Review

Semiochemicals for biting fly control: their identification and exploitation

James G Logan* and Michael A Birkett
Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Abstract: Insects that feed on the blood of vertebrates are difficult to control, and many previous efforts have been unsuccessful. This is becoming an ever increasing issue, not only in developing countries, but also in developed countries, as exemplified by the recent spread of West Nile virus by mosquitoes across the USA and recent dengue outbreaks in Singapore and Australia. Investigating the ways in which biting insects interact with each other, their environment and their hosts is providing valuable knowledge that will lead to the development of improved control technologies. For instance, recent advances in chemical ecology research have led to the identification of new semiochemicals that show great potential as control agents against biting insects. Exciting new chemical ecology tools and control technologies for the future are discussed.

Keywords: semiochemical; pheromone; olfaction; haematophagous; pest control

1 INTRODUCTION

The ability of some insects to transmit pathogens that cause disease was first confirmed in 1878 when the filarial worm, *Wucheria bancroftii* Cobbold, the causative organism of filariasis, was found to develop inside the mosquito, *Culex quinquefasciatus* Say.[1] Presently, many insects are of great medical and veterinary importance owing to their ability during the feeding process to vector (transmit) pathogens that cause infectious diseases in both human beings and livestock. Their irritating bites and the pathogens they transmit cause devastating loss of human and animal life and a significant cost to the economy of many countries. Presently, 14 000 species of biting insects are known to feed on the blood of vertebrates, and 300–400 of these feed on human beings.[2,3]

Although drugs have been developed to prevent or treat some insect-borne diseases, for others there are no vaccines or treatments. However, the most successful way to combat such diseases is to shorten the lifespan of the insect or prevent or suppress the interaction between the insects carrying the pathogen and their vertebrate host. Currently, such control methods include the use of insect repellents, trapping systems, larvicides and insecticides, which may often be used in conjunction with bed nets. However, owing to the sheer abundance of haematophagous (blood-feeding) insects, their excellent reproductive abilities, complex behaviours associated with finding blood and, for some, their ability to develop resistance against insecticides, complete control of such insects is difficult to achieve. Consequently, many control attempts fail to make a significant impact on populations using current methods.

During the location of a suitable host, haematophagous insects mainly use olfactory and visual cues. However, the detection of olfactory cues that emanate from the bodies and breath of vertebrate hosts dominates during this process. Many of these chemicals, which can also be called semiochemicals (behaviour- and physiology-modifying chemicals), are kairomones or attractants, but in some cases they can also be repellents. Owing to their widespread occurrence in nature, these semiochemicals are valuable tools and are rapidly becoming recognised as having the potential to improve on or exceed current control methods.

The aim of this review is to describe how semiochemical research, particularly on host-derived cues and insect pheromones, can be exploited to control biting insects, and it will mainly focus on dipterous flies as they represent the major group of insect disease vectors. The authors also explain how understanding the host location process, in particular the differential attractiveness of individual hosts to biting flies, is leading to the identification of useful semiochemicals. Finally, current methods used in semiochemical identification are described, as well as future prospects including new and emerging technologies for monitoring and control.

* Correspondence to: James G Logan, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
E-mail: james.logan@bbsrc.ac.uk
(*Received 29 September 2006; revised version received 20 February 2007; accepted 10 April 2007*)
Published online 4 June 2007; DOI: 10.1002/ps.1408

2 INSECTS AS VECTORS OF HUMAN AND ANIMAL DISEASES

Vector-borne disease (disease spread by vector insects) transmission in the human population and in farmed animals mainly involves members of the arthropod class Insecta. These insects predominantly comprise dipterous flies, including the families Culicidae, Muscidae, Ceratopogonidae, Glossinidae and Psychodidae. Mosquitoes (Diptera: Culicidae) act as vectors of pathogens of diseases such as malaria, dengue fever, dengue haemorrhagic fever and yellow fever, and are also responsible for the transmission of epidemic polyarthritis and several forms of encephalitis and filariasis.[4,5] The most famous and well-studied mosquito is the malaria mosquito, *Anopheles gambiae* Giles, which transmits the malaria parasite, *Plasmodium falciparum* (Welch), killing one child every 30 s. Midges (Diptera: Ceratopogonidae) of the genus *Culicoides* are vectors of livestock arboviruses, including blue tongue virus (BTV), African horse sickness virus (AHSV) and epizootic haemorrhagic disease (EHD) of deer.[6–10] Some species also vector the filarial nematode *Onchocerca cervicalis* (Railliet & Henry), which affects horses,[11] or cause 'sweet itch', which is not a disease but a distressing skin condition of horses caused by midge saliva.[12,13] Phlebotomine sandflies (Diptera: Psychodidae) spread protozoa belonging to the genus *Leishmania*, with approximately 12 million people affected by leishmaniasis worldwide. Tsetse flies (Diptera: Glossinidae) vector the *Trypanosoma* protozoa responsible for trypanosomiasis, or sleeping sickness, in both animals and human beings in sub-Saharan Africa, and this disease currently threatens over 60 million people in 36 countries of sub-Saharan Africa (WHO, 2006, http://www.who.int/mediacentre/factsheets/fs259/en/). Other haematophagous insects that bite farmed animals, such as head flies and horn flies (Diptera: Muscidae), also cause significant economic losses through disease incidence including myiasis, mastitis and pink eye conjunctivitis, and by causing reduced growth, milk production and fecundity through irritation.

There has been an increase in insect-borne disease prevalence in recent years, which means that the control of biting insects is becoming ever more important. Previously, control efforts have been aimed at developing countries, but, while these efforts must continue, attention is also required closer to home, as The Chief Medical Officer, Liam Donaldson, highlights in his recent consultative document on preparing a UK contingency plan for vector control, 'Emerging mosquito-borne diseases', and in the recent UK government Foresight project on 'Detection and Identification of Infectious Diseases' (http://www.foresight.gov.uk/Previous_Projects/Detection_and_Identification_of_Infectious_Disease.html). Increase in global travel, along with climate change and urbanisation, means that vector-borne disease prevalence in the developed world is also likely to increase. For example, West Nile virus has spread across the entirety of the USA[14] and is now also considered a threat to the UK, so that the UK Health Protection Agency (HPA) has set out a contingency plan to enhance surveillance, alert clinicians and control mosquito populations should the UK acquire an incursion of West Nile Virus (HPA, 2006, http://www.hpa.org.uk/infections/topics_az/west_nile/menu.htm). Also, dengue fever reappeared in Queensland, Australia, in the 1980s, and, although it is not considered to be endemic, there were several intensive outbreaks in 2003 and 2004.[15] There was also a severe outbreak of dengue in Singapore in 2005.[16] More recently, the Asian tiger mosquito, *Aedes albopictus* (Skuse), a carrier of dengue fever, was found in imported bamboo in a greenhouse in the Netherlands (reported by Willem Takken at the Society of Chemical Industry, London, 2005). In addition to this, climatic change since 1998 has resulted in six strains of bluetongue virus spreading across 12 European countries and 800 km further north in Europe than has previously been reported.[10] These recent developments in vector-borne disease incidence highlight the need for continuing research and deployment of control strategies in all types of environment and in many developing and developed countries.

3 BITING INSECTS AND SEMIOCHEMICALS

To exploit semiochemicals in control technologies, an understanding of the types of compound that are likely to be of use and how target insects interact with such compounds at the behavioural and physiological level is vital.

3.1 Insect olfaction

Haematophagous insects have a highly developed olfactory system and mainly use their antennae and, in some cases, maxillary palps, to detect semiochemicals. Semiochemicals can provide information about the location, suitability or physiological state of conspecifics, hosts or breeding sites.[17,18] Semiochemicals enter through pores on the sensilla on the antennae or maxillary palps, where they are transported across the sensillum lymph by odorant binding proteins (OBPs) to the olfactory receptors on the dendrites of the olfactory receptor neurones (ORNs).[18,19] The ORNs can have extreme sensitivity and specificity, particularly with pheromones, and, when stimulated, input information directly into the central nervous system, which induces a behavioural response in the insect.[19,20] Insect olfactory systems are sensitive not only to specific molecular structures but also to ubiquitous compounds and have sophisticated mechanisms such as coincidence detection, whereby blends of volatiles in specific ratios from a host plant are detected by insects within a complex background of volatiles from non-host plants. This might be facilitated by paired or clustered ORNs that allow fine-scale resolution of such complex signals. Odours from a host are

Pest Manag Sci **63**:647–657 (2007)
DOI: 10.1002/ps

likely to occur in 'pockets' emanating from a host, and therefore receptor cells that respond simultaneously may indicate the presence of a host, whereas the same receptor cells being stimulated with a delay would not.[21] Although this hypothesis has only been described for phytophagous (plant-feeding) insects thus far, coincidence detection might also occur in biting flies, considering the complexity of odours within their environments.

Olfactory stimuli are used by biting insects during various stages of their life cycle and thus offer more than one potential stage at which to apply control methods. During host location, mainly chemical stimuli, provided by vertebrate hosts, induce a series of behaviours that lead to the insect obtaining a blood meal.[22,23] Attaining a clear picture of the chemical basis for this process is a difficult task owing to the complexity of vertebrate odours. For example, the human body produces somewhere in the region of 300–400 volatile chemicals.[23] However, a good understanding of olfactory-mediated behaviour is crucial for the development of control strategies using host-derived semiochemicals.

Olfactory cues that stimulate upwind movement by orientation to the wind (odour-mediated anemotaxis) are thought to dominate during host location and thus offer great potential for exploitation as lures in traps.[24–27] As a result, many investigations have focused on the identification of such cues, also known as kairomones, from vertebrate hosts. These volatile kairomones mediate interspecific communication, where the responding insects gain a behavioural or physiological advantage, while the kairomone-emitting host does not. Many kairomones may play a major role in the 'host-seeking' process of haematophagous insects. Kairomones can emanate from various locations on the vertebrate, including breath, skin (which may include gland secretions and breakdown products of microorganisms), urine and faeces.

3.2 Semiochemical identification

Once an understanding is gained of whether semiochemicals are used by an insect in the relevant context, the compounds must be identified. This can be difficult, but various techniques have been developed to facilitate the process. Semiochemical isolation methods include solvent washing, solid-phase microextraction (SPME), vacuum distillation and air entrainment. Such methods have been used in the past to collect pheromones, plant volatiles and vertebrate volatiles.[28,29] Subsequent analytical chemistry techniques, such as high-resolution gas chromatography (GC) and coupled GC–mass spectrometry (GC-MS) can be applied to quantify and identify accurately compounds of interest. Combining GC with electroantennography (GC-EAG) or single-cell recordings (GC-SRC) provides a unique way of detecting individual, physiologically relevant (i.e. EAG-active) components within a complex array of chemicals in natural extracts. The detected compounds can then be tentatively identified by GC-MS and confirmed by GC coinjection with authentic samples,[30] and, where necessary, by nuclear magnetic spectroscopy (NMR).[31] Once identified, authentic samples of chemicals, either obtained from commercial sources or by chemical synthesis, are used to confirm both electrophysiological and behavioural activity.

The techniques described above, along with laboratory and field-based behavioural assays, can identify semiochemicals that can be exploited to control and monitor vector insects, as well as enhance the knowledge and understanding of their behaviour and evolution. The majority of semiochemicals with potential applications can be obtained from commercial sources and via chemical synthesis from fine chemical precursors. In the latter instance, however, cheap and efficient synthesis is sometimes difficult to achieve, and higher plants offer alternative cheap and renewable resources for semiochemical production, especially in resource-poor afflicted countries. For example, (*Z*)-5-hexadecenoic acid, a precursor to the *Cx. quinquefasciatus* oviposition pheromone (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, can be obtained from seed oil of a renewable plant resource, *Kochia scoparia* (L.) Roth (Chenopodiaceae).[32–34] The pheromone can be obtained efficiently from the precursor at a cost of $\$3\,g^{-1}$, which compares favourably with conventional synthetic materials, an example of whose production[35] has been costed at $\$15\,g^{-1}$.[33] Similarly, the sandfly pheromone, (*S*)-9-methylgermacrene-B, can be synthesised from germacrone, a major component of *Geranium macrorrhizum* L. (Geraniaceae) essential oil.[36] Although not directly related to biting fly chemical ecology, the most advanced example of plant-based semiochemical production is the aphid sex pheromone, (4a*S*, 7*S*, 7a*R*)-nepetalactone, which can be obtained by steam distillation from *Nepeta cataria* L. (Lamiaceae) plants at a cost 1000 times cheaper than its production from fine chemicals.[37] Although availability of semiochemicals will facilitate the development of control strategies, registration is also an important issue in the implementation of semiochemicals in control technologies, and can prove to be an expensive process. As a result, it can be difficult to exploit semiochemicals, especially those that are relevant to small markets (such as species-specific semiochemicals).

3.3 Using semiochemicals in traps

Traps can be used for monitoring populations of vector insects and are important for several reasons. Firstly, they provide early warning of insect activity to local authorities, which can facilitate rapid control measures and warnings to be issued. Secondly, they provide a better understanding of the relationship between arboviruses, vectors and their environment, which can aid and maximise control attempts. The development of trapping systems that utilise semiochemicals as lures depends upon the identification of behaviourally active compounds.[38] Such traps are relatively new for management of adult mosquito populations.[39] Currently, trapping systems baited with carbon dioxide are most widely used for monitoring and control. However, trapping systems that incorporate other volatile chemicals have been developed to include host-derived volatile compounds, pheromones and oviposition site cues. These traps have been mainly used to catch mosquitoes and *Culicoides* spp. biting midges and are described below.

The Centre for Disease Control (CDC) light trap attracts mosquitoes by emitting light (and sometimes carbon dioxide) and can be used in conjunction with human-baited bed nets.[40] Counterflow geometry (CFG) traps, originally developed by American Biophysics Corporation (ABC), are used to sample mosquitoes, and are efficient at trapping many species using various baits[41] such as (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, the *Cx. quinquefasciatus* oviposition pheromone,[42,43] grass infusions that are associated with breeding sites[44] and host odours such as carbon dioxide and 1-octen-3-ol.[45] The host odour compounds are used with great success to trap both mosquitoes and midges, and ABC now markets various models of the CFG trap (Mosquito Magnet®) globally. The suction motor on the trap is powered by burning butane, which conveniently also generates carbon dioxide to form part of an attractive lure alongside a racemic mixture of 1-octen-3-ol. Human landing catches (HBCs) have also been used successfully, and HBCs can be a reliable method for monitoring mosquito populations.[46,47] However, this method is now ethically unacceptable owing to the pathogen transmission potential. Alternatively, odour-baited entry traps[47–50] or light traps used in conjunction with bed nets can be used to evaluate the extent of contact between mosquitoes and human beings.[51]

Host-derived volatile semiochemicals, which have the potential to improve the efficacy of targets and traps for tsetse flies, have been identified from excretory products such as urine, dung or breath, or glandular secretions and exudates from hosts. Carbon dioxide, acetone and 1-octen-3-ol, which are present in exhaled ruminant breath, are known to be attractive,[52–55] and multicomponent baits including these compounds, along with butanone, 4-methylphenol and propylphenol, have been shown to be effective in trapping populations in the field.[56–58] Reports suggested that these baits are less than 50% as attractive as natural cattle odour when released at naturally occurring concentrations, implying that other unidentified attractive components of the host odour need to be identified.[56,59] However, by increasing their release to 10–100 times their natural dose, tsetse trap catches can be increased 20-fold.[56,59]

Tsetse fly attractants have also been evaluated for their ability to trap other Dipterous pests in the Muscidae, Tabanidae and Ceratopogonidae. For the stable fly *Stomoxys calcitrans* L., olfactory and behavioural responses to 1-octen-3-ol, acetone and cattle odour were observed.[60–63] 1-Octen-3-ol has also been shown to increase catches of *Stomoxys* spp.[64] Female *Haematococcus pluvialis* Flotow, *Tabanus bromius* L., *T. maculicornis* Zetterstedt, *Chrysops relictus* Meigen and *Hybomitra bimaculata* Macquart were also collected in significantly higher numbers in traps baited with 1-octen-3-ol than in traps baited with acetone or ammonium hydroxide, and some species were also attracted to a combination of acetone and aged horse urine.[65,66]

The traps described here are valuable tools for monitoring populations of insect vectors and can provide localised control of vector populations.[41,67] However, a trap that matches or exceeds the ability to lure as many insects as a natural vertebrate host does not exist, and most traps up until now have used carbon dioxide as part of the lure, which can be problematic in remote field locations. However, a new trap called the BioGents Sentinel trap, which does not use carbon dioxide, was recently developed and has been demonstrated to be extremely effective in catching mosquitoes (particularly *Aedes* sp.) many times. This trap releases lactic acid, ammonia and a fatty acid over a larger surface area and mimics convection currents from a human body.[67–72]

3.4 Semiochemicals and differential attraction

Biting insects may use several semiochemicals during the location of a suitable host. The numerous compounds that are known to play a role are not discussed here, but are summarised in Table 1. Instead, this section will focus on a particular aspect of host location that has received much attention recently. Many semiochemicals have recently been identified that are thought to be involved in the differential attraction of biting insects to individual vertebrate hosts. Research indicates that the host location process also involves the detection of repellents or 'non-host' compounds as well as attractants, especially during the discrimination between different hosts.[23,28,73–75] Many biting insects show preferences for certain host species and even individuals within a host species. By understanding this complex interaction, researchers have uncovered new semiochemicals with potential control applications.

Many studies have demonstrated that haematophagous insects show feeding preferences for certain host species, which has given rise to specialist and generalist species.[75,76] For example, recent studies are starting to elucidate the semiochemical basis of the natural differential attractiveness of certain vertebrate species by tsetse flies. The preference of *Glossina mortisans mortisans* Westwood and *G. pallipides* Austen for buffalo, *Syncerus caffer* (Sparrmann), and ox hosts, compared with the non-host waterbuck, *Kobus defassa* Rüppel, is caused by fewer aldehydes and more phenolic components, octalactone and a series of methyl ketones (C_8–C_{13}) in the non-hosts, which were not detected or only present in trace amounts in the two preferred hosts.[75] It

Pest Manag Sci **63**:647–657 (2007)
DOI: 10.1002/ps

Table 1. Reported semiochemicals for various biting insects

Chemical stimulus	Insects that bite humans	Insects that bite animals
Carbon dioxide	Mosquitoes: *Ae. aegypti; Cx. quinquefasciatus; An. quadrimaculatus*	Tsetse flies: *Glossina* spp.
	Midges: *C. impunctatus; C. furens; C. stellifer; C. mississippiensis*	Stable fly: *Stomoxys calcitrans*
	Tsetse flies: *Glossina* spp.	
Lactic acid	Mosquitoes: *Ae. aegypti; Cx. pipiens; Ae. atropalpus An. gambiae*	
	Midges: *C. impunctatus*	
	Triatoma bugs: *Triatoma infestans*	
Amino acids	Mosquitoes: *Ae. aegypti*	
Acetone	Midges: *C. impunctatus*	Tsetse flies: *Glossina* spp.
	Mosquitoes: *An. gambiae; An. stephensi*	Stable fly: *Stomoxys calcitrans*
		Midges: *C. nubeculosus*
Ammonia	Triatoma bugs: *Triatoma infestans*	Clegs: *Haematopota pluvialis; Hybomitra* expollicata
Carboxylic acids	Mosquitoes: *Ae. aegypti; An. gambiae*	
	Triatoma bugs: *Triatoma infestans*	
1-Octen-3-ol	Mosquitoes: *An. gambiae; C. impunctatus*, 15 *Aedes* spp. including *Ae. aegypti*	Tsetse flies: *Glossina* spp.
	5 *Anopheles* spp.; 10 *Culex* spp.; 2 *Culiseta* spp.; 2 *Mansonia* spp.; 5 *Psorophora* spp.; 2 *Wyeomyia* spp.	Clegs: *Haematopota pluvialis; Hybomitra expollicata*
	Midge: *C. impunctatus*	Midges: *C. nubeculosus*
	Sandfly: *L. longipalpis*	
	Triatoma bugs: *Triatoma infestans*	
Phenols	Mosquitoes: *An. gambiae*	Tsetse flies: *Glossina* spp.
	C. impunctatus	Stable fly: *Stomoxys calcitrans*
		Midges: Culicoides spp.
Other		
2-Oxopentanoic acid	Mosquitoes: *An. gambiae*	
Lysine, cadaverine, estradiol	Mosquitoes: *An. stephensi*	
Butanone	Midges: *C. impunctatus*	
7-Octenoic acid, (E) & (Z)-3-methyl-2-hexanoic acids	Mosquitoes: *An. gambiae*	
Methyl salicylate; isothiocyanates: allyl, butyl, phenyl, 2-phenyl	Midges: *C. impunctatus*	
6-Methyl-5-hepten-2-one; 2-pentanone; 3-pentanone; butanone; 4-hydroxy-4-methyl-2-pentanone,	Mosquitoes: *Ae. aegypti*	
4-methyl-2-pentanone.	Sandfly: *L. longipalpis*	

was proposed that the blend of waterbuck-specific components may function as long- or medium-range allomones against tsetse flies, and that the blend of aldehydes may be an unidentified part of the attraction system for these insects. In behavioural tests, *G. m. mortisans* were shown to be attracted to the host-specific aldehyde blend, but they preferred control stimuli when presented with the waterbuck-specific blend.[77] Lactic acid, 2-methoxyphenol and acetophenone could also play a role, as they have been shown to reduce trap catches of *G. pallipides* and *G. m. mortisans*.[78] Furthermore, the addition of pentanoic acid, hexanoic acid, acetophenone or 2-methoxyphenol to traps baited with an attractive odour blend substantially reduces trap catches.[79]

The differential attractiveness of vertebrate hosts within a single species, as opposed to that observed for tsetse flies, has been observed for cattle flies (Diptera: Muscidae).[80] The role of cattle-derived semiochemicals in differential attractiveness was established through isolation of volatiles from Holstein-Friesian heifers. Twenty-three compounds were identified by coupled GC-EAG.[28] Of these, 1-octen-3-ol, 6-methyl-5-hepten-2-one and 3-octanol were identified as attractants, whereas naphthalene, propyl butanoate and linalool were identified as repellents. When applied as slow-release formulations in the field, however, 6-methyl-5-hepten-2-one reduced fly populations on individual animals. The identification of attractants and repellents demonstrated the potential that these compounds have for monitoring and controlling cattle fly populations.

The host-seeking behaviour of the Culicidae and Ceratopogonidae has received much attention because of the close association between these insect families and human beings. In recent years, many scientists have focused on providing systematic evidence for the differential attraction of mosquitoes to human beings, which is well described anecdotally. Although there is now scientific evidence that this phenomenon may be mediated by chemical stimuli in mosquitoes,[49,81–87] so far investigations have merely scratched the surface in their attempts to explain the chemical basis for this complicated event. Differential attraction has also been demonstrated for sandflies[88] and for blackflies.[89]

Until recently, evidence for the involvement of specific olfactory cues in differential attraction of mosquitoes to human hosts was relatively scarce or contradictory. Some authors believe that a lack of 'attractive' stimuli or host kairomones is the main cause. For example, adding lactic acid to the skin of formerly non-attractive individuals increases mosquito responses towards them.[23,90–92] Lower levels of lactic acid contribute to the lesser attractiveness of non-human vertebrates to *An. gambiae* mosquitoes.[93] Lactic acid has also been shown to repel *Aedes* mosquitoes when applied to normally attractive human and mouse skin.[94] *Anopheles* mosquitoes, which normally respond behaviourally to sweat,[95,96] show no response to lactic acid when it is presented at the same concentration on its own.[97] Therefore, lactic acid could be involved in making a host more or less attractive to anthropophilic mosquitoes. Carbon dioxide has also been suggested to play a role in differential attraction. For example, the removal of exhaled air and standardising carbon dioxide has been shown to eliminate differential attraction of humans to blackflies and mosquitoes.[49,89] However, Costantini *et al.*[48] demonstrated that standardising carbon dioxide does not equalise responses of *An. gambiae* to individual human beings. Additionally, no study has accurately quantified carbon dioxide in odour profiles of individuals and related it directly to biting insect behaviour. Therefore, the true role of carbon dioxide in differential attraction is unknown. However, this type of compound is not expected to be associated with differential attraction, as it is a product of primary metabolism. Secondary metabolites comprising other volatile organic compounds are more likely to be involved, and are commonly found in animals and plants as defences against parasites, diseases and predators.

One study that attempted to quantify compounds in association with differential attraction indicated that several chemicals may have contributed to differential responses of *Ae. aegypti* to two volunteers or the same volunteer over time.[23] Some components were described as weak attractants or repellents, but the results were inconclusive owing to small sample sizes and the inability to distinguish between the chemicals that were physiologically relevant (i.e. EAG-active) to mosquitoes and the chemicals that simply comprised human odour, since electrophysiological techniques were not used. Additionally, an accurate quantification of the chemicals from the volunteers was not performed. However, the authors reported that lactic acid, butanone, 2-pentanone, 3-pentanone and 6-methyl-5-hepten-2-one were 'weak attractants' for *Ae. aegypti* mosquitoes and implied that these might be responsible for making people more attractive to this species. The remaining components tested did not elicit a response. However, the authors suggested that some of the chemicals could also be repellents.[23]

It is unknown whether the production of semiochemicals that negatively affect insect behaviour (i.e. repellents) has evolved from selective pressures or is a byproduct of metabolic processes.[98] If differential attraction has evolved as a natural defence trait in some human beings, the existence of genetic evidence for this might be expected. Although there is no evidence at present, in one study, 80% of malaria cases were found to be prevalent in only 20% of the population.[99] However, the extent to which differential attraction was involved in creating this statistic is unknown. There is evidence that the production of repellents in cattle is genetically determined. For livestock, this could lead to breeding programmes to select for unattractive individuals.[100] If this is also true for human beings, a screening process could identify the most vulnerable individuals, allowing for targeted control efforts.

Most chemicals that are described as causing positive behavioural responses in haematophagous insects, such as carbon dioxide, 1-octen-3-ol, lactic acid, ammonia, acetone and fatty acids, are found ubiquitously in all humans and in many other vertebrates. Therefore, chemicals such as these might comprise a basic 'core' suite of olfactory signals that, when present, convey information to an insect that a vertebrate is nearby. The addition or increase of certain other chemicals that either repel or 'mask' the activity of these core attractants could be a way by which inappropriate or unsuitable vertebrate hosts are avoided by host-seeking insects.

3.5 Pheromones

Pheromones which mediate interactions between members of the same species can be divided into different categories, depending upon the type of behaviour that is mediated, e.g. mating, aggregation, oviposition (egg laying) and invitation behaviour, and each class of pheromone has the potential to be utilised in traps. The function of sex pheromones, which are released by one sex, is to initiate either attraction or behaviour associated with mating in the opposite sex. Aggregation pheromones usually promote aggregation of both sexes, while oviposition pheromones promote egg-laying behaviour by gravid (egg-bearing) females.

Pheromones, although not always directly related to the vectoring component of the life cycle, represent a potentially potent means of vector detection through the deployment of pheromone baits in trapping systems. Such monitoring systems are typically highly species specific. *Culex quinquefasciatus* females utilise an oviposition pheromone, (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, which is released from egg rafts laid on the water surface.[33] Field trials in several countries and in areas where the pathogen is prevalent have demonstrated the efficacy of the synthetic pheromone in the field,[32,42,44] particularly when used in conjunction with site-derived oviposition cues found in grass infusions or soakage pit water, such as 3-methylindole (skatole).[32,42,101,102] This could be deployed effectively to monitor *Culex* spp. mosquito populations or even to control them if used in conjunction with environmentally benign larvicides, such as the insect growth regulator pyriproxyfen or larvae-specific pathogens, such as the fungus *Lagenidium gigantuem* Couch.[103] In spite of the demonstrated value of this pheromone in the trapping systems described above, it has not yet been exploited to a great extent in monitoring or control systems. However, the recent spread of West Nile virus by *Culex* species mosquitoes in the developed world means that there is renewed interest in this pheromone, which is now commercially available for trapping systems.

Sex pheromones produced by male sandflies of the *Lutzomyia longipalpis* (Lutz & Neiva) species complex are novel homosesquiterpenes characterised as 3-methyl-α-himachalene (*L. longipalpis* from State of Bahia, Brazil) and (*S*)-9-methylgermacrene-B (*L. longipalpis* from State of Minas Gerais, Brazil),[88,104–106] or a diterpene (*L. longipalpis* from State of Ceará, Brazil). The compounds act as sex pheromones to attract females and maintain species isolation.[88] More recently, (1*S*,3*S*,7*R*)-3-methyl-α-himachalene was shown also to cause male aggregation.[107] An oviposition pheromone has also been identified for *L. longipalpis* as dodecanoic acid.[108] These pheromones are not currently used in the control of sandfly populations but show potential. A female-produced sex pheromone has also been identified for the farmyard midge, *Culicoides nubeculosus* (Meigen), as *n*-heptadecane.[25] For the face fly, *Musca autumnalis* Deg., and the stable fly, *S. calcitrans*, sex pheromone components are straight-chain monoalkenes and mono- and dimethyl branched alkanes.[109] Contact sex pheromones have been reported for the tsetse fly *Glossina tachinoides* Westwood as comprising long-chain dimethylalkanes,[110] and for *G. austeni* Newstead 13,17-dimethylpentatriacont-1-ene is highly stimulatory to males. For the screwworm, *Cochliomyia hominivorax* Coq., a contact sex pheromone has been postulated but not identified.[111]

Some biting flies, including mosquitoes, blackflies and *Culicoides* midges, are believed to produce a pheromone while feeding on a host, although the semiochemicals involved have not yet been identified. These compounds are believed to be aggregation or 'invitation' pheromones that may attract conspecifics during feeding.[112–114]

Pheromones are an excellent resource as baits in trapping systems that target specific species. However, with the exception of the sandfly pheromone, sex pheromones often trap only males, the sex that usually does not bite. While this can give an indication of species populations as a whole, oviposition pheromones and host-derived kairomones have greater potential for monitoring and controlling the female vectors.

4 FUTURE PROPECTS

As stated above, semiochemicals and pheromones, although not always directly related to the vectoring part of the life cycle, could be a potentially extremely potent means of vector-borne disease control through the deployment of pheromone- and semiochemical-baited trapping systems or through personal protection. Monitoring systems, which are highly specific for the target insect, allow for mapping of populations with the potential to transmit diseases. As exemplified above, the role and identity of semiochemicals for major global insect vectors of pathogens has been investigated in many studies, and a number of chemical tools are already available for incorporation into vector trapping systems. Furthermore, rapid diagnostic techniques for pathogen presence in livestock disease vectors are already being used, e.g. for bovine arboviruses such as bluetongue in *Culicoides* spp. biting midges.[115]

For the development of vector trapping systems, it is critical that underpinning science is carried out in order fully to elucidate the role and identity of semiochemicals, as the incidence of diseases both in developing and developed countries through vector activity is high and is likely to increase. The underpinning science includes a greater understanding of vector chemical ecology throughout the entire life cycle, mainly concentrating on host location, but also mating and oviposition where pheromones are involved. This requires expertise in several scientific disciplines, all of which should preferably be linked directly through multidisciplinary groups at research institutes, or through well-established collaborative links with complementary areas of expertise. The disciplines include insect behaviour, neurophysiology and morphology, analytical and synthetic organic chemistry, insect behaviour, genetics, epidemiology and insect molecular biology.

Host-derived semiochemicals show greatest potential owing to their direct involvement in the process whereby a host gets bitten. Carbon dioxide and 1-octen-3-ol are most commonly utilised in traps for investigations, monitoring and localised control of some haematophagous insects, but few other semiochemicals are currently used on a large scale to control or monitor pest insects, although the new BioGents trap shows great promise. In spite of substantial research, a chemical lure that matches or exceeds the attractiveness of a natural host has not yet been discovered. This is likely to be due to the complexity of host odour profiles, meaning that a mixture of several chemicals in appropriate ratios is required. This can only be achieved by understanding more about the nature of olfactory mechanisms and their role in chemical ecology, which will be facilitated by utilising the state-of-the-art analytical chemistry techniques, behavioural assays and detection systems described above.

With the identification of semiochemicals that may have repellent or inhibitory effects, such as 7-octenoic acid, (*E*)- and (*Z*)-3-methyl-2-hexanoic acid and 6-methyl-5-hepten-2-one, semiochemicals may also be used as a form of personal protection for animals and human beings. Ultimately, the natural differential attraction involving attractants and repellents could be exploited to develop an artificial push–pull control strategy by using the semiochemicals involved. The chemicals could be used to prevent host location by 'pushing' biting insects away from human and animal hosts via inhibitory or repellent mechanisms. At the same time, chemicals such as carbon dioxide, 1-octen-3-ol or newly identified attractants can be used as baits in traps to 'pull' the insects into traps and away from potential hosts. The push–pull strategy could also be used against haematophagous insects that bite livestock, as described for cattle.[28] The differential attractiveness of biting insects to individuals could be exploited further by developing breeding programmes for animals that are less susceptible to being bitten and for screening human beings to determine those that are most susceptible. The latter could allow targeted control efforts against diseases such as malaria.

One single monitoring system or effective control method for all haematophagous insects would be very difficult to achieve. However, the use of permethrin on clothes,[116] in combination with DEET, can achieve almost 100% protection from mosquito bites.[117] The disadvantages to this are the expense and the safety-related risks mentioned previously. To be effective and economically viable (especially in developing countries), semiochemicals must be obtained with relative ease and low cost. This could be achieved by deriving compounds from renewable botanical resources as described above,[36] and the botanically derived oviposition pheromone for *Cx. quinquefasciatus* has already been shown to be effective in trapping gravid female mosquitoes in disease-afflicted areas.[32,44]

Understanding more about the molecular basis of insect olfaction will also play an important role in the future control of vector and disease control. It is already known that insect odorant binding proteins (OBPs) are likely to be involved in the recognition of odorants. Genes and cDNAs encoding the OBPs of many insect species have been cloned, and recombinant OBPs generated via a suitable expression system, e.g. *An. gambiae* s.s. OBPs[118] and *Cx. quinquefasciatus* oviposition PBP.[119] To determine the link between OBPs and semiochemicals, a range of experimental approaches, including conventional techniques such as displacement of fluorescent ligands and kinetic binding assays, can be used to study the interactions of the ligands with the OBPs. New approaches that can determine more specific interactions are also being developed. These include electrospray mass spectrometry and new techniques in NMR spectroscopy. The latter can include novel approaches to searching libraries of potential ligands against particular OBPs. If successful, this will allow the determination of simultaneous ligand/OBP interaction kinetics. Once the ligand/OBP interactions are established, the interactions with olfactory receptor proteins can be elucidated and interfered with to achieve control. Rapid diagnostic techniques, some of which are already established, e.g. real-time PCR, could be used in combination with traps to screen insect catches for the presence of pathogens, e.g. malaria parasites. Thus, information about the presence of a vector and pathogen can be achieved simultaneously. These screening systems are currently being developed for field use with mosquitoes (Lin Field, personal communication). Additionally, understanding such molecular mechanisms may lead to the development of biosensors for the detection of insect pheromones, which could create an important monitoring system for the presence of vector species.

Biosensors could also be developed for disease detection. As illustrated by recently emerging breakthroughs on disease detection,[120] it is likely that infection by disease pathogens leads to subtle changes in odour profiles of plants and animals. In this context, the pioneering studies on differential attractiveness of animals for haematophagous insects, and the ability to 'train' insects such as the honeybee, *Apis mellifera* L., to respond to volatiles collected from non-ecologically relevant situations,[17] clearly demonstrate that insects are able to detect such subtle changes in odour profiles, and therefore demonstrate the potential for using insects to detect subtle changes in relation to disease infection.

REFERENCES

1 Manson P, On the development of *Filaria sanguimis hominis*, and on the mosquito considered as a nurse. *J Linn Soc Zool London* **14**:304–311 (1878).
2 Adams TS, Haematophagy and hormone release. *Ann Entomol Soc Am* **92**:1–13 (1999).
3 Lehane MJ, *Biology of Blood-sucking Insects*. HarperCollins Academic, London (2005).
4 Christophers SR, *Aedes aegypti (L.): its Life History, Bionomics and Structure*. Cambridge University Press, Cambridge (1960).
5 Mark S and Fradin MD, Mosquitoes and mosquito repellents: a clinician's guide. *Ann Intern Med* **59**:595–596 (1998).
6 Mellor PS, The replication of Bluetongue virus in Culicoides vectors. *Curr Top Microbiol Immunol – Bluetongue Viruses* **163**:143–161 (1990).
7 Mellor PS, African horse sickness: transmission and epidemiology. *Vet Res* **24**:199–212 (1993).

8 Sellers RF and Maarouf AR, Possible introduction of epizootic hemorrhagic disease of deer virus (Serotype-2) and Bluetongue virus (Serotype-11) into British Columbia in 1987 and 1988 by infected *Culicoides* carried on the wind. *Can J Vet Res* **55**:367–370 (1991).
9 Kettle DS, *Medical and Veterinary Entomology*. Croom Helm, London (1984).
10 Purse BV, Mellor PS, Rogers DJ, Samuel AR, Mertens PPC and Baylis M, Climate change and the recent emergence of bluetongue in Europe. *Nat Rev Microbiol* **3**:171–181 (2005).
11 Mellor PS, *Studies on Onchocerca cervicalis (Railliet & Henry) and its Development in Culicoides (Latreille)*. University of London, London (1972).
12 Mellor PS and Mccaig J, Probable cause of sweet-itch in England. *Vet Rec* **95**:411–415 (1974).
13 Townley P, Baker KP and Quinn PJ, Preferential landing and engorging sites of the Culicoides species landing on a horse in Ireland. *Equine Vet J* **16**:117–120 (1984).
14 Spielman A, Andreadis TG, Apperson CS, Cornel AJ, Day JF, Edman JD, *et al.*, Outbreak of West Nile virus in North America. *Science (Washington)* **306**:1473–1473 (2004).
15 Russell RC and Kay BH, Medical entomology: changes in the spectrum of mosquito-borne disease in Australia and other vector threats and risks, 1972–2004. *Aust J Entomol* **43**:271–282 (2004).
16 Ooi EE, Goh KT and Gubler DJ, Dengue prevention and 35 years of vector control in Singapore. *Emerg Infect Dis* **12**:887–893 (2006).
17 Pickett J, Wadhams LJ and Woodcock CM, Mate and host location by insect model systems for exploiting olfactory interactions. *The Biochemist* pp. 8–13 (1998).
18 Mciver SB, Sensilla of Mosquitoes (Diptera, Culicidae). *J Med Entomol* **19**:489–535 (1982).
19 Zhou JJ, Huang WS, Zhang GA, Pickett JA and Field LM, 'Plus-C' odorant-binding protein genes in two *Drosophila* species and the malaria mosquito *Anopheles gambiae*. *Gene* **327**:117–129 (2004).
20 Hansson BS, A bug's smell – research into insect olfaction. *Trends Neurosci* **25**:270–274 (2002).
21 Bruce TJA, Wadhams LJ and Woodcock CM, Insect host location: a volatile situation. *Trends Plant Sci* **10**:269–274 (2005).
22 Takken W and Knols BGJ, Odor-mediated behaviour of Afrotropical malaria mosquitoes. *Annu Rev Entomol* **44**:131–157 (1999).
23 Bernier U, Kline DL, Schreck CE, Yost RA and Barnard DR, 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 (2002).
24 Cork A, Olfactory basis of host location by mosquitoes and other haematophagous Diptera. *Ciba F Symp* **200**:71–88 (1996).
25 Mordue (Luntz) AJ and Mordue W, Biting midge chemical ecology. *Biologist* **50**:159–162 (2003).
26 Colvin J, Brady J and Gibson G, Visually-guided, upwind turning behaviour of free-flying tsetse flies in odor-laden wind – a wind-tunnel study. *Physiol Entomol* **14**:31–39 (1989).
27 Carde RT, Odour plumes and odour-mediated flight in insects. *Ciba F Symp* **200**:54–70 (1996).
28 Birkett MA, Agelopoulos N, Jensen MV, Jespersen JB, Pickett JA, Prijs HJ, *et al.*, The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. *Med Vet Entomol* **18**:313–322 (2004).
29 Logan JG, Differential responses of the yellow fever mosquito, *Aedes aegypti*, and the Scottish biting midge, *Culicoides impunctatus*, to human host odours. *PhD Thesis*, University of Aberdeen (2006).
30 Pickett JA, Gas chromatography–mass spectrometry in insect pheromone identification: three extreme case histories, in *Chromatography and Isolation of Insect Hormones and Pheromones*, ed. by McCaffery AR and Wilson ID. Plenum Press, New York (1990).
31 Goldansaz SH, Dewhirst S, Birkett MA, Hooper AM, Smiley DWM, Pickett JA, *et al.*, Identification of two sex pheromone components of the potato aphid, *Macrosiphum euphorbiae* (Thomas). *J Chem Ecol* **30**:819–834 (2004).
32 Olagbemiro TO, Birkett MA, Mordue AJ and Pickett JA, Laboratory and field responses of the mosquito, *Culex quinquefasciatus*, to plant-derived *Culex* spp. oviposition pheromone and the oviposition cue skatole. *J Chem Ecol* **30**:965–976 (2004).
33 Olagbemiro TO, Birkett MA, Mordue AJ and Pickett JA, Production of (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, the mosquito oviposition pheromone, from the seed oil of the summer cypress plant, *Kochia scoparia* (Chenopodiaceae). *J Ag Food Chem* **47**:3411–3415 (1999).
34 Laurence BR and Pickett JA, Erythro-6-acetoxy-5-hexadecanolide, the major component of a mosquito oviposition attractant pheromone. *J Chem Soc – Chem Commun* : 59–60 (1982).
35 Dawson GW, Mudd A, Pickett JA, Pile MM and Wadhams LJ, Convenient synthesis of mosquito oviposition pheromone and a highly fluorinated analog retaining biological-activity. *J Chem Ecol* **16**:1779–1789 (1990).
36 Hooper AM, Farcet JB, Mulholland NP and Pickett JA, Synthesis of 9-methylgermacrene B, racemate of the sex pheromone of *Lutzomyia longipalpis* (Lapinha), from the renewable resource, *Geranium macrorrhizum* essential oil. *Green Chem* **8**:513–515 (2006).
37 Birkett MA and Pickett JA, Aphid sex pheromones: from discovery to commercial production. *Phytochemistry* **62**:651–656 (2003).
38 Njiru BN, Mukabana WR, Takken W and Knols BGJ, Trapping of the malaria vector *Anopheles gambiae* with odour-baited MM-X traps in semi-field conditions in western Kenya. *Malaria J* **5**:39 (2006).
39 Kline DL, Traps and trapping techniques for adult mosquito control. *J Am Mosquito Contr* **22**:490–496 (2006).
40 Mboera LEG, Kihonda J, Braks MAH and Knols BGJ, Short report: Influence of Centers for Disease Control light trap position, relative to a human-baited bed net, on catches of *Anopheles gambiae* and *Culex quinquefasciatus* in Tanzania. *Am J Trop Med Hyg* **59**:595–596 (1998).
41 Kline DL, Evaluation of various models of propane-powered mosquito traps. *J Vector Ecol* **27**:1–7 (2002).
42 Mboera LEG, Takken W, Mdira KY, Chuwa GJ and Pickett JA, Oviposition and behavioral responses of *Culex quinquefasciatus* to skatole and synthetic oviposition pheromone in Tanzania. *J Chem Ecol* **26**:1193–1203 (2000).
43 Laurence BR and Pickett JA, An oviposition attractant pheromone in *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bull Entomol Res* **75**:283–290 (1985).
44 Mboera LEG, Takken W, Mdira KY and Pickett JA, Sampling gravid *Culex quinquefasciatus* (Diptera: Culicidae) in Tanzania with traps baited with synthetic oviposition pheromone and grass infusions. *J Med Entomol* **37**:172–176 (2000).
45 Kline DL, Olfactory attractants for mosquito surveillance and control: 1-octen-3-ol. *J Am Mosquito Contr* **10**:280–287 (1994).
46 Mathenge EM, Omweri GO, Irungu LW, Ndegwa PN, Walczak E, Smith TA, *et al.*, Comparative field evaluation of the Mbita trap, the Centres for Disease Control light trap, and the human landing catch for sampling of malaria vectors in western Kenya. *Am J Trop Med Hyg* **70**:33–37 (2004).
47 Dia I, Diallo D, Duchemin JB, Ba Y, Konate L, Costantini C, *et al.*, Comparisons of human-landing catches and odor-baited entry traps for sampling malaria vectors in Senegal. *J Med Entomol* **42**:104–109 (2005).
48 Costantini C, Gibson G, Sagnon N, DellaTorre A, Brady J and Coluzzi M, Mosquito responses to carbon dioxide in a West African Sudan savanna village. *Med Vet Entomol* **10**:220–227 (1996).

49 Brady J, Costantini C, Sagnon N, Gibson G and Coluzzi M, The role of body odours in the relative attractiveness of different men to malarial vectors in Burkina Faso. *Ann Trop Med Parasitol* **91**:S121–S122 (1997).

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

51 Lines JD, Curtis CF, Wilkes TJ and Njunwa KJ, Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. *Bull Entomol Res* **81**:77–84 (1991).

52 Vale GA, 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 (1980).

53 Hall DR, Beevor PS, Cork A, Nesbitt BF and Vale GA, 1-Octen-3-ol – a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Sci Appl* **5**:335–339 (1984).

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

55 Vale GA and Hall DR, 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 (1985).

56 Vale GA and Torr SJ, Development of bait technology to control tsetse in *The Trapanosomiases*, ed. by Maudlin I, *et al*. CABI Publishing, pp. 509–523 (2004).

57 Spath J, Olfactory attractants for West African tsetse flies, *Glossina* spp (Diptera: Glossinidae). *Trop Med Parasitol* **46**:253–257 (1995).

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

59 Van den Bossche P and De Deken R, The application of bait technology to control tsetse in *The Trapanosomiases*, ed. by Maudlin I, *et al*. CABI Publishing, pp. 525–532 (2004).

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

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

62 Warnes ML and Finlayson LH, Electroantennogram responses of the stable fly, *Stomoxys calcitrans*, to carbon dioxide and other odors. *Physiol Entomol* **11**:469–473 (1986).

63 Warnes ML and Finlayson LH, Responses of the stable fly, *Stomoxys calcitrans* (L) (Diptera: Muscidae), to carbon dioxide and host odors. 2. Orientation. *Bull Entomol Res* **75**:717–727 (1985).

64 Mihok S, Kangethe EK and Kamau GK, Trials of traps and attractants for *Stomoxys* spp. (Diptera: Muscidae). *J Med Entomol* **32**:283–289 (1995).

65 Krcmar S, Response of horse flies (Diptera: Tabanidae) to different olfactory attractants. *Biologia* **60**:611–613 (2005).

66 Krcmar S, Hribar LJ and Kopi MA, Response of Tabanidae (Diptera) to natural and synthetic olfactory attractants. *J Vector Ecol* **30**:133–136 (2005).

67 Krockel U, Rose A, Eiras AE and Geier M, New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. *J Am Mosquito Contr* **22**:229–238 (2006).

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

69 Maciel-de-Freitas R, Eiras AE and Lourenco-de-Oliveira R, Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). *Mem I Oswaldo Cruz* **101**:321–325 (2006).

70 Ritchie SA, Moore P, Carruthers M, Williams C, Montgomery B, Foley P, *et al.*, Discovery of a widespread infestation of *Aedes albopictus* in the Torres Strait, Australia. *J Am Mosquito Contr* **22**:358–365 (2006).

71 Williams CR, Long SA, Russell RC and Ritchie SA, Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO_2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *J Am Mosquito Contr* **22**:296–300 (2006).

72 Geier M, Rose A, Gunewald J and Jones O, New mosquito traps improve the monitoring of disease vectors. *Internat Pest Cont* **48**:124–126 (2006).

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

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

75 Gikonyo NK, Hassanali A, Njagi PGN, Gitu PM and Midiwo JO, Odor composition of preferred (buffalo and ox) and non-preferred (waterbuck) hosts of some savannah tsetse flies. *J Chem Ecol* **28**:969–981 (2002).

76 Besansky NJ, Hill CA and Costantini C, No accounting for taste: host preference in malaria vectors. *Trends Parasitol* **20**:249–251 (2004).

77 Gikonyo NK, Hassanali A, Njagi PGN and Saini RK, Responses of *Glossina morsitans morsitans* to blends of electroantennographically active compounds in the odors of its preferred (buffalo and ox) and non-preferred (waterbuck) hosts. *J Chem Ecol* **29**:2331–2345 (2003).

78 Voskamp KE, Everaarts E and Den Otter CJ, Olfactory responses to attractants and repellents in tsetse. *Med Vet Entomol* **13**:386–392 (1999).

79 Torr SJ, Mangwiro TNC and Hall DR, Responses of *Glossina pallidipes* (Diptera: Glossinidae) to synthetic repellents in the field. *Bull Entomol Res* **86**:609–616 (1996).

80 Jensen KMV, Jespersen JB, Birkett MA, Pickett JA, Thomas G, Wadhams LJ, *et al.*, Variation in the load of the horn fly, *Haematobia irritans*, in cattle herds is determined by the presence or absence of individual heifers. *Med Vet Entomol* **18**:275–280 (2004).

81 Brouwer R, Variations in human body odour as a cause of individual differences of attraction for malaria mosquitoes. *V Trop Geogr Med* **12**:186–192 (1960).

82 Khan AA, Maibach HI, Strauss WG and Fisher JL, Increased attractiveness of man to mosquitoes with induced eccrine sweating. *Nature (London)* **223**:859–860 (1969).

83 Mayer MS and James JD, Attraction of *Aedes aegypti* (L) – responses to human arms carbon dioxide and air currents in a new type of olfactometer. *Bull Entomol Res* **58**:629–642 (1969).

84 Burkot TR, Non-random host selection by *Anopheline* Mosquitoes. *Parasitol Today* **4**:156–162 (1988).

85 Schreck CE, Kline DL and Carlson DA, Mosquito attraction to substances from the skin of different humans. *J Am Mosquito Contr* **6**:406–410 (1990).

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

87 Qiu YT, Smallegange RC, Hoppe S, van Loon JJ, Bakker EJ and Takken W, 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 (2004).

88 Hamilton JGC and Ramsoondar TMC, Attraction of *Lutzomyia longipalpis* to human skin odors. *Med Vet Entomol* **8**:375–380 (1994).

89 Schofield SW and Sutcliffe JF, Human individuals vary in attractiveness for host-seeking black flies (Diptera:

Simuliidae) based on exhaled carbon dioxide. *J Med Entomol* **33**:102–108 (1996).
90 Acree F, Turner RB, Gouck HK, Beroza M and Smith N, L-Lactic acid – a mosquito attractant isolated from humans. *Science (Washington)* **161**:1346–1347 (1968).
91 Smith CN, Smith N, Gouck HK, Weidhaas DE, Gilbert IH, Mayer MS, *et al.*, L-Lactic acid as a factor in attraction of *Aedes aegypti* (Diptera: Culicidae) to human hosts. *Ann Entomol Soc Am* **63**:760–770 (1970).
92 Steib BM, Geier M and Boeckh J, The effect of lactic acid on odour-related host preference of yellow fever mosquitoes. *Chem Senses* **26**:523–528 (2001).
93 Dekker T, Steib B, Carde RT and Geier M, L-lactic acid: a human-signifying host cue for the anthropophilic mosquito *Anopheles gambiae*. *Med Vet Entomol* **16**:91–98 (2002).
94 Shirai Y, Kamimura K, Seki T and Morohashi M, L-lactic acid as a mosquito (Diptera: Culicidae) repellent on human and mouse skin. *J Med Entomol* **38**:51–54 (2001).
95 Cork A and Park KC, Identification of electrophysiologically-active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat extracts. *Med Vet Entomol* **10**:269–276 (1996).
96 Meijerink J, Braks MAH and Van Loon JJA, Olfactory receptors on the antennae of the malaria mosquito *Anopheles gambiae* are sensitive to ammonia and other sweat-borne components. *J Insect Physiol* **47**:455–464 (2001).
97 Healy TP and Copland MJW, Human sweat and 2-oxopentanoic acid elicit a landing response from *Anopheles gambiae*. *Med Vet Entomol* **14**:195–200 (2000).
98 Rebollar-Tellez EA, Human body odor, mosquito bites and the risk of disease transmission. *Folia Entomol Mex* **44**:247–265 (2005).
99 Smith DL, Dushoff J, Snow RW and Hay SI, The entomological inoculation rate and *Plasmodium falciparum* infection in African children. *Nature (London)* **438**:492–495 (2005).
100 Thomas G, Prijs HJ and Trapman JJ, Factors contributing to differential risk between heifers in contracting summer mastitis, *Summer Mastitis*. Martinus Nijhof, Dordrecht (1987).
101 Blackwell A, Mordue (Luntz) AJ, Hansson BS, Wadhams LJ and Pickett JA, A behavioural and electrophysiological study of oviposition cues for *Culex quinquefasciatus*. *Physiol Entomol* **18**:343–348 (1993).
102 Mordue AJ, Blackwell A, Hansson BS, Wadhams LJ and Pickett JA, Behavioural and electrophysiological evaluation of oviposition attractants for *Culex quinquefasciatus* Say (Diptera: Culicidae). *Experientia* **48**:1109–1111 (1992).
103 Pickett JA and Woodcock CM, The role of mosquito olfaction in oviposition site location and in the avoidance of unsuitable hosts. *Ciba F Symp* **200**:109–123 (1996).
104 Hamilton JGC, Dawson GW and Pickett JA, 3-Methyl-alpha-himachalene: proposed structure for novel homosesquiterpene sex pheromone of *Lutzomyia longipalpis* (Diptera: Psychodidae) from Jacobina, Brazil. *J Chem Ecol* **22**:2331–2340 (1996).
105 Hamilton JGC, Dawson GW and Pickett JA, 9-Methylgermacrene-B: proposed structure for novel homosesquiterpene from the sex pheromone glands of *Lutzomyia longipalpis* (Diptera: Psychodidae) from Lapinha, Brazil. *J Chem Ecol* **22**:1477–1491 (1996).
106 Hamilton JGC, Hooper AM, Ibbotson HC, Kurosawa S, Mori K, Muto SE, *et al.*, 9-Methylgermacrene-B is confirmed as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Lapinha, Brazil, and the absolute stereochemistry defined as S. *Chem Comm* 2335–2336 (1999).
107 Spiegel CN, Jeanbourquin P, Guerin PM, Hooper AM, Claude S, Tabacchi R, *et al.*, (1*S*,3*S*,7*R*)-3-Methyl-alpha-himachalene from the male sandfly *Lurzomyia longipalpis* (Diptera: Psychodidae) induces neurophysiological responses and attracts both males and females. *J Insect Physiol* **51**:1366–1375 (2005).
108 Dougherty M and Hamilton G, Dodecanoic acid is the oviposition pheromone of *Lutzomyia longipalpis*. *J Chem Ecol* **23**:2657–2671 (1997).
109 Sonnet PE, Uebel EC and Miller RW, Sex pheromone of face fly and compounds influencing pheromone activity. *Environ Entomol* **4**:761–764 (1975).
110 Carlson DA, Offor II, El Messoussi S, Matsuyama K, Mori K and Jallon JM, Sex pheromone of *Glossina tachinoides*: isolation, identification, and synthesis. *J Chem Ecol* **24**:1563–1575 (1998).
111 Hammack L, Sex pheromone communication in the screwworm, *Cochliomyia hominivorax* – ontogenic and strain effects. *J Chem Ecol* **17**:2143–2154 (1991).
112 Blackwell A, Dyer C, Mordue (Luntz) AJ, Wadhams LJ and Mordue W, Field and laboratory evidence of a volatile pheromone produced by parous females of the Scottish biting midge, *Culicoides impunctatus*. *Physiol Entomol* **19**:251–257 (1994).
113 McCall PJ and Lemoh PA, Evidence for the 'invitation effect' during blood feeding by blackflies of the *Simulium damnosum* complex (Diptera: Simuliidae). *J Ins Behav* **10**:299–303 (1997).
114 Charlwood JD, Smith T, Kihonda J, Heiz B, Billingsley PF and Takken W, Density-independent feeding success of malaria vectors (Diptera, Culicidae) in Tanzania. *Bull Entomol Res* **85**:29–35 (1995).
115 Ohashi S, Yoshida K, Yanase T, Kato T and Tsuda T, Simultaneous detection of bovine arboviruses using single-tube multiplex reverse transcription-polymerase chain reaction. *J Virol Meth* **120**:79–85 (2004).
116 Goddard J, Health risks and benefits of insect repellents. *Infect Med* **19**:256–264 (2002).
117 Fradin MS, Mosquitoes and mosquito repellents: a clinician's guide. *Ann Intern Med* **128**:931–940 (1998).
118 Li ZX, Pickett JA, Field LM and Zhou JJ, Identification and expression of odorant-binding proteins of the malaria-carrying mosquitoes *Anopheles gambiae* and *Anopheles arabiensis*. *Arch Ins Biochem Physiol* **58**:175–189 (2005).
119 Ishida Y, Cornel AJ and Leal WS, Identification and cloning of a female antenna-specific odorant-binding protein in the mosquito *Culex quinquefasciatus*. *J Chem Ecol* **28**:867–871 (2002).
120 Phillips M, Cataneo RN, Ditkoff BA, Fisher P, Greenberg J, Gunawardena R, *et al.*, Prediction of breast cancer using volatile biomarkers in the breath. *Breast Cancer Research and Treatment* **99**:19–21 (2006).