

Review

Human attractive cues and mosquito host-seeking behavior

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Female mosquitoes use chemical and physical cues, including vision, smell, heat, and humidity, to orient toward hosts. Body odors are produced by skin resident bacteria that convert metabolites secreted in sweat into odorants that confer the characteristic body scent. Mosquitoes detect these compounds using olfactory receptors in their antennal olfactory receptor neurons. Such information is further integrated with the senses of temperature and humidity, as well as vision, processed in the brain into a behavioral output, leading to host finding. Knowledge of human scent components unveils a variety of odorants that are attractive to mosquitoes, but also odor-triggering repellency. Finding ways to divert human-seeking behavior by female mosquitoes using odorants can possibly mitigate mosquito-borne pathogen transmission.

Volatiles produced by human-skin resident microbiota attract mosquitoes

Mosquito host-seeking behavior is a multistep process triggered by different host cues [1]. Host-seeking behavioral steps include activation, odor-mediated long-range attraction, hovering, and landing [1]. The chemical cues that trigger these behavioral steps encompass carbon dioxide and **skin odors** (see [Glossary](#)) [1]. Visual stimuli act as a guide to orient the olfactory-mediated behavior towards the host, playing an important role in landing behavior [2], whereas temperature and humidity are thought to be close-range attractants [3]. Carbon dioxide (CO₂) has been shown to activate resting mosquitoes [4,5] and drive long-range attraction (orientation; >1 m). Once close to a human host (<1 m), female mosquitoes hover around until they find the most attractive body part on which to land [6]. This short-range attraction can be induced by sweat-derived host odors [7,8] produced by the resident **skin microbiota** (Box 1; [9]).

The olfactory system of **anthropophilic** mosquito species is tuned to the formulation of human sweat [9]. Among the components of human sweat, L-(+)-lactic acid is a key **synergist** of CO₂ and an attractive human cue for some mosquito species [4,10]. For other species, ammonia is the main attractive odorant that, along with CO₂, ignites attraction to humans [11]. These mosquitoes prefer aged sweat, which has higher levels of ammonia produced upon the consumption of L-(+)-lactic acid by the growing skin microbiota [11]. Short-chain carboxylic acids are highly abundant on the human skin [12] and these compounds make mosquito attraction to L-(+)-lactic acid, ammonia, and CO₂ even stronger [13].

In light of the development of insecticide resistance in mosquitoes [14] and drug resistance in malaria parasites [15], and filarial worms [16], new strategies to control mosquito-borne disease transmission are urgently needed. Despite significant advances in the identification of odor blends attractive to the main mosquito vectors [17,18], controlling mosquito populations using odor-baited traps seems to be a task nearly impossible to achieve. DEET has been used as a mosquito repellent for many decades [19], yet high cost and human health issues preclude its widespread use [20]. This review describes multiple aspects of the mosquito neuroethology that drive human-

Highlights

Mosquito host-seeking is mediated by attraction to physical and chemical cues released in the breath and by the skin.

Most of the skin volatiles are produced by the host skin microbiota, and differences in microbiota composition correlate with human attractiveness.

Quantitative and qualitative discrimination of skin volatiles may be an important driver of host preference for anthropogenic mosquito species.

Differences in skin odor composition are detected by the peripheral olfactory system and discriminated by sequence and expression polymorphisms of olfactory receptors.

Designing new repellents and re-engineering the human skin microbiota to alter the composition of the human scent may possibly reduce mosquito bites and prevent the spread of mosquito-borne diseases.

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seeking behavior and highlights the odor-based approaches that effectively reduce mosquito bites. Based on this information, novel approaches could be developed to potentially reduce mosquito bites and prevent mosquito-borne diseases.

Chemical and physical cues attractive to the main mosquito vectors

Anopheles gambiae females are attracted to compounds found in skin odor in the presence of CO₂ [4,21,22]. The need for these mosquitoes to have contact with CO₂ to trigger host-seeking behavior seems to be an important adaptation to life indoors where these mosquitoes are in constant exposure to skin odors even in the absence of a host [23]. In this sense, such mosquitoes could rest while the human subjects are absent and attack when the subjects are found indoors releasing CO₂ [23]. Along these lines, in the absence of CO₂, female *An. gambiae* is weakly attracted to ammonia alone, and the addition of L-(+)-lactic acid to ammonia has no additive attractive effect (Figure 1A and Table 1) [24]. In the presence of CO₂, by contrast, female attraction is enhanced by addition of ammonia and L-(+)-lactic acid [24,25]. Addition of seven specific carboxylic acids only enhances attraction to a blend of CO₂, L-(+)-lactic acid, and ammonia, but not to a similar blend lacking L-(+)-lactic acid (Figure 2A and Table 1) [13,17,24–27].

Combinations of skin odor with CO₂ synergistically affect *Aedes aegypti* attractive behavior (Figure 1B and Table 2) [28]. Female *Ae. aegypti* are not attracted to L-(+)-lactic acid alone [10,28], and they are weakly attracted to CO₂ or human skin odor alone [28]. Furthermore, combinations of L-(+)-lactic acid or skin odor with CO₂ synergistically affect attractive behavior [28]. A blend with CO₂ and odorants presented on the human skin [L-(+)-lactic acid, ammonia, and short (C1–C3) and middle (C5–C8) length carboxylic acids] was shown to be nearly as attractive as CO₂ along with human skin odor [7]. Female *Ae. aegypti* mosquitoes are also attracted to different combinations of L-(+)-lactic acid with specific ketones, sulfides, and/or chloroalkanes, indicating that such odorants can replace CO₂ as an attractant [29–31]. Some of these combinations are

Box 1. Sweat, skin microbiota, mosquito attraction

Sweat is produced on the skin in mammals by three distinct glands: eccrine, apocrine, and sebaceous [102]. Eccrine is the most abundant gland in humans; it is present all over the body but with the highest density on hands and feet [102]. Apocrine glands are very limited and are found mostly on the human axilla and genitals [102]. Sebaceous glands are evenly distributed all over the body (Figure 1; [102]). The sweat of eccrine glands consists of salts, proteins, amino acids, urea, ammonia, and L-(+)-lactic acid. *Staphylococcus* bacteria convert the aliphatic amino acids from eccrine gland secretions on the feet into short-chain carboxylic acids [9]. Apocrine secretions consist mostly of lipids, steroids, and proteins [9]. Sebaceous gland sweat consists primarily of lipids [9]. These lipids are converted into long-chain carboxylic acids by skin bacteria of the genera *Propionibacterium*, *Corynebacterium*, and *Staphylococcus*, which are, in turn, converted into short-chain carboxylic acids by *Corynebacterium* species [9]. As the different sweat glands are more concentrated on specific body parts [9], the skin volatiles isolated from each part also exhibit different compositions [102]. Whereas specific aldehydes and ketones are more abundant in hand and forearm odor bouquets [33], alkenes and carboxylic acids are more frequently found in foot and axilla odor bouquets [102].

The volatile bouquet collected from cultures of human foot microbiota revealed 15 predominant odorants [103], out of which five odorants were also present in the bouquet volatile of a reference strain of the predominant skin bacterium, *Staphylococcus epidermidis* [103]. Both human foot microbiota and *S. epidermidis* cultures were attractive to *Anopheles gambiae* [103]. The bouquet volatile of the other three bacterial species present in the human skin microbiome are also attractive to *An. gambiae* females [27]. These species encompass *Corynebacterium minutissimum*, *Brevibacterium epidermidis*, and *Bacillus subtilis* [27].

Differences in sweat gland distribution and skin microbiota composition have been observed amongst humans and apes [104–106]. Whereas the ratio of eccrine to apocrine glands in primates ranges between 50:50 and 60:40, eccrine glands are very limited in the human body [9]. Even though humans have the lowest quantity and richness of skin microbiota [104,106], *Corynebacterium*, *Staphylococcus*, and *Pseudomonas* bacteria have the highest relative abundance on the human skin compared with apes [104,105]. Compared with other primates, human skin surfaces display much higher amounts of L-(+)-lactic acid [9]; free carboxylic acids are nearly absent on the skin of other primates whereas these compounds are very abundant on the human skin [9].

Glossary

Anthropophagic: feeding on humans.

Anthropophilic: attraction to and/or preference for feeding on humans.

Anthropo(zoo)philic: attraction to and/or preference for humans and other animals.

Coreceptor: a protein subunit that pairs with other receptors' subunits, assembling into fully functional receptors.

Edited (gene editing): a molecular technique that modifies the genetic material by means of deletions (knockouts) and insertions of specific DNA sequences (knockins).

Genetic drivers: genetic components responsible for controlling gene expression, such as promoters and enhancers. Promoters can be inserted into the genetic material (gene editing) to control the expression of exogenous genes (knockins).

Glomeruli (olfactory): structures of the antennal lobes where synapses form between olfactory receptor neurons, projection neurons, and local interneurons. Whereas local interneurons have modulatory roles, projection neurons transmit the signals to the higher brain centers.

Habituation: weakening of behavioral responses due to repeated stimulation.

Heated blood meal: blood warmed at the human body temperature (37°C) and offered to mosquitoes using feeding devices.

Higher brain centers: regions of the insect brain responsible for learning and memory (mushroom bodies) and innate behavior (lateral horn).

Kairomones: chemical cues released by an animal species that evoke mosquito behavioral responses.

Mosquito host choice (host preference): the range of host species a mosquito species is typically attracted to and/or feeds on.

Mosquito human discrimination

(host specificity): the degree of selectivity exhibited by a mosquito species in choosing a host.

Odor-based learning behavior: the association of an odorant with a reward, leading to increased behavioral responses towards such an odorant.

Olfactory receptor neurons (ORNs): the set of sensory neurons that perceive odorants in the antennae's sensilla and transmit the information to other brain centers.

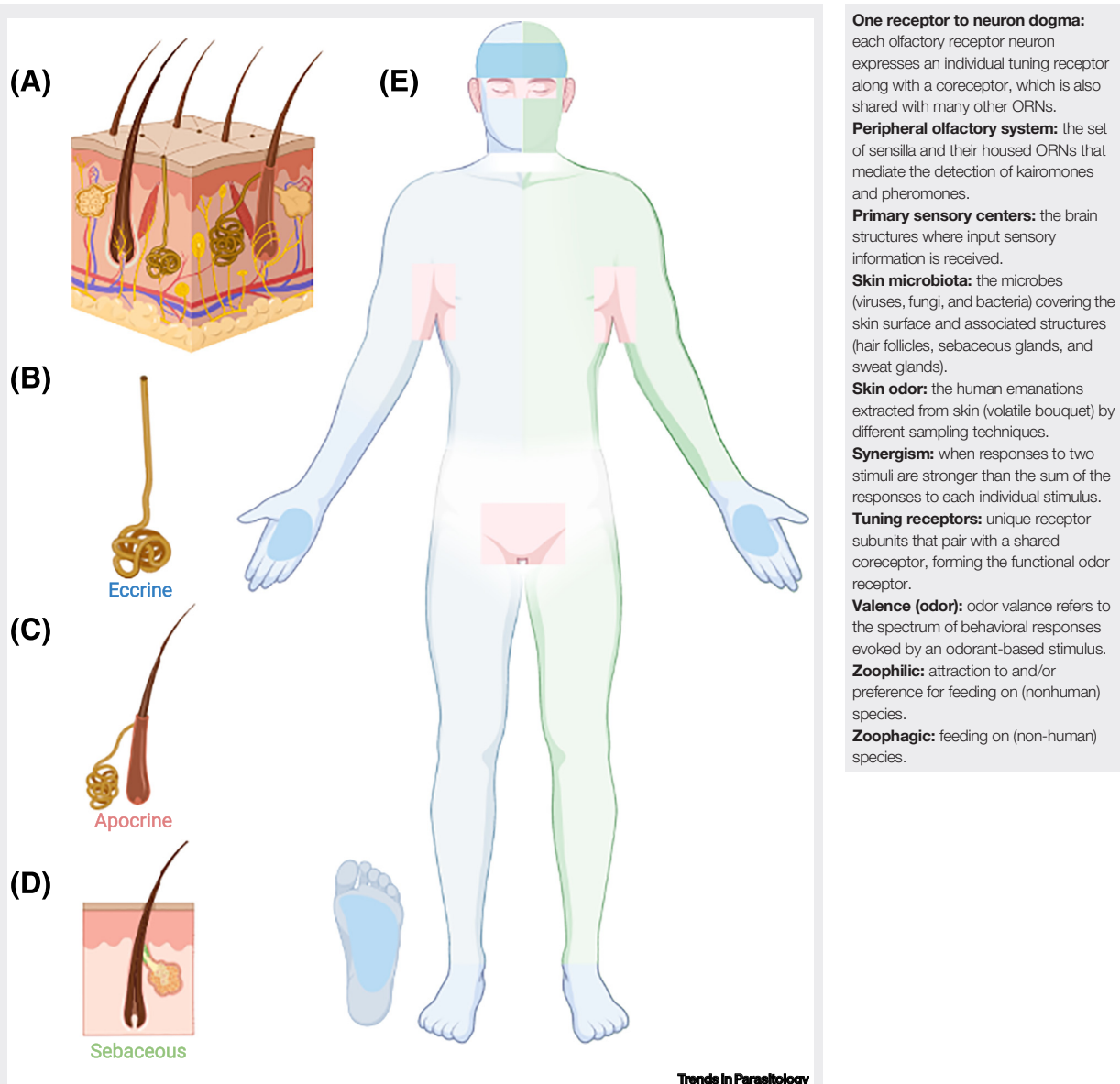
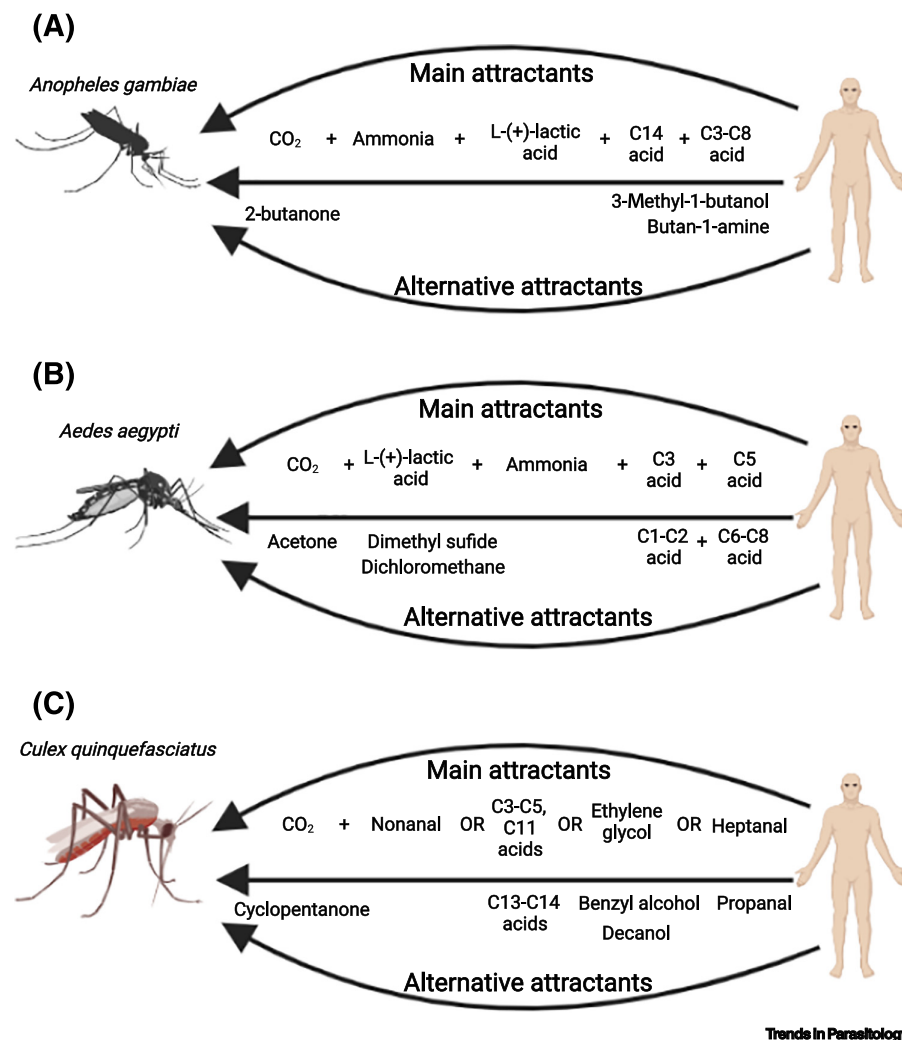


Figure 1. Sweat gland distribution in the human skin. (A) Section of the human skin showing the sweat glands: (B) eccrine, (C) apocrine, and (D) sebaceous. (E) Eccrine glands are distributed all over the body (depicted on the right-half of the body in light blue), and more densely distributed on the palms, soles, and forehead (dark blue). Apocrine glands are mostly located on the axilla and genital regions, as highlighted in beige. Sebaceous glands are found all over the body (highlighted on the left side of the body in light green), with higher densities on the face (dark green). Figure created with BioRender.

more attractive to these mosquitoes than human skin odor or mixtures of CO₂ and L-(+)-lactic acid [18]. Specific carboxylic acids and sulfur compounds can alone induce landing for this mosquito species (Figure 1B and Table 2) [32].



Trends in Parasitology

Figure 1. Human odorants attractive to female mosquitoes. (A) Attraction of female *Anopheles gambiae* is evoked by sensing carbon dioxide (CO₂) and ammonia, followed by the detection of L-(+)-lactic acid that triggers the perception of different carboxylic acids. Attractive behavior is similarly evoked when CO₂ is replaced by 2-butanone, and short-chain carboxylic acids are replaced by 3-methyl-1-butanol along with butan-1-amine. (B) Combinations of CO₂, L-(+)-lactic acid, ammonia, one C1–C3 as well as one C5–C8 carboxylic acid are sufficient to trigger *Aedes aegypti* female attraction. The substitutive odorants acetone and dimethyl sulfide or dichloromethane can play the roles of CO₂ and L-(+)-lactic, respectively. (C) Even though less comprehensively investigated, *Culex quinquefasciatus* female attractive behavior can be evoked by a combination of CO₂ and nonanal, and individually by short-chain (C1), middle-chain (C6–C11) and long-chain (C13–C14) carboxylic acids, specific alcohols, and aldehydes. Figure created with BioRender.

Human skin odor, particular specific carboxylic acids and sulfur compounds were found to be very important landing cues for *Culex quinquefasciatus* (Figure 1C and Table 3) [6,22,32]. Nonanal, found at high abundance in human skin odor, can synergize with CO₂ to attract these mosquitoes to baited traps [33]. *Cx. quinquefasciatus* females are also attracted to a variety of carboxylic acids (C3, C6–C11, and C13–C14), alcohols (ethylene glycol, benzyl alcohol, cholesterol, and decanol), as well as aldehydes (heptanal, propanal, and nonanal) present in the human skin volatile bouquet (Figure 1C and Table 3) [34].

Table 1. Odor blends and single odorants attractive or aversive to *Anopheles gambiae* females

<i>Anopheles gambiae</i>				
Odor blend	Dose/concentration	Effect	Behavior (assay)	Refs
Carbon dioxide ^a Ammonia L-(+) lactic acid	0–1030 parts per million (ppm) 2.5% 90%	Attraction	Activation Long-range attraction (wind tunnel – dual port)	[22]
Ammonia L-(+) lactic acid Carboxylic acids (C3–C8 and C14)	2.5% Pure Pure	Attraction 3mC4 reduces attraction to blend	Short-range attraction/ Trap-catch (wind tunnel – dual port; MM-X trap)	[13,24]
Carbon dioxide Ammonia L-(+) lactic acid Carboxylic acids: C3 C4 C5 C7 C8 C14	500 ml/min 2.5% 85% 0.1% 1% 0.01% 0.01% 0.01%	Attraction ^a 3mC4 inert to blend	Trap-catch (MM-X trap - semi-field)	[17]
1-Butanol 2,3-Butanedione 2-Methyl-1-butanol 2-Methylbutanal 2-Methylbutanoic acid 3-Hydroxy-2-butanone 3-Methyl-1-butanol 3-Methylbutanal 3-Methylbutanoic acid benzene ethanol	Pure Pure Pure Pure Pure Pure Pure Pure Pure Pure Pure	Attraction	Trap-catch (MM-X trap)	[103]
Ammonia L-(+) lactic acid Carboxylic acid (C14) + 2-Methylbutanoate OR butyl butyrate OR butyl acetate OR butyl isobutyrate OR dimethylsulfide	2.5% Pure Pure 1% 1% 1% 0.1% 0.01–0.001%	Attraction ^a 2-Pentadecanone (1%) reduces attraction to blend	Short-range attraction (wind tunnel – dual port)	[27]
Ammonia L-(+) lactic acid Carboxylic acid (C14) + 3-Methyl-1-butanol OR 3-methylbutanal	25% Pure Pure 0.01% 1%	Attraction	Short-range attraction (wind tunnel – dual port)	[26]
Carbon dioxide Ammonia L-(+) lactic acid Carboxylic acid (C14) + 3-Methyl-1-butanol OR 3-methylbutanoic acid	4.5% 25% Pure Pure 0.1% (pure for traps) 0.1%	Attraction	Trap-catch/ Short-range attraction (MM-X trap - semi-field; wind tunnel – dual port; MM-X trap – open-field)	[25,26,107]
Carbon dioxide Ammonia L-(+) lactic acid Carboxylic acid (C14) + butan-1-amine	4.5% 25% Pure Pure 0.00001–1%	Attraction	Short-range attraction (wind tunnel – dual port)	[26]

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Table 1. (continued)

<i>Anopheles gambiae</i>				
Odor blend	Dose/concentration	Effect	Behavior (assay)	Refs
Carbon dioxide Ammonia L-(+) lactic acid Carboxylic acid (C14) + 3-methyl-1-butanol + butan-1-amine	4.5% 25% Pure Pure 0.01% or 0.001% 0.01 to 0.004%	Attraction	Short-range attraction (wind tunnel – dual port)	[26]
Carbon dioxide Ammonia L-(+) lactic acid Carboxylic acid (C14) 3-Methyl-1-butanol + butan-1-amine OR 1-dodecanol	63 ml/min 2.5% 85% 0.00025% 0.000001% 0.0000001% 0.0001%	Attraction	Trap-catch (MM-X trap – semi-field)	[108]
Ammonia L-(+) lactic acid Carboxylic acid (C14) 3-Methyl-1-butanol butan-1-amine 2-Butanone	2.5% 85% 0.00025% 0.000001% 0.001% Pure	Attraction	Trap-catch (MM-X trap – semi-field)	[40]
Individual odor	Dose/concentration	Effect	Behavior	Refs
Ammonia	13.7–13 700 ppm	Attraction	Short-range attraction (wind tunnel – dual port)	[24]

^aTurbulent plume.

An interesting aspect of the olfactory system of mosquitoes is the fact that some skin odorants can also bind to the CO₂ receptor, evoking neuronal responses and inducing behavior [35–39]. Skin odors and their chemical analogs have been used to activate, superactivate, and inhibit the CO₂ receptor and, in turn, manipulate the host-seeking behavior of mosquitoes (Tables 1–3) [36,37,40,41]. At nonphysiological concentrations the human skin odor 2,3-butanedione triggers prolonged firing of the CO₂ neuron, disorienting the host-seeking behavior of *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes [36]. Cyclopentanone and ethyl pyruvate are two odorants with a chemical structure similar to that of skin odors and capable of activating and inhibiting the CO₂ receptor neuron, respectively [37]. Whereas the former odorant acts as an attractant, the latter can reduce CO₂ source attraction of female *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes [37]. Along the same lines, acetone is another CO₂ receptor neuron activator [39], and *Ae. aegypti* females show similar attraction to acetone and L-(+)-lactic acid as to CO₂ and L-(+)-lactic acid [18,29]. Additionally, a human body odor called spermidine inhibits CO₂ activation and reduces *Ae. aegypti* landing evoked by human skin odor [41]. Lastly, replacing CO₂ in an attractive blend of odorants with the CO₂ receptor activator 2-butanone [36] sustains similar rates of trap catches of *An. gambiae* and *Anopheles funestus* in field settings in Kenya [40].

Physical cues, such as heat and humidity, are also perceived by female mosquitoes, playing important roles in short-range attraction [1]. Upon activation by CO₂, female *Ae. aegypti* mosquitoes are attracted to heat [2,28], without any evidence of **habituation** [42]. These mosquitoes respond to heat at 2.5°C above ambient temperatures and remain attracted to temperatures up to 40°C [42]. Attraction to warmed objects is further enhanced when humidity is increased [3], triggering the hovering of mosquitoes over a 6–8 cm distance [2].

Visual cues are also involved with female mosquito short-range attraction and landing behaviors [1]. *Ae. aegypti* is aided by visual cues, or heat, to land when activated by CO₂ [2,28,42]. When

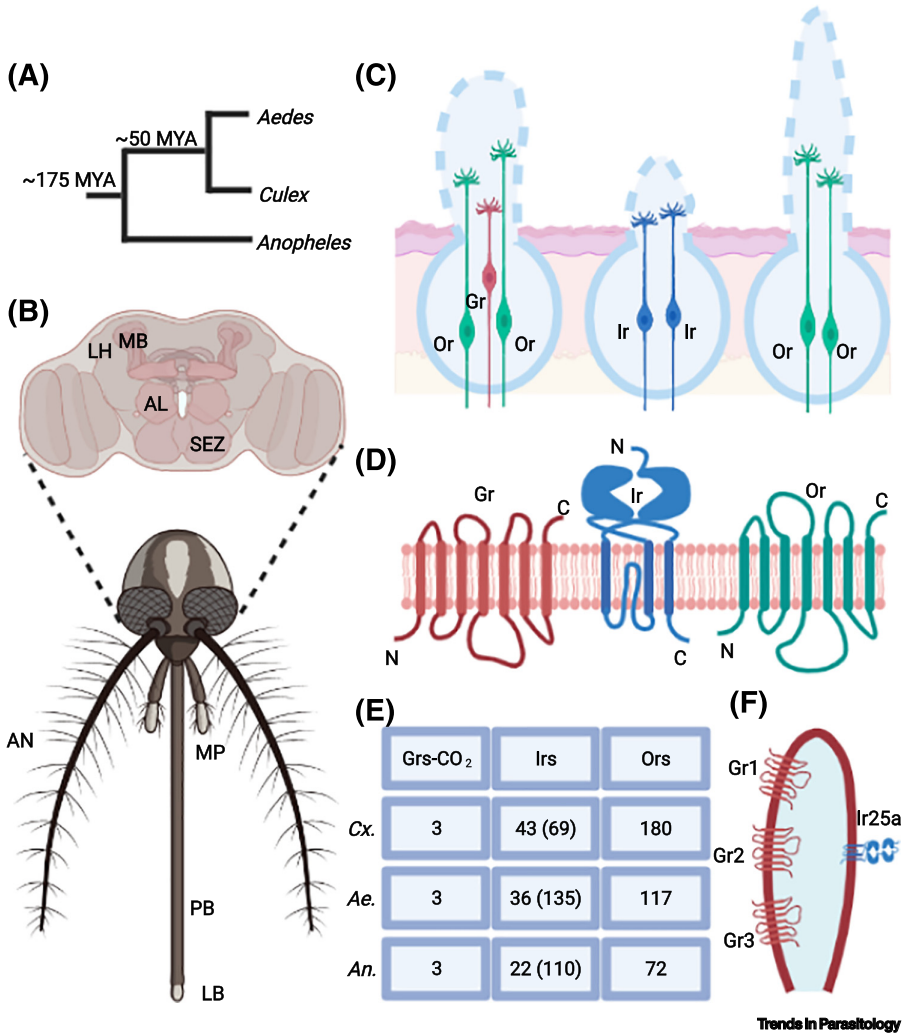


Figure 2. Mosquito olfactory receptor families expressed in odorant receptor neurons (ORNs) and housed in distinct sensilla. (A) Cladogram showing the divergence time between Culicini and Aedini subtribes and Anophelinae and Culicinae subfamilies (B) Head of a Culicinae mosquito exhibiting the olfactory appendages [antennae (AN), maxillary palps (MP), and labella (LB)], along with the brain's primary olfactory and taste centers [antennal lobes (AL), subesophageal zone (SEZ)], and high-order centers [lateral horns (LH) and mushroom bodies (MB)]. (C) Classical view of the olfactory system showing one receptor per neuron. Left. In mosquitoes, capitata-peg sensilla on the maxillary palps house the cpA neuron that expresses the CO₂ sensors Gr1–3 (or Gr22–24). These sensilla also house two other neurons expressing predominantly Ors. On the antennae, grooved peg (middle) and trichoid (right) sensilla house ORNs expressing either Ors or Irs or both. The exact distribution of such neurons in the different sorts of antennal sensilla is not known. (D) Molecular structures of Gr (left), Ir (middle) and Or (right). Whereas Ors and Grs are seven-pass transmembrane proteins that resemble G-protein-coupled receptors but function as ion channels, Irs emerged from the ionotropic glutamate receptor (iGluRs) gene family [63]. (E) The number of genes belonging to the gustatory receptor (Gr - CO₂ sensors), ionotropic receptors (Ir), and odorant receptors (Or) in *Culex quinquefasciatus* (Cx.), *Aedes aegypti* (Ae.), and *Anopheles gambiae* (An.) is depicted. For the Irs, genes expressed in olfactory appendages along with the number of genes in the genome (parenthesis) are shown. (F) Modern view of the olfactory system depicting more than one receptor per neuron for the mosquito capitata peg sensillum neuron cpA [75]. Figure created with BioRender. Abbreviation: MYA, million years ago.

Table 2. Odor blends and single odorants attractive or aversive to *Aedes aegypti* females[illegible]

Table 2. (continued)

Individual odor	Dose/concentration	Effect	Behavior	Refs
Methyl propyl disulfide	200 µg/1 µg	Attraction	Short-range attraction/ landing on perfumed object (Y-olfactometer; Cage assay)	[32]
Butanoic acid	1 µg	Attraction	Landing on perfumed object (Cage assay)	[32]
3-Methyl butanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Heptanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Tetradecanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Hexadecanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Octadecanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Benzoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
2-hydrobenzoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Spermidine (human odorant analog)	10%	Repellent (reduced landing on skin odor)	Landing on perfumed object (cage assay)	[41]
6-Methyl-5-hapten-2-one (sulcatone)	10 ng 1 µg	Reduced probing activity	Short-range attraction/ probing on perfumed hand (Y-olfactometer)	[53]
Octanal	10 ng 100 ng 1 µg 10 µg	Reduced attraction	Short-range attraction/ probing on perfumed hand (Y-olfactometer)	[53]
Nonanal	1 µg 10 µg	Reduced attraction	Short-range attraction/ probing on perfumed hand (Y-olfactometer)	[53]
Decanal	1 µg 10 µg 100 µg	Reduced attraction	Short-range attraction/ probing on perfumed hand (Y-olfactometer)	[53]
Geranylacetone	10 ng (probing only) 100 ng 1 µg 10 µg 100 µg (attraction only)	Reduced attraction and probing activity	Short-range attraction/ probing on perfumed hand (Y-olfactometer)	[53]

heat and visual cues are present, females still prefer warmed dark objects, yet this attraction is only induced when the warmed object exhibits a high contrast against the background [2]. Visual cues can induce landing without CO₂ activation, even though CO₂ switches attention from visual cues and helps mosquitoes to focus on the thermal cue [43]. In the presence of CO₂, mosquitoes also discriminate against color hues [44]. *Ae. aegypti* shows preference for cyan, orange, and red objects; *Cx. quinquefasciatus* prefers blue and red objects; *Anopheles stephensi* is more attracted to black and red objects [44].

As aforementioned, the integration of multiple host cues triggers a synergistic effect regarding mosquito host-finding [1]. Heat along with skin odors evoked a higher *An. gambiae* landing

Table 3. Odor blends and single odorants attractive or aversive to *Culex quinquefasciatus* females

<i>Culex quinquefasciatus</i>				
Odor blend	Dose/concentration	Effect	Behavior (assay)	Refs
Carbon dioxide	4%	Attraction	Activation Long-range attraction (wind tunnel)	[6]
2,3Butanedione 1-Hexanol 1-Butanal 1-Pentanal	3% 3% 3% 3%	Repellent (reduced attraction to carbon dioxide source)	Short-range repellency (MM-X trap – semi-field)	[36]
Carbon dioxide Nonanal	Released from dry ice 0.1 mg	Attraction	Trap-catch (MM-X trap – open-field)	[33]
Individual odor	Dose/concentration	Effect	Behavior	Refs
L-(+)-lactic acid	200 µg	Attraction	Short-range attraction (Y-olfactometer)	[32]
Acetic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Hexadecanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Octadecanoic acid	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Methyl sulfide (dimethyl sulfide)	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Methyl propyl disulfide	1 µg	Attraction	Landing on perfumed object (cage assay)	[32]
Propanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Hexanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Heptanoic acid	1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Octanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Nonanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Decanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Undecanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Tridecanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Tetradecanoic acid	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Pentadecanoic acid	10 µg	Repellent	Short-range attraction (Y-olfactometer)	[34]
Hexapentanoic acid	0.1 µg, 1 µg, 10 µg	Repellent	Short-range attraction (Y-olfactometer)	[34]
Heptapentanoic acid	0.1 µg, 1 µg (10 µg)	Attractant (repellent)	Short-range attraction (repellency) (Y-olfactometer)	[34]
Octapentanoic acid	0.1 µg, 1 µg, 10 µg	Repellent	Short-range attraction (Y-olfactometer)	[34]

Table 3. (continued)

Individual odor	Dose/concentration	Effect	Behavior	Refs
Glycerol	0.1 µg, 1 µg	Repellent	Short-range attraction (Y-olfactometer)	[34]
Ethylene glycol	1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Benzyl alcohol	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Cholesterol	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Decanol	0.1 µg (10 µg)	Repellent (attractant)	Short-range repellency (attraction) (Y-olfactometer)	[34]
Heptenal	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Propanal	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Nonanal	0.1 µg, 1 µg, 10 µg	Attraction	Short-range attraction (Y-olfactometer)	[34]
Cyclopentanone (human odorant analog)	5–20%	Attraction	Trap-catch (MM-X trap – semi-field)	[37]
Ethyl butyrate (human odorant analog)	10%	Repellent (reduced attraction to carbon dioxide source)	Trap-catch (MM-X trap – semi-field)	[37]

response than such cues alone [45], and the combination of heat, skin odor, and vision increases trap catches drastically [46]. CO₂ gates *Ae. aegypti* attraction to heat and skin odor [28]. Also, CO₂ gates *An. gambiae* responses to skin odor [4], evoking landing behavior [23]. By contrast, *Cx. quinquefasciatus* females land on warmed beads perfumed with skin odor regardless of contact with CO₂ [6]. This species also lands into individual skin odors without previous exposure to CO₂ [32,34].

The multistep behavioral output that leads to host finding in different species is triggered by a few similar components, such as CO₂, visual cues, heat, humidity, and skin odor. Nonetheless, skin odor is differentially sensed by different mosquito species. These findings highlight the evolutionary differences amongst mosquito species, which share common ancestors approximately 145–200 million years ago (MYA) between the subfamilies Anophelinae and Culicinae [47] and 52–54 MYA between the subtribes Aedini and Culicini (Figure 2A) [48,49]. This evolutionary divergence led to the differences in behavioral responses to olfactory cues and likely defined the ecological niche of each mosquito species [50].

Mosquito host choice

Mosquitoes can exhibit species-specific or seasonal attraction to humans, and such behaviors are triggered by human-skin volatiles [33,51]. Different subspecies of *Ae. aegypti* show distinct **mosquito host choice** (host preferences) [51], with the domestic subspecies *Ae. aegypti aegypti* displaying a strong preference to feed on humans, and the forest-dwelling subspecies *Ae. aegypti formosus* exhibiting **zoophilic behavior** [51]. Such preferences may be associated with the ability of these subspecies to sense the human-skin-emanating volatile sulcatone [51]. This volatile is present in much greater amounts in human skin volatiles than in the scent of other animals [51], and the **olfactory receptor neurons (ORNs)** of the anthropophilic

subspecies display a higher sensitivity to sulcatone than do the olfactory neurons of the zoophilic subspecies [51].

Differences in human-host perception are also observed between the anthropophilic mosquito *Ae. aegypti* and the **anthropo(zoo)philic** mosquito *Cx. quinquefasciatus* [33,52]. *Ae. aegypti* shows stronger attraction to human odors than to other animals [52]. Such attraction is mediated by the enrichment of specific ketones (sulcatone and geranylacetone) and long-chain aldehydes (decanal) in the human scent, contrasting with the greater abundance of short-chain aldehydes (hexanal and heptanal) in the scent of other animals [52]. As decanal activates the *Ae. aegypti* olfactory system at levels released by human skin, it possibly mediates mosquito attractive behavior towards humans [52]. By contrast, *Cx. quinquefasciatus* prefers to feed on both humans and birds, switching hosts upon season and availability [33]. To do so, these mosquitoes rely upon chemical volatiles that are commonly present in both the human and bird volatile bouquets [33]. Among the volatiles commonly found in the chicken and pigeon odor bouquets, nonanal was also found at higher proportions in human skin emanations [33]. This compound evokes strong responses by specific classes of olfactory sensilla and synergizes with CO₂ in attracting this mosquito species to traps [33].

Mosquito human discrimination

Mosquitoes exhibit differential attraction to different human individuals [53,54], and quantitative and qualitative differences in skin microbiota and skin odors among individuals are likely the factors tuning attractiveness [53,54]. Differences in human attractiveness by *An. gambiae* are correlated with the quantity and diversity of the skin microbiota [54]. A greater abundance of *Staphylococcus* spp. in a less diverse skin microbiome is associated with highly attractive individuals whereas enrichment with *Pseudomonas* spp. in a more diverse skin microbiome correlates with reduced attractiveness to *An. gambiae* females [54]. In addition, reduced attractiveness to human hands has been associated with increased releases of specific human skin volatiles [53]. While perfuming a human hand with different quantities of sulcatone were inert to *Ae. aegypti* females, wearing specific doses of octanal, nonanal, decanal, and geranylacetone on the skin reduced attractiveness [53]. In fact, decanal can even reduce mosquito attractiveness to a human hand to levels similar to that of clean air, and geranylacetone is capable of reducing even probing activity [53].

Odorant-based learned behavior

Mosquito host attraction and avoidance can also be tuned by skin **odor-based learning behavior** [55,56]. Exposure of *Ae. aegypti* to individual odorants, followed by a reward (**heated blood meal**) can change the **valence** of neutral odorants, such as L-(+)-lactic acid and 1-octen-3-ol, inducing attractive behavior [55]. Even training with the aversive odorants (Z)-hex-3-en-1-ol or the repellent DEET combined with a heated blood meal reward results in a partial to complete valence switch, inducing *Ae. aegypti*'s attractive behavior towards known repellents [55]. Conversely, exposure of *Ae. aegypti* to an aversive stimulus, along with human skin odor, changes the odor's valence, inducing aversive behavior towards skin odor [56]. The degree of aversiveness in this instance was different amongst skin odors from different individuals [56], and this phenomenon is also observed against rat scent, but not with chicken scent. Altogether, these findings indicate that odor-based mosquito learning behavior is a property of certain chemicals in an odor scent [56]. Whereas **kairomones** like 1-octen-3-ol evoked aversive learned behavior upon previous exposure to an aversive stimulus, nonanal was neutral, and L-(+)-lactic acid showed even stronger attractive behavior [56]. Hence, mosquitoes can learn to avoid, or be attracted to, specific compounds in the host skin odor based on previous negative (host defense behavior) or positive (blood meal) experiences with those compounds [56]. Understanding mosquito

host preference has uncovered important clues about the olfactory signals that lead to human attractiveness [33,51,53,56].

Molecular basis of mosquito attraction to humans

The mosquito **peripheral olfactory system** perceives chemical volatiles via ORNs housed in the olfactory sensilla present in the antennae, maxillary palps, and proboscis' labella (Figure 2B) [35,57–59]. Such sensilla are innervated by two or three ORNs that express members of the odorant receptor gene families (Figure 2C) [60]. These encompass the odorant receptor (*Or*) [57,58,61] and the ionotropic receptor (*Ir*) gene families (Figure 2D,E) [62–64]. Whereas the **tuning receptors** of the former gene family form heteromeric tetramers with the **coreceptor** Orco [57,58,61,65], members of the later family pair with any one or two of the three coreceptors, named Ir25a, Ir8a, and Ir76b [66]. Both gene families encode ligand-gated ion channels [62–64,67]. The Ors perceive mostly aromatic and heterocyclic odorants in *Anopheles* mosquitoes [68]; the Irs are mostly tuned to amines and carboxylic acids as seen in fruit flies [62–64,66]. By contrast, CO₂ is detected by a specific class of neurons (cpA) housed in the maxillary palp capitae peg sensilla (Figure 2B,C) [35]. In mosquitoes, the cpA neurons express three members of the gustatory receptor gene families (Grs), named Gr1–3 in Culicinae and Gr22–24 in Anophelinae (Figure 2D,E) [35]. These proteins assemble into a trimeric receptor that detects mostly CO₂ but also skin odors [35–39].

Most of the axons of the ORNs extend to the primary olfactory center of the deutocerebrum called the antennal lobe (Figure 2B) [69]. The axons of multiple neurons of the same class converge to specific regions of the antennal lobe called the olfactory glomeruli (Figure 2B) [69]. The antennal lobes of mosquitoes bear between 65 and 81 glomeruli in *Ae. aegypti* [70,71] and 67–70 in *An. gambiae* females [60]. Since males and females exhibit the same number of glomeruli (67–70 in *Anopheles* mosquitoes), then the host-seeking ability of females is likely related to differences in antennal lobe volume (2:1 female over male; [60]) as well as sexual dimorphism in the expression of the transcription factor *fruitless* in the brain and peripheral ORNs [72]. As male *Ae. aegypti* are also attracted to humans in the field [73], further investigation is needed to unveil the factors triggering host finding. By 24 h after adult emergence, the mosquito peripheral olfactory system exhibits complete activation of olfactory receptor gene expression, with olfactory related gene expression increasing as female mosquitoes mature and develop host-seeking abilities [74].

In contrast to the **one receptor to neuron dogma** postulated based on observations of the *Drosophila melanogaster* olfactory system [69], fluorescent labeling of *Ae. aegypti* olfactory glomeruli using **genetic drivers** for the odorant receptor coreceptors, Orco, Ir25a, Ir8a, and Ir76b, and the CO₂ receptor subunit Gr3, identified significant neuronal overlap in the expression of the different odorant receptor gene families [75], as observed in fruit flies [76]. These findings agreed with the greater number of odorant receptors in the *Ae. aegypti* genome [77] as compared to the number of olfactory glomeruli, breaking the one-receptor-to-neuron dogma in mosquitoes [75]. The CO₂ receptor neurons also express the Ir25a receptor (Figure 2E), which is activated by amines [75], and the glomeruli innervated by CO₂ receptor neurons respond to trimethylamine in Gr3^{-/-} mosquitoes [75]. Interestingly, trimethylamine can replace CO₂ and attract Gr3^{-/-} *Ae. aegypti* mosquitoes when associated with L-(+)-lactic acid [75]. Furthermore, wild-type *Ae. aegypti* females are only weakly attracted to a blend of trimethylamine and L-(+)-lactic acid [75]. As the correspondent olfactory neurons of Orco and Gr3 knockout mosquitoes are devoid of spontaneous activities [28,78,79], and CO₂ receptor knockouts are unresponsive to multiple skin odors [79], the expression of ionotropic receptors in Orco and CO₂ receptor neurons might play a modulatory role, fine-tuning the neuronal responses of such neurons.

Some of the genes encoding odorant coreceptors have been **edited** in mosquitoes, unveiling their roles in mosquito host-seeking behavior [28,42,78–81]. The first odorant receptor knocked out was the odorant receptor coreceptor *Orco* in *Ae. aegypti* [78]. Both male and female *Orco* knockouts lose attraction to honey [78]. In the absence of CO₂, female *Orco* knockouts lose attraction to human skin odor, which is mildly attractive to wild-type mosquitoes [78]. By contrast, activation by CO₂ strongly enhances attraction to human skin odor in both wild-type and *Orco* knockout female mosquitoes, demonstrating that CO₂ does not gate *Orco*-driven behavior, and other olfactory pathways can only be activated in the presence of CO₂ [78]. Similarly, *An. gambiae* (*Anopheles coluzzii*) female *Orco* knockouts partially lose attraction to human hands [81].

Gene knockout of the *Orco* receptor in *Ae. aegypti* demonstrated that *Or*-expressing neurons play an important role in host discrimination [78]. Whereas wild-type mosquitoes showed strong attraction to human subjects, as opposed to guinea pigs, *Orco* mutants partially lose such preferred human attraction [78]. Comparisons of the olfactory systems between (sub)species of anthropophilic (**anthropophilic**) and zoophilic (**zoophilic**) mosquitoes reveal both sequence and expression differences in the olfactory system [51,82,83]. Although the anthropophilic *An. gambiae* and the zoophilic *Anopheles quadriannulatus* display very similar chemosensory gene repertoires, divergences at sequence and transcriptional levels are evident [82]. Such divergences encompass a few receptors evolving under positive selection, most of the *Irs* genes showing differential expression, and a subset of the *Ors* genes of *An. quadriannulatus* predominantly expressed in the antennae of *An. gambiae* [82]. Between the anthropophilic *Ae. aegypti* and the zoophilic *Ae. aegypti formosus*, *Or4* plays a major role in host preferences [51,83]. *Or4* is among the highest expressed odorant receptor genes in anthropophilic mosquitoes [51]. Also, the alleles of the *Or4* receptor show clear genetic structuring between anthropophilic and zoophilic mosquitoes [51,83], and these differences parallel the strength of *Or4* neuronal activation by the human skin odorant sulcatone [51].

Knocking out the ortholog genes encoding the subunits of the heteromeric CO₂ receptor in *An. gambiae* [79], or expression of different combinations of these subunits of *Ae. aegypti* [84] and *Cx. quinquefasciatus* [85] using heterologous expression systems, demonstrated a conserved role of each subunit in CO₂ sensing [79,84,85]. Whereas knockouts of the *An. gambiae* Gr23 and Gr24 subunits demonstrated that these subunits are essential for CO₂ detection, knockouts of the Gr22 displayed only reduced sensitivity [79]. Further, *Ae. aegypti* knockouts of a CO₂ receptor subunit (Gr3) bore a nonfunctional CO₂ receptor and lost their attraction to CO₂ in cage experiments [28]. The synergy in attraction evoked by the combination of CO₂ with L-(+)-lactic acid or human skin odor or heat were also lost in Gr3 knockouts [28]. Interestingly, the Gr3 knockout females exhibited similar attraction to human skin odor alone and to a human arm as wild-type mosquitoes [28]. Thus, CO₂ seems to be essential for long-range host attraction, yet mosquitoes can find human hosts by sensing and integrating other cues such as heat, skin odor, and humidity [28].

When the ionotropic receptor coreceptor *Ir8a*, which is predominantly expressed in the antennae, is knocked out in female *Ae. aegypti*, these mosquitoes lose the ability to detect odor cues abundant on the human skin, such as L-(+)-lactic acid and other carboxylic acids [80]. In the absence of CO₂, *Ir8a* mutants showed quite reduced attraction to human skin odors [80], as observed for the *Orco* mutants, indicating that both receptors contribute to skin odor attraction [80]. Upon CO₂ activation, *Orco* knockouts display similar attraction to human skin odors as wild-type mosquitoes [28,80] whereas *Ir8a* knockouts exhibit reduced attraction to the same cues [80], indicating that CO₂ is necessary to gate skin odor attraction through the *Ir8a* neuronal pathways

but not through the Orco pathways [80]. Interestingly, double knockouts of Orco/Ir8a do not completely abrogate attraction to human skin and skin odors in the presence or absence of CO₂, pointing to the existence of still other receptors and neural pathways responsible for skin odor detection [80].

Besides odorants, the peripheral sensory system of mosquitoes also perceives physical cues, such as heat and humidity [86,87]. Even though humidity and dryness receptors are yet to be pinpointed for mosquitoes, heat is perceived by the Ir21a receptor [88] and via transient receptor potential (TRPA1) channels [86]. A thermoregulator mediating mosquito heat-seeking was identified in *An. gambiae* female mosquito antennae [88]. Female Ir21a-receptor knockouts lose most of their attractiveness to a surface warmed at the human body temperature as well as preference for a warmed blood meal [88]. Conversely, female *Ae. aegypti* knockouts for the *trpa1* gene, which encodes a thermosensor and receptor of noxious compounds, are still attracted to warmed objects, yet lose their normal avoidance to stimuli at temperatures higher than the human body [42]. These mosquitoes also lose their avoidance to feed on noxious compounds detected by the TRPA1 channel [42] and to the smell of the mosquito repellent herb catnip [89].

Integration of olfactory information in the olfactory, taste, and visual brain centers

The mosquito olfactory glomerulus is innervated by neurons coexpressing different odorant coreceptors [75] but it also can receive innervations from different classes of olfactory neuron [75]. In addition, specific glomeruli innervated by Ir8a-expressing neurons are activated exclusively by exposure to a combination of CO₂ and L-(+)-lactic acid, but not by either of these kairomones alone [90]. As such neurons display very reduced expression of other odorant receptor coreceptors [75] it is possible that activation of the glomerulus innervated by CO₂ receptor neurons disinhibits olfactory neurons innervating other glomeruli [90]. A similar phenomenon is observed in floral scent discrimination by *Ae. aegypti* [91]. The release of higher amounts of the attractive odor nonanal by the preferred orchid leads to activation of the LC2 glomerulus and inhibition of the AM2 glomerulus via GABAergic (γ -aminobutyric acid) interneurons [91]. The latter glomerulus detects odors that are aversive to *Ae. aegypti* [91].

The signals detected by such olfactory and thermal receptors responsible for sensing human chemical and physical cues are transduced to the **primary sensory centers** in the mosquito brain [92], where the information is integrated and decoded by the **higher brain centers** to trigger the behavioral responses [56,60,93,94]. CO₂ modulates visual responses in the optical lobe, potentially coupling host identification and host location [94]. Host attraction and avoidance can also be modulated by odor-based mosquito learning behavior, which involves a food-based reward and an aversive stimulus, respectively [56]. Physical evidence for this sort of phenomenon, by contrast, was demonstrated by the axon projections of olfactory neurons on the proboscis' labella to the subesophageal zone, which is the brain region responsible for taste coding (Figure 2B). These axonal connections potentially integrate taste and smell into the perception of flavor [60]. Together, these findings highlight the initial steps in the elucidation of the complex organization of the primary sensory centers in the mosquito antennal lobes and the subesophageal ganglion, and the complexity of neuronal coding used by mosquitoes to decipher host-choice and drive host-seeking behavior.

Concluding remarks

Disrupting the neural coding that drives mosquito attraction to humans can lead to a reduction in mosquito-borne disease transmission. As only a handful of odorants (DEET, picaridin, eucalyptol, and *N*-acetylpyridines) have been shown to effectively repel mosquitoes [95–98], the quest for

Outstanding questions

What are the main odors igniting *Culex quinquefasciatus* human attraction? Does it follow similar patterns as *Anopheles gambiae* and *Aedes aegypti*? How about the other mosquito vectors?

Are there other skin volatiles defining human discrimination by mosquitoes besides sulcatone? Is sulcatone the key odorant driving human attractiveness in other mosquito vectors?

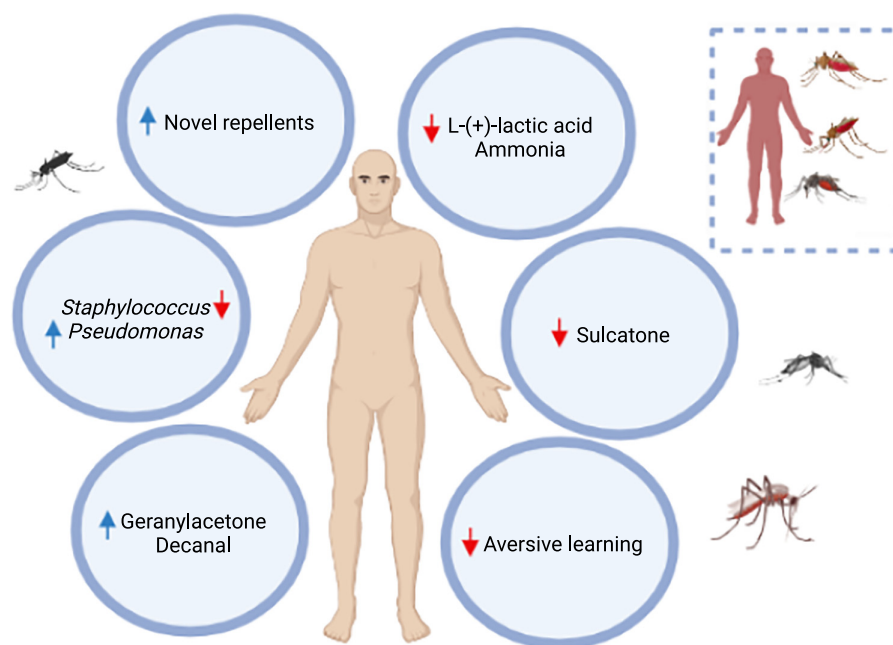
Can mosquito learning be used as a strategy to induce effective mosquito repellency behavior?

Can we completely abrogate mosquito host-seeking behavior by shutting down all olfactory pathways?

Will re-engineering the human skin microbiota be a strategy effective against all mosquito species? Or should this strategy be designed for each main mosquito species?

novel effective mosquito repellents has taken the search of huge databases using computational tools involving chemoinformatics [99] and machine learning [100]. Testing DEET substitutes found through chemoinformatics [99] across fruit fly species showed conserved repellency [101], suggesting that such novel repellents might also repel mosquitoes. In addition, previous findings associating odor volatiles with manipulation of mosquito behavior support the potential application of novel chemical ecology approaches in mosquito control (Figure 3):

- (i) Differences in skin microbiota composition are associated with human attractiveness [54]. So, inducing shifts in the human skin microbiota composition might reduce mosquito bites without affecting the skin homeostasis.
- (ii) *Aedes* mosquitoes are only attracted to other human body odors and CO₂ in the presence of L-(+)-lactic acid [28]. The same role is played by the combination of ammonia and L-(+)-lactic acid in *Anopheles* mosquitoes [13,24]. Hence, reducing production of both ammonia and L-(+)-lactic acid might reduce mosquito attraction and bites.
- (iii) Increasing the quantities of attractive odors on the human skin can evoke repellency behavior in mosquitoes and even reduce probing activity, as shown for the skin odorants decanal and geranylacetone [53].



Trends in Parasitology

Figure 3. Odorant-based applications to reduce mosquito attraction. Reducing mosquito attraction to a human host may be achieved by the development of novel repellents. Also, applying an attractive odorant, such as decanal and geranylacetone, on the human skin can also result in mosquito aversive behavior. Alternative strategies to reduce mosquito attraction include training mosquitoes to avoid a human host by integrating an odorant to host-averse behavior, changing the human skin microbiota profile to a less attractive one, reducing the emission of odorants that ignite mosquito attraction, for example, L-(+)-lactic acid and ammonia, as well as odorants linked to human attractiveness, for instance sulcatone. *Inset.* Without any intervention, mosquitoes are highly attracted to humans, on which they feed and transmit pathogens. Blue and red arrows point to increasing or decreasing, respectively, the chemical volatiles released or the densities of specific skin resident bacteria. Figure created with BioRender.

- (iv) The skin odorant sulcatone drives the mosquito's preference for human odor [51]; so, reducing sulcatone on the human skin might deflect anthropophilic mosquitoes from biting humans.
- (v) Mosquitoes can also learn to avoid hosts by associating skin odors with the host aversive behavior [56]. Applying such odors on the human skin might also cause a deficit in mosquito learning, reducing human bites.

Designing strategies to modify the profile of human skin odor volatiles appears to be pivotal to reduce mosquito bites. Even though odorant coreceptor and heat-sensing knockout mosquitoes are yet attracted to human hosts [28,78,88], re-engineering the native human skin microbiota with bacteria secreting lower amounts of attractive and higher quantities of aversive odorants might completely abrogate mosquito attraction to human hosts (see Outstanding questions). If successful, this strategy opens the doors to a variety of ways in which mosquito host-seeking behavior can be manipulated, leading potentially to a reduction in mosquito bites and preventing the transmission of mosquito-borne diseases.

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Declaration of interests

O.S.A is a founder of Agragene, Inc. with equity. All other authors declare no competing interests.

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