276.

Grieco JP, Achee NL, Sardelis MR, Chauhan KR and Roberts DR (2005) A novel high-throughput screening system to evaluate the behavioral response of adult mosquitoes to chemicals. J Am Mosq Control Assoc 21: 404-411.

Haathi EOA and Fales HM (1967) The uropygiols: identification of the unsaponifiable constituent of a diester wax from chicken preen glands. J Lipid Research 8: 131-137.

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

Haddow AJ and Ssenkubuge Y (1973) The mosquito of bwamba county, Uganda. IX. Further studies on the biting behaviour of an outdoor population of the *Anopheles gambiae* Giles complex. Bull Entomol Res 62:407-414.

Hallem EA, Dahanukar A and Carlson JR (2006) Insect odor and taste receptors. Annu Rev Entomol 51: 113-135.

Hallem EA, Fox AN, Zwiebel LJ and Carlson JR (2004) Olfaction - Mosquito receptor for human-sweat odorant. Nature 427: 212-213.

Hao H, Wei J, Dai J and Du J (2008) Host-seeking and blood-feeding behavior of *Aedes albopictus* (Diptera: Culicidae) exposed to vapors of geraniol, citral, citronellal, eugenol, or anisaldehyde. J Med Entomol 45: 533-539.

Harrington LC (2001) Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? J Med Entomol 38: 411-422.

Hasegawa Y, Yabuki M and Matsukane M (2004) Identification of new odoriferous compounds in human axillary sweat. Chem Biodivers 1: 2042-2050.

Hassanali A, Herren H, Khan ZR, Pickett JA and Woodcock CM (2008) Integrated pest management: the push–pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. Phil Trans R Soc B 363: 611-621.

Haze S, Gozu Y, Nakamura S, Khono Y, Sawano K, Ohta H and Yamzaki K (2001) 2-Nonenal newly found in human body odor tends to increase with aging. J Invest Dermatol 116: 520-524.

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

Healy TP and Copland MJW (1995) Activation of *Anopheles gambiae* mosquitoes by carbon dioxide and human breath. Med Vet Entomol 9: 33 1-336.

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

Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, Wincker P, Clark AG, Ribeiro JM, Wides R, Salzberg SL, Loftus B, Yandell M, Majoros WH, Rusch DB, Lai Z, Kraft CL, Abril JF, Anthouard V, Arensburger P,

Atkinson PW, Baden H, de Berardinis V, Baldwin D, Benes V, Biedler J, Blass C, Bolanos R, Boscus D, Barnstead M, Cai S, Center A, Chaturverdi K, Christophides GK, Chrystal MA, Clamp M, Cravchik A, Curwen V, Dana A, Delcher A, Dew I, Evans CA, Flanigan M, Grundschober-Freimoser A, Friedli L, Gu Z, Guan P, Guigo R, Hillenmeyer ME, Hladun SL, Hogan JR, Hong YS, Hoover J, Jaillon O, Ke Z, Kodira C, Kokoza E, Koutsos A, Letunic I, Levitsky A, Liang Y, Lin JJ, Lobo NF, Lopez JR, Malek JA, McIntosh TC, Meister S, Miller J, Mobarry C, Mongin E, Murphy SD, O'Brochta DA, Pfannkoch C, Qi R, Regier MA, Remington K, Shao H, Sharakhova MV, Sitter CD, Shetty J, Smith TJ, Strong R, Sun J, Thomasova D, Ton LQ, Topalis P, Tu Z, Unger MF, Walenz B, Wang A, Wang J, Wang M, Wang X, Woodford KJ, Wortman JR, Wu M, Yao A, Zdobnov EM, Zhang H, Zhao Q, Zhao S, Zhu SC, Zhimulev I, Coluzzi M, della Torre A, Roth CW, Louis C, Kalush F, Mural RJ, Myers EW, Adams MD, Smith HO, Broder S, Gardner MJ, Fraser CM, Birney E, Bork P, Brey PT, Venter JC, Weissenbach J, Kafatos FC, Collins FH and Hoffman SL (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298: 129-149.

Hurd H (2003) Manipulation of medically important insect vectors by their parasites. Annu Rev Entomol 48: 141-146.

Issachar D, Holland JF and Sweeley CC (1982) Metabolic profiles of organic acids from human plasma. Analytical Chemistry 54: 29-32.

Jawara M, Smallegange RC, Jeffries D, Nwakanma DC, Awolola TS, Knols BGJ, Takken W and Conway DJ (2009). Optimising odour baited trap methods for collecting mosquitoes during the malaria season in The Gambia. PLoS ONE 12: e8167.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL and Daszak P (2008) Global trends in emerging infectious diseases. Nature 451: 990-993.

Jordan AM (1986) Trypanosomiasis control and African rural development. Longman Group Ltd., New York, USA.

Justice RW, Biessmann H, Walter MF, Dimitratos SD and Woods DF (2003) Genomics spawns novel approaches to mosquito control; Bioassays 25: 1011-1020.

Kennedy JS (1978) The concepts of olfactory 'arrestment' and 'attraction'. Physiol. Entomol 3: 91-98.

Killeen G, Kihonda J, Lyimo E, Oketich F, Kotas M, Mathenge E, Schellenberg J, Lengeler C, Smith T and Drakeley C(2006) Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. BMC Infect. Dis. 6:161.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP and Daszak P (2006) West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol 4: e82.

Kline DL, Allan SA, Bernier UR and Posey KH (2006) Olfactometer and large cage evaluation of a solid phase technology for the controlled production of CO2. J Am Mosq Control Assoc 22: 378-381.

Kline DL (2006) Traps and trapping techniques for adult mosquito control. J Am Mosq Control Assoc 22: 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, Bernier UR, Posey KH, and Barnard DR (2003) Olfactometric evaluation of spatial repellents for *Aedes aegypti*. J Med Entomol 40: 463-467.

Klowden MJ (1986) Effects of sugar deprivation on the host-seeking behaviour of gravid *Aedes aegypti* mosquitoes. J Insect Physiol 32: 479-483.

Klowden MJ (1995) Blood, sex and mosquito. The mechanism that control mosquito blood feeding behavior. Bioscience 45: 326-331.

Klowden MJ (1996) Endogenous factors regulating mosquito host-seeking behaviour. In: Bock GR and Cardew G (eds) Olfaction in mosquito interactions. Wiley, Chichester, UK, pp 212-225.

Klowden and Lea (1979) Humoral inhibition of host-seeking in Aedes aegypti during oocyte maturation. J Insect Physiol 25: 231–235.

Klun JA, Kramer M, Debboun M: (2005) A new *in vitro* bioassay system for discovery of novel human-use mosquito repellents. J Am Mosq Control Assoc 21:64-70.

Knols BG, Njiru BN, Mathenge EM, Mukabana WR, Beier JC and Killeen GF (2002) MalariaSphere: A greenhouse-enclosed simulation of a natural *Anopheles gambiae* (Diptera: Culicidae) ecosystem in western Kenya. Malar J 1: 19.

Knols BG, Takken W and De Jong R (1994a) Influence of human breath on selection of biting sites by *Anopheles albimanus*. J Am Mosq Control Assoc 10: 423-426.

Knols BGJ, De Jong R and Takken W (1994b) Trapping system for testing olfactory responses of the malaria mosquito *Anopheles-gambiae* in a wind-tunnel. Med Vet Entomol 8: 386-388.

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

Knols BGJ, Takken W, Charlwood D and De Jong R (1995) Species-specific attraction of *Anopheles* mosquitoes (Diptera: Culicidae) to different humans in South Tanzania. Proceedings of Experimental and Applied Entomology, N.E.V., Amsterdam 6: 201-206.

Knols BGJ, Van Loon JJA, Cork A, Robinson RD, Adam W, Meijerink J, De Jong R and Takken W (1997) Behavioural and electrophysiological responses of the female malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) to Limburger cheese volatiles. Bull Entomol Res 87: 151-159.

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

Krotoszynski B, Gabriel G and O'Neill H (1977) Characterization of human expired air: A promising investigative and diagnostic technique. J Chromatogr Sci 15: 239-244.

Leal WS, Barbosa RMR, Xu W, Ishida Y, Syed Z, Latte N, Chen AM, Morgan TI, Cornel AJ and Furtado A (2008) Reverse and conventional chemical ecology approaches for the development of oviposition attractants for *Culex* mosquitoes. PLos ONE 3: e3045.

Lefèvre T, Gouagna L-C, Dabire KR, Elguero E, Fontenille D, Costantini C and Thomas F (2009) Evolutionary lability of odour-mediated host preference by the malaria vector *Anopheles gambiae.* Trop Med Int Health 14:228-236.

Lehane MJ (1991) Biology of blood-sucking insects. Harper Collins Academic, London, UK. 288 pp.

Leyden JJ, McGinley KJ, Holzle E, Labows JN and Kligman AM (1981) The microbiology of the human axilla and its relationship to axillary odor. J Investig Dermatol 77: 413-416.

Lindsay SW, Adiamah JH, Miller JE, Pleass RJ and Armstrong JR (1993) Variation in attractiveness of human subjects to 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 Manage 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.

Lu T, Qiu YT, Wang G, Kwon JY, Rutzler M, Kwon HW, Pitts RJ, Van Loon JJA, Takken W, Carlson JR and Zwiebel LJ (2007) Odor coding in the maxillary palp of the malaria vector mosquito *Anopheles gambiae*. Current Biology 17: 1533-1544.

Maxwell CA, Wakibara J, Tho S and Curtis CF (1998) Malaria-infective biting at different hours of the night. Med Vet Entomol 12:325-327.

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 Huisman PWT (1998) Olfactory responses of female *Culex quinquefasciatus* Say (Diptera: Culicidae) in a dual-choice olfactometer. J Vector Ecol 23: 107-113.

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

Mboera LEG and Takken W (1999) Odour-mediated host preference of *Culex quinquefasciatus* in Tanzania. Entomol Exp Appl 92: 83-88.

McCall PJ, Harding JR, Roberts J and Auty B (1996) Attraction and trapping of *Aedes aegypti* (Diptera: Culicidae) with host odors in the laboratory. J Med Entomol 33:177-179.

McCall PJ and Eaton G (2001) Olfactory memory in the mosquito *Culex quinquefasciatus*. Med Vet Entomol 15: 197-203.

McIver SB: Host preferences and discrimination by the mosquitoes *Aedes aegypti* and *Culex tarsalis* (Diptera: culicidae). J Med Entomol 1968, 5:422-428.

Mehr ZA, Rutledge LC, Buescher MD, Gupta RK and Zakaria MM (1990) Attraction of mosquitoes to diethyl methylbenzamide and ethyl hexanediol. J Am Mosq Control Assoc 6: 469-476.

Meijerink J, Braks MAH, Brack AA, Adam W, Dekker T, Posthumus MA, Van Beek TA and Van Loon JJA (2000) Identification of olfactory stimulants for *Anopheles gambiae* from human sweat samples. J Chem Ecol 26: 1367-1382.

Morrison AC, Zielinski-Gutierrez E, Scott TW and Rosenberg R (2008) Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. Plos Med 5: e68.

Mukabana WR, Takken W, Coe R and Knols BG (2002) Host-specific cues cause differential attractiveness of Kenyan men to the African malaria vector *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: 8.

Muturi EJ, Muriu S, Shililu J, Mwangangi JM, Jacob BG, Mbogo C, Githure J and Novak RJ (2008) Blood-feeding patterns of *Culex quinquefasciatus* and other culicines and implications for disease transmission in Mwea rice scheme, Kenya. Parasitol Res 102: 1329-1335.

Natsch A, Derrer S, Flachsmann F and Schmid J (2006) A broad diversity of volatile carboxylic acids, released by a bacterial aminoacylase from axilla secretions, as candidate molecules for the determination of human-body odor type. Chem Biodivers 3: 1-20.

Nene V, Wortman JR, Lawson D, Haas B, Kodira C, Tu ZJ, Loftus B, Xi Z, Megy K, Grabherr M, Ren Q, Zdobnov EM, Lobo NF, Campbell KS, Brown SE, Bonaldo MF, Zhu J, Sinkins SP, Hogenkamp DG, Amedeo P, Arensburger P, Atkinson PW, Bidwell S, Biedler J, Birney E, Bruggner RV, Costas J, Coy MR, Crabtree J, Crawford M, Debruyn B, Decaprio D, Eiglmeier K, Eisenstadt E, El-Dorry H, Gelbart WM, Gomes SL, Hammond M, Hannick LI, Hogan JR, Holmes MH, Jaffe D, Johnston JS, Kennedy RC, Koo H, Kravitz S, Kriventseva EV, Kulp D, Labutti K, Lee E, Li S, Lovin DD, Mao C, Mauceli E, Menck CF, Miller JR, Montgomery P, Mori A, Nascimento AL, Naveira HF, Nusbaum C, O'Leary S, Orvis J, Pertea M, Quesneville H, Reidenbach KR, Rogers YH, Roth CW, Schneider JR, Schatz M, Shumway M, Stanke M, Stinson EO, Tubio JM, Vanzee JP, Verjovski-Almeida S, Werner D, White O, Wyder S, Zeng Q, Zhao Q, Zhao Y, Hill CA, Raikhel AS, Soares MB, Knudson DL, Lee NH, Galagan J, Salzberg SL, Paulsen IT, Dimopoulos G, Collins FH, Birren B, Fraser-Liggett CM and Severson DW (2007) Genome sequence of *Aedes aegypti*, a major arbovirus vector. Science 316: 1718-1723.

Nicolaides N, C. FH and Rice GR (1968) The skin surface lipids of man compared with those of eighteen species of animals. J. Investigative Dermatology 51: 83-89.

Njiru BN, Mukabana WR, Takken W and Knols BG (2006) Trapping of the malaria vector *Anopheles gambiae* with odour-baited MM-X traps in semi-field conditions in western Kenya. Malar 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 (2010) Development and field evaluation of a synthetic mosquito lure that is more attractive than humans. PLoS ONE 5: e8951.

Pates HV, Takken W and Curtis CF (2005) Laboratory studies on the olfactory behaviour of *Anopheles quadriannulatus*. Entomol Exp Appl 114: 153-159.

Pates HV, Takken W, Curtis CF, Huisman PW, Akinpelu O and Gill GS (2001a) Unexpected anthropophagic behaviour in *Anopheles quadriannulatus*. Med Vet Entomol 15: 293-298.

Pates HV, Takken W, Stuke K and Curtis CF (2001b) Differential behaviour of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) to human and cow odours in the laboratory. Bull Entomol Res 91: 289-296.

Pelletier J and Leal WS (2009) Genome analysis and expression patterns of odorant-binding proteins from the southern house mosquito *Culex pipiens quinquefasciatus*. PLoS ONE 4: e6237.

Penn DJ, Oberzaucher E, Grammer K, Fischer G, Soini HA, Wiesler D, Novotny MV, Dixon SJ, Xu Y and Brereton RG (2007) Individual and gender fingerprints in human body odour. J R Soc Interface 4: 331-340.

Perry TL, Hansen S, Diamond S, Bullis B, Mok C and Melançon SB (1970) Volatile fatty acids in normal human physiological fluids. Clin Chim Acta 29: 369-374.

Philips M (1997) Method for the collection and assay of volatile organic compounds in breath. Anal Biochem 247: 272-278.

Posey KH, Barbard DR and Schreck CE (1998) Triple cage olfactometer for evaluating mosquito (Diptera: Culicidae) attraction responses. J Med Entomol 35: 330-334.

Puri SN, Mendki MJ, Sukumaran D, Ganesan K, Prakash S and Sekhar K (2006) Electroantennogram and behavioral responses of *Culex quinquefasciatus* (Diptera: Culicidae) females to chemicals found in human skin emanations. J Med Entomol 43: 207-213.

Qiu YT (2005) Sensory and behavioural responses of the malaria mosquito *Anopheles gambiae* to human odours. PhD thesis, Wageningen University, Wageningen, the Netherlands, 208 pp.

Qiu YT, Smallegange RC, Hoppe S, Van Loon JJA, Bakker EJ and Takken W (2004a) Behavioural and electrophysiological responses of the malaria mosquito *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae) to human skin emanations. Med Vet Entomol 18: 429-438.

Qiu YT, Smallegange RC, Smid H, Van Loon JJA, Galimard AMS, Posthumus MA, Beek TAv and Takken W (2004b) GC-EAG analysis of human odours that attract the malaria mosquito *Anopheles gambiae sensu strict*. Proc Exp Appl Ent, N.E.V., Amsterdam 15: 59-64.

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

Qiu YT, Spitzen J, Smallegange RC and Knols BGJ. (2007b). Monitoring systems for adult insect pests and disease vectors. In: Takken W and Knols BGJ (eds) Emerging pests and vector-borne diseases in Europe. Ecology and control of vector-botne diseases, vol. 1. Wageningen Academic Publishers, Wageningen, the Netherlands, pp 329-354.

Qiu YT, Smallegange RC, Van Loon JJA, Ter Braak CJF and Takken W (2006a) Interindividual variation in the attractiveness of human odours to the malaria mosquito *Anopheles gambiae s. s.* Med Vet Entomol 20: 280-287.

Qiu YT, van Loon JJA, Takken W, Meijerink J and Smid HM. 2006b. Olfactory coding in antennal neurons of the malaria mosquito, *Anopheles gambiae*. Chem Senses 31:845-863.

Richards SL, Ponnusamy L, Unnasch TR, Hassan HK and Apperson CS (2006) Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to availability of human and domestic animals in suburban landscapes of central North Carolina. J Med Entomol 43: 543-551.

Robertson HM and Wanner KW (2006) The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. Genome Res 16: 1395-1403.

Rudolfs W (1922) Chemotropism of mosquitoes. Bull NJ Agric Exp Stn 367: 4-23.

Rutledge LC and Gupta RK (2004) Evaluation of an *in vitro* bloodfeeding system for testing mosquito repellents. J Am Mosq Control Assoc 20: 150-154.

Rutzler M and Zwiebel LJ (2005) Molecular biology of insect olfaction: recent progress and conceptual models. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191: 777-790.

Sardelis MR, Turell MJ, Dohm DJ and O'Guinn ML (2001) Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. Emerging Infectious Diseases 7: 1018-1022.

Sastry SD, Buck KT, Janak J, Dressler M and Preti G (1980) Volatiles emitted by humans. In: Waller GR and Dermer OC (eds) Biochemical applications of mass spectrometry. John Wiley and Sons, New York, USA, pp 1085-1127.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK, Anderson M, Charnetzky D, McMillen L, Unnasch EA and Unnasch TR (2007) Host choice and West Nile virus infection rates in blood-fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis and Shelby County, Tennessee, 2002-2003. Vector Borne Zoonotic Dis 7: 365-386.

Schaub GA (2006) Parasitogenic alterations of vector behaviour. Int J Med Microbiol 296: 37-40.

Schofield S and Brady J (1997) Effects of carbon dioxide, acetone and 1-octen-3-ol on the flight responses of the stable fly, *Stomoxys calcitrans*, in a windtunnel. Physiol Entomol 22: 380-386.

Schofield S, Witty C and Brady J (1997) Effects of carbon dioxide, acetone and 1-octen-3-ol on the activity of the stable fly, *Stomoxys calcitrans*. Physiol Entomol 22: 256-260.

Schofield S, Cork A and Brady J (1995) Electroantennogram responses of the stable fly, *Stomoxys calcitrans*, to components of host odor. Physiol Entomol 20: 273-280.

Schmied WH, Takken W, Killeen GF, Knols BGJ 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.

Schreck CE, Kline DL and Carlson DA (1990) Mosquito attraction to substances from the skin of different humans. J Am Mosq Control Assoc 6: 406-410.

Schreck CE, Smith N, Carlson DA, Price GD, Haile D and Godwin DR (1981) A material isolated from human hands that attracts female mosquitoes. J Chem Ecol 2: 429-438.

Scott TW, Githeko AK, Fleisher A, Harrington LC and Yan GY (2006) DNA profiling of human blood in anophelines from lowland and highland sites in western Kenya. Am J Trop Med Hyg 75: 231-237.

Shelley WWB, Hurley HHJ Jr., Nichols AAC (1953) Axillary odor; experimental study of the role of bacteria, apocrine sweat, and deodorants. *AMA Arch Derm Syphilol* 68: 430-446.

Shirai Y, Kamimura K, Seki T and Morohashi M (2001) L-lactic acid as a mosquito (Diptera: Culicidae) repellent on human and mouse skin. J Med Entomol 38: 51-54.

Silva IM, Eiras AE, Kline DL and Bernier UR (2005) Laboratory evaluation of mosquito traps baited with a synthetic human odor blend to capture *Aedes aegypti*. J Am Mosq Control Assoc 21: 229-233.

Smallegange RC, Geier M and Takken W (2002) Behavioural responses of *Anopheles gambiae* to ammonia, lactic acid and a fatty acid in a y-tube olfactometer. Proc Exp Appl Ent, N.E.V., Amsterdam 13: 147-152.

Smallegange RC, Knols BGJ and Takken W (2010) Effectiveness of synthetic versus natural human volatiles as attractants for *Anopheles gambiae* (Diptera: Culicidae) *sensu stricto*. J Med Entomol 47: 338-344.

Smallegange RC, Qiu YT, Bukovinszkiné-Kiss G, Van Loon JJA and Takken W (2009) The effect of aliphatic carboxylic acids on olfaction-based host-seeking of the malaria mosquito *Anopheles gambiae sensu stricto*. J Chem Ecol 35: 933-943.

Smallegange RC, Qiu YT, Galimard AMS, Posthumus MA, Van Beek TA, Van Loon JJA and Takken W (2003) Why humans are attractive to malaria mosquitoes. Entomol Berichten 63: 50-53.

Smallegange RC, Qiu YT, Van Loon JJA 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.

Smith CN, Smith N, Gouck HK, Weidhaas DE, Gilbert IH, Mayer MS, Smittle BJ and Hofbauer A (1970) L-Lactic acid as a factor in the attraction of *Aedes aegypti* (Diptera: Culicidae) to human hosts. Ann Entomol Soc Am 63: 760-770.

Spinhirne JP, Koziel JA and Chirase NK (2003) A Device for Non-invasive On-site Sampling of Cattle Breath with Solid-Phase Microextraction. Biosystems Engineering 84: 239–246.

Spinhirne JP, Koziel JA and Chirase NK (2004) Sampling and analysis of volatile organic compounds in bovine breath by solid-phase microextraction and gas chromatography-mass spectrometry. J Chromatogr A 1025: 63-69.

Spitzen J, Smallegange RC and Takken W (2008) Effect of human odours and positioning of CO2 release point on trap catches of the malaria mosquito *Anopheles gambiae sensu stricto* in an olfactometer. Physiol Entomol 33: 116-122.

Strickman D (2007) Area repellent products. In: Debboun M, Frances SP and Strickman D (eds) Insect repellents. Principles, methods, and uses. CRC Press, Boca Raton, FL, USA, pp 385-395.

Syed Z and Leal WS (2008) Mosquitoes smell and avoid the insect repellent DEET. PNAS 105: 13598-13603.

Syed Z and Leal WS (2009) Acute olfactory response of *Culex* mosquitoes to a human- and bird-derived attractant. PNAS 106: 18809-18814

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

Takken W (1996) Synthesis and future challenges: the response of mosquitoes to host odours. In: Bock GR and Cardew G (eds) Olfaction in mosquito host interactions. John Wiley and Sons Ltd, Chichester, UK, pp 302-320.

Takken W, Dekker T and Wijnholds YG (1997) Odor-mediated flight behavior of *Anopheles gambiae* Giles *sensu stricto* and *An. stephensi* Liston in response to CO2, acetone, and 1-Octen-3-ol (Diptera: Culicidae). J Insect Behav 10: 395-407.

Takken W, Eling W, Hooghof JDT, Hunt R and Coetzee M (1999) Susceptibility of *Anopheles quadriannulatus* Theobald (Diptera: Culicidae) to *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 93: 578-580.

Takken W and Kline DL (1989) Carbon dioxide and 1-octen-3-ol as mosquito attractants. J Am Mosq Control Assoc 5: 311-316.

Takken W and Knols BGJ (1999) Odor-mediated behavior of afrotropical malaria mosquitoes. Ann Rev Entomol 44: 131-157.

Takken W and Knols BGJ (2007) Emerging pests and vector-borne diseases in Europe. Ecology and control of vector-borne diseases Vol. 1. Wageningen Academic Publishers, Wageningen, the Netherlands, 499 pp.

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

Takken W, Van Loon JJA and Adam W (2001) Inhibition of host-seeking response and olfactory responsiveness in *Anopheles gambiae* following blood feeding. J Insect Physiol 47: 303-310.

Tomberlin J, Rains G, Allan S, Sanford M and Lewis W (2006) Associative learning of odor with food- or blood-meal by *Culex quinquefasciatus* Say (Diptera: Culicidae). Naturwissenschaften 93:551-556.

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.

Torr SJ, Hall DR and Smith JL (1995) Responses of tsetse flies (Diptera: Glossinidae) to natural and synthetic ox odours. Bull Entomol Res 85: 157-166.

Turell MJ, Dohm DJ, Sardelis MR, guinn ML, Andreadis TG and Blow JA (2005) An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J Med Entomol 42: 57-62.

Vale GA (1980) Field studies of the responses of tsetse flies (Glossinidae) and other Diptera to carbon dioxide, acetone and other chemicals. Bull Entomol Res 70: 563-570.

Vale GA (1993) Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. J Med Entomol 30: 831-842.

Vale GA and Hall DR (1985b) The use of 1-octen-3-ol, acetone and carbon dioxide to improve baits for tsetse flies, *Glossina* spp. (Diptera: Glossinidae). Bull Entomol Res 75: 219-231.

Vale GA and Hall DR 1985a The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to ox odour. Bull Entomol Res 75: 209-217.

Vale GA, Hall DR and Gough AJE (1988) The olfactory responses of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to phenols and urine in the field. Bull Entomol Res 78: 293-300.

Van der Goes van Naters W and Carlson JR (2006) Insects as chemosensors of humans and crops. Nature 444: 302-307.

Van Thiel P (1937) Quelles sont les excitations incitant l'*Anopheles maculipennis atroparvus* a visiter et a piquer l'homme ou le batail? Bulletin Soc Path Exot 30: 193-203.

Vazeille M, Jeannin C, Martin E, Schaffner F and Failloux AB (2008) Chikungunya: a risk for Mediterranean countries? Acta Trop 105: 200-202.

Verhulst NO, Beijleveld H, Knols BGJ, Takken W, Schraa G, Bouwmeester HJ and Smallegange RC (2009). Cultured skin microbiota attracts malaria mosquitoes. Malar J 8: 302.

Waka M, Hopkins RJ, Glinwood R and Curtis C (2006) The effect of repellents *Ocimum forskolei* and deet on the response of *Anopheles stephensi* to host odours. Med Vet Entomol 20: 373-376.

Wang G, Carey AF, Carlson JR and Zwiebel LJ (in press) The molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. PNAS (in press).

Wang ZY, Mo JC and Zhang SM (2006) Laboratory and field evaluations of potential human host odors for *Aedes albopictus* Skuse (Diptera: Culicidae). J Agric Urban Entomol 23: 57-64.

Waterhouse RM, Wyder S and Zdobnov EM (2008) The *Aedes aegypti* genome: a comparative perspective. Insect Mol Biol 17: 1-8.

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

White GB (2007) Terminology of insect repellents. In: Debboun M, Frances SP and Strickman D (eds) Insect repellents. Principles, methods, and uses. CRC Press, Boca Raton, FL, USA, pp 31-46.

WHO (2009) Malaria. WHO factsheet No 94. World Heath Organisation, Geneva, Switzerland.

WHO (2008) Dengue and dengue haemorrhagic fever. WHO factsheet No 117. World Heath Organisation, Geneva, Switzerland.

Williams CR, Bergbauer R, Geier M, Kline DL, Bernier UR, Russell RC and Ritchie SA (2006a) Laboratory and field assessment of some kairomone blends for host-seeking *Aedes aegypti*. J Am Mosq Control Assoc 22: 641-647.

Williams CR, Kokkinn MJ and Smith BP (2003) Intraspecific Variation in odor-mediated host preference of the mosquito *Culex annulirostris*. J Chem Ecol 29: 1889-1903.

Williams CR, Long SA, Russell RC and Ritchie SA (2006b) Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. J Am Mosq Control Assoc 22: 296-300.

Williams CR, Ritchie SA, Russell RC, Eiras AE, Kline DL and Geier M (2006c) Geographic variation in attraction to human odor compounds by *Aedes aegypti* mosquitoes (Diptera: Culicidae): a laboratory study. J Chem Ecol 32: 1625-1634.

Young S, Hardie J and Gibson G (1993) Flying insects in the laboratory. In: Wratten SD (ed) Video techniques in animal ecology and behaviour. Chapman and Hall, London, UK, pp 17-32.

Zeng C, Leyden JJ, Spielman AI, and Preti G (1996) Analysis of characteristic human female axillary odors: qualitative comparison to males. J Chem Ecol 22: 237-257.

Zeng XN, Leyden JJ, Lawley HJ, Sawano K, Nohara I and Preti G (1991) Analysis of characteristic odors from human male axillae. J Chem Ecol 17: 1469-1493.

Zinser M, Ramberg F and Willott E (2004) *Culex quinquefasciatus* (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: blood meal analysis indicates feeding on both humans and birds. J Insect Sci 4: 20.

Zwiebel LJ and Takken W (2004) Olfactory regulation of mosquito-host interactions. Insect Biochem Mol Biol 34: 645-652.