Chemical ecology and olfaction in arthropod vectors of diseases

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Hematophagous arthropods (ticks and insects, collectively hereafter referred to as vectors) transmit various life threatening diseases resulting in over one million human deaths annually. Exploiting vertebrates for blood demanded extensive sensory and behavioral adaptations that are apparent across the evolutionary range of vector species, from primitive ticks to advanced dipterans. Since animal senses are biological features that have been shaped by natural selection to promote adaptive behavior, a variety of exciting patterns are apparent in what they sense and how. Vectors display robust olfactory driven behaviors. A distinct yet limited range of volatile organic compounds are parsimoniously used as major cues for tracking in various contexts. These chemicals elicit behaviors such as attraction or repulsion/avoidance while vectors seek habitats, hosts, mates, or oviposition sites. Interestingly, there is a substantial consensus among olfactory structures and function in arthropod vectors, which is also reflected in the parsimonious use of chemical ligands. A detailed analysis of chemosensory signals and reception by these arthropod vectors can be exploited to identify natural ligands that can be used as baits to manipulate vector behaviors.

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Chemical codes

The human ‘volatilome’ consisting of volatile organic compounds (VOCs) derived from exhaled breath, skin emanations, urine, feces and saliva contains over 1700 chemicals [10]. One of the significant discoveries in demonstrating how a single human derived chemical can act as a strong stimulus inducing attraction in host-seeking *Aedes aegypti* mosquitos came with the discovery of 1-lactic acid, isolated from human skin washings [11]. This had led to an early assumption that a single compound on its own can potentially act as an attractant. Subsequent studies however are revealing that complex repertoires of host derived chemicals are often needed to elicit attraction. Currently, over 200 compounds are being suggested as putative attractants for *Ae. aegypti* [12,13]. Extensive analytical work has been directed toward finding chemical attractants for the malaria mosquito, *Anopheles gambiae*. Human feet washing and its derivatives [14] as well as sweat and its constituent chemicals [15,16] are demonstrated as strong chemostimuli in various behavioral assays. Building on the early work by Nicolaides et al., that demonstrated a distinct odor/chemical fingerprint among humans [17], there has been a recent interest in how a diverse human skin microbiota [18] directly impacts the relative attraction of malaria mosquitoes toward particular individuals [19].

A significant development in identifying natural ligands for vectors was the coupling of high resolution chromatography with electrophysiological recordings to selectively isolate biologically active constituents from within a myriad of host derived volatile organic compounds (VOCs). This technique is termed gas chromatography linked electroantennographic detection (GC-EAD), wherein insect antennae are used as sensing elements to isolate a handful of constituents from complex odors as they elute from a high resolution GC capillary column. GC-EAD was developed for the identification of sex pheromones in moths [20], and it was first used to study...
Vectors and medical and veterinary entomology

vector olfaction at the Natural Resource Institute UK (now part of Greenwich University) in tsetse flies, *Glossina* spp. One alcohol constituent from cow odors, 1-octen-3-ol, generated a strong antennal signal [21]. Subsequent behavioral studies in a laboratory wind tunnel and later field testing demonstrated how this single VOC elicited significant behavioral responses both in laboratory bioassays and field trap captures. Enigmatically, 1-octen-3-ol remains the most common chemostimulus eliciting varied behaviors in almost every hematophagous arthropod studied so far, ranging from ticks, triatomines, mosquitoes, midges, stable flies and sand flies. The GC-EAD method has since been used to isolate and identify important chemostimuli for mosquitoes, triatomines, and tsetse flies with the resulting chemostimuli proving to be behaviorally active [22].

Though the GC-EAD technique offered a remarkable advantages over purely chemical-analytical tools by significantly shortening the process of isolating and identifying chemostimuli, its use was challenging in arthropods that have primitive olfactory structures composed of only a small number of olfactory sensilla (bed bugs and triatomines) or do not have a defined antenna (ticks). In these instances, GC was linked to single unit recordings from olfactory sensilla in ticks, bed bugs and triatomines [23]. GC linked single sensillum recordings (GC-SSR) have since been extended to more advanced dipterans with well-developed antenna to search for novel natural ligands, such as in mosquitoes and sand flies [24]. Most recently, olfactory receptor genes (Ors) from various vectors have been heterologously expressed into an ‘empty neuron’ in *Drosophila melanogaster* [25] using the GAL4-UAS driver system [26]. Studies utilizing this method have since been used to deorphanize *An. gambiae* ORs by challenging them offline (not connected to GC) with over 100 chemicals implicated as potential chemostimuli [27,28], and most recently GC-SSR method has been applied to identify a key human odor constituent, sulcatone, that selectively activates an OR from *Ae. aegypti* [29]. The screening revealed only a handful of compounds (1-octen-3-ol, 2,3-butanediol, 2-ethylphenol and indole) that appeared to be strong chemostimuli. It is worth mentioning that two ORs, each responding to 1-octen-3-ol (OR8) or indole (OR2), are the most sensitive of the receptors deorphanized from mosquitoes. These two ORs responded with the highest selectivity and sensitivity, and they are among the most highly conserved between Culicine and Anopheline species. While the existence of a highly complex chemical landscape in and around arthropod vectors is apparent, the salience of only a handful of these VOCs suggests multiple roles for these chemostimuli in a vector’s life history.

Parsimony in signaling

GC-EAD and GC-SSR studies from a large variety of hematophagous arthropods such as ticks, triatomines, bugs, mosquitoes, and sandflies consistently revealed three major classes of biologically active chemostimuli: 1 — short chain carboxylic acids, 2 — aldehydes and 3 — low molecular weight nitrogenous compounds such as ammonia and alkyl amines. Two other VOCs that also emerge from multiple analyses are 1-octen-3-ol and indole. Finally, a major host metabolite, carbon dioxide (CO₂), induces attraction in every arthropod studied so far, including triatomines bugs, bedbugs, ticks, tsetse flies, sand flies, and mosquitoes. Specialized ORNs detecting CO₂ in various arthropod vectors have been identified [30,31]. It is also worth mentioning that conspecific mating signals, especially pheromones, are not widely recorded in vectors, except in ticks [32] and sand flies [33].

Thus a high conservation in signaling molecules (Figure 1) leads to the intriguing question of why there is such numerical and structural redundancy in VOCs used by vectors. Generally in arthropods, a strong adaptation pressure to use the same chemicals as signals for different purposes is quite common, a trend termed as chemical parsimony [34]. This trend can range from a simple system wherein the same chemical signal can encode different referents based on its concentrations, or simply one chemical serving in a different context. For example, nonanal serves as an attractant in host seeking mosquitoes, while in gravid females it signals as a cue for seeking a suitable site to oviposit; the same chemical also contributes in the recognition of sugar-feeding site (flowers). An elegant example of plasticity can be found in host seeking *Ae. aegypti* that are attracted to human skin washings. Adding lactic acid to the skin extract incrementally increased the attraction, and this chemical alone made totally unattractive animal washings (lacking lactic acid) attractive [35]. It has long been argued that blood from different host sources does not differ much in its nutritive content [36]. Not surprisingly, the host choice for feeding in vectors is quite plastic. An extensive evaluation of the associations between host, habitat and tick spp. associations revealed that host choice in ticks is determined largely by adaptation to a particular habitat type and not by adaptation to a particular host taxon. Many tick species parasitize phylogenetically distantly related hosts with similar nesting or perching habitats, such as bat ticks that are also found on cave swallows. Bat ticks were therefore considered ‘habitat specialists rather than host specialists’ [37]. Studies of selection in *An. gambiae* mosquitoes showed divergent feeding preferences for humans or cows within five to six generations [38] and cross-mating of zoophilic *Ae. simpsoni* and anthropophilic *Ae. aegypti* generates offspring of intermediate preference [39]. And finally, individual experience and learning in a population has been clearly demonstrated when a first successful blood meal was shown to modulate mosquito preference for the subsequent second blood meal [40]. Though shown to have a genetic basis, host preference of
mosquitoes is characterized by high plasticity [41]. Thus, it appears advantageous for hematophagous arthropods to utilize a limited set of chemostimuli.

Sensory correlates of chemical codes

The differences among species in the design of sensory systems reflect the effects of evolution [42,43]. Blood sucking arthropods feed from a wide range of hosts including mammals, birds, fish, amphibians, reptiles, insects, arachnids, and annelids [36]. The evolution of hematophagy over 100 million years ago in arthropods, and no fewer than 12 times independently within diptera alone [44], has resulted in diverse combinations of morphological and neuroethological adaptations for host seeking and blood feeding.

The exquisite olfactory sense in arthropods is conferred by hair like epicuticular structures, called sensilla, which are concentrated on the main olfactory organs, such as antennae and palps. Each sensillum houses ORNs which are responsible for sensitivity and selectivity to chemostimuli [45**]. Sensilla in vector arthropods vary in size, shape and numbers (Figure 2). An analysis of these chemosensory sensilla is largely reflective of the mode of life. There is a strong correlation in sensory capabilities of vectors between the number of ORNs and the host-locating distance undertaken. For example, the bedbug, Cimex sp., with a restricted life style possesses only 200 ORNs per antenna, while a long range explorer, Simulium californicum has 12 000 ORNs. Interestingly, T. infestans has ~3000 ORNs [46], and the density of the chemosensory sensilla on antenna becomes progressively simplified in accordance with increasing habitat stability within Triatoma complex [47]. Non-blood feeding and autogenous Wyomyia smithii has fewer antennal olfactory sensilla than the blood-feeding W. aponomata [48].

Synchronous evolution of volatile codes and sensation

Studies correlating the dynamic equilibrium wherein olfactory signaling and reception co-evolve either as a consequence of, and/or leading to ecological specialization are emerging. We recently began to test this co-evolutionary process of signaling and reception in the evolution of oviposition decisions, since less-than-perfect signaling in oviposition decisions severely lowers the reproductive success of individuals and fitness of the progeny [4,5].

The first unequivocal evidence of the occurrence of an oviposition pheromone in any disease vector was in Culex
4 Vectors and medical and veterinary entomology

Diversity of olfactory structures in arthropod vectors. (a) Distal antennal segment on the antenna of Triatoma spp. (T. dimidiata). Inset Grooved peg sensillum that responds to acids, amines and nonanal. (b) Antennal segment of a mosquito (An. gambiae) inset from left to right: i — Trichoid sensilla are present in both culicine and anpheline mosquitoes and respond to indole, 1-octen-3-ol, various aldehydes and alcohols, ii — Grooved peg sensilla detect polar compounds such as ammonia, alkyl amines, carboxylic acids and iii — Coeloclonic sensilla (small) are only seen in anpheline and there is no clear indication as to what they detect (suspected to be mostly polar compounds). (c) Antennal segment of a sand fly (P. papatasii). Inset is an ascoid sensillum that detects alcohols and pheromone components. (d) Maxillary palps in mosquitoes (Cu. quinquemaculatus) are adorned with only one type of sensillum (basiconica type) in both anpheline and Culicine mosquitoes inset are details of the sensillum. Each sensillum houses three ORNs, one detects carbon dioxide and the second one detects 1-octen-3-ol with extreme sensitivity.

mosquitoes that was identified as crythro-6-acetoxy-5-hexadecanolide, commonly referred to as Mosquito Oviposition Pheromone (MOP) [49]. By comparing the apical egg droplet composition from Culex populations originating from six global regions and their reception, we observed neuroethological correlates that define and separate each population [50]. Similar analysis of VOCs from Brindley and metasternal glands, implicated to influence communication between conspecifics and heterospecifics in T. dimidiata complex, sorted closely related populations into distinct subpopulations [51]. An analysis of the antennal chemosensory sensilla in these T. dimidiata populations essentially resolved them into subpopulations that reflected the pattern of discriminant clusters resolved on the basis of VOC constituent from the glands [52]. In other example of strong co-evolution between signal and reception, most abundant olfactory sensilla on the antenna of Cu. quinquemaculatus were those housing highly sensitive ORNs to nonanal, the most abundant compound in bird odors (primary hosts) and a major constituent of human skin odors (alternate host) [53]. Thus, it remains an exciting area to further explore the reciprocal evolutionary relationship between signaling and reception.

**Molecular correlates of olfaction in vectors**

The encoding of olfactory signals from a complex chemical landscapes is mediated by a large number of protein families [54,55]. The most well studied family of chemosensory proteins thus far is the olfactory receptors (ORs) which are selectively expressed in a subset of ORNs. Comparison of the ORs in the sequenced genomes of the three major mosquito species, An. gambiae, Ae. Aegypti and Cu. quinquemaculatus [56–58] revealed an interesting pattern: while the ORs in An. gambiae are limited to ~70 receptors, the Ae. aegypti and Cu. quinquemaculatus genomes show significant expansions, with an approximate 68% and 140% increase respectively in predicted ORs. Changes in the Or repertoire, such as expansions by gene duplication events or reductions, have been correlated with habitat variability in the Drosophila model, wherein flies known to feed on a limited number of hosts have a reduced repertoire of ORs compared to flies that exploit a wide variety of resources [59–61]. Such studies are just emerging in the vector field. Recent sequencing of 16 anpheline species, known to occupy varied ecological niches and exploiting different hosts for blood feeding, allowed the comparative analysis of the chemosensory gene repertoires, which appear to be relatively stable throughout the evolution of these anpheline [62]. It is to be noted that while the genome of multiple anpheline spp. and the ongoing sequencing of the proposed 5000 insect species — including many vector arthropods — is providing unique evolutionary insights (http://arthropodgenomes.org/wiki/5k), recent advances in analyzing whole transcriptome using high-throughput sequencing is providing additional insights. The added advantage of studying whole transcriptome is the ability to sample the varying quantity of all the transcripts at each developmental stage or physiological condition. A common challenge however, is to identify low abundance transcripts, such as ORs, from the whole transcriptome that is overwhelmingly dominated by sequencing reads that are derived from a small subset of genes. These challenges can partly be overcome by employing commercial kits to remove the sequences of highly transcribed genes such...
as ribosomal and mitochondrial origin. In addition, tissue specific enrichment design helps to isolate low-abundance but differentially expressed transcripts.

A comparison of the antennal transcriptomes of *An. gambiae* before and after blood-feeding identified a small sub set of differentially regulated ORs that proved to be directly involved in modulating oviposition response in laboratory behavioral experiments [63]. Cross-species comparison of whole transcriptomes between a highly anthropophilic *An. gambiae* and zoophilic *An. quadrannulatus* revealed a differential transcript abundance of ORs and GRs potentially implicated in their unique chemosensory behavior [64,65]. One final thought is the ‘frequently disregarded’ aspect of temporal components in sensory biology since signaling and reception appear to be synchronized in time in vectors [66]. Whole transcriptome analysis of rhythmic gene expression in *An. gambiae* under circadian and diel [observed under light:dark (LD) cycle] conditions revealed that almost 20% of the genome is expressed rhythmically, including a large number of chemosensory genes [67**]. Further studies have recently characterized over a dozen of these rhythmically expressed chemosensory genes. Transcript modulation correlated with rhythmic protein levels (as measured by targeted quantitative proteomics) and the behavioral and electrophysiological sensitivity [68*]. These studies are beginning to provide novel insights into the dynamic modulation of the olfactory system that coordinate with the circadian (e.g. nocturnal versus diurnal) niche of vector arthropods.

**Conclusion**

High degree of parsimony in use of volatile chemical signals in hematophagous arthropods is also reflected in the relatively conserved sensory structures that detect them with high sensitivity and selectivity. Evolution of hematology over 100 million years ago, at no fewer than 12 times within diptera alone, appears to have followed the model of synchronous evolution of signaling and reception. Thus, isolating and identifying chemical signals (natural ligands) from rich chemical landscapes, and the concurrent adaptations in olfactory systems that control behaviors is a fascinating filed. Understanding such processes will lead, and contribute to the ongoing vector management strategies.

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**References**

8. Carey AF, Carlson JR: Insect olfaction from model systems to disease control. *Proc Natl Acad Sci U S A* 2011, 108:12987-12995. This review provides exciting insights into the neurogenetics of olfaction in the model system, Drosophila and how such understanding can be sought and applied in the vector arthropods.
6 Vectors and medical and veterinary entomology


This work introduced the ‘empty neuron’ model for heterologous expression of ORs. A true tour de force of techniques in insect olfactory work.


A comprehensive study that deorphanized malaria mosquito ORs and analyzed the presented how the chemosensory maps generated in the periphery of a mosquito differ from fruit fly.


This exciting work provided a solid understanding of the contribution of a single OR gene polymorphism contributing to host shifts.


Most comprehensive treatise on the development, structure and function of olfactory cilia (senillia) in insects.


This work isolated and identified nonanal as a major chemostimuliant for Culex mosquitoes. This aldehyde was the dominant constituent of bird and human odors and captured significant number of mosquitoes in combination with CO2 in field trials.


This comparative transcriptive work describes the changes in profile upon blood feeding and how such understanding can be exploited to test novel hypothesis.


This pioneering work demonstrated how a large part of the malarial mosquito transcriptome is rhythmic.


This comprehensive study reported multiple rhythmically expressed chemosensory genes that correspond with rhythmic protein levels as measured by quantitative proteomics. Electrophysiological measurement further revealed time-of-day-specific differences in olfactory sensitivity of antennae to major host-derived odorants.