Odour-mediated Host Preference in Mosquitoes: The Role of the Maxillary Palps in Host Recognition

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Abstract

The mechanisms underlying host preference in mosquitoes are ultimately a result of optimal foraging on currently available hosts and historical patterns of host availability. The proximate mechanisms are regulated by available host cues and the nature of the mosquitoes' responses to these cues. Although mosquito host preference has a genetic background and the response to specific host cues are adaptive, mosquitoes exhibit a high degree of plasticity in their host preference. The purpose of this study was to analyse the extent of this variability in the disease vectors *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*, and if their behavioural response is constrained by qualitative and quantitative differences in host cues.

I investigated the sensory mechanisms and constraints regulating mosquito behaviour in response to various carbon dioxide (CO_2) concentrations, within the range emitted from vertebrate hosts. The temporal capacity of the CO_2-ORNs in *Ae. aegypti* and *An. gambiae* is higher than in *Cu. quinquefasciatus*, which translated into behavioural constraints leads to an increased time to take off at high concentrations. The altered sensitivity of CO_2-ORNs in transient elevated background levels of CO_2 also constrain the host-seeking behaviour in *Ae. aegypti*. Take off flight was impeded, correlating with a behavioural threshold in the net CO_2-ORN response. Hence, sensory limitations of CO_2-ORNs induce behavioural constraints at higher CO_2 concentrations, which may be partially responsible for species-specific host preferences.

The specificity and sensitivity of host volatile blends play a key role in mosquito host recognition and non-host avoidance. I determined that *Ae. aegypti* and *An. gambiae* consider (*R*)-1-octen-3-ol as a host cue, whereas *Cu. quinquefasciatus* perceives it as a non-host cue, when not in the context of a preferred host. Hence, host recognition is regulated by the perception of specific volatiles in the context of a blend. This specificity appears to be consistent with host preference, as *Ae. aegypti* is attracted by a wide range of (*R*)-1-octen-3-ol concentrations, while in *Cu. quinquefasciatus* it elicits no attraction or even repels at higher concentrations. The behavioural and physiological specificity and sensitivity to (*R*)-1-octen-3-ol is the output of the sensitivities of the orthologous mosquito odorant receptors, the OR8s. The functional tuning of these receptors and their cognate neurons was reflected in minor differences in the receptors amino acid structure. These findings provide a substrate with which to examine how the sensory system of a mosquito interprets host and non-host odour cues and thus produces host recognition behaviour.

Keywords: 1-octen-3-ol, mosquitoes, odorant receptor, enantiomers, heterologous expression, calcium imaging, olfaction, carbon dioxide, mechanism.

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Dedication

To my brothers, sisters and especially my lovely parents

Almighty Allah said:

"O people, an example is presented, so listen to it. Indeed, those you invoke besides Allah will never create [as much as] a fly, even if they gathered together for that purpose. And if the fly should steal away from them a [tiny] thing, they could not recover it from him. Weak are the pursuer and pursued"

(Surah al-baqarah, 2:26)

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Majeed S, Hill SR, Dekker T, Birgersson G and Ignell R**. Context is not everything- sensory constraints also regulate host-seeking behaviour of mosquitoes. (manuscript).

II **Cook JI, Majeed S, Ignell R, Pickett JA, Birkett MA and Logan JG** (2011). Enantiomeric selectivity in behavioural and electrophysiological responses of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. *Bulletin of Entomological Research* 101: 541-550.

III **Hill SR, Majeed S, Ignell R**. Proximate mechanism behind enantiomeric selectivity and sensitivity of an odorant receptor. (manuscript).

IV **Majeed S, Hill RS, Ignell R**. Impact of elevated CO_2 background levels on the host-seeking behaviour of *Aedes aegypti*. (submitted).

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The contribution of Shahid Majeed to the papers included in this thesis was as follows:

I Designed, conducted and analysed the behavioural and electrophysiological studies and wrote the manuscript together with co-authors.

II Conducted, analysed, and wrote the electrophysiological studies.

III Conducted and analysed the electrophysiological studies.

IV Conducted and analysed the behavioural and electrophysiological studies and wrote the manuscript together with co-authors.

Abbreviations

AL	Antennal lobe
GC-EAD	Gas chromatography-electroantennographic detection
GC-MS	Gas chromatography coupled mass spectrometry
GOBP	General odorant binding protein
GR	Gustatory receptors
iGluR	Ionotropic glutamate receptor
IR	Ionotropic receptors
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
MOP	Mosquito oviposition pheromone
OBP	Odorant binding protein
ODE	Odorant degrading enzyme
OR	Odorant receptor
Orco	Odorant receptor co-receptor
ORN	Olfactory receptor neuron
PBP	Pheromone binding protein
RNA	Ribonucleic acid
SNPs	Single nucleotide polymorphisms
SSR	Single sensillum recording

1 Objectives

The main objective of this thesis was to analyse the proximate mechanisms regulating host selection and discrimination in the disease vectors *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus*. Specifically, to identify the role of qualitative and quantitative differences in host volatiles in mosquito host choice, and its sensory correlate, with the aim to provide directives for future vector control methods.

2 Introduction

Mosquitoes (Culicidae: Diptera) are generally considered a nuisance due to their blood-feeding behaviour, which affects humans and livestock (Lehane, 2005). This problem is caused by female mosquitoes that require blood to complete their oogenesis (Clements, 1999). The close interaction between mosquitoes and their hosts can also be detrimental since mosquitoes may transmit harmful diseases, including malaria, dengue, West Nile fever and lymphatic filariasis, during blood feeding. The effect of these diseases on public health can have a considerable socio-economic impact on society (Otranto *et al.*, 2013; Guzman *et al.*, 2010; Snow *et al.*, 2005). Today, mosquito-borne diseases affect the everyday life of more than 50% of the human population globally and human morbidity and mortality is on the rise (WHO, 2012). In addition, mosquito-borne diseases have a significant effect on the world's GDP, where the estimated annual cost of malaria alone, in 2011, was 1.66 billion USD (Korenromp *et al.*, 2013). So far, no permanent cure is available for any of the infectious diseases transmitted by mosquitoes (Wilder-Smith *et al.*, 2010; WHO, 2011; Van Ooij, 2009). However, current mosquito control measures, including insecticide-treated nets (ITNs) and indoor residual spraying (IRS), are being effective in reducing the transmission of these perilous diseases in many regions (Okumu *et al.*, 2011). Recent studies, however, have shown that mosquito populations may adapt to ITNs and IRS by developing resistance against the insecticide used or changing its behavioural characteristics thereby avoiding contact with the insecticide. As a result, there is an increasing realisation that no single intervention is likely to halt disease transmission, and that a multipronged approach is required to overcome the problem. The current circumstance has called for worldwide, integrated efforts to prevent further deterioration of the situation (Tolle, 2009; Sutherst, 2004). One such effort exploits the behaviour and ecology of the mosquitoes to reduce their contact with human hosts. Understanding the proximate mechanisms regulating host-species choice in mosquitoes and how these could be manipulated could identify novel disease control strategies based on altering vector behaviour.

The chemical ecology of mosquitoes is recognised as an important area on which future control strategies can depend, since mosquitoes are highly dependent on odour cues for successful nectar and blood feeding as well as oviposition (Takken & Verhulst, 2013; Takken & Knols, 1999). These behaviours form an integral part of the vectorial capacity of most mosquito species (Takken & Lindsay, 2003). In recent time, we have accrued a better understanding of mosquito behaviour, due to advances in physiological and molecular investigations. However, we still lack a fundamental knowledge of the ecological relevance of some of the key kairomones currently used in vector control.

In this thesis, I review the current understanding of the ultimate and proximate mechanisms regulating host preference of mosquitoes. Special attention is given to how qualitative and quantitative variation in host kairomone cues regulate host selection, and how differences in host preference are reflected at various levels in the olfactory system. In light of this, I present novel data on how variation in CO_2 and 1-octen-3-ol, key host kairomone cues currently used in vector control, affect the host-seeking behaviour of the disease vectors *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus*.

3 Investigated species and their host preferences

3.1 The yellow fever mosquito, *Aedes aegypti*


Photo by Vivien Lettry

Aedes aegypti transmits some of the most prevalent arthropod-borne viruses (arboviruses), including dengue and yellow fever, affecting humans in tropical and sub-tropical regions of the world today (WHO, 2012). Dengue is caused by a zoonotic arbovirus, which primarily is maintained in a sylvatic cycle between non-human primates and mosquitoes (Kyle & Harris, 2008). This viral disease affects an estimated 2.5 billion people (40% of the worlds' population) and is endemic in 100 countries, worldwide (WHO, 2012). Approximately 500,000 cases of dengue fever are registered annually, with a fatality rate of *circa* 5%, mostly among children (WHO, 2012). The situation has worsened in recent years due to factors such as urbanization, population growth and tourism (Guzman & Istúriz, 2010), and is predicted to deteriorate (Kyle & Harris, 2008). Efforts to eliminate the transmission by vector control have failed and no antiviral or specific treatment has been established, although early medical care is thought to lower the fatality rates (WHO, 2012). Similar to dengue, yellow fever is a haemorrhagic disease that is widespread in countries within Africa and South America, where it affects 900 million people (WHO, 2012). Approximately 200,000 yellow fever cases are reported annually, with a fatality rate of approximately 15% (WHO, 2012). Currently, the worst outbreak in 20 years is ongoing in Darfur, Sudan, in which 849 cases of yellow fever have been confirmed and 171 people have died (20% fatality rate). Vaccination is one of the most effective measures to control yellow fever. The vaccine is safe, affordable and provides effective immunity that lasts for 10 years after vaccination (WHO, 2011).

The yellow fever mosquito is closely associated with humans and their surrounding environment. It is endophagic (feeds indoors), endophilic (rests indoors) and day-biting (Scott & Takken, 2012). The diurnally active *Ae. aegypti* can be effectively controlled by insecticide-treated materials including insecticide-treated nets and window curtains as well as using covers for domestic water containers to significantly reduce the dengue vector densities (Lenhart *et al.*, 2008; Kroeger *et al.*, 2006; Vanwambeke *et al.*, 2006). Indoor residual insecticide treatments have rarely been used to protect against *Ae. aegypti*, nor are they currently recommended (WHO 2006). Many field studies have shown that *Ae. aegypti* preferentially and frequently imbibes human blood over that of dogs, bovines and chickens (Ponlawat & Harrington, 2005; Harrington *et al.*, 2001; Scott *et al.*, 2000; Clements, 1999; Scott *et al.*, 1993). More than 90% of female mosquitoes imbibe blood from humans and no geographical variation in host preference has been observed (Scott and Takken, 2012). Furthermore, behavioural analysis in the laboratory has shown that *Ae. aegypti* prefer humans over other animals (Barrera *et al.*, 2012; Steib *et al.*, 2001).

3.2 The malaria mosquito, *Anopheles gambiae*

Photo by Vivien Lettry

Malaria is the most perilous and infectious vector-borne disease of humans, and is widespread in tropical, subtropical and some temperate regions. The disease is caused by human protozoan parasites, primarily *Plasmodium falciparum* and *P. vivax,* and is transmitted by *Anopheles* mosquitoes (Datta & Singh Chauhan, 2012). One of the most efficient vectors of the disease is *An. gambiae sensu stricto* (*s.s.*) (hereafter referred to as *An. gambiae*), which is endemic to Sub-Saharan Africa (Snow *et al.*, 2005). In 2010, 1.24 million people died from malaria, of which 91% were reported in Africa (Murray *et al.*, 2012). Approximately 0.7 million of the fatalities were children below the age of 5 years. Currently no vaccine is available, and the best available treatment is antimalarial drugs. Besides drugs, ITNs and IRS are currently the most effective control methods. Recently, however, it has been shown that *An. gambiae* can develop resistance against intensively used insecticide treatments e.g. ITNs and IRS.

Anopheles gambiae is closely associated with humans due to its endophagic and endophilic behaviour (Scott & Takken, 2012). In addition, the species has a high survival rate and is highly susceptible to parasite infection, which increases its vector capacity (Coetzee *et al.*, 2000). Blood meal analysis have revealed that *An. gambiae* prefer humans over other hosts, such as cattle (Takken & Verhulst, 2013), although host preference may vary geographically (Duchemin *et al.*, 2001).

3.3 The Southern house mosquito, *Culex quinquefasciatus*


Photo by Vivien Lettry

Culex quinquefasciatus transmits human lymphatic filariasis (*Wuchereria bancrofti*) in the tropics, and West Nile fever caused by the West Nile arbovirus, in the Americas, Europe, Africa, the Middle East and Western Asia. Human lymphatic filariasis, commonly known as elephantiasis, is caused by parasitic nematodes (Simonsen & Mwakitalu, 2013). Currently 1.3 billion people in 72 countries worldwide are at risk of contracting the disease, with 120 million people reported being infected, 65% of them living in Southeast Asia and 30% in Africa (WHO, 2012). The disease itself is not fatal, but inflicts a considerable socio-economic impact due to human disability and ostracization (Simonsen & Mwakitalu, 2013). The recommended treatment for the disease is microfilaricides, which has no affect on adult nematodes. Since the vectors exhibit indoor resting behaviours, the use of ITNs and IRS are also recommended to both prevent the contraction and the transmission of the disease. The West Nile virus is a zoonotic arbovirus, which is maintained in an enzootic cycle between birds and mosquitoes. The West Nile virus was first identified in 1937 in Uganda (Smithburn *et al.*, 1940), is now widespread, and has caused recent outbreaks in North America, North Africa and Europe (Suthar *et al.*, 2013). Furthermore, the virus appeared in the United States at the turn of the millennium, and outbreaks have so far resulted in 5,387 human clinical infections and more than 200 fatalities (CDC, factsheet, 2012). There is no vaccine or specific treatment available for this viral infection, except the supportive treatment including intravenous fluids and help with breathing.

The Southern house mosquito, as well as many other *Culex* species prefers to feed on birds (Kilpatrick *et al.*, 2006; Clements, 1999). However, this preference can shift temporally from avian to mammalian hosts, when birds migrate, causing a paucity in the availability of hosts (Lyimo & Ferguson, 2009; Kilpatrick *et al.*, 2006). In other regions, the host preference of *Culex* mosquitoes has been shown to switch temporally from mammals to avian hosts due to a change in the rainy seasons (Chandler *et al.*, 1977). Host preference has also been shown to differ geographically, with populations of *Cu. quinquefasciatus* preferring humans in Arizona, USA (Zinser *et al.*, 2004), India (Samuel *et al.*, 2004; Kumar *et al.*, 2002), Tanzania (Mboera & Takken, 1999) and Kenya (Beier *et al.*, 1990); and birds in the Mediterranean (Calistri *et al.*, 2010) and the Yucatán, Mexico (Garcia-Rejon *et al.*, 2010). Hence, host preference of *Cu. quinquefasciatus* appears to be innately regulated, but highly dependent on host availability.

4 Evolutionary and ecological aspects of host preference in mosquitoes

The association between mosquitoes and humans have increased dramatically following the agricultural revolution within the past 11,000 years, and humans today suffer from numerous infectious diseases similar to those of the wild primate hosts (Wolfe *et al.*, 2007; Hume *et al.*, 2003). Available data suggest that the tendency of mosquito feeding on humans evolved because of a shift in land-use activities (Brown *et al.*, 2011; Chaves *et al.*, 2010; Lyimo & Ferguson, 2009). Deforestation and the intensification of rural agriculture have been ascribed as important factors regulating this interaction (Lyimo & Ferguson, 2009). A drastic increase in mosquito interaction with humans have also been shown to affect mosquito behaviour, leading to a switch in blood feeding from non-human vertebrates to humans, as well as a change from exophilic to endophilic behaviour (Costantini & Diallo, 2001; Coluzzi, 1999). Such extreme ecological overlap and strong association between humans and mosquitoes has an important implication for public health and disease transmission.

Evolution of host preference in mosquitoes is a continuous process, through time and space, under divergent natural selection pressures (Ayala & Coluzzi, 2005). Mosquitoes have evolved different host preferences as a consequence of various adaptations to the environment, interactions with other organisms, limitations imposed by past evolutionary history and adaptive foraging behaviours (Ayala & Coluzzi, 2005). These circumstances have favoured the existence of both specialists and generalists (Egas *et al.*, 2004). Host specialists are predicted to evolve during the process of trade-off between fitness and resources, i.e. gained sufficient net energy by consuming limited resources. In contrast, host generalists are predicted to evolve when there is moderate difference in gained energy among resources (Lyimo & Ferguson, 2009; Egas *et al.*, 2004). For a more extensive review of the ultimate selective forces responsible for driving the evolution of host species choice in mosquitoes, see Lyimo and Ferguson (2009).

5 External and internal determinants of host choice

Host preference of mosquitoes is affected by both intrinsic and extrinsic factors. Inherent factors are determined by the physiology of the mosquitoes or may be driven by selection and therefore have a genetic background. Despite a genetic basis, host preference of mosquitoes is characterized by high plasticity mediated by the density of host species, which by their abundance form a readily accessible source of blood. Other external factors, e.g. spatial and temporal distribution of hosts, may also affect host preference. A short overview of the external and internal determinants of mosquito host choice is given below. For more extensive reviews, please see Takken and Verhulst (2013) and Lyimo and Ferguson (Lyimo & Ferguson, 2009). In this overview, I pay special attention to odorants as an external determinant of host choice. The reason for this is the important role these chemicals have been shown to play in host selection and discrimination (Zwiebel & Takken, 2004; Bowen, 1991).

5.1.1 Physiology

Newly emerged female mosquitoes require nectar as a source of metabolic energy (Foster & Takken, 2004; Foster, 1995). The competence to blood feed only develops after mating, 24-48 hours after pupal emergence (Takken & Verhulst, 2013), and coincides with the onset of the host-seeking behaviour and response to host volatiles, as observed in *Ae. aegypti* and *An. gambiae* (Foster & Takken, 2004; Hancock & Foster, 1993). At this time, the innate preference for a specific host is expressed, as shown in e.g. the highly anthropophilic *An. gambiae* (Foster & Takken, 2004). However, the innate host preference may be overruled by the physiological state of the mosquitoes, including the nutritional state, and host abundance to safeguard reproduction, and ultimately fitness (Takken & Verhulst, 2013).

5.1.2 Genetics

Little is known about the genetic determinants of host preference in mosquitoes. In a pioneering study, Gillies (1964) analysed the genetic determinants of the innate host preference of *An. gambiae* through backcrossing and selection experiments. F1 progeny of selected *An. gambiae* mosquitoes, with behavioural preference for either humans or cows, were screened to determine whether the offspring preferred the same host as their parents. The results of this study showed that it is possible to select for a specific host preference within a few generations, and that this selection may operate by genetic polymorphism. Lefevre *et al.* (2009a) recently confirmed this mechanism in a field experiment, and indicated that differences in odour-mediated host preferences likely reflect genetic differences between various *An. gambiae* populations. Other studies on the genetic determinants of host preference have involved the analysis of species strains or hybrids of closely related species, to determine the extent by which genes involved in host preference become fixed under natural conditions. In a study by Mukwaya (1977), a cross of two strains each of *Ae. aegypti* and *Ae. simpsoni* with different host preferences revealed that interstrain hybrids and their backcrosses were intermediate in their host preferences between their parental strains. This finding confirmed that behavioural differences between species strains could be controlled genetically, although, host selection was not strongly fixed in these populations. However, this was not the case when the highly anthropophilic *An. gambiae* was backcrossed with zoophilic *An. quadriannulatus* (Pates, 2002), suggesting that the anthropophilic behaviour of *An. gambiae* is fixed in the natural population. This has also been confirmed in various field studies showing that the anthropophilic behaviour of *An. gambiae* is a dominant character (Takken & Knols, 1999; Costantini *et al.*, 1993). Besides the genetic determinants described above, chromosomal inversions have been suggested to regulate behavioural expression, including host preference and endophilic behaviour, of *Anopheles* mosquitoes (Takken & Verhulst, 2013; Coluzzi *et al.*, 1977).

5.1.3 Plasticity in host choice – the role of learning and experience

It is unclear whether plasticity in host preference of mosquitoes is be influenced by learning under natural conditions. Available laboratory studies show that *Cu. quinquefasciatus* (Tomberlin *et al.*, 2006) and *An. gambiae* (Chilaka *et al.*, 2012) are able to learn to respond to unconditioned stimuli in association with a positive stimulus, such as blood meal, as a reward, while, *Ae. aegypti* is capable of associative learning in response to aversive stimuli (Menda *et al.*, 2012). In the study by Menda *et al.*, *Ae. aegypti* learned to associate the host kairomone cue, 1-octen-3-ol, with other stimuli and demonstrated aversive behaviour to this attractant. Such learned aversion to host odour may explain the heterogeneous distribution of *Ae. aegypti* among host individuals and may provide an avenue to target this disease vector (McCall & Kelly, 2002). Besides laboratory studies, semi-field experiments have shown that, *Culex* mosquitoes (Mwandawiro *et al.*, 2000) and *An. gambiae* (Lefévre pers. Comm.) feed on hosts similar to that which they have been exposed to previously. However, learning in host preference does not seem to be sustained in the next generation (Mwandawiro *et al.*, 2000), suggesting that behavioural conditioning in a background of phenotypic plasticity, rather than genetic variability, is involved in the generation of individual host preference.

5.1.4 Host abundance

Host abundance often determines host choice, especially if the mosquito species is opportunistic (Takken & Verhulst, 2013). However, a decline in host population may also affect a species with a more specific preference (Lefèvre *et al.*, 2009b; Wekesa *et al.*, 1997). Such shifts in host preference have been observed in *Culex* mosquitoes that shift to mammalian hosts, including humans, when their preferred avian hosts migrate (Simpson *et al.*, 2009; Kilpatrick *et al.*, 2006). In addition, the highly anthropophilic *An. gambiae* may select other hosts than humans, when the preferred host is not readily available (Lefèvre *et al.*, 2009b).

5.1.5 Spatial distribution of hosts

Variation in mosquito host preference is dependent on the geographical distribution of the host with respect to the mosquitoes' natural habitat, as shown for allopatric populations of *Culex* and *Aedes* mosquitoes, as well as of *An. gambiae* (Lyimo & Ferguson, 2009; Williams *et al.*, 2003; Sousa *et al.*, 2001). Vertical spatial variation in host preference has also been shown for various mosquito species (Darbro & Harrington, 2006; Anderson *et al.*, 2004). Using bird sentinel traps, a number of studies have shown that some mosquito species, including *Culex*, feed at both ground and canopy level as a result of host availability, whereas others have adapted an ornithophilic preference and only feed at canopy level (Darbro & Harrington, 2006).

5.1.6 Temporal distribution of hosts

The host preference of mosquitoes has been shown to shift between seasons. For example, *Culex* mosquitoes have been shown to shift their host preference from birds, during spring and early summer, to mammals, during late summer and autumn (Kilpatrick *et al.*, 2006). Shifts in climatic conditions may also regulate host preference, as shown for *Cu. univittatus* (Chandler *et al.*, 1977).

6 Odour-mediated host selection in mosquitoes

The principle of odour-mediated host preference in mosquitoes was first described by Crumb (1922) and Rudolfs (1922). Several subsequent studies have suggested a role of host volatiles in host preference of *Aedes*, *Anopheles* and *Culex* mosquitoes e.g. (Suom *et al.*, 2010; Simpson *et al.*, 2009; Darbro & Harrington, 2006; Komar, 2001; Mboera & Takken, 1999; Snow, 1983; Haddow, 1942; Van Thiel *et al.*, 1939). These studies, however, used live hosts, hence did not dissociate host odour from other external host-associated stimuli. Recent field and semi-field studies, on the other hand, have validated Crumb's and Rudolfs' principle and showed that mosquitoes are attracted to human and animal odours from a distance (Takken, 1991). Furthermore, dual-choice assays in the field have showed that mosquitoes are capable of differentiating between volatiles emanating from preferred and non-preferred hosts (Costantini *et al.*, 1993). For example, *An. gambiae* and *Ae. aegypti* have been shown to prefer human odour to that of cattle (Costantini & Diallo, 2001; Costantini *et al.*, 1998; Costantini *et al.*, 1996; Costantini *et al.*, 1993). In addition, behavioural studies in the laboratory have showed that *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus* are attracted to the odour bouquet of their hosts, as well as some of their individual components (Allan *et al.*, 2006; Puri *et al.*, 2006; Qiu *et al.*, 2006a; Qiu *et al.*, 2004; Braks *et al.*, 2001; Dekker *et al.*, 2001; Pates *et al.*, 2001; Braks & Takken, 1999; Mboera *et al.*, 1998; Geier *et al.*, 1996).

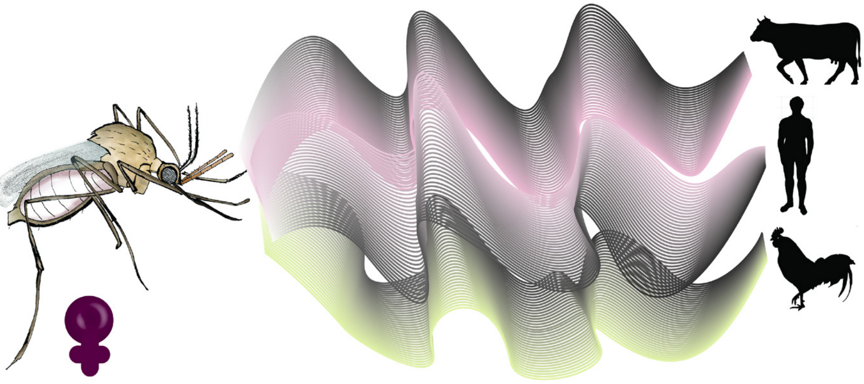


Figure 1. Schematic representation of host-seeking behaviour of female mosquito towards the odours of their specific hosts. Overlapping of the colour scheme represents the shared compounds among the host species.

The host-seeking behaviour of mosquitoes is thought to be regulated by both ubiquitous and host-specific host volatiles (Takken, 1991). In order to analyse the chemical volatile profile of mosquito host species, a variety of sampling methods have been used in conjunction with gas chromatography coupled mass-spectrometry analysis. Although the majority of studies have been made on human emanations (Gallagher *et al.*, 2008; Curran *et al.*, 2007; Penn *et al.*, 2007; Curran *et al.*, 2005; Haze *et al.*, 2001; Healy & Copland, 2001; Bernier *et al.*, 2000; Meijerink *et al.*, 2000; Cork & Park, 1996), emanations of other animals, including cattle (Gikonyo *et al.*, 2002; Steullet & Guerin, 1994; Bursell *et al.*, 1988; Hall *et al.*, 1984) and birds (Campagna *et al.*, 2012; Bernier *et al.*, 2008; Cooperband *et al.*, 2008; Douglas III *et al.*, 2001) have received some attention. These studies reveal both quantitative differences and qualitative differences in the chemical volatile profile of the various hosts, hence providing a chemical substrate on which host selection potentially can act. The chemical profile of humans and cattle share several compounds, e.g. carboxylic acids (acetic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid and pentanoic acid), alcohols (1-octen-3-ol), aldehydes (heptanal, hexanal, octanal, nonanal, decanal, dodecanal and benzaldehyde), aromatics (4-methylphenol) and ketones (acetone, 2-decanone and 6-methyl-5-hepten-2-one) that, however, differ in quantity (Tchouassi *et al.*, 2012). In addition, human-specific, e.g. lactic acid, hexadecenoic acid, octadecenoic acid and butanone, and cattle-specific compounds, e.g. 3-methylphenol, 3- and 4-ethylphenol, 3- and 4-n-propylphenol and 2-methoxyphenol have been found. Furthermore, the chemical volatile profile of humans and birds share similar compounds, including carboxylic acids (propanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, dodecanoic acid, octadecanoic acid and hexadecanoic acid), aldehydes (2-methylbutanal, nonanal, decanal and benzaldehyde), alcohols (2-hexadecanol and 2-heptadecanol), alkanes (octane, decane, heptadecane and octadecane). In addition, human-specific, e.g. lactic acid, 6-methyl-5-hepten-2-one and butanone, and bird-specific, e.g. 2,3-n-alkanediol, 2-ethylhexanoic acid, 2-methylheptadecane, (*Z*)-heptadec-7-ene, and tetradecyl decanoate, compounds have been found (Campagna *et al.*, 2012; Curran *et al.*, 2007; Curran *et al.*, 2005; Bernier *et al.*, 2000).

In addition to the interspecific differences in volatile composition described above, intraspecific quantitative differences have been reported in both humans and cattle (Gallagher *et al.*, 2008; Curran *et al.*, 2005; Bernier *et al.*, 2002; Phillips, 1997). These differences have been associated with differential attractiveness among individual hosts, as shown in behavioural experiments. For example, Logan et al. (2008) showed that a high relative amount of 6-methyl-5-hepten-2-one, octanal, nonanal, decanal and geranylacetone was correlated with a decreased attraction of *Ae. aegypti* to humans. An increased level of 6-methyl-5-hepten-2-one in cattle was also found to repel the horn fly, *Haematobia irritans* (Birkett *et al.*, 2004).

To analyse how the neural response space is related to the host preferences of mosquitoes, various techniques, including electroantennography (EAG) and single sensillum recordings (SSRs) alone or coupled with gas chromatography (GC), have been used (Ghaninia *et al.*, 2008; Logan *et al.*, 2008; Meijerink & van Loon, 1999; van den Broek & den Otter, 1999; Cork & Park, 1996). These studies have enabled the identification of bioactive compounds in host emanations, as well as revealed species-specific detection of host volatiles (Table 1).

Carbon dioxide is the best-described host-species cue in terms of chemical identity and amounts released into the host-species airstream (Guerenstein & Hildebrand, 2008; Mboera & Takken, 1997). As a result, CO_2 has received the most attention in terms of sensory physiology and behaviour (Guerenstein & Hildebrand, 2008; Bowen, 1991). Carbon dioxide is a strong behavioural activator and attractant for several mosquito species, including *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus* (Spitzen *et al.*, 2008; Dekker *et al.*, 2005; Mboera & Takken, 1997; Costantini *et al.*, 1996). The presence of CO_2 also synergises the behavioural response of these mosquitoes to other host-species volatiles (Table 1) (Dekker *et al.*, 2002; Mboera *et al.*, 2000; Mboera *et al.*, 1997; Geier *et al.*, 1996). For example, field studies have shown that *Aedes* and *Anopheles* mosquitoes are significantly more attracted to lactic acid or 1-octen-3-ol when combined with CO_2, than to the individual compounds alone (Kline et al., 1990; Kline et al., 2007; Russell, 2004; Takken and Kline, 1989; Kline and Lemire 1995; Burkett et al., 2001). Similarly, trap catches of *Cu. quinquefasciatus* are enhanced when nonanal is combined with CO_2 (Syed & Leal, 2009). Furthermore, both laboratory and field studies have shown that various carboxylic acids in the presence of CO_2 enhance the behavioural response of both *Ae. aegypti* and *An. gambiae* (Okumu *et al.*, 2010; Smallegange *et al.*, 2009; Smallegange *et al.*, 2005; Bosch *et al.*, 2000). These studies also show that the capture of mosquitoes increase with the complexity of the volatile blend. To gain a better understanding of how mosquitoes perceive host-species volatile blends, additional understanding of their olfactory system is required.

Table 1. Overview of the chemical compounds that have been tested for their impact on the host-seeking behaviour of mosquitoes.

	Behaviour	***Aedes aegypti***	***Anopheles gambiae***	***Culex quinquefasciatus***	**References**
CO_2	Attractant	Weak	Weak	Little or none	Braks *et al.*, 1999 Dekker *et al.*, 2002 Geier and Boeckh, 1999 Mboera *et al.*, 1998
	Synergist	Skin extract, lactic acid	Skin extract	None	
	Concentration dependent	Dependent	None	Repellent at high concentration	
1-octen-3-ol	Attractant	Weak	Weak	Weak	Kline *et al.*, 1990 Takken *et al.*, 1997 Cook *et al.*, 2011
	Synergist	With CO_2	With CO_2	With CO_2	
	Concentration dependent	NA	NA	NA	
Lactic acid	Attractant	Weak	Weak	Strong	Braks *et al.*, 2001 Geier *et al.*, 1999 Dekker *et al.*, 2002 Allan *et al.*, 2006
	Synergist	With CO_2, Skin extract & Ammonia	With CO_2, Host odour, Ammonia & Carboxylic acids	NA	
	Concentration dependent	NA	None	NA	
Ammonia	Attractant	Weak	Yes	NA	Braks *et al.*, 2001 Geier *et al.*, 1999
	Synergist	With lactic acid & CO_2	With lactic acid, carboxylic acid	NA	
	Concentration dependent	Dependent	Attractant/Repellent depending on concentration	NA	
Carboxylic acids	Attractant	Yes	Yes	Yes	Bosch *et al.*, 2000 Smallegange *et al.*, 2005 Puri *et al.*, 2006
	Synergist	With lactic acid	With lactic acid, ammonia & CO_2	NA	
	Concentration dependent	NA	NA	NA	
Lactic acid+ Ammonia+ Carboxylic acids	Attractant	Yes	Yes	Yes	Bosch *et al.*, 2000 Smallegange *et al.*, 2005, 2009 Puri *et al.*, 2006
	Synergist	Yes	Yes	Yes	
	Concentration dependent	NA	NA	NA	

7 Peripheral olfactory system

In mosquitoes, the olfactory organs consist of the antennae, maxillary palps and proboscis (Kwon *et al.*, 2006; Pitts & Zwiebel, 2006; Mclver, 1982). Various forms of cuticular hair-like structures, called sensilla (Figure 1), cover the surface of these appendages, and are the basic functional units in the olfactory system. The olfactory sensilla are divided into two distinct morphological types, single- and doubled-walled sensilla, based on their external features (McIver & Charlton, 1970; McIver, 1970). These sensilla contain either pore channels (single-walled sensilla) or spoke channels (double-walled sensilla) that are the supposed site of entry of the odour molecules (Steinbrecht, 1997; Keil, 1982). The cuticular surface of the sensilla is hydrophobic, allowing predominantly lipophilic odour molecules into the sensillum lymph (Hansson, 1999; Steinbrecht, 1997). Olfactory sensilla contain one or more bipolar olfactory receptor neurons (ORNs), which extend their dendrites, branched or unbranched, into the sensillum lymph (Jacquin-Joly & Merlin, 2004). The axons of these ORNs project towards the antennal lobe (AL), the primary olfactory centre in brain. Olfactory, including odorant, ionotropic and gustatory, receptors are expressed on the membranes of the dendrites, and have been shown to regulate the functional characteristics of the ORNs (Benton *et al.*, 2009; Vosshall *et al.*, 1999). Auxiliary cells, including the thecogen, tormogen and trichogen cells, are involved in the sensillum development, and regulate the ionic composition of the sensillum lymph (Figure 1) (Hansson, 1995; Schneider, 1964). In addition, the tormogen and trichogen cells produce and excrete odorant-binding proteins (OBPs) into the sensillum lymph (Schneider, 1964). These proteins have been shown to play a role in olfactory perireceptor events, and have been suggested to directly or indirectly interact with the odorant receptors (Vogt, 2003).

7.1.1 The antennae

Female mosquito antennae are composed of 13 flagellomeres (Pitts & Zwiebel, 2006; McIver, 1978; McIver, 1970), with approximately 90% of the total number of olfactory sensilla evenly distributed over these antennal sub-segments (Mclver, 1982; McIver, 1978). Both single-walled, including trichoid sensilla, and double-walled sensilla, including grooved peg and coeloconic sensilla, have been described in mosquitoes (Mclver, 1982). Of these, both trichoid and grooved peg sensilla are conserved in Culicine and Anopheline mosquitoes (Hill *et al.*, 2009; Boo, 1980; McIver, 1978; McIver, 1974; McIver, 1970), whereas coeloconic sensilla are only present in Anopheline mosquitoes (Mclver, 1982).

Numerous studies have been conducted to investigate the correlation between the number of olfactory sensilla and host-seeking behaviour (Isberg E *et al.*, 2013; Pitts & Zwiebel, 2006; Mclver, 1982). An important aspect to consider in this type of analysis is the phylogenetic relationship of the species, as this may significantly confound the interpretation of sensillum counts (Mclver, 1982). Hence, analysis of sensillum numbers in relation to host preference is often made at the genus level.

Comparative studies, at genus level, have consistently shown that mosquitoes and biting midges (Ceratopogonidae: Culicoides) with an ornithophilic host preference have a significantly higher number of specific sub-types of trichoid sensilla as well as grooved peg sensilla (Mclver, 1982) (Isberg E *et al.*, 2013). For example, ornithophilic *Culex* species, including *Cu. pipens* and *Cu. quinquefasciatus*, have been shown to have higher numbers of trichoid sensilla compared to the opportunistic poikilothermic *Cu. territans* and opportunistic homoeothermic *Cu. tarsalis* (Table 2a,b) (Hill *et al.*, 2009; Mclver, 1982; McIver, 1970). Furthermore, grooved peg sensilla are present in higher numbers in ornithophilic biting midges (Isberg E *et al.*, 2013), and in *Culex* mosquitoes compared to *Ae. aegypti* and *Anopheles* mosquitoes (Mclver, 1982) (Table 2a). A higher number of olfactory sensilla may increase the sensitivity of the olfactory system. In addition, a greater number of sensilla may increase the number of functional sensillum types, thus increasing the number of possible response patterns, resulting in a greater capacity for discriminating among the host odours (Mclver, 1982).

In contrast to the ornithophilic species, opportunistic species do not display clear differences in the number of sensilla. For example, the two opportunistic species, *Cu. territans* and *Cu. tarsalis*, have an intermediate number of antennal olfactory sensilla, yet display a significant difference in host preference (Table 2a,b). Similarly, anthropophilic *An. gambiae* and zoophilic *An. quadriannulatus* mosquitoes have the same number of sensilla

(Pitts & Zwiebel, 2006). This suggests that host discrimination is regulated differently in these species. A possible mechanism for this could be differences in the functional characteristics of the morphologically indistinguishable sensillum types, as shown in closely related *Anopheles* mosquitoes (van den Broek & den Otter, 1999; Ghaninia and Ignell, pers. comm.). These studies indicate that host preferences of opportunistic, or polyspecies, mosquitoes is regulated by the differential tuning of their ORNs to specific host odours.

7.1.2 The maxillary palp

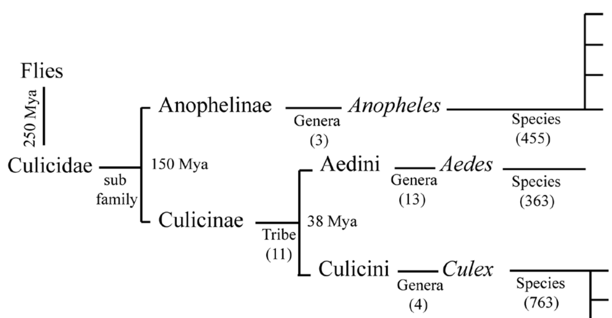
The maxillary palps bear only one type of olfactory sensilla, so called capitate pegs (McIver & Charlton, 1970). These sensilla cover the fourth segment of the maxillary palp in *Aedes* and *Culex* females (Mclver, 1982), whereas they are found on the ventral side of the second to fourth segments in female *Anopheles* species (McIver & Siemicki, 1975). Numerous studies have been conducted to investigate the number of capitate peg sensilla on the maxillary palps of mosquitoes (Mclver, 1982) (Table 2a) and biting midges (Isberg E *et al.*, 2013; Mckeever *et al.*, 2008). These studies have revealed that ornithophilic species possess a larger number of capitate peg sensilla compared to species that feed on large vertebrates (Table 2a). One possible explanation for this discrepancy in numbers is that mosquito species that feed on small or dispersed animals require higher sensitivity to host volatiles, e.g. CO_2 that is detected by ORNs in the capitate pegs, compared to those that feed on large gregarious animals.

7.1.3 The proboscis

The proboscis has primarily been thought to process gustatory information during food intake. However, Kwon et al. (2006) showed that *An. gambiae* may use the labellum at the tip of the proboscis to detect odour components. These authors postulated that mosquitoes use the proboscis to detect low volatile host cues at close proximity, in order to receive critical olfactory information as part of the penultimate steps in the blood-feeding behaviour.

Table 2. (A) Intuitive phylogeny of mosquito genera based on morphology reproduced as a cladogram (Harbach, 2007; Nene *et al.*, 2007) in relation to host preferences and number of olfactory sensilla on the antennae and maxillary palps (Hill *et al.*, 2009; Pitts & Zwiebel, 2006; Mclver, 1982) (B) Host preferences of different mosquito species.

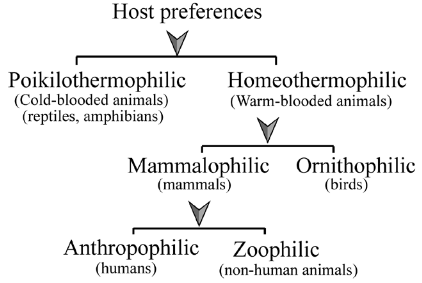
(A)



	Host preferences	Maxillary palp	Antenna		
		Capitate peg	Trichodea	Grooved peg	Large coeloconica
An. gambiae	Anthropophilic	67	629	84	33
An. arabiensis	Oppotunistic	NA	NA	NA	NA
An. quadriannulatus	Zoophilic	71	792	114	29
An. stephensi	Zoophilic	98	573	107	32
Ae. aegypti	Anthropophilic	29	532	105	0
Cu. restuans	Ornithophilic	89	902	348	0
Cu. pipiens	Ornithophilic	78	901	265	0
Cu. quinquefasciatus	Ornithophilic	NA	1124	234	0
Cu. fatigans	Ornithophilic	70	970	233	0
Cu. territans	Poikilothermophilic	39	537	275	0
Cu. tarsalis	Homeothermophilic	72	605	272	0

NA, not available

(B)



8 Odorant reception, activation and degradation

The ability of the peripheral olfactory system to detect, receive and rapidly inactivate trace odorants in the foundation of olfaction. This early olfactory processing involves odorant uptake, transport, receptor activation and rapid odorant inactivation. At each stage, odorants interact with sensillar proteins, in the lymph and on the dendrites of the ORNs, to lead to the transduction of the chemical into an electrical signal to be passed to the higher brain centres. These initial events begin after the hydrophobic odour molecules have been adsorbed onto the waxy surface of the sensillum cuticle and travel through pores to the sensillum lymph (Vogt, 2003). Here, odour molecules bind to the odorant-binding proteins (OBPs) for transport to the olfactory receptors, i.e. odorant receptors (ORs), ionotropic receptors (IRs) and gustatory receptors (GRs) (Pelosi *et al.*, 2006), on the dendritic membrane of ORNs. The OBPs may simply deliver the odorant to the olfactory receptor, or it may make a complex with the odorant that together activates the olfactory receptor. After conveying their message to the olfactory receptors, odorants are rapidly inactivated by odorant-degrading enzymes (ODEs) and scavenger proteins. A model describing the contributions of each of these proteins to the peripheral olfactory system, as a whole, is beginning to emerge.

8.1.1 Odorant binding proteins (OBPs)

In insects, the OBPs were identified for the first time in male antennae from the moth *Antheraea polyphemus* (Vogt & Riddiford, 1981). This breakthrough led to the identification of families of OBPs in other insects, including mosquitoes

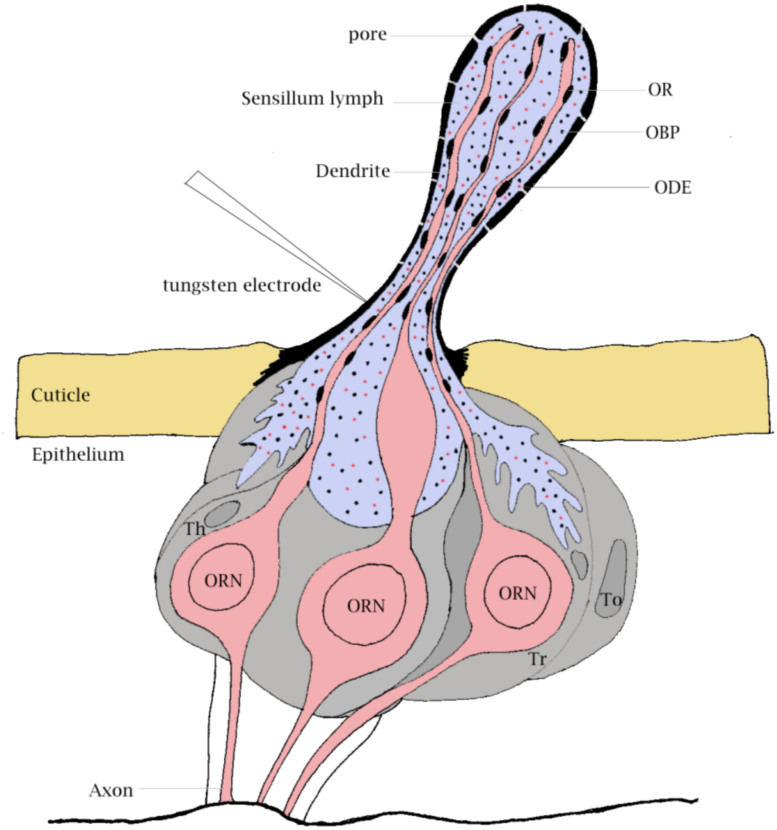


Figure 2. Schematic representation of the structure of a capitate peg sensillum. Single sensillum recording, to record the action potentials generated by the olfactory receptors of the olfactory receptor neurons (ORNs). OBP (black dots), ODE (red dots) and the auxiliary cells; thecogen (Th), trichogen (Tr), tormogen cells (To). Modified after Jacquin-joly and Merlin, 2004.

(Zhou *et al.*, 2008; Li *et al.*, 2005; Vogt *et al.*, 2002). Identified OBPs differ in number among species, e.g. *D. melanogaster* (38), *An. gambiae* (66), *Ae. aegypti* (57) and *Cu. quinquefasciatus* (53) (Pelletier *et al.*, 2010; Nene *et al.*, 2007; Graham & Davies, 2002; Holt *et al.*, 2002). It has been postulated that OBPs can be either specific, e.g. pheromone binding proteins (PBPs), or generalist e.g. general odorant binding proteins (GOBPs) (Vogt & Riddiford, 1981). What it is that accounts for this ligand affinity is not known. However, a few studies have found some common structural features among OBPs, e.g. that the OBP alpha-helix structure forms a hydrophobic binding pocket for ligands (Sandler *et al.*, 2000). Ligand binding and release has also been shown to be regulated by pH-dependent conformational change (Leal, 2012).

Besides the biochemical and structural information, the functional characteristics of OBPs remain elusive. The advancement of genetic tools in *D. melanogaster*, and its recent expansion to include other insects, has contributed considerably to understanding the function of OBPs in insect olfaction. Pheromone binding proteins appear to be required for pheromone detection under natural conditions (Vogt, 2003). The pheromone binding OBP in *D. melanogaster*, LUSH, has been shown to be required to evoke activity of pheromone-sensitive neurons to 11-*cis* vaccenyl acetate, following knockdown studies (Xu *et al.*, 2005). Similarly, the sensitivity to the main pheromone component of *Bombyx mori*, bombykol, was significantly enhanced when BmorOR1 was co-expressed with silkworm PBP, BmorPBP1, in the "empty neuron system" in *D. melanogaster* (Syed *et al.*, 2006). In the malaria mosquito *An. gambiae*, the sensitivity of the antennae to the mosquito oviposition pheromone (MOP) was significantly reduced by RNA interference (RNAi)-mediated knockdown of CqOBP1 (Pelletier *et al.*, 2010), previously shown to bind MOP (Laurence & Pickett, 1982). Not only known PBPs, but OBPs as well, appear to enhance the sensitivity of olfactory receptors to their ligands. The most notable example in mosquitoes is the abolished antennal response to the kairomones indole and 3-methyl indole following the RNAi-mediated knockdown of AgOBP1 in *An. gambiae* (Biessmann *et al.*, 2010).

The link between the reduction of OBPs and the decrease in sensitivity in the ORNs described a mechanism that may regulate the sensitivity of ORNs under natural conditions, such as a change in physiological state. In mosquitoes, a subset of OBPs reduce their expression following a blood meal (Biessmann *et al.*, 2005; Justice *et al.*, 2003), indicating that these OBPs may be involved in host-seeking behaviour. The reduction of OBP expression, an energy costly process, following a blood meal appears to be an elegant mechanism for decreasing olfactory sensitivity to the host, which also saves energy during a time when the majority of a mosquitoes' energy is focussed on egg maturation.

8.1.2 Olfactory receptors (ORs)

The first OR gene was identified in *D. melanogaster* (Clyne *et al.*, 1999; Gao & Chess, 1999; Vosshall *et al.*, 1999). Since then, the identification of the fly's entire set of OR genes, together with the availability insect genome databases, has opened the door to identify the OR gene repertoires in disease vector mosquitoes (Bohbot *et al.*, 2007; Fox *et al.*, 2001). Bioinformatic and molecular analyses of the mosquito genomes has identified 100 candidate OR genes in *Ae. aegypti* (Bohbot *et al.*, 2007), 150 OR genes in *Cu.*

quinquefasciatus (Arensburger *et al.*, 2010) and 79 OR genes in *An. gambiae* (Hill *et al.*, 2002). In insects, each ORN typically expresses only one type of conventional OR along with the conserved odorant receptor co-receptor (Orco). The exception came when Goldman *et al.* (2005) demonstrated the expression of two functional ORs along with Orco in a single type of ORN in *D. melanogaster*.

Insects ORs are a novel family of seven transmembrane (7TM) domain proteins and have no sequence similarities with vertebrate ORs (Hansson & Stensmyr, 2011). Furthermore, insect ORs have an inverted topology compared with other 7TM domain proteins, i.e. the location of the N-terminal is inside and the C-terminal is outside the cell membrane (Benton *et al.*, 2006). Insect ORs function as a heterodimer complex composed of highly diverged ORs and a highly conserved Orco (Vosshall & Hansson, 2011). Mainly the OR-Orco complex functions to facilitate the binding of odorants during the process of signal transduction and promote the responsiveness to odorants without affecting the ligand specificity (Benton, 2006; Larsson *et al.*, 2004). So far, the mechanism of odorant binding is not yet fully characterised. There are two proposed signal transduction pathways; first, the ionotropic pathway, in which the heterodimeric complex acts as ligand gated ion channel independent of G-protein signalling. This describes the rapid response kinetics of ORs (Sato *et al.*, 2008). Second is a slow prolonged metabotropic pathway, involving the conventional OR, activating a G-protein pathway that induces an increase in intracellular cAMP to activate the Orco cation channel (Wicher *et al.*, 2008). The currently espoused model involves both the ionotropic and metabotropic pathways in inducing the electrical current that results in the initiation and maintenance of ORN membrane depolarisation (Sato *et al.*, 2008; Wicher *et al.*, 2008).

8.1.3 Ionotropic receptors (IRs)

Recently, an antennally enriched subfamily of ionotropic glutamate receptors (iGluRs), the ionotropic receptors (IRs) has been identified as a new family of olfactory receptors in *D. melanogaster* (Benton *et al.*, 2009). Comprehensive analysis have showed that the IRs are expressed in coeloconic ORNs (Benton *et al.*, 2009), a subset of orphan olfactory sensilla with only one previously known olfactory receptor expressed (OR35a-Orco) in one ORN type (Couto *et al.*, 2005; Yao *et al.*, 2005). In *D. melanogaster*, 66 IR genes have been identified (Croset *et al.*, 2010). Out of these selectively expressed IRs, two, IR25a and IR8a, are broadly expressed in coeloconic ORNs, and are hypothesised to work in a similar manner to Orco in the OR repertoires

(Benton *et al.*, 2009; Benton, 2006; Larsson *et al.*, 2004). In contrast, to OR-expressing neurons, however, up to three IR genes can be expressed by each ORN (Benton *et al.*, 2009). Ionotropic receptors are highly conserved compared to other receptor repertoires and function as a multimeric complex (Benton *et al.*, 2009). Mis-expression analysis reveals that the IRs function as chemosensory receptors (Benton *et al.*, 2009). Although IRs appear to be ligand-gated ion channels (Kaupp, 2010; Benton *et al.*, 2009), biochemical assays *in vitro* are still required to confirm this hypothesis (Kaupp, 2010; Benton *et al.*, 2009).

8.1.4 Gustatory receptors (GRs)

At the end of the last century a new set of chemosensory receptors, the gustatory receptors (GRs), were identified in the labella of *Drosophila*, suggesting a role as taste receptors (Clyne *et al.*, 2000). Over the next few years, 68 GRs, encoded by 60 GR genes, have been found to be expressed in the cells of antennae, maxillary palp, labial palp, labrum, cibarium, tibiae, tarsi and anterior wing margin of *Drosophila* (Robertson *et al.*, 2003; Dunipace *et al.*, 2001; Scott *et al.*, 2001). That some of these receptors were expressed in typical olfactory organs suggested that some GRs may have olfactory function. Similar to ORs, GRs are 7TM domain proteins and function as a heteromeric complex with an inverted topology, i.e. intracellular N-terminal and extracellular C-terminal (Clyne *et al.*, 2000). In contrast to ORs, Orco is not required in GRs in Drosophila (Jones *et al.*, 2007), nor has a "Grco" yet been identified.

A few *Drosophila* GRs have been functionally classified, e.g. courtship contact pheromone-sensitive GR68a (Bray & Amrein, 2003), sugar-sensitive GR5a and bitter-sensitive GR66a (Wang *et al.*, 2004; Chyb *et al.*, 2003; Dahanukar & Foster, 2001; Ueno *et al.*, 2001). However only one pair of GRs, GR21a and Gr63a, has been shown to have an olfactory function. These GRs were found to act as heterodimers and to be sensitive to CO_2 (Kent *et al.*, 2008; Jones *et al.*, 2007; Scott *et al.*, 2001). In *situ hybridisation* assays revealed that Gr21a and Gr63a genes are expressed in *Drosophila* antennae and electrophysiological assays confirmed that these GR expressing neurons are involved in the detection of CO_2 (Jones *et al.*, 2007).

The homologues of the Drosophila CO_2-sensitive GRs (CO_2-GRs) have been found to be expressed in the maxillary palps of *An. gambiae* (Kent *et al.*, 2008; Jones *et al.*, 2007), *Ae. aegypti* (Robertson & Kent, 2009; Kent *et al.*, 2008) and *Cu. quinquefasciatus* (Robertson & Kent, 2009). In *Ae. aegypti* and *Cu. quinquefasciatus*, these homologous GRs (AaGR1 and 3; CqGR1 and 3) are also CO_2-sensitive, as revealed by RNAi-knockdown assays, and are probably expressed as heterodimers (Erdelyan *et al.*, 2012). Interestingly, there is a paralogue to AaGr1 and CqGR1, which is expressed in the maxillary palps but whose knockdown in expression does not affect the mosquito's response to CO_2 (Erdelyan *et al.*, 2012). The function of the paralogue, if indeed it is functional, is unknown. Of note in a discussion of the importance of CO_2 reception in host-seeking is that the mosquito CO_2-GRs are differentially expressed between the sexes (Erdelyan *et al.*, 2012). The expression of CO_2-GRs is enhanced in females over males in *Cu. quinquefasciatus* as would be predicted by the supposed increased need for CO_2 sensitivity for host-seeking in females. However, this trend is reversed in *Ae. aegypti*, suggesting that the selective pressures on mosquito sensitivity to CO_2 may not be so clear cut (Erdelyan *et al.*, 2012).

8.1.5 Odorant-degrading enzymes (ODEs)

Chemical stimuli in the form of general odours or pheromones are xenobiotic substances in the olfactory environment. Within a few milliseconds after interacting with the odour-sensitive receptors, these substances are rapidly inactivated (Leal, 2012; Vogt, 2005). There are two proposed mechanisms for signal inactivation: 1) three decades of investigation supports the degradation of odorants using enzymes; while 2) using a molecular trap to scavenge these chemical substances has been proposed, but has little experimental support (Leal, 2012). The presence and number of ODEs has been established (Ishida *et al.*, 2004; Vogt, 2003), whereas, the scavengers have been proposed as an additional role for OBPs (Kaissling, 2001). Previously, it has been shown in *A. polyphemus* and *Bombyx. mori* that the degrading enzymes are species-specific esterases present in the sensillum lymph, where they inactivate the pheromone components by degradation (Vogt & Riddiford, 1981). In addition to the specific estrases for pheromone degradation, it has been shown that the same ODE, SlCXE7, could efficiently hydrolyse both the pheromone as well as green leaf volatiles (Durand *et al.*, 2011). Subsequently, it has been shown in lepidopterans that various types of pheromone compounds are degraded by antennal aldehyde oxidases, aldehyde dehydrogenases, epoxide hydrolases, glutathione-S-transferases, and cytochrome P450 (Vogt, 2005).

8.1.6 Translation from gene to behaviour

Both genetic and environmental factors can modulate host recognition (Richgels & Rollmann, 2012; Rollmann *et al.*, 2010). However, only the genetic factors are heritable and thus can lead to the modulation of host choice across generations. The underlying genetic architecture that governs host recognition is largely unknown. However, recent investigations into the structural and functional variation within the odorant receptor gene family provide insights into the relationship among gene structure, receptor tuning and odour-mediated behaviour (Richgels & Rollmann, 2012; Rollmann *et al.*, 2010; Wang *et al.*, 2007).

The genetic basis delimiting an insect's odour space relies on the family of Or genes that are expressed in the peripheral olfactory system (Carey *et al.*, 2010; Wang *et al.*, 2010; Hallem & Carlson, 2006; Dobritsa *et al.*, 2003). The tuning of these receptors constrains the insect's ability to perceive odour blends. This is done by limiting the cues perceived to those that can be detected by the receptors. The tuning of individual Ors, across the spectrum from narrow to broad, also appears to play a role in determining the information content of the cues detected (Carey *et al.*, 2010; Wang *et al.*, 2010; Hallem & Carlson, 2006).

Narrowly tuned receptors appear to carry information about major biologically salient odours through specialist channels (Carey *et al.*, 2010; Wang *et al.*, 2010; Hallem & Carlson, 2006). The most narrowly tuned receptors, with a high ligand affinity ($<10^{-7}$ dilution), appear to play a pivotal role in host preference (Wang *et al.*, 2010; Bohbot & Dickens, 2009). In *An. gambiae*, the majority of these receptors (e.g. AgOr1, 2, 5, 8 and 35) respond to odorants in human sweat, breath and urine (e.g. 4-methylphenol, indole, 2,3-butanedione, 1-octen-3-ol and geranyl acetone) (Carey *et al.*, 2010; Wang *et al.*, 2010). Similarly, narrowly tuned receptors in *D. melanogaster* (e.g. DmOr35a, 85b, 22a and 67a) respond primarily to the principle volatiles (e.g. esters) from their feeding and oviposition substrate, rotting fruit (Hallem & Carlson, 2006).

On the other hand, broadly tuned receptors may indicate the presence of the odorants, rather than discriminate among them (Carey *et al.*, 2010; Wang *et al.*, 2010). The two most broadly tuned receptors in *An. gambiae*, (AgOr30 and 57) respond to 14-15 chemically diverse human derived odorants, including acids, ketones, aromatics, heterocyclics, and alcohols (Carey *et al.*, 2010; Wang *et al.*, 2010). In contrast, the four most broadly tuned receptors in *D. melanogaster* (DmOr35a, 85b, 22a and 67a) were each found to be most sensitive to structurally similar odorants (Hallem & Carlson, 2006). Thus, Or tuning provides with an indication of the relative value to the insect of individual volatiles, as well as groups of related compounds, in host odour blends.

The combination of the Or tuning profiles describes a species-specific odour space. The overall pattern of response to these odours appears to reflect the biological relevance of specific chemical classes to individual species (Carey *et al.*, 2010; Hallem & Carlson, 2006). One example compares the Or response profiles of *D. melanogaster* with *An. gambiae*. The mosquito is able to discriminate among more aromatics, key compounds in human emanations, than esters, which comprise the main volatiles emitted from rotting fruit. This is in contrast to fruit fly, which discriminates among the esters more efficiently (Carey *et al.*, 2010; Wang *et al.*, 2010), thus mirroring relevance of each of these chemical classes to each species. It is important to note that the overall odour space of an insect includes response profiles of more than only the odorant receptors. Whereas in *D. melanogaster* Ors respond to the carboxylic acids, in *An. gambiae* it is not the Ors but possibly the ionotropic glutamate receptors (Irs) expressed in grooved peg sensilla that respond to the carboxylic acids (Qiu *et al.*, 2006b). Consequently, it is the overall functional profile of olfactory receptors that provides one potential underlying genetic mechanism that enables insects to recognise their particular host species in an ecological environment.

So, it is established that the overall tuning of the olfactory receptors correlates with host preference, but what is the genetic architecture that underpins the tuning of a receptor, and thus the odour-mediated behaviour of the insect? One postulate states that small changes in the structure of an Or gene can alter the sensitivity of the receptor to its key ligand, and thus modulate such odour-induced behaviour (Rollmann *et al.*, 2010). Recently, a small number of single nucleotide polymorphisms (SNPs) in Or genes were found to be directly associated with the variability in behavioural response to key odorants (e.g. benzalaldehyde) by *D. melanogaster* (Richgels & Rollmann, 2012; Rollmann *et al.*, 2010; Wang *et al.*, 2010). This illustrates that minor variations in Or gene structure that arise over evolutionary time can contribute directly to variations in olfactory-mediated behaviour and may give rise to shifts in host choice.

9 Summary of results

9.1 Context is not everything-sensory constraints also regulate host-seeking behaviour of mosquitoes (Paper-I)

During the process of evolution, mosquitoes evolve their olfactory system for locating and detecting suitable host species. Evidence of this is the specifically tuned and highly sensitive CO_2 and (*R*)-1-octen-3-ol ORNs in *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus*. In this study, I analysed the electrophysiological and behavioural responses (Box 1,4) of these mosquitoes to CO_2. This analysis showed that *Ae. aegypti* and *An. gambiae* are able to detect and track pulsed CO_2 stimuli, whereas the behavioural responses of *Cu. quinquefasciatus* is constrained due to sensory adaptation, at high CO_2 concentrations (Figure 3A,B). Using a landing bioassay (Box 2), I also analysed how mosquitoes with different host preferences perceive different host species' volatile blends (Figure 3C). This analysis showed that blend perception plays a key role in host-species recognition and non-host species avoidance. In my behavioural analysis I show that single host-species volatile compounds can change blend perception. *An. gambiae* and *Ae. aegypti* use the ubiquitous mammalian volatile cue, (*R*)-1-octen-3-ol, as an indicator of a suitable host species, even when presented in the background of a non-preferred host-species volatile blend. In contrast, *Cu. quinquefasciatus* perceive (*R*)-1-octen-3-ol as a non-host cue. Hence, context appears to play a major role for the insect's blend perception, and therefore determination of host suitability.

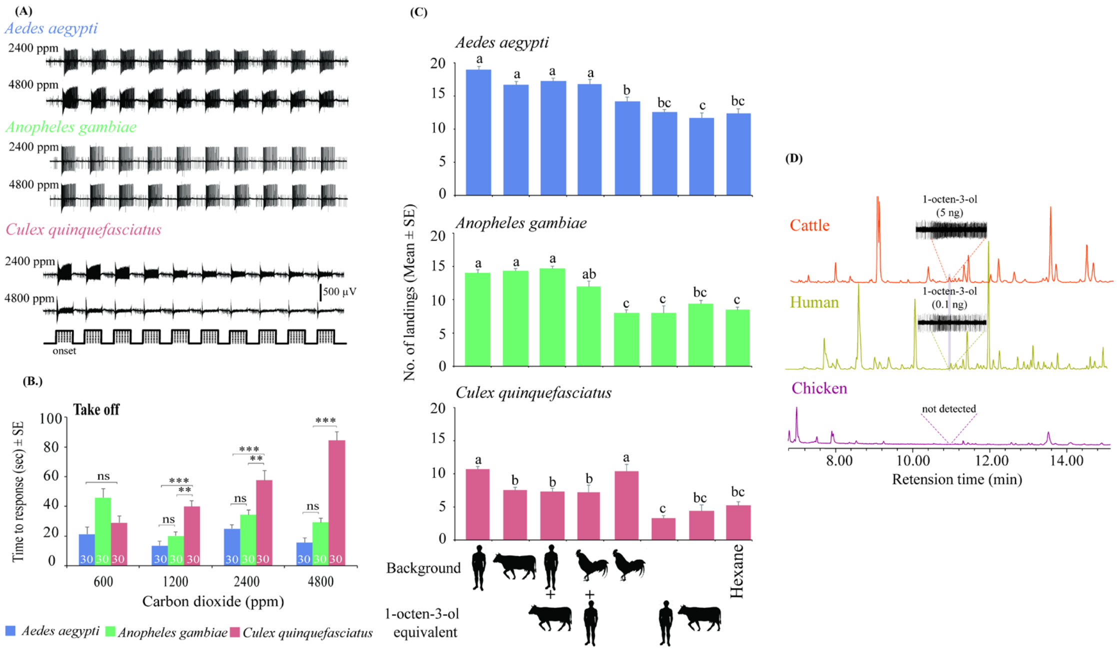
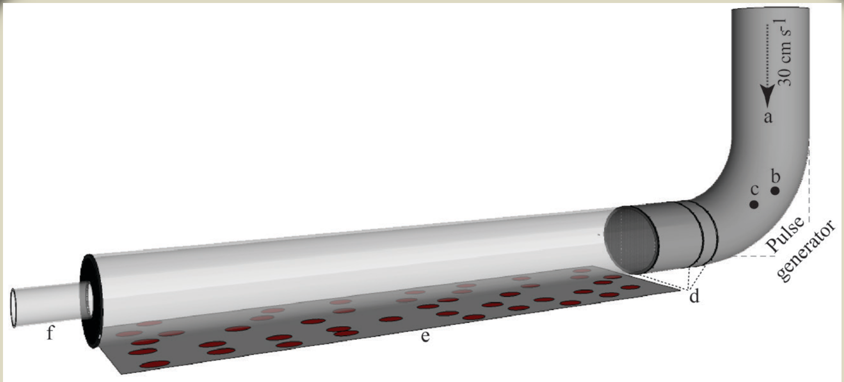
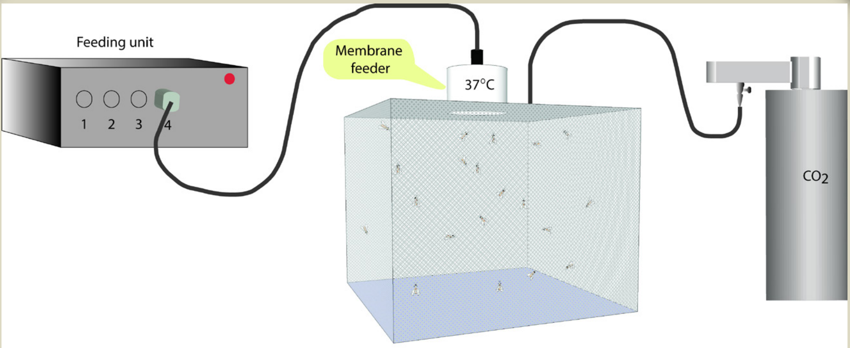


Figure 3. The temporal coding capacity of CO_2 detection and behavioural response to (*R*)-1-octen-3-ol in female *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus*. (A) Detection of pulsed stimuli by the CO_2-sensitive neuron over increasing concentrations. (B) The attraction of female mosquitoes in flight tunnel towards pulsed stimuli of the indicated concentrations of CO_2 (n = 30 each species). Asterisks indicate the significant differences among the species (*Kruskal-Wallis*, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). (C) Landing bioassays were performed to observe the role of (*R*)-1-octen-3-ol in host seeking behaviour (n = 10). Different extracts were tested alone and in combination with human and cattle equivalent (*R*)-1-octen-3-ol (0.1 and 5 ng, respectively). Letters above the bars denote significant difference between treatments within species (GLM ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). (D) Representative odorant profiles as generated by gas chromatograph (GC) of cattle, human and chicken extracts. The grey bar represents the identified compound (*R*)-1-octen-3-ol present in the cattle and human extracts but not detected (nd) in the chicken extracts. Traces above the human and cattle extract represent the elicited responses from the 1-octen-3-ol sensitive neuron (B cell) using GC-single sensillum recording (SSR).



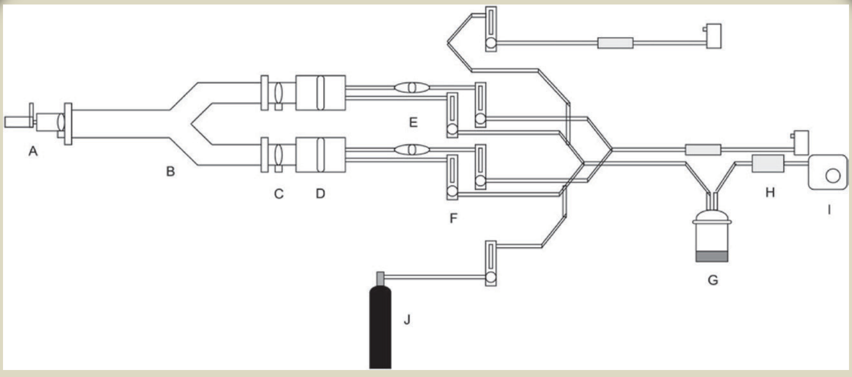
Box 1. Female mosquito behaviour was assessed in a wind tunnel assay, schematic diagram shown with labels including: (a) charcoal filtered and humidified air, (b) pressurized air inlet, (c) stimulus inlet into which the odours were blown to travel through (d) stainless steel mesh plume diffusers, (e) and into the glass flight tunnel (f) towards release chamber.



Box 2. Group of female mosquito behaviour was assessed in landing bioassay. Temperature of membrane feeder is regulated by through feeding unit. Extracts and synthetic compounds were applied onto the membrane of the membrane feeder and exposed to the mosquitoes. Carbon dioxide was delivered through CO_2 cylinder.

9.2 Enantiomeric selectivity of olfactory receptor neuron to 1-octen-3-ol (Paper II)

1-octen-3-ol is one of the most extensively studied host-species kairomones used by haematophagous insects, including mosquitoes. Here, we investigated the behavioural and electrophysiological responses of *Ae. aegypti* and *Cu. quinquefasciatus* to (*R*)-1-octen-3-ol, (*S*)-1-octen-3-ol and racemic mixtures thereof (Box 3). For the first, time enantiomeric selectivity is shown for *Ae. aegypti* using electrophysiology in vivo. We show that an olfactory receptor neuron (ORN) in the maxillary palp sensilla of both mosquito species (Figure 4A,B,C,D), responds to enantiomeric and racemic 1-octen-3-ol in a dose dependent manner. The ORNs of both mosquito species are more sensitive to (*R*)-1-octen-3-ol, than to racemic 1-octen-3-ol and (*S*)-1-octen-3-ol (Figure 4C,D). Behaviourally (Box 3), (*R*)-1-octen-3-ol, but not the racemic 1-octen-3-ol or (*S*)-1-octen-3-ol, elicits a significant increase in flight activity and relative attraction, at 10^{-7} g μl^{-1} and 10^{-5} g μl^{-1}, in *Ae. aegypti* (Figure 4E). In contrast, none of the tested treatments elicited attraction in *Cu. quinquefasciatus* (Figure 4F). However, a reduced relative attraction response was observed in *Cu. quinquefasciatus*, to (*R*)-1-octen-3-ol enantiomer, either singly or in the racemic mixture, when presented at 10^{-3} g μl^{-1} (Figure 4F). Combined, our results show that the natural isomer of 1-octen-3-ol, (*R*)-1-octen-3-ol, elicits differential behavioural responses in mosquitoes, and that perception of this compound is regulated at the central nervous system.



Box 3. Y-tube olfactometer bioassay design. Female insects were collected in pre-release chambers (A) and allowed to acclimatize for two hours. These chambers were designed to easily attach to the main Y-tube olfactometer (B). Air was pumped (I) through a charcoal filter (H) and humidified in a glass bell jar containing dH_2O (G). The airflow was regulated by flow meters (F). Chemicals were applied to filter papers and placed in sintered glass vessels (E). Where necessary, CO_2 was introduced from a cylinder located in the bioassay room (J).

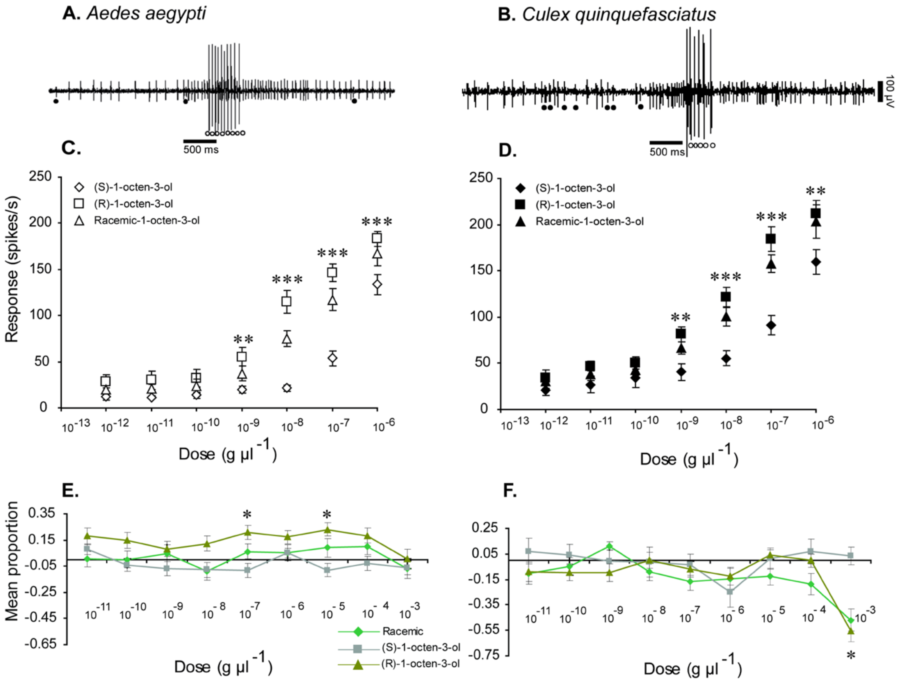
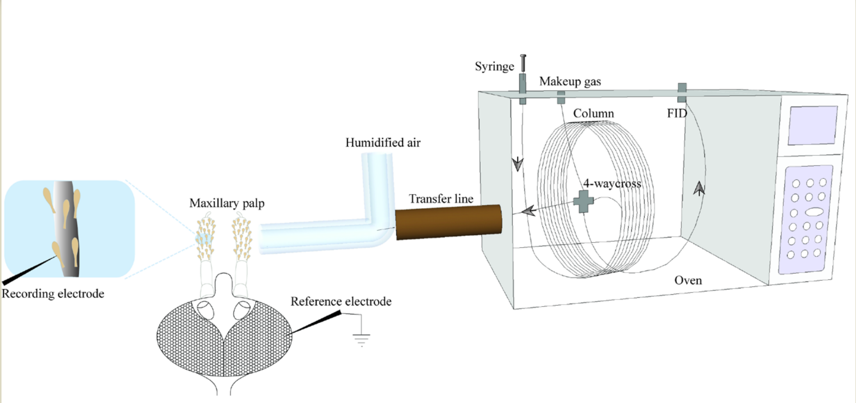


Figure 4. Dose response relationships of *Ae. aegypti* and *Cu. quinquefasciatus* female mosquitoes. 1-Octen-3-ol and its enantiomers elicit dose-dependent responses in the B neuron of the maxillary palp capitate pegs of *Ae. aegypti* and *Cu. quinquefasciatus* female mosquitoes. (A), (B) Response of the B neuron to (*R*)-1-octen-3-ol, and of the A neuron (open circle) to ambient CO_2 trapped in the stimulus cartridge in *Ae. aegypti* and *Cu. quinquefasciatus*, respectively. Closed circles indicate the smallest spiking neuron, the C neuron. Unmarked intermediate spikes are from the B neuron. (C), (D) The B neurons in *Ae. aegypti* and *Cu. quinquefasciatus*, respectively, display significantly different responses to (*R*)- and (*S*)-1-octen-3-ol, and to the racemic mixture of 1-octen-3-ol (two-way repeated measures ANOVA; $*P<0.05$, $**P<0.01$, $***P<0.001$, n=10; mean±SEM). (E), (F) The behavioural response to (*R*)- and (*S*)-1-octen-3-ol, and to the racemic mixture of 1-octen-3-ol is shown for *Ae. aegypti* and *Cu. quinquefasciatus*, respectively.

9.3 Proximate mechanisms behind enantiomeric selectivity and sensitivity of an odorant receptor (Paper III).

The host-seeking behaviour of mosquitoes is regulated by the specificity and sensitivity to host odours at the peripheral olfactory level. While, the ultimate mechanism of mosquito host selection and speciation over an evolutionary timescale has been investigated, little effort has been made to understand the proximate mechanism. We have performed SSR in vivo and calcium imaging of ORs expressed in an Sf9 cell-line in vitro (Box 5) to investigate the functional properties of ORs with minor structural differences, and their effect on their cognate ORNs. We found that the ORNs of each mosquitoes species, *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus*, are more sensitive to (*R*)-1-octen-3-ol than to (*S*)-1-octen-3-ol (Figure 5A,B,C). Similarly, orthologous odorant receptors, the Or8s, from each mosquito species, AgOr8, AaOr8 and CqOr118, were significantly more sensitive to (*R*)1-octen-3-ol than (*S*)-1-octen-3-ol. The paralogous CqOr113, however, did not show differential selectivity among the enantiomers (Figure 6A,B,C,D). We found that this variable functional tuning was reflected in minor differences in the receptor's amino acid structure. In particular, we found that Or8 enantiomeric specificity relies on a subset of the c-terminal amino acids encoded by the final exon of the gene, i.e. the receptor CqOr113 codes 20 c-terminal amino acids that are not orthologous to those of the other Or8s (Figure 6E,F). In addition, we found that Or8 sensitivity to (*R*)-1-octen-3-ol appears to rely on up to 14 amino acids, only three of which are situated to interact directly with the odorant ligand. The other nine amino acids are located in regions indicating possible interactions with cell signalling pathways and the Orco ion channel. Amino acid substitution polymorphisms in each of these regions have the potential to alter the sensitivity of the receptors through established mechanisms.



Box 4. Gas chromatography coupled single sensillum recording (GC-SSR) technique for mosquitoes: extracts are injected into the injector port via syringe and onto the GC column, which is situated in an oven; as the oven temperature increases, the components of the extract elute and reaches a 4-waycross splitter. From the spliter, half of the sample goes to a flame ionization detector (FID) and the rest of the sample passes through a transfer line into a glass tube, which passes humidified synthetic air without carbon dioxide over the maxillary palps. Simultaneous single sensillum recordings were performed by poking reference electrode into the eye and recording electrode onto the base or shaft of the sensilla to record the action potential of bioactive compounds. Modified after Ghaninia *et al*. (2008).

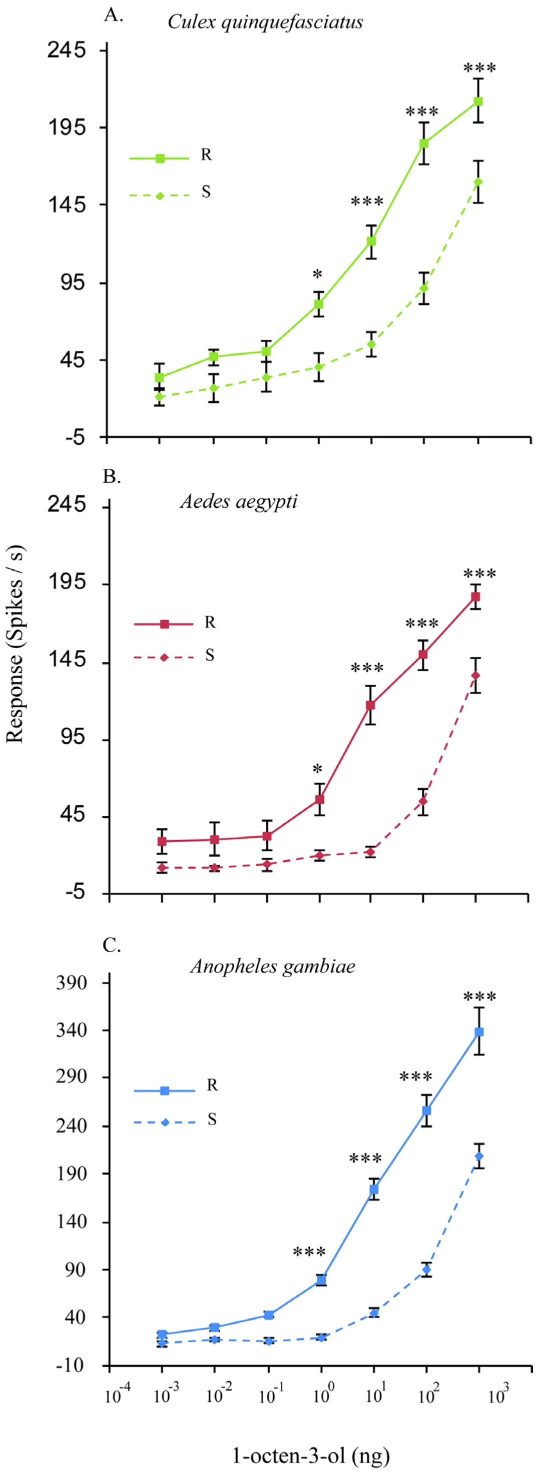


Figure 5. Enantiomeric specificity of 1-octen-3-ol sensitive neurones in the maxillary palp basiconic sensillum from female *Culex quinquefasciatus* (A), *Aedes aegypti* (B) and *Anopheles gambiae* (C) mosquitoes. Asterisks indicate level of statistical significance between the single sensillum recording responses to S-(+)-1-octen-3-ol and R-(-)-1-octen-3-ol as determined by two-way ANOVA (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

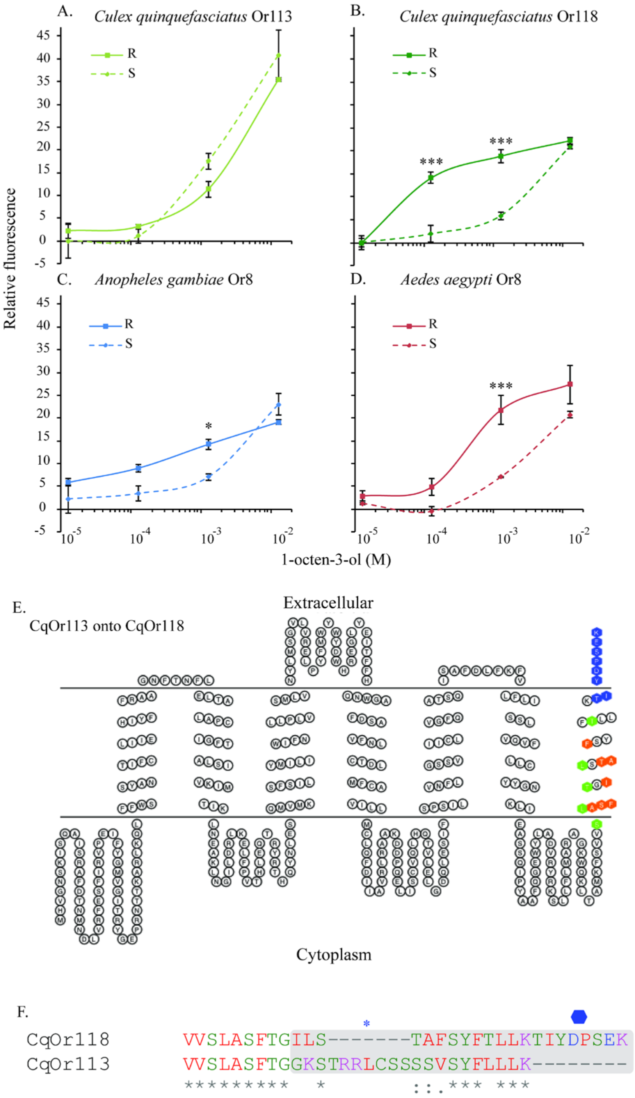
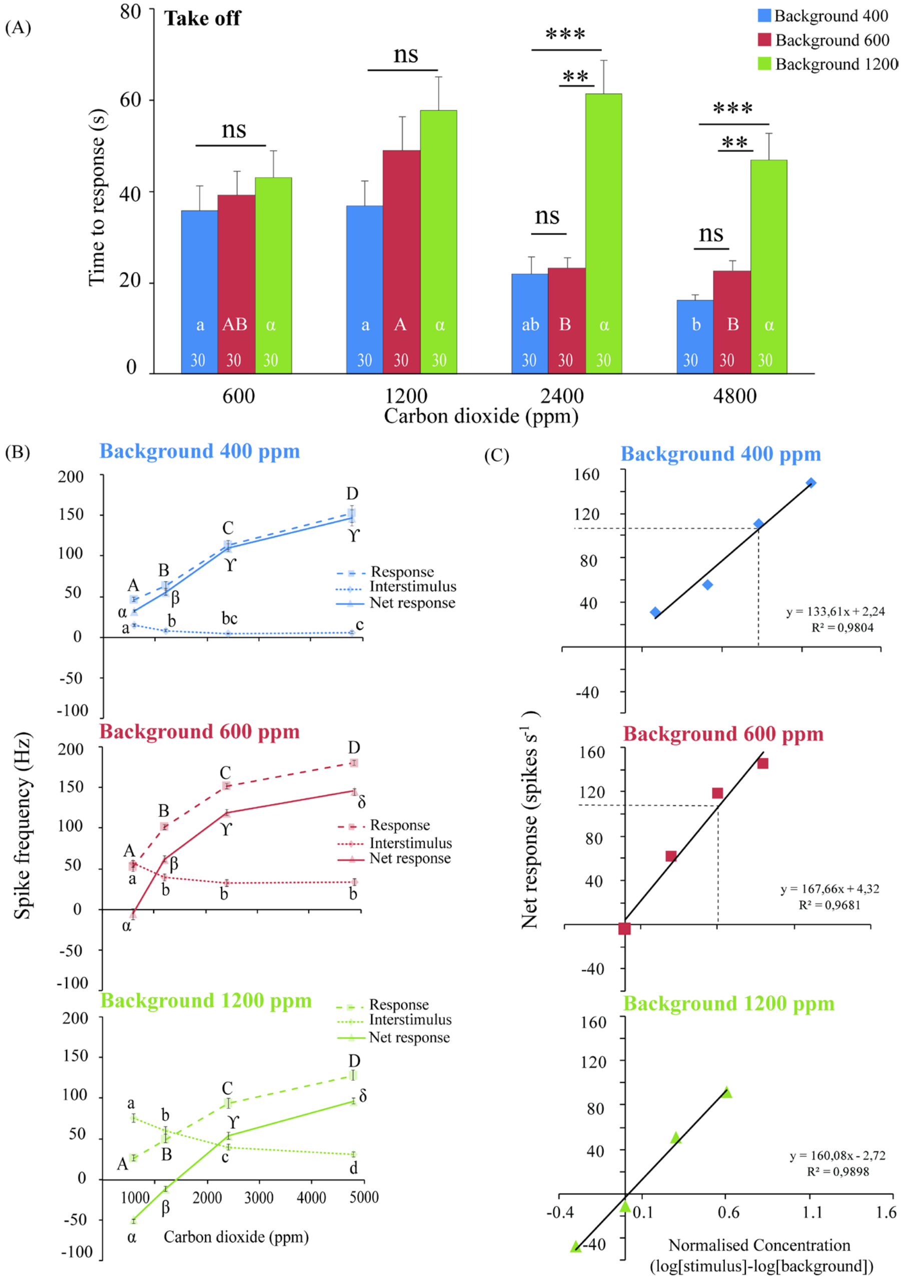


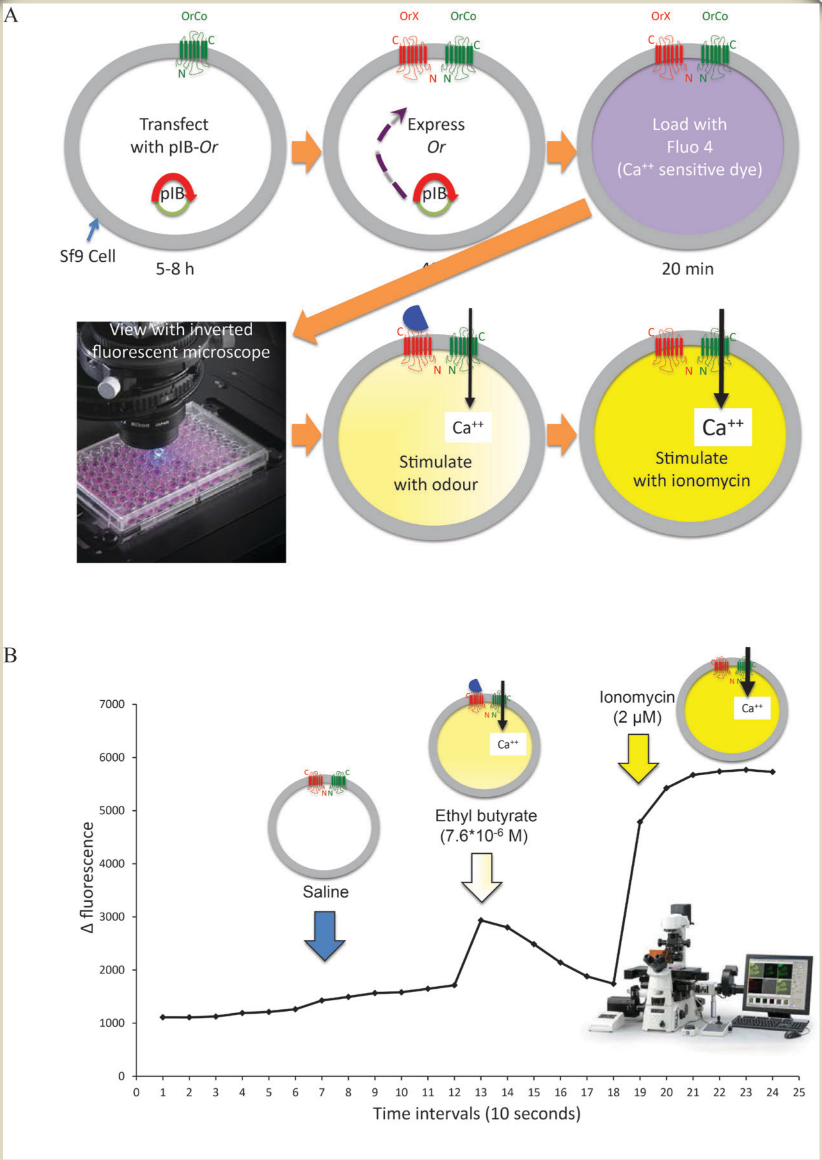
Figure 6. Specificity of heterologously expressed Or8 family members from *Culex quinquefasciatus* (A and B), *Anopheles gambiae* (C) and *Aedes aegypti* (D) to enantiomers of 1-octen-3-ol. Asterisks indicate level of statistical significance between the intensity of fluorescent responses from S-(+)-1-octen-3-ol and R-(-)-1-octen-3-ol as determined by two-way ANOVA (*$P<0.05$, **$P<0.01$, ***$P<0.001$). Figure 3: Differences in amino acid sequence between the enantiomer selective CqOR118 and the non-selective CqOR113. (E) A sequence-based model depicting the protein orientation and transmembrane domains of CqOr118. Colours indicate those amino acids that differ from CqOr113: conserved substitutions (orange hexagons), non-conserved substitutions (green hexagons) and amino acids missing from CqOr113 (blue hexagons and an asterisk). (F) Clustal alignment of transmembrane domain 7 and the C-terminus of CqOr118 and CqOr113. The grey box indicates the amino acids coded by the final exon of CqOr118 and CqOr113.

9.4 Impact of elevated CO_2 on mosquito behaviour (Paper IV)

Carbon dioxide is a ubiquitous kairomone produced by all vertebrates, on which mosquitoes rely to orient towards their blood hosts, as shown in paper I. However, variable and rapid fluctuations of atmospheric CO_2 concentrations may impact the ability of mosquitoes to regulate their host-seeking behaviour. Here, we examined the effect of transient elevated background levels of CO_2 on *Ae. aegypti* host-seeking behaviour and the physiological characteristics of the CO_2-sensitive ORNs. To enquire into the role of elevated background levels of CO_2 in forging behaviour of these mosquitoes, we performed wind-tunnel bio-assays on host-seeking females (Box 1) and SSRs on the CO_2-sensitive ORN housed in the capitate peg sensilla on the maxillary palp (Box 4). We demonstrate a significant impact of transient elevated background levels of CO_2 on the behaviour of *Ae. aegypti*. Our study showed that the take off and upwind flight behaviour of *Ae. aegypti* is impeded in background level of 1200 ppm CO_2 as a result of a physiological masking of the stimulus signal (Figure 7A). The mechanism involved in masking the stimulus signal is one of the sensory constraint. At a background level of 1200 ppm CO_2, the overall net response of the CO_2-ORNs was significantly reduced by the increased interstimulus activity caused by the elevated background level of CO_2 (Figure 7B). The resulting behavioural constraint is explained by a threshold in CO_2-ORN net response($\geq$ 110 spikes s^{-1}), which when exceeded, results in reducing the time to take off flight (Figure 7C).

Figure 7. The temporal characteristics of CO_2 chemosensation in female *Ae. aegypti* in different background levels of CO_2. (A) Female mosquitoes' attraction towards pulsed stimuli of ascending CO_2 concentrations in the wind tunnel at different CO_2 background levels (n=30 for each background level). Numbers inside the bars represent the number of tested individuals that took off or flew upwind towards the source. Asterisks indicate the significance among the different CO_2 backgrounds (*Kruskal-Wallis*, $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). Letters inside the bars indicate significant differences within the background levels over increasing concentrations. (B) Averaged spike frequency (n=10) of response, interstimulus activity and net response of the CO_2-ORNs in different CO_2 backgrounds. Letters and symbols denote significant differences within the response, interstimulus activity and net response over increasing concentration. (C) Take off flight behaviour in *Ae. aegypti* females is correlated with the net physiological response of the CO_2-ORNs to normalised net change in CO_2 concentrations. The dotted lines identify the behavioural threshold in net physiological response, i.e. at which take off flight were significantly reduced.


(A)
Take off
Time to response (s)
80
60
40
20
0
Background 400
Background 600
Background 1200
ns
ns
***
**
ns
***
**
ns
a
AB
α
a
A
α
ab
B
α
b
B
α
30
600
1200
2400
4800
Carbon dioxide (ppm)
(B)
Background 400 ppm
Spike frequency (Hz)
200
150
100
50
0
-50
-100
A
B
C
D
a
b
bc
c
α
β
ϒ
Response
Interstimulus
Net response
Background 600 ppm
A
B
C
D
a
b
b
b
α
β
ϒ
δ
Background 1200 ppm
A
B
C
D
a
b
c
d
α
β
ϒ
δ
1000
2000
3000
4000
5000
Carbon dioxide (ppm)
(C)
Background 400 ppm
Net response (spikes s⁻¹)
160
120
80
40
-40
y = 133,61x + 2,24
R² = 0,9804
Background 600 ppm
y = 167,66x + 4,32
R² = 0,9681
Background 1200 ppm
y = 160,08x - 2,72
R² = 0,9898
-0.4
0.1
0.6
1.1
1.6
Normalised Concentration
(log[stimulus]-log[background])



Box 5. (A) Transfection agent along with the purified plasmid DNA to Sf9 cells and incubate for 5-8 hours. Transfeced cells shifted into new media and incubated for 48 hours prior to calcium imaging. Transformed Sf9 cell lines were placed in individual wells, loaded with CA^{+2} sensitive dye and screened in response to the odorants. (B) Response of transfected Sf9 cell to saline, ethyl butyrate and ionomycin.

10 Conclusion and future prospective

In this thesis, I have described the proximate mechanisms underpinning the host selection and discrimination in three mosquito species, *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus*, using two known mosquito kairomones, CO_2 and 1-octen-3-ol. Although numerous studies have shown the attraction of host-seeking mosquitoes to CO_2 and 1-octen-3-ol in the laboratory and field, this is the first body of work to demonstrate their role in host discrimination.

First, I investigated the sensory mechanisms and constraints regulating mosquitoes' behaviour in response to CO_2. The sensory limitations described by the temporal capacity of CO_2-sensitive ORNs correlates with the behavioural constraints on mosquito activation (time to take off) in response to CO_2 (paper-I). The activation of *Ae. aegypti* in response to a range of CO_2 concentrations similar to host emission rates was shown to be constrained by a behavioural threshold in net response of CO_2-ORNs during elevated background CO_2 levels. Therefore, the CO_2-sensitive ORNs in these mosquitoes appear to be highly adapted to pulsed stimuli, translating this sensory limitation, as a behavioural output that is, in part, responsible for their host preferences.

In the following part of study, I investigated the specificity and sensitivity to (*R*)-1-octen-3-ol and its perception by *Ae. aegypti*, *An. gambiae* and *Cu. quinquefasciatus* as a host and non-host volatile cue. Host discrimination in mosquitoes appears to be, at least partly, dependent on their physiological and behavioural sensitivity to host specific compounds. When the host volatile context was altered by artificially increasing the amount of (*R*)-1-octen-3-ol in host extracts, I found that *Ae. aegypti* and *An. gambiae* use (*R*)-1-octen-3-ol as a host cue, whereas *Cu. quinquefasciatus* perceives it as a non-host cue (paper-I). This observation is supported by my findings that *Ae. aegypti* are attracted by a wide range of (*R*)-1-octen-3-ol concentrations, while *Cu. quinquefasciatus* appear to ignore low concentrations of (*R*)-1-octen-3-ol, but are repelled at higher concentrations, reflecting the (*R*)-1-octen-3-ol emissions of their preferred host species (paper-II). These behavioural and physiological sensitivities to (*R*)-1-octen-3-ol are reflected in the sensitivities of the orthologous mosquito 1-octen-3-ol-sensitive odorant receptors, the OR8s (paper-III). The OR8 receptors, their cognate ORNs and the resulting 1-octen-3-ol-induced behavioural response differentiate between the two enantiomers of 1-octen-3-ol (papers-II and III). In all instances, but one, (*R*)-1-octen-3-ol is more active than its enantiomer (*S*)-1-octen-3-ol. The orthologous odorant receptors, AgOr8, AaOr8 and CqOr118, are significantly more sensitive to (*R*)-1-octen-3-ol than (*S*)-1-octen-3-ol, whereas CqOr113, the paralogue of CqOr118 does not differentiate between the enantiomers. The receptor CqOr113 differs in amino acid sequence from orthologous receptors at the c-terminus. This suggests that the c-terminus of the OR8s are responsible for their enantiomeric selectivity, and demonstrates that changes in OR structure during the evolutionary time can result in differential receptor tuning.

Combined, these studies provide an understanding of how the olfactory system of mosquitoes translates host and non-host odour cues into host recognition behaviour. These studies also reveal that the host-seeking behaviour of mosquitoes is constrained by sensory limitations. Understanding these constraints will be of utmost importance in the development of future odour-based vector control strategies.

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