Title
Reception of odors and repellents in mosquitoes

Author
Ray, Anandasankar

Publication Date
2015-10-01

DOI
10.1016/j.conb.2015.06.014

Peer reviewed

Published in final edited form as:
Curr Opin Neurobiol. 2015 October ; 34: 158–164. doi:10.1016/j.conb.2015.06.014.

Reception of odors and repellents in mosquitoes

Anandasankar Ray
Department of Entomology, Center for Disease Vector Research, University of California Riverside, Riverside, CA 92521, USA

Anandasankar Ray: anand.ray@ucr.edu

Abstract

Mosquitoes use their sense of smell to find hosts, nectar, and oviposition sites, and to avoid repellents. A small number of mosquito species are adapted to feed on humans and have a major impact on public health by transmitting malaria, dengue, filariasis, etc. The application of odorants for behavioral control has not been fully realized yet due to complexity of the mosquito olfactory system. Recent progress in molecular and computational tools has enabled rigorous investigations of the mosquito olfactory system function and has started to reveal how specific receptors contribute to attractive and aversive behaviors. Here we discuss recent advances in linking odors to receptors and in exploiting this knowledge in finding attractants and repellents for mosquitoes.

INTRODUCTION

Odorants can be used to control mosquitoes in three complimentary ways: repellents that "push" mosquitoes away, "maskers" that block attraction to humans, and attractants that "pull" mosquitoes into traps placed away from humans. Each of these methods can potentially reduce disease transmission by preventing mosquito-human interactions. Limited field studies evaluating odor-based control find that repellents are generally better at reducing biting pressure as compared to traps [1,2]. However, repellents and traps tested together in an integrated push-pull is more effective than either alone [3,4].

Despite the potential for reducing disease transmission there are considerable drawbacks in the attractants and repellents available currently for public health use. Repellents like DEET have very limited use in disease-inflicted tropical countries, likely for aversion to use a non-natural compound, the need to apply high concentrations on skin, relatively high costs, and poor cosmetic qualities (dissolves nylons and plastics, smells unpleasant, oily feel). Similarly, the current mosquito traps are extremely expensive and bulky, since they need a CO_2 (lure) source, and also may contain other synergists that smell unpleasant. There is an urgent need for new classes of improved repellents and lures for mosquito control globally.

Conflict of Interest Statement:
A. Ray is an equity holder at Olfactor Labs Inc and a founder of Sensorygen Inc.

The challenges of finding behaviorally active odorants of practical utility are enormous. The mosquitoes have extremely sophisticated olfactory systems with hundred of receptors proteins from three different families: odorant receptor (Or), ionotropic receptor (Ir) and gustatory receptor (Gr) families [5]. These receptors are likely expressed in similar numbers of classes of odorant receptor neurons (ORNs) housed in sensilla on the antenna, maxillary palps and proboscis. The axons of the ORNs project to the antennal lobe (AL) in the brain's deutocerebrum, where they innervate glomeruli, likely sorting according to their expressed receptors [6–8]. The *Or* family is the largest, most diverse, with ligands known for a majority of members in *Anopheles gambia* [9,10]. Their comprehensive deorphanization in the *Drosophila* "empty neuron" system and *Xenopus* oocytes enabled a systems-level understanding of odor detection by the *Or* family and identified several odorants that the mosquitoes detect strongly. However, to design repellents and attractants this knowledge is most useful if we understand how the multitudes of olfactory receptors contribute to odor valence and behavior generation. The highly distributed pattern of multiple Ors sensing an odorant, and the complex connectivity patterns in the brain of ORNs, make it very difficulty to identify neurons and receptors that convey attraction or aversion.

Identifying attractive odorants, receptors and developing lures for "pull"

Host-seeking mosquitoes usually undertake several distinct behavioral steps: activation to fly upwind, navigation of the odor plume using olfactory cues and optomotor anemotaxis, navigating along odor plumes through surging and casting, close-range navigation towards skin, and landing [11]. Along the way, they continuously discriminate host odors from background, and select amongst multiple acceptable hosts. These distinct behavioral steps are likely to be guided by distinct sets of olfactory cues that are detected by independent olfactory pathways (FIGURE 1). At close range, mosquitoes also integrate non-olfactory attraction cues such as humidity, temperature, and visual stimuli for landing. While these steps are challenging to study, they also offer many different opportunities to reduce host-seeking behavior.

When a female mosquito in flight or resting on a walls inside a house comes in contact with a turbulent plume of CO_2, she immediately turns upwind and increases speed or takes off in a process described as activation [12]. The CO_2-receptor (Gr1, Gr2, Gr3) in the cpA neurons of the maxillary palps also detects whole-skin odor weakly [13], so when a female *Aedes aegypti* enters a plume of undiluted skin odor, she turns upwind and increases flight speed just as she does in a plume of CO_2 [12,14]. Diluted skin odor however is not sufficient to activate a mosquito or induce an upwind surge however it is highly attractive after a mosquito is activated by a momentary pulse of CO_2, through mechanisms that are not well understood [12,14]. After activating upwind navigation the mosquito surges forward every time it contacts a CO_2 plume, closer towards the emitting source, and when contact is lost she casts across the direction of airflow maximizing chances of encountering another plume from the source [12].

CO_2 is therefore routinely used in mosquito traps for vector surveillance and control [reviewed in 15]. This general vertebrate-host cue also attracts many other hematophagous insects. However, because of the costs of traps themselves and the logistical difficulties of obtaining CO_2 (burning butane from pressurized cylinders or evaporating dry ice), alternative strategies are required. Fermenting sugar or molasses with yeast that generate CO_2 has been used [16,17]. More conveniently, volatile odorants that activate the CO_2 receptor could also be used [13]. Many of these odorants were first found in the model system *Drosophila melanogaster* that shares a conserved CO_2 receptor [18]. Subsequently, activators were identified in mosquitoes using electrophysiology, many from human skin sources such as microbial degradation products [13,19]. A high-throughput chemical informatics approach was developed to identify structural features of known actives and screened hundreds of thousands of chemicals *in silico* that identified hundreds of candidate chemicals, from activators with improved usability features such as better smell, safety and affordability could be found [13]. One of these chemicals, cyclopentanone, was found to capture similar numbers of mosquitoes as a CO_2 lure when used with a counter-flow design trap inside a greenhouse [13]. Several additional odorants remain to be tested as lures and the method is tractable for continuous improvement.

The catch rates of CO_2 lures can be increased significantly by the addition of odorants that can act synergistically, often identified from skin, such as carboxylic acid mixtures, lactic acids, ammonia, 1-octen-3-ol, nonanal etc. These chemicals by themselves are poor lures in traps, suggesting that they may enhance the catch rate by acting along with the CO_2 neuronal circuit in the nervous system. 1-octen-3-ol is detected by Or8 in the neuron neighboring the CO_2-sensitive ORN in the same sensillum [20–22] and it is >2 orders of magnitude more sensitive to the (R)-(−) than to the (L)-(+) enantiomer, increasing in sensitivity as an adult mosquito matures and begins host seeking [20,23]. At least 10 other mosquito ORs also detect 1-octen-3-ol and may also influence behavior [10]. Nonanal activate several known Ors. Other odorants like the acids and ammonia are expected to activate conserved Ir family receptors that are known to detect these odors in *Drosophila melanogaster* [24]. Ammonia-sensitive ORNs have been observed in grooved-peg sensilla and trichoid sensilla of mosquitoes [25]. It was recently shown in *Drosophila* that the ammonium transporter *Amt* is required for the ac1 neuron to detect ammonia [26]; this gene is also expressed in the mosquito antenna and confers ammonium responses in cell culture, which is consistent with a role in mosquito olfaction as well [27]. Odorants that effectively lure mosquitoes have also been identified from plants, a sugar feeding substrate, such as Linalool oxide [28]. It is not known which mosquito ORNs or receptors determine behavior towards any of these odorants yet, but recently developed genetic tools could enable systematic design of improved synergists using chemical informatics and neurophysiology.

When used by itself, or with the known synergists, CO_2 activates host-seeking mosquitoes and induces them to fly upwind toward the trap. However, it is not sufficient to induce a mosquito to enter a trap, necessitating powered fans to suck them in. Odorants from skin are expected to play a major role in close-range attraction and landing. Interestingly, when a CO_2 and a skin odor plumes are presented to a mosquito simultaneously, she will ignore the CO_2 plume and navigate toward the human odor source [29]. Female *A. gambiae* respond very weakly to human skin odor in landing assays, which is dramatically increased by exposure to even low concentrations of CO_2 [30]. Skin odors are also expected to play an important role in host selection and preference. Mosquitoes prefer to bite some potential host species over others [reviewed in 31]. Mutant *A. aegypti* lacking *orco* no longer prefer odor from a human as strongly over odor from a guinea pig, suggesting that one or more members of the Or family are involved in discriminating between these host odors [32]. One proposed candidate is AaOr4, which detects 6-methyl-5-hepten-2-one (also called sulcatone). Expression levels and allelic sensitivity of this receptor to 6-methyl-5-hepten-2-one vary across two subspecies of *A. aegypti* that differ in their preference for humans and correlate positively with preference for human hosts across populations and hybrids of the two subspecies [33]. However, others studies have reported that 6-methyl-5-hepten-2-one is a potent natural repellent found in skin [34,35], and additional studies are required to understand these confounding results.

A few chemicals from skin such as oxopentanoic acid and to a smaller degree lactic acid can increase landing by a small degree [36]. Lactic acid is detected by ORNs in grooved peg sensilla on the antennae, however the receptors expressed in these ORNs have not yet been identified, despite extensive screening of Ors [9,10]. Mosquito grooved peg sensilla appear to be homologous to fly coeloconic sensilla, which express Ir-family chemoreceptors and respond to acids [37] so it is likely that the mosquito lactic acid receptor is also a member of this family. In *A. aegypti*, heat and moisture together are sufficient to induce landing behavior and subsequent probing, but neither stimulus by itself is sufficient [38]. Human odor in the absence of these cues is also sufficient to induce landing, which is enhanced with added heat [39]. Sweat also enhances the attractiveness of a warm, moist cue in *A. gambiae*, and this has been attributed at least partly to the presence of C4–C6 2-oxocarboxylic acids [40,41]. The receptor(s) involved in this pathway are not known, although Ir family members are likely to be involved. This would be consistent with the observation that *orco* mutant *A. aegypti* females retain strong attraction to human skin odor alone [13,32]. A rigorous analysis of skin-odor detection by mosquitoes from a close range is necessary for identification of odorant receptors, neurons and odorants that induce strong landing behaviors as observed towards a skin odor blend. The findings would facilitate development of fan-less traps as well as inhibitors that could block attraction to human skin.

Inhibitory odorants to mask attraction towards hosts

CO_2 is one of the best-known compounds that attract mosquitoes from a distance. In addition, it activates upwind flight and increases attraction towards skin odorants and body temperature heat source. This points to mosquito CO_2 detection as a key target in the identification of inhibitory volatiles for reduction of host-attraction. Volatiles associated with ripened fruits were initially identified as CO_2 receptor inhibitors in *D. melanogaster*, and these were found to affect the conserved mosquito receptor as well [18,19]. A thorough structure-activity analysis identified several different chemical classes of inhibitors that are likely associated with allosteric sites on the receptor [13]. A powerful chemical informatics method identified physicochemical features associated with CO_2 receptor activation or inhibition based on a training-set of known ligands, and screened *in silico* ~230,000 chemical structures to identify stronger, safer and pleasant smelling inhibitors. Ethyl pyruvate is one strong inhibitor that reduced the number of mosquitoes lured into a CO_2-baited counter-flow trap. Additionally, ethyl pyruvate inhibited CO_2 receptor-mediated detection of a skin odor blend, and reduced attraction of mosquitoes towards a human arm inserted into a cage of *A. aegypti* females [13].

An unusual class of odorants that induced an ultra-prolonged activation of the mosquito CO_2 receptor was also identified. These compounds induced a tonic response in the cpA neuron that persisted for several minutes beyond the end of the odor stimulus [19], and disabled subsequent cpA responses to CO_2. Brief pre-exposure to an ultra-prolonged activating odorant blend significantly hampered mosquito navigation in a wind tunnel, as constant CO_2 detection is required for upwind travel along a turbulent CO_2 plume. In a “Malariasphere” greenhouse, the ultra-prolonged activating blend significantly reduced entry of *Culex quinquefasciatus* mosquitoes into a hut that contained a CO_2-baited trap [19].

To investigate the behavioral functions of the receptor more closely, Zn-finger nuclease technology was used to generate a *Gr3* mutant strain of *A. aegypti* that did not show responses to CO_2 [39]. A chemical genetics strategy was also developed in which exposure to a reactive inhibitory ligand (butyryl chloride) knocked down activity of the cpA neuron for hours [13]. Both methods of silencing the cpA neuron led to marked reduction in behavioral activation induced by CO_2 or skin odor in a wind tunnel. The *Gr3* mutant mosquitoes show reduced ability to obtain a blood meal from a mouse in a moderately-sized cage, but are only mildly impaired at finding a mouse in a small cage, or a human in a greenhouse enclosure [39]. Similarly, mosquitoes lacking cpA function that do activate stochastically are able to navigate efficiently to a source of skin odor in the wind tunnel [13]. These observations indicate that additional, unidentified olfactory pathways are also involved in attraction towards skin odor. In this context, it is interesting to speculate as to why an inhibitory odorant like ethyl pyruvate is effective in lowering attraction to skin. As a wildtype mosquito approaches treated skin, its cpA neuron will progressively fire fewer action potentials due to increasing proximity to inhibitory ethyl pyruvate. It is not known whether progressive inhibition of an attractive circuit is interpreted by the insect as undesirable, leading to aversion. Alternatively, ethyl pyruvate could also inhibit additional unknown skin odor-detecting receptors or activate additional repellent pathways.

Identifying olfactory receptors for repellent odorants, leading to aversion

The most commonly used mosquito repellent, DEET (N,N-diethyl-meta-toluamide), was discovered in the 1940s through empirical testing of thousands of chemicals. There is an urgent need to find new affordable and safe repellents that can overcome the disadvantages of DEET, especially for adoption in parts of the world that suffer most from mosquito borne diseases. The identification of insect olfactory receptors and neurons that detect DEET and cause avoidance could directly enable rational design of improved repellents, but this has proved to be an extremely challenging task. Like host-attraction behavior, avoidance to repellents such as DEET is also complex and multimodal. DEET has a strong fixative effect, lowering volatilization of odorants from skin where it is applied [42]. It is detected by the mosquito olfactory system from close proximity as an aversive volatile cue, potentially by two different classes of receptors (*Irs* and *Ors*) [32,43]. If a mosquito lands on DEET-treated skin, its gustatory system detects the chemical as a bitter aversive cue [44]. DEET has widespread neurological effects on ion channels and acetylcholinesterase [45], which could also disrupt insect behavior toward skin.

Several studies have focused on the identification of receptors, neurons, and molecular mechanisms underlying DEET repellency. Initial experiments in *Drosophila* showed that the response of the Or59b odorant receptor to 1-octen-3-ol (a vertebrate odor) was reduced by the addition of DEET, suggesting an inhibitory, odor-masking mechanism [46]. It was later demonstrated that the reduction in ORN activity observed in the study resulted from the fixative effect of DEET that prevented evaporation of 1-octen-3-ol when added to the same odor-loaded filter paper [42]. In fact, application of DEET to a human arm substantially reduced the emission of skin volatiles, revealing a fixative effect on human skin volatiles as well. However, DEET also repelled mosquitoes from sugar stations, and DEET-activated olfactory neurons in the *Culex quinquefasciatus* antenna, supporting an additional mechanism for direct detection [42]. It was later proposed that *Drosophila* Or42a responded to DEET and other repellents [47], but this response too was later found to be an artifact of hexane used as the solvent [43].

The development of *orco* mutant *Aedes aegypti* mosquitoes aided in understanding the involvement of the *Or* family in DEET repellency [32]. *Orco* mutants did not feed on a DEET-covered human arm inserted into a cage, suggesting that repellency was maintained. However, an arm presented 2.5 cm outside the cage repelled wildtype but not orco mosquitoes from that area of the cage [32]. Experimenters proposed that *orco* mutants were unable to avoid DEET in a non-contact manner, and the protection observed for the treated arm in the mutants was solely due to contact repellency mediated by the gustatory system upon landing [32]. However, the Or gene family is extremely divergent, whereas DEET repellency is highly-conserved across tested insect species, including arthropods such as ticks, which lack the Or family members. In addition, a mixture of attractant, repellent, and neutral odorants is emitted from the skin [34], and the fixative effect of DEET applied directly to the forearm could alter the balance between attractant and repellent odorants. It is therefore difficult to interpret whether the *orco* mutants could not detect from 2.5 cm the repellent DEET, or certain repellent skin odorants such as for geranylacetone and 6-methyl-5-hepten-2-one [34]. Indeed, a less complex attraction source demonstrated that both wildtype and *orco*- mutant *Aedes aegypti* retain strong non-contact (6 mm gap) aversion to DEET placed over an attractive heat pad [48]. This suggests that DEET detection and avoidance is maintained to a significant extent in the *orco* mutants in this context, and non-Or family receptors are sufficient to cause repellency at a 6 mm distance without contact.

Recent studies have revealed a highly-conserved *Ir40a* receptor pathway that mediates non-contact DEET repellency [43]. An unbiased DEET activity screen with a CaLexA transgenic system [49] identified neurons in the sacculus of *Drosophila melanogaster* antennae, and the axonal innervation pattern to the characteristic column glomerulus identified these as *Ir40a*-expressing ORNs. The ORNs responded to DEET in highly sensitivity Ca^{2+} imaging experiments on a confocal microscope, and cell silencing using Tetanus toxin or *IR40a* expression knockdown using RNAi reduced avoidance of *Drosophila* to DEET [43]. The *Ir40a* ORNs also co-express *Ir93a* and *Ir25a* [50], however the composition of the heteromeric receptor that detects DEET has not been ascertained yet via reconstitution.

Nevertheless, the properties of the Ir receptor can potentially explain aspects of the repellent effect of DEET: 1) Irs are highly conserved across insects, as is DEET repellency; 2) it responds to high concentrations of DEET, as is used in repellent formulations; 3) it should be functional in *orco-* mosquitoes that still avoid DEET in non-contact assays; and 4) it is activated by strong repellents that work on both flies and mosquitoes. However, a recent paper using *Culex quinquifasciatus* mosquitoes proposed that volatile DEET detection in that species relies solely on the *CquiOr136* receptor [51] and not the *Ir40a* [43] pathway. It is important to note that only partial reduction of *Ir40a* was achieved using RNAi, raising the likelihood that residual Ir40a protein is sufficient for DEET detection. The *CquiOr136 RNAi* mosquitoes were not repelled by 0.1% DEET, which is 100–1000 fold lower than in repellent formulations (~10%–100%), raising the possibility that formulation concentrations would still be avoided via *Ir* receptors that detect higher DEET concentrations. In addition, the *CquiOr136* receptor is not conserved in other mosquito species such as *Aedes* and *Anopheles*, suggesting that there would be little utility in developing new repellents targeted solely towards this receptor.

The challenges in identifying new repellents extend beyond identification of receptors, and the main ones are the lack of a high-throughput chemical screening platform and very high estimated costs for regulatory approval of new chemicals for use on human skin. A chemical informatics approach was developed to address these challenges in which 34 carboximides and 34 N-acylpiperidines were predicted from quantitative-structure-activity models (CODESSA PRO) using USDA archived repellency data for DEET derivatives. These compounds were synthesized and behaviorally tested to show greater biological efficacy than DEET [52–54]. In a related approach, a specific set of physiochemical DRAGON descriptors was identified from the repellents in these studies and used to train a Support Vector Machine [43]. The trained-model then screened the structure of >400,000 chemicals *in silico* to predict nearly 1000 new repellents, including >100 that are naturally occurring. A small set of 10 compounds from the natural set was tested behaviorally on flies, resulting in an 80% predictive success rate. Of these compounds, the anthranilates showed improved toxicity profiles, do not dissolve nylons and plastics, and improved cosmetic profiles (their smell is mild and pleasant) as compared to DEET. Further testing showed a strong *Ir40a*-dependent repellency to the anthranilates in *A. aegypti* mosquitoes [43], and differential repellency in flying versus oviposition assays [55]. The identification of new repellent chemical classes through activity screening of the *Ir40a* neuron in *Drosophila* is another extremely valuable alternative to current methods that rely on structural similarity to DEET and other known repellents. The efficacy of this activity-based discovery method is demonstrated by the structurally unrelated, high-volatility repellent, 4-methylpiperidine, which protects a greater spatial zone than DEET [48].

Natural repellents have also been identified from a variety of plant sources, and more recently, from volatiles found in the skin of humans found to be minimally attractive to mosquitoes [35,56]. Identical twins are more similarly attractive to mosquitoes than are fraternal twins, suggesting a genetic basis [57]. A chemical ecology screen for components of human odor that correlate with preference identified 33 candidate repellents for *Ae. aegypti*, five of which were selected for behavioral experiments. Of those, decanal was confirmed as a possible repellent [34]. Two other candidates, geranylacetone and 6-methyl-5-hepten-2-one, show strong repellency to *A. aegypti*, particularly when mixed 1:1 [35,56]. Plant-derived repellents such as citronella, p-methane-3,8-diol are also highly effective as repellents, albeit for shorter time periods than DEET. While these odorants have been developed for use as mosquito repellents, identification of their specific olfactory receptors will reveal additional repellent pathways that can be used to develop better repellents.

Identification of target receptors to screen for new repellents is still difficult due to limited genetic tools in mosquitoes. One strategy that seeks to overcome this difficulty is the use of Orco co-receptor agonists such as VUAA1 to broadly activate all Or family olfactory receptors [58,59]. While VUAA1 has low volatility and has not yet been behaviorally tested for repellency in adult mosquitoes, it is anticipated that this broad activation will inadvertently activate repellent classes of Or-neurons, as well as confound host recognition through broad neuronal activity patterns. Identifying volatile and natural substitutes for VUAA1 would likely have substantial utility, as it would make formulations and regulatory approval more practical.

Pyrethroid insecticides are another class of highly effective compounds widely used in tropical countries for blocking biting behavior. Used mostly indoors, they are evaporated from heated devices or burning coils, or dispensed by devices with fans. The efficacy in behavioral control is related to the compounds' neurotoxic insecticidal properties, which cause hyperactivity in neuronal axons through the blockade of Na^{2+} channels. There is a risk that resistance to pyrethroids in mosquitoes could spread rapidly and render them ineffective [60], and these compounds pose risks to the environment. New generations of safer alternatives and derivatives that act via a similar mechanism could provide an added line of behavioral control of mosquitoes.

While the complexity of the mosquito olfactory system presents enormous challenges in the discovery of target receptors and new generations of repellents, it also provides an incredible opportunity to find safe and affordable approaches to prevent disease transmission. The development of insect control strategies for the future will require that we surpass traditional methods of chemical ecology and apply the powerful new technologies that are prevalent in drug discovery, that have yielded so many life-saving medications.

Acknowledgments

I would like to thank Genevieve Tauxe, Crystal Pontrello and Sarah Perry for comments on the manuscript. Research in A Ray's laboratory is supported by grants from the National Institutes of Health (R01AI087785, R01DC014092, R21NS085765 and 1U19AI089672), HHMI (HCIA),

References and recommended reading

1. Revay EE, Kline DL, Xue RD, Qualls WA, Bernier UR, Kravchenko VD, Ghattas N, Pstygo I, Muller GC. Reduction of mosquito biting-pressure: spatial repellents or mosquito traps? A field comparison of seven commercially available products in Israel. Acta Trop. 2013; 127:63–68. [PubMed: 23545129]
2. Van Roey K, Sokny M, Denis L, Van den Broeck N, Heng S, Siv S, Sluydts V, Sochantha T, Coosemans M, Durnez L. Field evaluation of picaridin repellents reveals differences in repellent

sensitivity between Southeast Asian vectors of malaria and arboviruses. PLoS Negl Trop Dis. 2014; 8:e3326. [PubMed: 25522134]

3. Wagman JM, Grieco JP, Bautista K, Polanco J, Briceno I, King R, Achee NL. The field evaluation of a push-pull system to control malaria vectors in Northern Belize, Central America. Malar J. 2015; 14:184. [PubMed: 25925395]
4. Menger DJ, Omusula P, Holdinga M, Homan T, Carreira AS, Vandendaele P, Derycke JL, Mweresa CK, Mukabana WR, van Loon JJ, et al. Field evaluation of a push-pull system to reduce malaria transmission. PLoS One. 2015; 10:e0123415. [PubMed: 25923114]
5. Suh E, Bohbot J, Zwiebel LJ. Peripheral olfactory signaling in insects. Curr Opin Insect Sci. 2014; 6:86–92. [PubMed: 25584200]
6. Couto A, Alenius M, Dickson BJ. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. Curr Biol. 2005; 15:1535–1547. [PubMed: 16139208]
7. Ghaninia M, Hansson BS, Ignell R. The antennal lobe of the African malaria mosquito, *Anopheles gambiae* - innervation and three-dimensional reconstruction. Arthropod Structure & Development. 2007; 36:23–39. [PubMed: 18089085]
8. Ghaninia M, Ignell R, Hansson BS. Functional classification and central nervous projections of olfactory receptor neurons housed in antennal trichoid sensilla of female yellow fever mosquitoes, *Aedes aegypti*. European Journal of Neuroscience. 2007; 26:1611–1623. [PubMed: 17880395]
9. Wang G, Carey AF, Carlson JR, Zwiebel LJ. Molecular basis of odor coding in the malaria vector mosquito Anopheles gambiae. Proc Natl Acad Sci U S A. 2010
10. Carey AF, Wang G, Su C-Y, Zwiebel LJ, Carlson JR. Odorant reception in the malaria mosquito *Anopheles gambiae*. Nature. 2010; 464:66–71. [PubMed: 20130575]
11. Cardé, RT.; Gibson, G. Long-distance orientation of mosquitoes to host odours and other host-related cues. In: Takken, W.; Knols, BGJ., editors. Ecology of Vector-Borne Diseases. Vol. 2. Wageningen Academic Publishers; 2010. p. 115-141.
12. Dekker T, Cardé RT. Moment-to-moment flight manoeuvres of the female yellow fever mosquito (*Aedes aegypti* L) in response to plumes of carbon dioxide and human skin odour. Journal of Experimental Biology. 2011; 214:3480–3574. [PubMed: 21957112]

**13. Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A. Targeting a dual detector of skin and CO2 to modify mosquito host seeking. Cell. 2013; 155:1365–1379. Identifies the mosquito CO_2 receptor as a broadly tuned receptor for a variety of skin odors, uses chemical informatics to identify ligands of the receptor. An agonist shows activity as a lure and antagonist masks attraction to CO_2 and skin odor. [PubMed: 24315103]

14. Dekker T, Geier M, Cardé RT. Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. Journal of Experimental Biology. 2005; 208:2963–2972. [PubMed: 16043601]
15. Mukabana, WR.; Olanga, EA.; Knols, BGJ. Host-seeking behaviour of Afrotropical anophelines: field and semi-field studies. In: Takken, W.; Knols, BGJ., editors. Olfaction in Vector-Host Interactions. Wageningen Academic Publishers; 2010. p. 181-202.
16. Mweresa CK, Omusula P, Otieno B, van Loon JJA, Takken W, Mukabana WR. Molasses as a source of carbon dioxide for attracting the malaria mosquitoes *Anopheles gambiae* and *Anopheles funestus*. Malaria Journal. 2014; 13:160. [PubMed: 24767543]
17. Smallegange RC, Schmied WH, van Roey KJ, Verhulst NO, Spitzen J, Mukabana WR, Takken W. Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito Anopheles gambiae. Malar J. 2010; 9:292. [PubMed: 20973963]
18. Turner SL, Ray A. Modification of CO2 avoidance behaviour in Drosophila by inhibitory odorants. Nature. 2009; 461:277–281. [PubMed: 19710651]

*19. Turner SL, Li N, Guda T, Githure J, Carde RT, Ray A. Ultra-prolonged activation of CO2-sensing neurons disorients mosquitoes. Nature. 2011; 474:87–91. Identifies inhibitors of the mosquito CO_2 neuron, and ultra-prolonged activators. A blend containing both classes of compounds can disrupt mosquito navigation upwind to CO_2. [PubMed: 21637258]

20. Bohbot JD, Dickens JC. Characterization of an enantioselective odorant receptor in the yellow fever mosquito *Aedes aegypti*. PLoS ONE. 2009; 4

21. Lu T, Qiu YT, Wang G, Kwon Jae Y, Rutzler M, Kwon H-W, Pitts RJ, van Loon JJA, Takken W, Carlson JR, et al. Odor Coding in the Maxillary Palp of the Malaria Vector Mosquito *Anopheles gambiae*. Current Biology. 2007; 17:1533–1544. [PubMed: 17764944]

22. Syed Z, Leal WS. Maxillary Palps Are Broad Spectrum Odorant Detectors in *Culex quinquefasciatus*. Chemical Senses. 2007; 32:727–738. [PubMed: 17569743]

23. Bohbot JD, Durand NF, Vinyard BT, Dickens JC. Functional Development of the Octenol Response in *Aedes aegypti*. Frontiers in Physiology. 2013; 4:39. [PubMed: 23471139]

24. Min S, Ai M, Shin SA, Suh GS. Dedicated olfactory neurons mediating attraction behavior to ammonia and amines in Drosophila. Proc Natl Acad Sci U S A. 2013; 110:E1321–1329. [PubMed: 23509267]

25. Qiu YT, van Loon JJA, Takken W, Meijerink J, Smid HM. Olfactory Coding in Antennal Neurons of the Malaria Mosquito, *Anopheles gambiae*. Chemical Senses. 2006; 31:845–863. [PubMed: 16963500]

26. Menuz K, Larter NK, Park J, Carlson JR. An RNA-Seq Screen of the *Drosophila* Antenna Identifies a Transporter Necessary for Ammonia Detection. PLoS Genetics. 2014; 10:e1004810. [PubMed: 25412082]

27. Pitts RJ, Derryberry SL Jr, Pulous FE, Zwiebel LJ. Antennal-Expressed Ammonium Transporters in the Malaria Vector Mosquito *Anopheles gambiae*. PLoS ONE. 2014; 9:e111858. [PubMed: 25360676]

28. Nyasembe VO, Tchouassi DP, Kirwa HK, Foster WA, Teal PE, Borgemeister C, Torto B. Development and assessment of plant-based synthetic odor baits for surveillance and control of malaria vectors. PLoS One. 2014; 9:e89818. [PubMed: 24587059]

*29. Lacey ES, Ray A, Cardé RT. Close encounters: contributions of carbon dioxide and human skin odour to finding and landing on a host in *Aedes aegypti*. Physiological Entomology. 2014; 39:60–68. A 3D analysis of mosquito flight behavior to CO_2 or human skin odor, revealing characteristic flight behavior. [PubMed: 24839345]

30. Webster B, Lacey ES, Carde RT. Waiting with bated breath: opportunistic orientation to human odor in the malaria mosquito, Anopheles gambiae, is modulated by minute changes in carbon dioxide concentration. J Chem Ecol. 2015; 41:59–66. [PubMed: 25572756]

31. Takken W, Verhulst NO. Host preferences of blood-feeding mosquitoes. Annual Review of Entomology. 2013; 58:433–453.

*32. DeGennaro M, McBride CS, Seeholzer L, Nakagawa T, Dennis EJ, Goldman C, Jasinskiene N, James AA, Vosshall LB. orco mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. Nature. 2013; 498:487–491. First report of a mutant mosquito line done on the orco co-receptor. Orco mutants still navigate towards a human skin odor source. [PubMed: 23719379]

33. McBride CS, Baier F, Omondi AB, Spitzer SA, Lutomiah J, Sang R, Ignell R, Vosshall LB. Evolution of mosquito preference for humans linked to an odorant receptor. Nature. 2014; 515:222–227. [PubMed: 25391959]

34. Logan JG, Birkett MA, Clark SJ, Powers S, Seal NJ, Wadhams LJ, Mordue AJ, Pickett JA. Identification of human-derived volatile chemicals that interfere with attraction of Aedes aegypti mosquitoes. Journal of Chemical Ecology. 2008; 34:308–322. [PubMed: 18306972]

35. Logan JG, Stanczyk NM, Hassanali A, Kemei J, Santana AE, Ribeiro KA, Pickett JA, Mordue Luntz AJ. Arm-in-cage testing of natural human-derived mosquito repellents. Malar J. 2010; 9:239. [PubMed: 20727149]

36. Healy TP, Copland MJ. Human sweat and 2-oxopentanoic acid elicit a landing response from Anopheles gambiae. Med Vet Entomol. 2000; 14:195–200. [PubMed: 10872864]

37. Ai M, Min S, Grosjean Y, Leblanc C, Bell R, Benton R, Suh GS. Acid sensing by the Drosophila olfactory system. Nature. 2010; 468:691–695. [PubMed: 21085119]

38. Klun JA, Kramer M, Debboun M. Four simple stimuli that induce host-seeking and blood-feeding behaviors in two mosquito species, with a clue to DEET's mode of action. Journal of Vector Ecology. 2013; 38:143–153. [PubMed: 23701619]

**39. McMeniman CJ, Corfas RA, Matthews BJ, Ritchie SA, Vosshall LB. Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans. Cell. 2014;

156:1060–1071. Electrophysiology and behavioral analysis of *Gr3* mutant *Aedes aegypti* to test the role of CO_2 in host-seeking behavior. [PubMed: 24581501]

40. Healy TP, Copland MJW. Human sweat and 2-oxopentanoic acid elicit a landing response from *Anopheles gambiae*. Medical and Veterinary Entomology. 2000; 14:195–200. [PubMed: 10872864]

41. Healy TP, Copland MJW, Cork A, Przyborowska A, Halket JM. Landing responses of *Anopheles gambiae* elicited by oxocarboxylic acids. Medical and Veterinary Entomology. 2002; 16:126–132. [PubMed: 12109705]

42. Syed Z, Leal WS. Mosquitoes smell and avoid the insect repellent DEET. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:13598–13603. [PubMed: 18711137]

**43. Kain P, Boyle SM, Tharadra SK, Guda T, Pham C, Dahanukar A, Ray A. Odour receptors and neurons for DEET and new insect repellents. Nature. 2013; 502:507–512. Identifies Ir40a as a receptor for volatile DEET, and undertakes a chemical informatics assay to find hundred's of new ligands. [PubMed: 24089210]

44. Lee Y, Kim SH, Montell C. Avoiding DEET through Insect Gustatory Receptors. Neuron. 2010; 67:555–561. [PubMed: 20797533]

45. Swale DR, Sun B, Tong F, Bloomquist JR. Neurotoxicity and mode of action of N, N-diethyl-meta-toluamide (DEET). PLoS ONE. 2014; 9:e103713. [PubMed: 25101788]

46. Ditzen M, Pellegrino M, Vosshall LB. Insect odorant receptors are molecular targets of the insect repellent DEET. Science. 2008; 319:1838–1842. [PubMed: 18339904]

47. Syed Z, Pelletier J, Flounders E, Chitolina RF, Leal WS. Generic insect repellent detector from the fruit fly Drosophila melanogaster. PLoS ONE. 2011; 6:e17705. [PubMed: 21436880]

*48. Guda, T.; Kain, P.; Sharma, KR.; Pham, CK.; Ray, A. Repellent compound with larger protective zone than DEET identified through activity-screening of Ir40a neurons, does not require Or function. 2015. http://dx.doi.org/10.1101/017145. Idenitfies a new volatile mosquito repellent that can protect a greater spatial zone than DEET

49. Masuyama K, Zhang Y, Rao Y, Wang JW. Mapping neural circuits with activity-dependent nuclear import of a transcription factor. J Neurogenet. 2012; 26:89–102. [PubMed: 22236090]

50. Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB. Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell. 2009; 136:149–162. [PubMed: 19135896]

51. Xu P, Choo YM, De La Rosa A, Leal WS. Mosquito odorant receptor for DEET and methyl jasmonate. Proc Natl Acad Sci U S A. 2014; 111:16592–16597. [PubMed: 25349401]

52. Katritzky AR, Wang Z, Slavov S, Tsikolia M, Dobchev D, Akhmedov NG, Hall CD, Bernier UR, Clark GG, Linthicum KJ. Synthesis and bioassay of improved mosquito repellents predicted from chemical structure. Proc Natl Acad Sci U S A. 2008; 105:7359–7364. [PubMed: 18508970]

53. Katritzky AR, Wang Z, Slavov S, Dobchev DA, Hall CD, Tsikolia M, Bernier UR, Elejalde NM, Clark GG, Linthicum KJ. Novel carboxamides as potential mosquito repellents. J Med Entomol. 2010; 47:924–938. [PubMed: 20939392]

54. Oliferenko PV, Oliferenko AA, Poda GI, Osolodkin DI, Pillai GG, Bernier UR, Tsikolia M, Agramonte NM, Clark GG, Linthicum KJ, et al. Promising Aedes aegypti repellent chemotypes identified through integrated QSAR, virtual screening, synthesis, and bioassay. PLoS One. 2013; 8:e64547. [PubMed: 24039693]

55. Afify A, Horlacher B, Roller J, Galizia CG. Different repellents for Aedes aegypti against blood-feeding and oviposition. PLoS One. 2014; 9:e103765. [PubMed: 25079819]

56. Logan JG, Birkett MA, Clark SJ, Powers S, Seal NJ, Wadhams LJ, Mordue Luntz AJ, Pickett JA. Identification of human-derived volatile chemicals that interfere with attraction of Aedes aegypti mosquitoes. J Chem Ecol. 2008; 34:308–322. [PubMed: 18306972]

57. Fernandez-Grandon GM, Gezan SA, Armour JA, Pickett JA, Logan JG. Heritability of attractiveness to mosquitoes. PLoS One. 2015; 10:e0122716. [PubMed: 25901606]

58. Jones PL, Pask GM, Rinker DC, Zwiebel LJ. Functional agonism of insect odorant receptor ion channels. Proc Natl Acad Sci U S A. 2011; 108:8821–8825. [PubMed: 21555561]

59. Jones PL, Pask GM, Romaine IM, Taylor RW, Reid PR, Waterson AG, Sulikowski GA, Zwiebel LJ. Allosteric antagonism of insect odorant receptor ion channels. PLoS One. 2012; 7:e30304. [PubMed: 22272331]

60. Kitau J, Oxborough R, Matowo J, Mosha F, Magesa SM, Rowland M. Indoor residual spraying with microencapsulated DEET repellent (N, N-diethyl-m-toluamide) for control of Anopheles arabiensis and Culex quinquefasciatus. Parasit Vectors. 2014; 7:446. [PubMed: 25249021]

Highlights

- Mosquitoes sense exhaled CO_2 and skin odor to find humans
- Attractive odorants can be used as lures in mosquito traps
- Inhibitors of the mosquito CO_2 receptor can mask attraction.
- Insect repellents like DEET are detected by multiple sensory pathways
- Discovery of new repellents can be improved using chemical informatics.

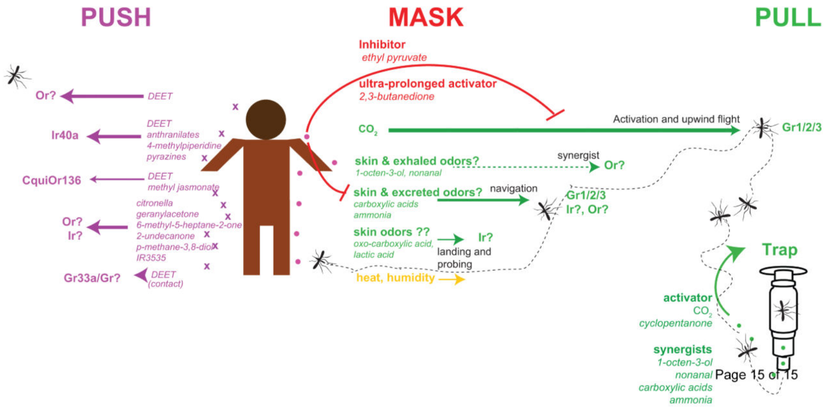
PUSH
MASK
PULL
Or?
DEET
Ir40a
DEET
anthranilates
4-methylpiperidine
pyrazines
CquiOr136
DEET
methyl jasmonate
Or?
Ir?
citronella
geranylacetone
6-methyl-5-heptane-2-one
2-undecanone
p-methane-3,8-diol
IR3535
Gr33a/Gr?
DEET
(contact)
Inhibitor
ethyl pyruvate
ultra-prolonged activator
2,3-butanedione
CO_2
Activation and upwind flight
Gr1/2/3
skin & exhaled odors?
1-octen-3-ol, nonanal
synergist
Or?
skin & excreted odors?
carboxylic acids
ammonia
navigation
Gr1/2/3
Ir?, Or?
skin odors ??
oxo-carboxylic acid,
lactic acid
Ir?
landing and
probing
heat, humidity
Trap
activator
CO_2
cyclopentanone
synergists
1-octen-3-ol
nonanal
carboxylic acids
ammonia
Page 15 of 15

Figure 1.