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# 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 consilience 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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## Introduction

Signals and reception rarely arise *de novo* [1], and this reciprocal evolutionary relationship in chemical communication between insects and their host choice was elegantly demonstrated 50 years ago by Ehrlich and Raven in their classical synthesis of evidence from a wide range of insects and their host utilization [2,3]. They coined the term ‘co-evolution’ to describe the evolution of secondary host metabolites and the accompanying step-wise adaptive

responses by insects. Less than optimal signaling severely lowers insect reproductive success and the fitness of their progeny [4,5]. As the most successful and abundant animal group on earth, arthropods display a highly sophisticated system of chemical communication. While this exquisite system has been studied with great enthusiasm within various groups of arthropods from evolutionary and functional perspectives [6,7], recently there has been considerable interest to potentially exploit this understanding of insect olfaction to manipulate vector populations to our advantage [8,9].

## 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* mosquitoes came with the discovery of L-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

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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-butanedione, 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.

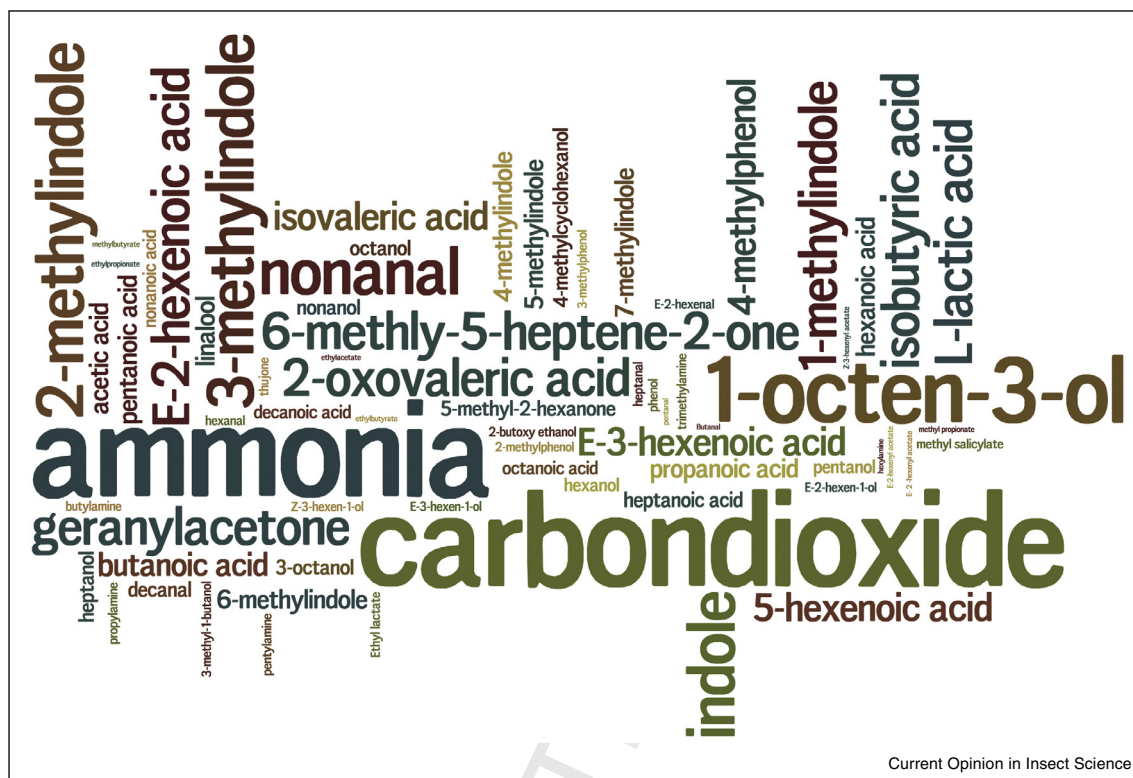
### Parsimony in signaling

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 (CO<sub>2</sub>), induces attraction in every arthropod studied so far, including triatomines bugs, bedbugs, ticks, tsetse flies, sand flies, and mosquitoes. Specialized ORNs detecting CO<sub>2</sub> 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 distant 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

Figure 1



Hematophagous arthropods detect and respond to a variety of host and habitat derived chemostimuli. An approximate depiction of the reported frequency and strength of the chemostimuli based on *in vivo* electrophysiological recordings and behavioral analyses.

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 calcitrans* 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 *Wyeomyia smithi* has fewer antennal olfactory sensilla than the blood-feeding *W. aporonoma* [48].

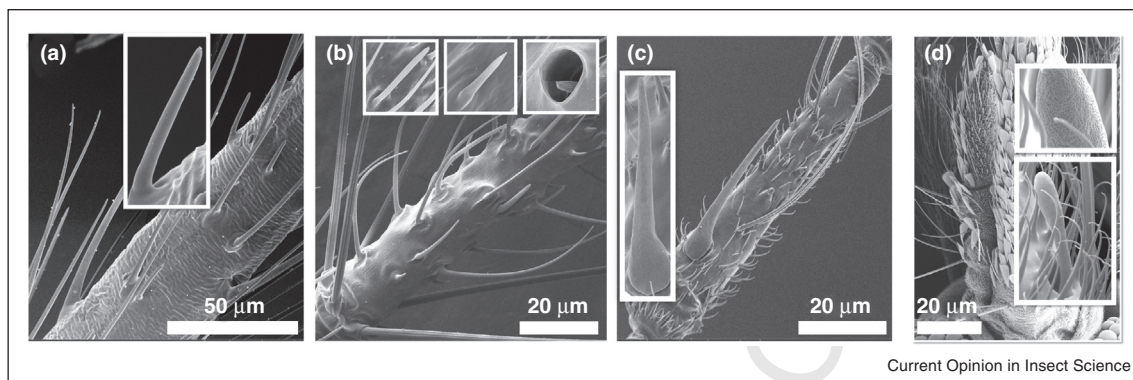
### Synchronous evolution of volatile codes and sensation

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*

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Figure 2



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 anopheline 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 – Coeloconic sensilla (small) are only seen in anopheline 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. papatasi*). *Inset* is an ascoid sensillum that detects alcohols and pheromone components. **(d)** Maxillary palps in mosquitoes (*Cu. quinquefasciatus*) are adorned with only one type of sensilla (basiconica type) in both anopheline 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-3ol with extreme sensitivity.

262 mosquitoes that was identified as erythro-6-acetoxy-5-  
263 hexadecanolide, commonly referred to as Mosquito Ovi-  
264 position Pheromone (MOP) [49]. By comparing the apical  
265 egg droplet composition from *Culex* populations originat-  
266 ing from six global regions and their reception, we ob-  
267 served neuroethological correlates that define and  
268 separate each population [50]. Similar analysis of VOCs  
269 from Brindley and metasternal glands, implicated to  
270 influence communication between conspecifics and het-  
271 erospecifics in *T. dimidiata* complex, sorted closely related  
272 populations into distinct subpopulations [51]. An analysis  
273 of the antennal chemosensory sensilla in these *T. dimi-*  
274 *diata* populations essentially resolved them into  
275 subpopulations that reflected the pattern of discriminant  
276 clusters resolved on the basis of VOC constituent from the  
277 glands [52]. In other example of strong co-evolution  
278 between signal and reception, most abundant olfactory  
279 sensilla on the antenna of *Cu. quinquefasciatus* were those  
280 housing highly sensitive ORNs to nonanal, the most  
281 abundant compound in bird odors (primary hosts) and  
282 a major constituent of human skin odors (alternate host)  
283 [53]. Thus, it remains an exciting area to further explore  
284 the reciprocal evolutionary relationship between signal-  
285 ing and reception.

### Molecular correlates of olfaction in vectors

287 The encoding of olfactory signals from a complex chemi-  
288 cal landscapes is mediated by a large number of protein  
289 families [54,55]. The most well studied family of chemo-  
290 sensory proteins thus far is the olfactory receptors (ORs)  
291 which are selectively expressed in a subset of ORNs.  
292 Comparison of the ORs in the sequenced genomes of the  
293 three major mosquito species, *An. gambiae*, *Ae. Aegypti* and

294 *Cu. quinquefasciatus* [56–58] revealed an interesting pat-  
295 tern: while the ORs in *An. gambiae* are limited to ~70  
296 receptors, the *Ae. aegypti* and *Cu. quinquefasciatus* genomes  
297 show significant expansions, with an approximate 68%  
298 and 140% increase respectively in predicted ORs.  
299 Changes in the *Or* repertoire, such as expansions by gene  
300 duplication events or reductions, have been correlated  
301 with habitat variability in the *Drosophila* model, wherein  
302 flies known to feed on a limited number of hosts have a  
303 reduced repertoire of ORs compared to flies that exploit a  
304 wide variety of resources [59–61]. Such studies are just  
305 emerging in the vector field. Recent sequencing of  
306 16 anopheline species, known to occupy varied ecological  
307 niches and exploiting different hosts for blood feeding,  
308 allowed the comparative analysis of the chemosensory  
309 gene repertoires, which appear to be relatively stable  
310 throughout the evolution of these anopheline [62]. It is  
311 to be noted that while the genome of multiple anopheline  
312 spp. and the ongoing sequencing of the proposed 5000 in-  
313 sect species — including many vector arthropods — is  
314 providing unique evolutionary insights ( [http://](http://arthropodgenomes.org/wiki/i5K)  
315 [arthropodgenomes.org/wiki/i5K](http://arthropodgenomes.org/wiki/i5K)), recent advances in ana-  
316 lyzing whole transcriptome using high-throughput se-  
317 quencing is providing additional insights. The added  
318 advantage of studying whole transcriptome is the ability  
319 to sample the varying quantity of ‘all’ the transcripts at each  
320 developmental stage or physiological condition. A common  
321 challenge however, is to identify low abundance tran-  
322 scripts, such as ORs, from the whole transcriptome that  
323 is overwhelmingly dominated by sequencing reads that are  
324 derived from a small subset of genes. These challenges can  
325 partly be overcome by employing commercial kits to  
326 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<sup>\*</sup>]. Cross-species comparison of whole transcriptomes between a highly anthropophilic *An. gambiae* and zoophilic *An. quadriannulatus* 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<sup>\*\*</sup>]. 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<sup>\*</sup>]. 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 hematophagy 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 field. Understanding such processes will lead, and contribute to the ongoing vector management strategies.

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