



Peripheral olfactory signaling in insects

Eunho Suh¹, Jonathan D Bohbot² and Laurence J Zwiebel¹

Olfactory signaling is a crucial component in the life history of insects. The development of precise and parallel mechanisms to analyze the tremendous amount of chemical information from the environment and other sources has been essential to their evolutionary success. Considerable progress has been made in the study of insect olfaction fueled by bioinformatics-based utilization of genomics along with rapid advances in functional analyses. Here we review recent progress in our rapidly emerging understanding of insect peripheral sensory reception and signal transduction. These studies reveal that the nearly unlimited chemical space insects encounter is covered by distinct chemosensory receptor repertoires that are generally derived by species-specific, rapid gene gain and loss, reflecting the evolutionary consequences of adaptation to meet their specific biological needs. While diverse molecular mechanisms have been put forth, often in the context of controversial models, the characterization of the ubiquitous, highly conserved and insect-specific Orco odorant receptor co-receptor has opened the door to the design and development of novel insect control methods to target agricultural pests, disease vectors and even nuisance insects.

Addresses

¹ Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, United States

² Department of Entomology, The Hebrew University, Rehovot 76100, Israel

Corresponding author: Zwiebel, Laurence J (l.zwiebel@vanderbilt.edu)

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Introduction

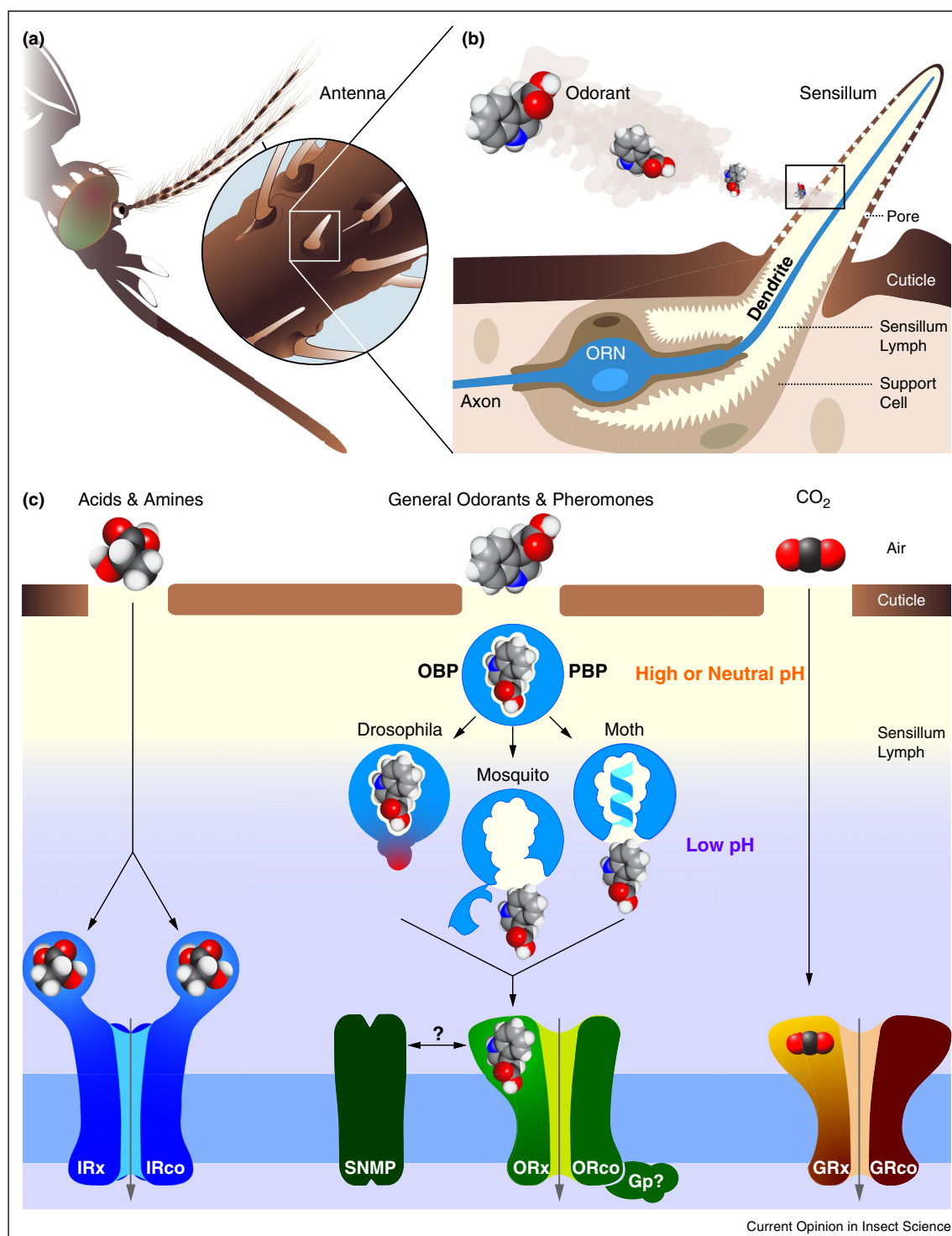
Chemosensation plays an essential role throughout the life cycles of insects that respond to a diverse array of biological and environmental chemical signals/cues to locate and select food, mates, oviposition sites and avoid predators. For insects, the precise discrimination and interpretation of what is an essentially unlimited spectrum of chemical information impacts numerous behavioral decisions that directly contribute to their success and in many cases, survival. To interpret these signals

insects utilize a range of molecular components that center around several large families of chemosensory receptors which are housed in a diverse array of hair-like structures called sensilla that are non-randomly distributed across peripheral appendages such as the antennae, maxillary palps, or labials (reviewed in [1]). The number and type of sensilla present on chemosensory appendages vary according to species as well as their developmental stage (e.g., larvae vs. adults), and gender. While it remains unclear how sensilla-specific ultrastructure contributes to the functionality of each sensilla type, the high degree of diversity may reflect significant evolutionary consequences for high sensitivity in odor trapping and phylogenetic and/or developmental constraints evolved with physical environment [2]. Odorants are thought to diffuse through numerous pores located on the sensilla surface after which they enter an aqueous lymph that must be traversed in order to reach the spectrum of molecular receptors present on the dendrites of olfactory receptor neurons (ORNs) [3]. Activation of a diverse set of chemosensory receptors by odorants provides a range of both excitatory and inhibitory inputs for the generation of ORN action potentials that are further processed across several layers of downstream neural pathways. At the same time, the timely termination and desensitization of peripheral signaling is required to promote sensitivity and selectivity of ORN-based signaling although the precise elucidation of the underlying mechanisms responsible for this essential component is still undefined. Here, we summarize recent advances derived from the study of model and non-model systems as well as our current understanding of how insects detect odors with a focus on sensory reception and signal transduction, and discuss evolutionary considerations regarding the development of diverse modalities of insect olfactory reception.

Odorant binding proteins and odorant degrading proteins

Because odorants are typically hydrophobic they require solubilization to traverse the sensillum lymph. This role is presumed to be carried out by a suite of odorant binding proteins (OBPs [4]) (Figure 1). OBPs are globular, water-soluble proteins (14–20 kDa) that contain six α -helical domains. In mosquitoes, classic OBPs are conserved with 6 cysteine residues and multiple cysteine variants are reported as PlusC (12 cysteines), MinusC (4 cysteines) and atypical (elongated C-terminus) [5]. The total number of OBPs for *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* are now reported as 69, 111, and 109, respectively [6]. OBPs are typically the most highly expressed genes in peripheral olfactory tissues and accordingly are well studied in a wide range of insects

Figure 1



(A) Insect chemosensory organs and molecular models in signal transduction. Insect chemosensory appendages such as the antennae, maxillary palps and labials are covered by sensilla. **(B)** An olfactory sensillum housing support cells and an ORN (blue); odorants encounter the ORN dendrite across sensillum lymph after penetration via cuticular pores. **(C)** Distinct classes of odorants activate specific groups of chemoreceptors and other components with diverse mechanistic models: *Drosophila* may use the bound OBP to activate the receptors. Moth pheromone-binding proteins (PBPs) eject their odorant load through a pH-induced conformational change including the formation of an α -helix that occupies the binding pocket. Mosquito odorant-binding proteins (OBPs) also eject their ligands through a pH-induced conformational change including the formation of a 'lid'.

(as reviewed in [7]). OBPs use several mechanisms to release odorants and activate ORs. OBPs have been hypothesized to use several, not necessarily consistent, mechanisms to release odorants and activate ORs. In *Drosophila* for example, it has been suggested that OR67d (present in T1 neurons) is activated by the OBP LUSH, bound to cis-vaccenyl acetate (cVA) sex pheromone [8,74], although a recent study contradicts this model to show that cVA directly activates OR67d [9**]. Moth PBPs [10] and mosquito OBPs [11] eject odorants through pH-dependent conformational changes at close proximity to the membrane. In addition to OBPs, a range of odorant degrading enzymes (ODEs) play an important role in the termination of odor-based signal transduction [12,13]. In particular, several esterases and cytochrome P450s act as ODEs and pheromone degrading enzymes (PDEs) and are abundant in sensilla lymph where they rapidly degrade odorants and pheromones [14–17].

ORN chemosensory receptors Odorant receptors (ORs)

After intensive effort, a large family of over 60 ORs was first identified and characterized in *D. melanogaster* by the laboratory of John Carlson using novel bioinformatics-based methods [18]. Subsequent studies confirmed OR localization on the dendritic membrane of *Drosophila* ORNs as well as unexpectedly revealing an inverted topology where the N terminus is intracellular relative to mammalian ORs [19]. This departure from mammalian OR paradigms was further strengthened as it became clear that insects ORs act as ligand-gated ion channels [20,21]. Insect OR ion channels are composed of heteromeric complexes of as yet unknown stoichiometry of two subunits, one of which is highly conserved and found in all OR complexes and the other being highly divergent and variable [22–26]. In light of its highly conserved sequence and constitutive presence within OR complexes, the first of these subunits is now known as the OR co-receptor (Orco; [27]). The other OR subunit(s) are highly divergent both in terms of number and primary amino acid sequence across insect taxa and are often termed tuning ORs reflecting the hypothesis they are responsible for recognizing odorant ligands. Consistent with mammalian paradigms, typically only one tuning OR/Orco complex is expressed in each ORN although co-expression of two tuning *Or* genes have been occasionally observed together with Orco [28].

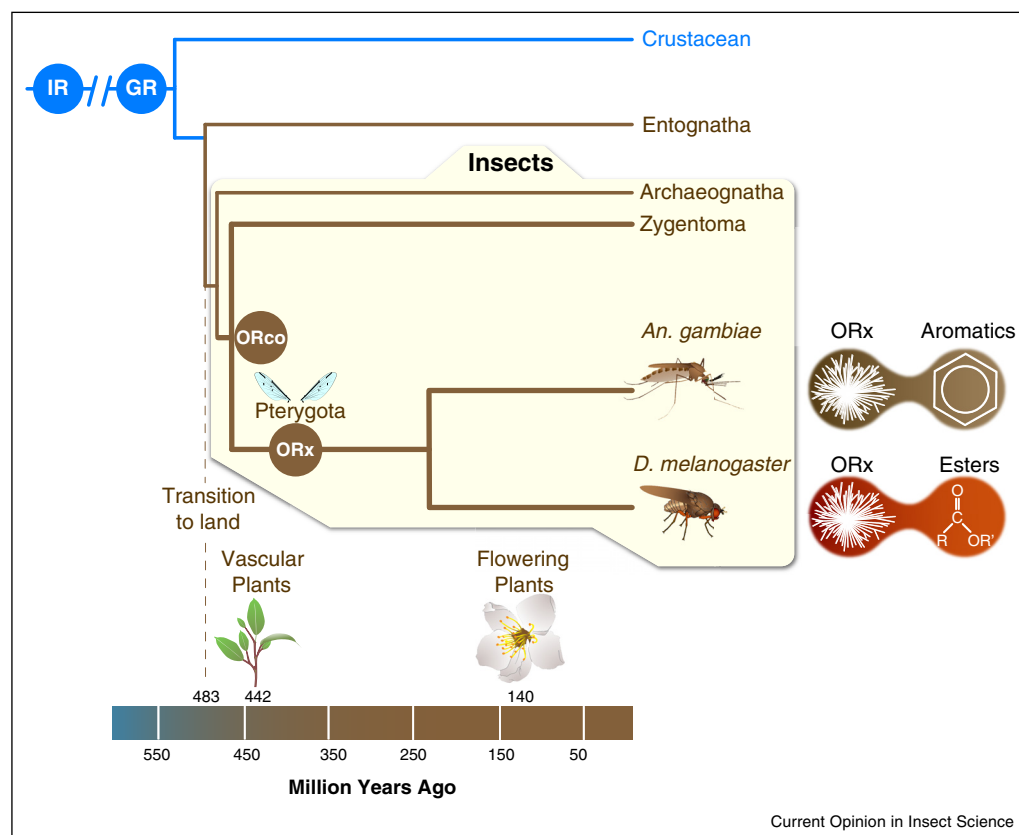
Two models have been put forth for the molecular mechanisms responsible for insect OR-based signal transduction. In the first model, OR/Orco complexes form odorant-activated ionotropic cation channels [20]. In the second, Orco itself is the ionotropic cation channel that is activated by fast, odor-dependent pathways as well as being affected by a slow, metabotropic mechanism involving G-proteins and adenylyl cyclases [21]. In addition to mechanistic considerations, the comprehensive functional study of heterologously expressed OR/Orco complexes have revealed

the response profiles of individual tuning ORs to biologically relevant insect odorants. Comprehensive ‘deorphanization’ studies have been carried out in both *D. melanogaster* [29–31] and *An. gambiae* [32–35] in which panels of biologically relevant odors were tested against OR/Orco complexes using the ‘empty neuron’ paradigm established in *Drosophila* or adopting well-established systems such as *Xenopus* oocytes in a two electrode voltage clamp approach. In addition a large number of other insect OR complexes have been similarly deorphanized [36–39]. The distinctive shift in odor space covered by *D. melanogaster* focusing on esters compared to that of *An. gambiae* focusing more on aromatics (Figure 2) represents an example of the species-specific evolution of OR repertoires specialized for detecting and discriminating odors that convey ecologically relevant information utilized for oviposition, nutrient localization and, in the case of *An. gambiae*, vertebrate host seeking for blood meals [35].

Taken together these studies reveal a general principle of insect odor coding that is consistent with mammalian models [40] whereby combinatorial signal transduction by multiple tuning ORs respond to a wide range of chemicals. Specifically, individual odorants can activate specific groups of receptors while individual receptors also can respond to overlapping groups of odorants. Some receptors broadly respond to a large number of odorants to act as ‘generalists’ while ‘specialist’ ORs respond to unitary or small sets of odorants [41]; in this paradigm ORs specially tuned to pheromones would be considered ‘ultra-specialists’ that also reflect their novel interaction with other membrane components including the sensory neuron membrane protein SNMP and PBP accessory proteins. SNMPs are related to the CD36 family of receptors that are highly conserved in multiple insect families particularly in holometabolous species [42]. Two subfamilies (SNMP1 and SNMP2) were found in dipterans and lepidopterans [43], respectively. SNMPs have been suggested to play an essential role in mediating the interactions between pheromone ligands and ORs in *D. melanogaster* [44], although their functional mechanisms and precise role in general signal transduction remains to be determined (Figure 1).

Although *in vivo* Orco is believed to act exclusively as a co-receptor that is not directly involved in odorant binding/recognition, recent high-throughput screens have identified a novel class of small molecules that act as Orco agonists and antagonists [45–47]. In light of Orco’s wide conservation and ubiquitous expression across insect taxa [22–25], the discovery of powerful and selective Orco agonists has important implications in the design of novel, broadly effective insect control methods that utilize an excito-repellent paradigm. Moreover, the availability of a suite of Orco-specific reagents has facilitated studies that have revealed several fundamental aspects of insect OR structure–function that impact our understanding of the

Figure 2



Evolution of chemosensory receptor gene families including lineage specific evolution of the insect Orco gene family and Pterygota-specific ORx gene family.
Modified from [53**].

role of Orco and tuning ORs in mediating ORN signaling [48*,49]. Other studies have revealed an additional level of odor coding beyond odorant recognition wherein each tuning OR contributes to the formation of a spectrum of diverse OR ion channels each with unique ionotropic characteristics; this suggests that odor coding exists in peripheral ORNs which respond differentially depending upon their OR/Orco complex [50]. Lastly, the functional characterization of a subset of ORs that act in the chemical-based activation of insect sperm preceding fertilization represents a new aspect in our appreciation of the importance of ORs in insect life cycles and suggests even more novel target sites for insect control methods by disrupting important signaling pathways in the reproductive cycle of disease vectors or pests [51**].

The origin of the *Or* gene family in insects is presumably the result of adaptations to terrestrial life [52] as well as the emergence of winged insects adapting to the rapid spread and evolution of vegetation [53**] (Figure 2). In general, the molecular evolution of tuning *Or* gene families reflects species-specific expansions and contractions resulting in highly divergent *Or* gene families with

no apparent orthologs across insect orders (Figure 2; reviewed in [2]). Indeed, insect *Or* gene family repertoires range from ten *Or* genes in lice (Phthiraptera) [54] to over 400 in ants (Hymenoptera) [55**]. The extensive gene gain and loss are thought to be a consequence of adaptation of a species to a specific environmental condition reflecting different biological needs in the life history of each insect species [55**].

Gustatory receptors (GRs) predate the divergence of crustacean and insects are generally denoted as taste or contact receptors belonging to the same general superfamily as insect ORs (Figure 2, [56]). While there is a relative paucity of information on GR specific ligands, several *D. melanogaster* GRs have been directly linked to sugar responses [57,58] as well as bitter tastants including caffeine [59–61], and interestingly volatile CO₂ [62]. In the vector mosquitoes *An. gambiae* and *Ae. aegypti*, a trio of highly conserved GRs co-expressed in a single non-ORN neuron specific to the maxillary palp have also been shown to collectively act as a CO₂ chemoreceptor [32,63] that plays an important role in host seeking behaviors [64].

Ionotropic receptors

Ionotropic receptors (IRs), the most ancient family of chemoreceptors (Figure 2) [65] that were first described in *D. melanogaster*, are evolutionarily derived from ionotropic glutamate receptors (iGluRs) and accordingly are thought to act as ligand gated ion channels [66]. In *Drosophila*, IRs are expressed in coeloconic sensilla where OR/Orco complexes are usually not present [67] and which specifically respond to amines or acid-based odorants that are largely ignored by ORs [30,68,69]. IR positive neurons functionally express two to four *Ir* genes representing a mix of odorant sensing IRs and generally one or more co-receptor [69,70]. For example, in *Drosophila* Ir64a forms ligand-gated cation channels together with the *Ir8a* co-receptor to drive responses to acids and protons [71,72]. These studies support a model in which IR-based signaling is required for odor coding of amines, acids and other odorants that are not covered by ORs. While understudied relative to ORs, highly conserved families of IRs have been identified in the genomes of many insect taxa [65] and one member has been functionally characterized in *An. gambiae* [73].

Conclusions

In insects, sensory reception involves diverse and parallel molecular components to process a nearly infinite spectrum of chemical information. While multiple, and not necessarily incompatible, models persist as to the underlying mechanisms and precise functional roles of each element in these diverse signal transduction paradigms, there is general consensus around the idea that precise and temporally restricted odor sensing is required for many aspects of insect success and survival. Indeed, the evolution of insects is, in part, a story of rapid adaptations to various environments by continuously upgrading OR repertoires to maximize overall fitness, sometimes resulting in the establishment of so called 'labeled lines' for specialized pathways such as pheromonal signaling. While the further elucidation of insect signaling pathways will continue to inform our understanding of these evolutionary relationships, recent advances revealing the unique aspects of the insect olfactory system are likely to be utilized in the design of novel approaches to manipulate salient behaviors of economically and medically important insects.

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