The narrowing olfactory landscape of insect odorant receptors

Jonathan D. Bohbot[1]* and Ronald J. Pitts[2]

[1] Department of Entomology, The Hebrew University, Rehovot, Israel, [2] Department of Biological Sciences and Institute for Global Health, Vanderbilt University, Nashville, TN, USA

The molecular basis of odorant detection and its corollary, the task of the odorant receptor, are fundamental to understanding olfactory coding and sensory ecology. Based on their molecular receptive range, olfactory receptors have been classified as pheromone and non-pheromone receptors, which are respectively activated by a single pheromone component ("specialist") or by multiple odorant ligands ("generalist"). This functional distinction is unique among ligand-gated ion channels and has shaped how we model olfactory coding both at the peripheral and central levels. Here, we revisit the long-standing combinatorial theory of olfaction and argue, based on physiological, pharmacological, evolutionary, and experimental grounds that the task of the odorant receptor is not different from that of neurotransmitter receptors localized in neuronal synapses.
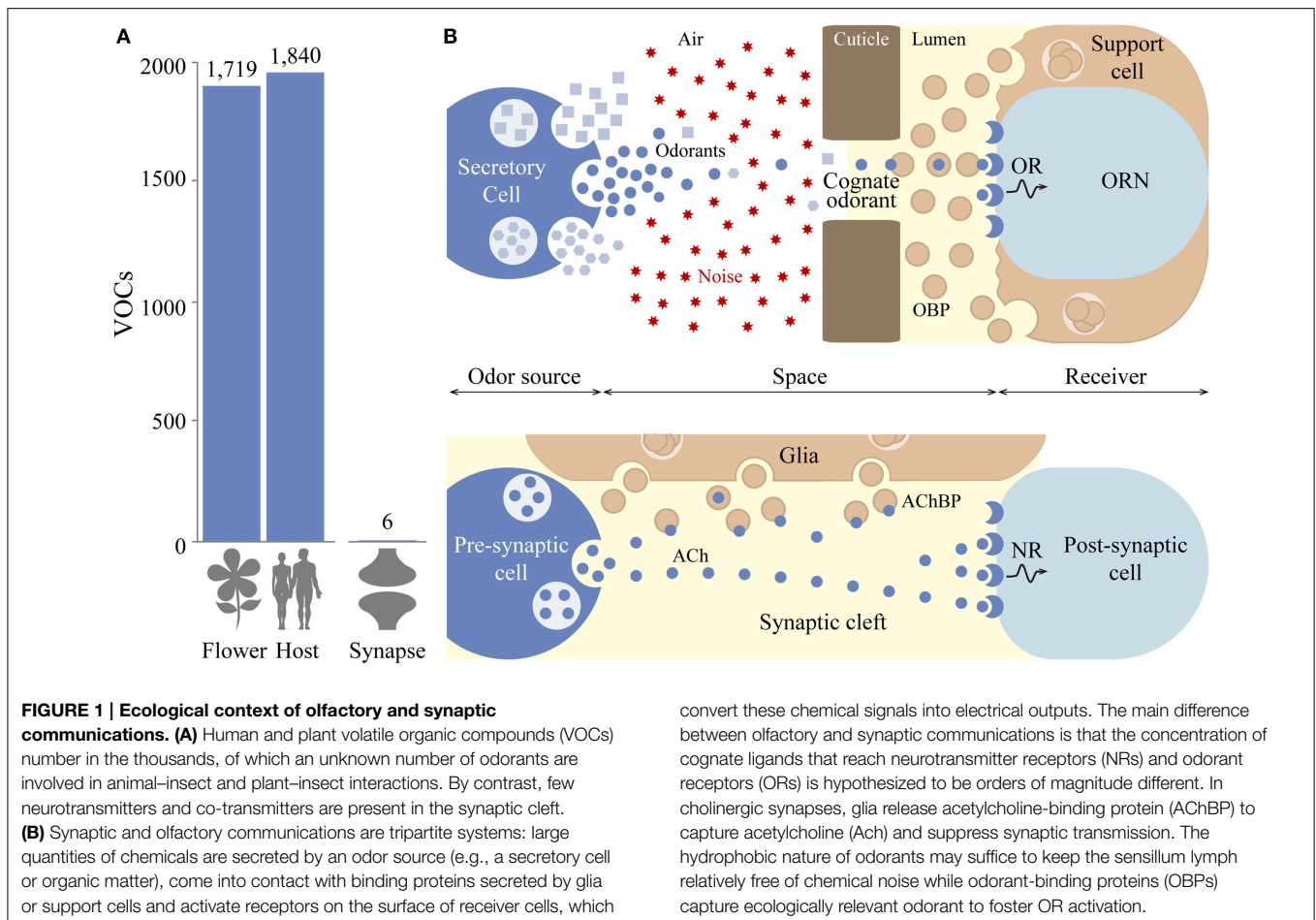
Keywords: odorant receptor, ligand-gated ion channel, synaptic communication, olfactory communication, neurotransmitter receptors

Introduction

How insects process odorants is a central question in the field of olfactory neurobiology. The number of green leaf volatiles (GLVs) produced by plants and volatile organic compounds (VOCs) emitted by animals or other sources (rotting fruits and excretion products) are not well-defined. Most recent studies report that GLVs (Dudareva et al., 2006; Knudsen et al., 2006) and VOCs number just below 2000 chemicals (Penn et al., 2007; Phillips et al., 2013; de Lacy Costello et al., 2014) (**Figure 1A**), which is likely an underestimate. Insects rely on odorant receptor (*Or*), ionotropic receptor (*Ir*), and the CO_2-sensing gustatory receptor (*Gr*) gene families for the long-range detection of airborne chemical cues (Suh et al., 2014). Until now, the *Or* clade has been the most extensively studied from both evolutionary and functional standpoints, and will be the focus of this theory article.

Odorant Receptors (ORs) are at the front line of odorant detection and much like neurotransmitter receptors (NRs), their task is to convert chemical signals into electrical outputs (**Figure 1B**) thereby ensuring the continuity of information flowing from the environment to the brain. Functional ORs are heteromeric complexes composed of an odorant-sensing unit belonging to a large and diverse family and a conserved *OR co*-receptor named ORco (for review, see Suh et al., 2014). These OR complexes are localized in the dendrites of olfactory receptor neurons (ORNs) embedded in sensilla, which project from the insect cuticular surface on olfactory appendages.

Insect OR repertoires vary greatly in number, ranging from 0 in the bristletail (Missbach et al., 2014), 110 in the yellow fever mosquito *Aedes aegypti* (Bohbot et al., 2007), 170 in the honeybee *Apis mellifera* (Robertson and Wanner, 2006) to over 400 ORs in eusocial ants (Zhou et al., 2012). Despite such a limited number of ORs, insects navigate complex chemical environments by

FIGURE 1 | Ecological context of olfactory and synaptic communications. (A) Human and plant volatile organic compounds (VOCs) number in the thousands, of which an unknown number of odorants are involved in animal–insect and plant–insect interactions. By contrast, few neurotransmitters and co-transmitters are present in the synaptic cleft. **(B)** Synaptic and olfactory communications are tripartite systems: large quantities of chemicals are secreted by an odor source (e.g., a secretory cell or organic matter), come into contact with binding proteins secreted by glia or support cells and activate receptors on the surface of receiver cells, which convert these chemical signals into electrical outputs. The main difference between olfactory and synaptic communications is that the concentration of cognate ligands that reach neurotransmitter receptors (NRs) and odorant receptors (ORs) is hypothesized to be orders of magnitude different. In cholinergic synapses, glia release acetylcholine-binding protein (AChBP) to capture acetylcholine (Ach) and suppress synaptic transmission. The hydrophobic nature of odorants may suffice to keep the sensillum lymph relatively free of chemical noise while odorant-binding proteins (OBPs) capture ecologically relevant odorant to foster OR activation.

exhibiting remarkable olfactory sensitivity, considering that some of these airborne VOCs are present in the air in the picomolar range (Phillips, 1997). The impact of this information on insect fitness, both in terms of survival and reproduction cannot be overstated. Host species must be located, conspecifics recognized, trails followed, and potential dangers avoided during many stages of the insect life cycle. Thus, it is tempting to surmise that ORs have evolved high sensitivity and selectivity capabilities for the detection of ecologically meaningful odorants.

The combinatorial theory of olfaction (Malnic et al., 1999) explains how this limited set of "generalist" ORs, thereafter referred to as non-pheromone receptors (nPRs), encode thousands of odorants. This weak shape theory (Rinaldi, 2007), a "relaxed" version of the stereochemical model of olfaction (Moncrieff, 1949; Amoore, 1963), postulates that an nPR only recognizes a part of the odorant and therefore can accommodate a variety of odorants provided they share common chemical features. Functional studies on *Drosophila melanogaster* (Hallem et al., 2004) and *Anopheles gambiae* (Carey et al., 2010; Wang et al., 2010) support the theory that nPRs exhibit broad molecular receptivity. However, moth pheromone receptors (PRs) function according to a more rigid lock-and-key mechanism as they are activated by single pheromone components (Große-Wilde et al.,

2007; Miura et al., 2009; Wang et al., 2011; Leary et al., 2012; Sun et al., 2013). Together, nPRs and PRs allow the peripheral olfactory system to encode the identity and quantity of odorants over a wide range of molecules and concentrations. This model continues to have important implications on the conceptual roles of the peripheral and central nervous systems in terms of signal filtering and olfactory coding, respectively.

In recent years, the discovery of an increasing number of specialized nPRs in moths and mosquitoes has broken the pharmacological monopoly of PRs in terms of sensitivity and specificity. This recent development is cause for revisiting the concepts of molecular receptive range and olfactory tuning as well as their respective contributions to the theory of combinatorial receptor codes for odorants in insects. This article continues an earlier discussion on the task of the OR (Bohbot and Dickens, 2012a). Here, we have compared the physiological contexts and pharmacological properties of ORs and NRs by discussing the notions of receptive range and olfactory tuning from the perspective of chemical ecology and evolution and review the experimental designs supporting the notion of generalist ORs, which ultimately have shaped the combinatorial theory of olfaction. Finally, we comment on the respective roles of the peripheral and central nervous systems in olfactory coding and propose strategies for testing our ideas.

Olfactory and Synaptic Communications

The foundation of our discussion begins with a deceptively simple question: is the task of an insect OR different from that of a NR (Firestein, 2001)? Although not the focus of this article, ionotropic glutamate receptors provide a clear example of an evolutionarily ancient mechanism linking olfactory and synaptic communications (Croset et al., 2010). In both cases, a chemical signal travels through space to interact with binding-proteins and degrading enzymes (Vogt and Riddiford, 1981; Smit et al., 2001), which modulate the signal before being detected by an ionotropic receptor (**Figure 1B**). Despite these similarities, the chemical constraints on ORs and NRs differ in one fundamental way. In the case of synaptic neurotransmission, millimolar concentrations of water-soluble neurotransmitters are released in the synaptic cleft (Kuffler and Yoshikami, 1975; Clements, 1996; Karayannis et al., 2010), a space several nanometers wide (Stocker and Nuesch, 1975; Felten and Olschowka, 1987) whose chemical content is regulated by the local cellular environment. Synaptic clefts may contain one or more neurotransmitters and co-transmitters (**Figure 1B**), but their exact compositions remain largely unknown (Burnstock, 2004). Considering that these conditions are optimal for synaptic transmission, it may be sufficient that NRs act as low-sensitivity receptors (millimolar range) much like the *Bombyx mori* GR9, a highly selective sugar receptor activated by millimolar concentrations of D-fructose that functions as an ionotropic receptor (Sato et al., 2011) (**Figure 2A**). Moreover, low-sensitivity in the synapse reduces potential noise associated with spontaneous neurotransmitter release (Faisal et al., 2008).

By comparison, ORs potentially face a far more complex chemical environment. With greater distances form the emitting sources and due to the chaotic nature of turbulent air, the occurrence of any particular cognate odorant reaching an OR is orders of magnitude lower than the probability of neurotransmitters interacting with NRs. The presence of odorant molecules competing for the recognition sites of ORs is a matter of conjecture but it is likely that due to their hydrophobic nature, most odorants do not cross the sensillum lymph barrier unless helped by transporter proteins such as odorant-binding proteins (**Figure 1B**). Provided that these odorant-binding proteins are selective, the sensillum lymph and synaptic cleft may therefore be similar in terms of chemical complexity. To increase selective OR-odorant binding probability events, insects have evolved several anatomical and biochemical adaptations including elongated porous sensillae and binding proteins to facilitate the transport of cognate odorants through the sensillum lymph. Based on these parameters, we suggest that ORs are likely to be more sensitive and equally specific toward their cognate ligands than NRs.
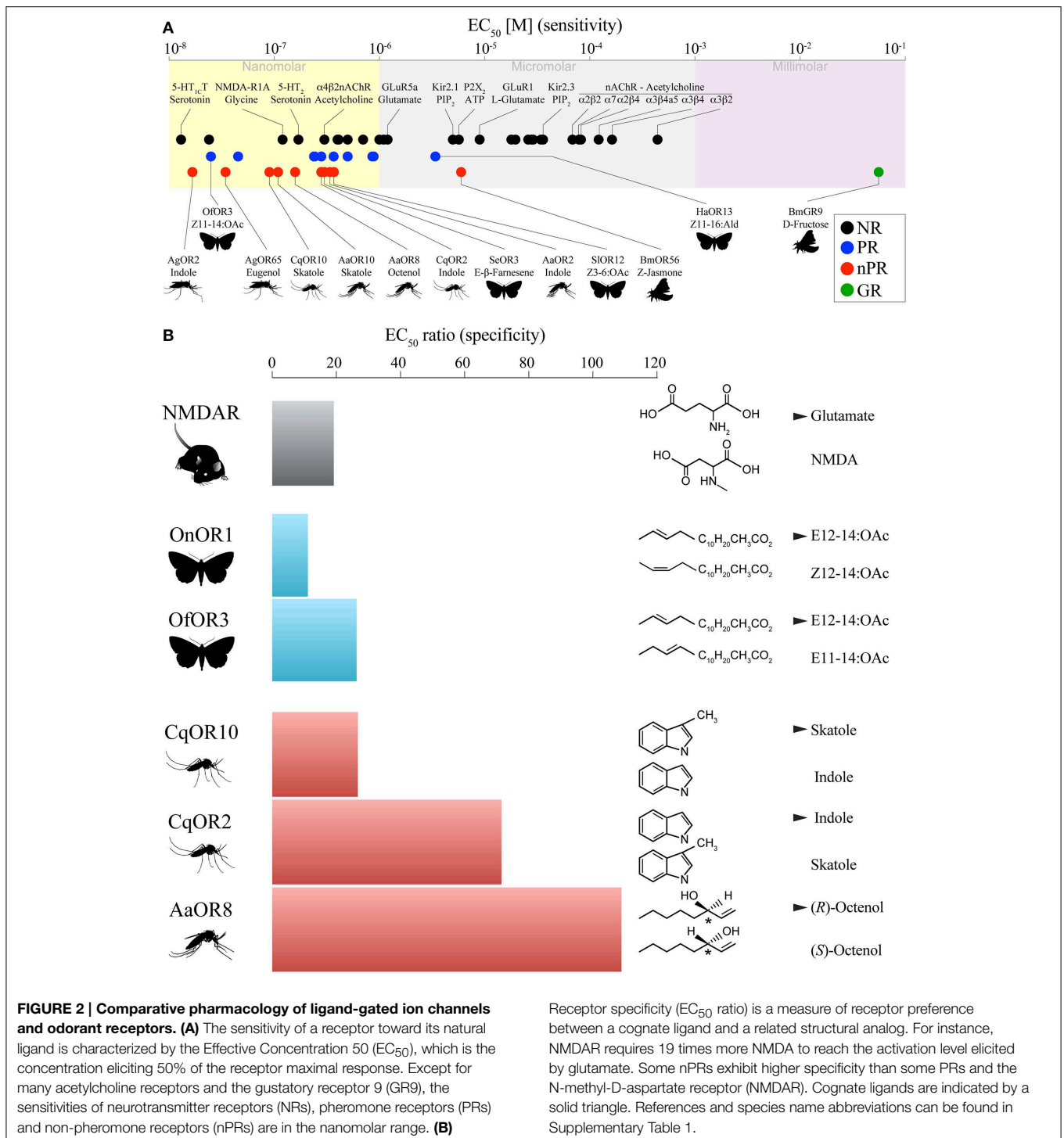
Comparative Pharmacology of ORs and NRs

How do ligand-receptor interactions differ in terms of sensitivity and specificity in the context of olfactory and synaptic communications? To address this question, we have compared these pharmacological features between ORs and other ligand-gated ion channels (LGICs) including cys-loop receptors, ionotropic glutamate receptors, and ATP-gated channels. We surveyed the scientific literature and compared 50 data points representing the EC_{50}-values (concentration of ligand that elicits 50% of maximum receptor activation) of NRs, nPRs, PRs, and one sugar GR (Supplementary Table 1) expressed in *Xenopus laevis* oocytes (**Figure 2A**). This expression system provided the largest EC_{50} dataset of LGICs.

NRs exhibit sensitivities in the nanomolar and micromolar range. Nicotinic acetylcholine receptors (nAChRs) have the lowest and broadest range of sensitivities to acetylcholine, which is likely caused by the use of non-native subunit combinations (Chavez-Noriega et al., 1997; Wonnacott and Barik, 2007). Ligands for the serotonin, glutamate, GABA, and N-methyl-D-aspartate receptors display relatively narrower potencies in the nanomolar range (**Figure 2A**), which, contrary to our assumption, shows that LGICs are highly sensitive to their cognate ligand.

The majority of PRs also exhibit EC_{50}-values in the nanomolar range with the moths *Ostrinia furnicalis* OR3 showing the highest sensitivity to (Z)-11-tetradecenyl acetate (Leary et al., 2012) and *Heliothis armigera* OR13 showing the lowest sensitivity to (Z)-11-hexadecenal (Liu et al., 2013) (**Figure 2A**). All the nPRs for which cognate ligands have been identified using sensory physiology and behavior exhibit EC_{50}-values in the nanomolar range as well, suggesting that the distinction between PRs and non-PRs on the bases of sensitivity is unwarranted provided that the cognate ligands are used. Recently, a "generalist" OR has been shown to detect the GLV E-β-farnesene in the nanomolar range demonstrating at the molecular level that GLV-sensing ORs can be highly sensitive and specific (Liu et al., 2014).

Receptor specificity is the ability to distinguish between a cognate odorant ligand and its closest structural analog. We used EC_{50}-values to benchmark the discriminative power of PRs and nPRs, an aspect of LGIC pharmacology that has been scarcely studied, which limits our ability to draw general conclusions. Nonetheless, we find examples of ORs that display clear differential sensitivities ranging from 1 to 2 orders of magnitude (**Figure 2B**), suggesting that these receptors have evolved high stereospecificity, which likely reflect their ecological relevance (Bohbot and Dickens, 2009). Several mosquito ORs show superior recognition abilities (Hughes et al., 2010; Pelletier et al., 2010) compared to some moth PRs, which discriminate between geometric pheromone isomers (Wanner et al., 2010; Leary et al., 2012). Considering how little to no recognition PRs and nPRs display toward structural analogs of the cognate odorant, it is interesting to note that some PRs are more robustly activated by the formate analogs of known aldehyde constituents of moth pheromones (Xu et al., 2012). This observation might suggest that "super" ligands may be discovered by exploring close structural analogs of known cognate ligands. However, the selectivity of the octenol receptor (OR8) from *A. aegypti* suggests that any modifications to the cognate ligands elicit little to no receptor activation (Bohbot and Dickens, 2009). Whatever the case may be, detailed studies of formate pheromone derivatives will help clarify our understanding of receptor-ligand molecular relationships and may lead to the development of novel

FIGURE 2 | Comparative pharmacology of ligand-gated ion channels and odorant receptors. (A) The sensitivity of a receptor toward its natural ligand is characterized by the Effective Concentration 50 (EC_{50}), which is the concentration eliciting 50% of the receptor maximal response. Except for many acetylcholine receptors and the gustatory receptor 9 (GR9), the sensitivities of neurotransmitter receptors (NRs), pheromone receptors (PRs) and non-pheromone receptors (nPRs) are in the nanomolar range. **(B)** Receptor specificity (EC_{50} ratio) is a measure of receptor preference between a cognate ligand and a related structural analog. For instance, NMDAR requires 19 times more NMDA to reach the activation level elicited by glutamate. Some nPRs exhibit higher specificity than some PRs and the N-methyl-D-aspartate receptor (NMDAR). Cognate ligands are indicated by a solid triangle. References and species name abbreviations can be found in Supplementary Table 1.

bioactive molecules affecting the behavior of agricultural and medical pests.

ORs as Ecological Adaptations

The combinatorial receptor coding scheme posits that the majority of ORs, with the exclusion of PRs, are promiscuous in terms of odorant recognition. While it provides an attractive model to encode a wide variety of odorant cues, it is counterintuitive from an evolutionary standpoint. Such an olfactory system would potentially be exposed to continuous overstimulation and thus lack the ability to distinguish important signals from background noise. In addition, the central nervous system would be required to filter this information into a useful code. What evolutionary

mechanism would explain how olfactory systems develop such a state?

Distinctive features of insect ORs include high evolutionary rates (Neafsey et al., 2015), lineage-specific expansions (Hill et al., 2002; Robertson and Wanner, 2006; Zhou et al., 2012; Cande et al., 2013) and large variations in gene repertoires (Nei et al., 2008; Sánchez-Gracia et al., 2009). The major mechanism of evolution of the *Or* gene family follows the birth-and-death model (Sánchez-Gracia et al., 2009) whereby genes multiply via tandem gene duplication events and are removed by deletion (Gardiner et al., 2008). Studies on *Or* evolution in *Drosophila* (McBride, 2007; McBride et al., 2007; Gardiner et al., 2008; Sánchez-Gracia et al., 2009; Stensmyr et al., 2012) and *Anopheles* mosquitoes (Neafsey et al., 2015) indicate that the birth-and-death of *Or* genes is not random (McBride et al., 2007) but that genetic diversity and variability are principally acted upon by purifying selection. Despite these observations, *Or* genes exhibit some of the highest level of positive selection in many lineage specific expansions suggesting functional divergence associated with host specialization (McBride et al., 2007; Neafsey et al., 2015), and mate selection (Leary et al., 2012).
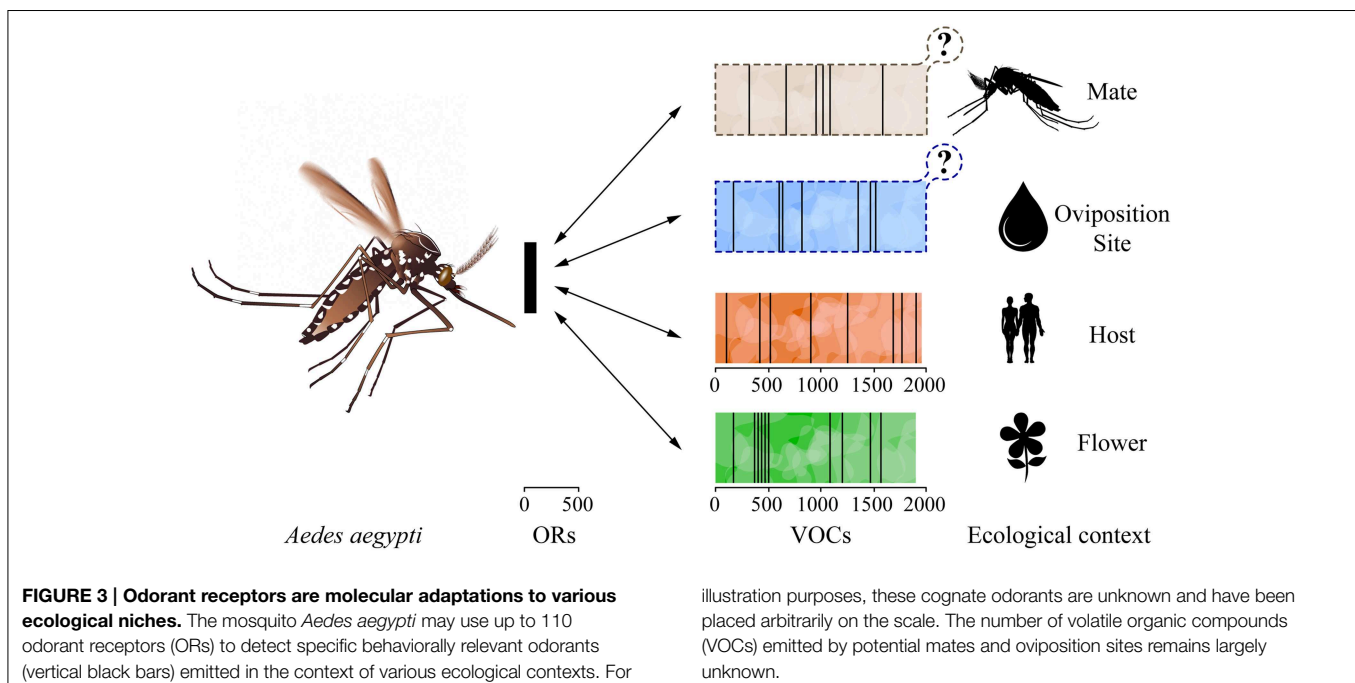
The mosquito-specific indole receptors show remarkable sequence conservation across the Culicinae and Anophelinae families (Bohbot et al., 2007), indicating that they fulfill fundamental olfactory functions critical to the life cycle of these insects. These receptors diversified through several instances of duplication events followed by positive selection, which diversified their tuning range toward indole analogs, functions that have subsequently been maintained by purifying selection. It appears that although the evolutionary mechanisms differ, lineage-specific ORs are associated with ecological adaptations (**Figure 3**). The evolution of the mosquito *A. aegypti* OR4 illustrates how these evolutionary forces also act on a short time scale: alleles of OR4

in *A. aegypti* sub-species exhibit different sensitivities toward the cognate human odorant sulcatone, which is associated with human host preference (McBride et al., 2014). Mate selection is also driving odorant tuning in closely related noctuid species, where positive selection is acting on discrete amino-acid residues of PRs (Leary et al., 2012).

The constant tuning of ORs occurring over short and long time scales, as well as the conservation of specific lineages via purifying selection, runs counter to the notion that these receptors possess promiscuous binding sites. Such a broad peripheral filter would provide the central nervous system with the greater task of sorting out this crude input. However, there is little evidence that the CNS and the antennal lobe accomplish this task (Sachse and Galizia, 2003). In fact, one study has demonstrated that the discriminatory capabilities of the antennal lobes diminish when stimulated by high concentrations of odorants (Silbering et al., 2008). Rather, we concur with previous authors that ORs are better chemical detectors than previously assumed and that the burden of extracting behaviorally relevant odorant signals from the environment is largely their task (Hansson and Stensmyr, 2011). If so, the role of the antennal lobes would be to integrate olfactory inputs from hardwired lines associated with the detection of cognate odorants.

Molecular Receptive Range and Olfactory Tuning

The inherent advantages of the two-electrode voltage clamp system (Kvist et al., 2011) has facilitated the use of oocytes for assaying OR-odorant interactions (see References in Supplementary Table 1). Despite these efforts, the oocyte system is limited in the number of chemicals that can be tested and is most efficiently

FIGURE 3 | Odorant receptors are molecular adaptations to various ecological niches. The mosquito *Aedes aegypti* may use up to 110 odorant receptors (ORs) to detect specific behaviorally relevant odorants (vertical black bars) emitted in the context of various ecological contexts. For illustration purposes, these cognate odorants are unknown and have been placed arbitrarily on the scale. The number of volatile organic compounds (VOCs) emitted by potential mates and oviposition sites remains largely unknown.

used for target validation studies or studies of receptor-ligand qualities such as concentration responsiveness and comparative efficacies (Kvist et al., 2011). Where oocytes have been used as a screen for previously uncharacterized insect ORs, dozens of receptors and tens of odorants have been used, providing hundreds of receptor–ligand comparisons (Wang et al., 2010). In stark contrast are functional studies in Human Embryonic Kidney cells, which can be used to screen tens of thousands of ligand–receptor combinations (Rinker et al., 2012). Both systems suffer from the inherent requirement to solubilize odorants in aqueous solutions, making it difficult to include VOCs that are often highly insoluble. This limitation can be circumvented by using an *in vivo* system such as the *Drosophila* empty neuron where VOCs can be delivered via airstream to OSNs expressing heterospecific ORs (Dobritsa et al., 2003), but again this system lacks truly high throughput capacity.

Two of the most critical limitations of OR heterologous functional screens relate to the lack of chemical ecological context. The first limitation is the unavoidable gap in *a priori* information regarding potentially meaningful relationships between ORs and their cognate ligands. This usually means that OR functional studies are carried out in more or less random fashion with ORs being targeted by VOCs that happen to be readily available.
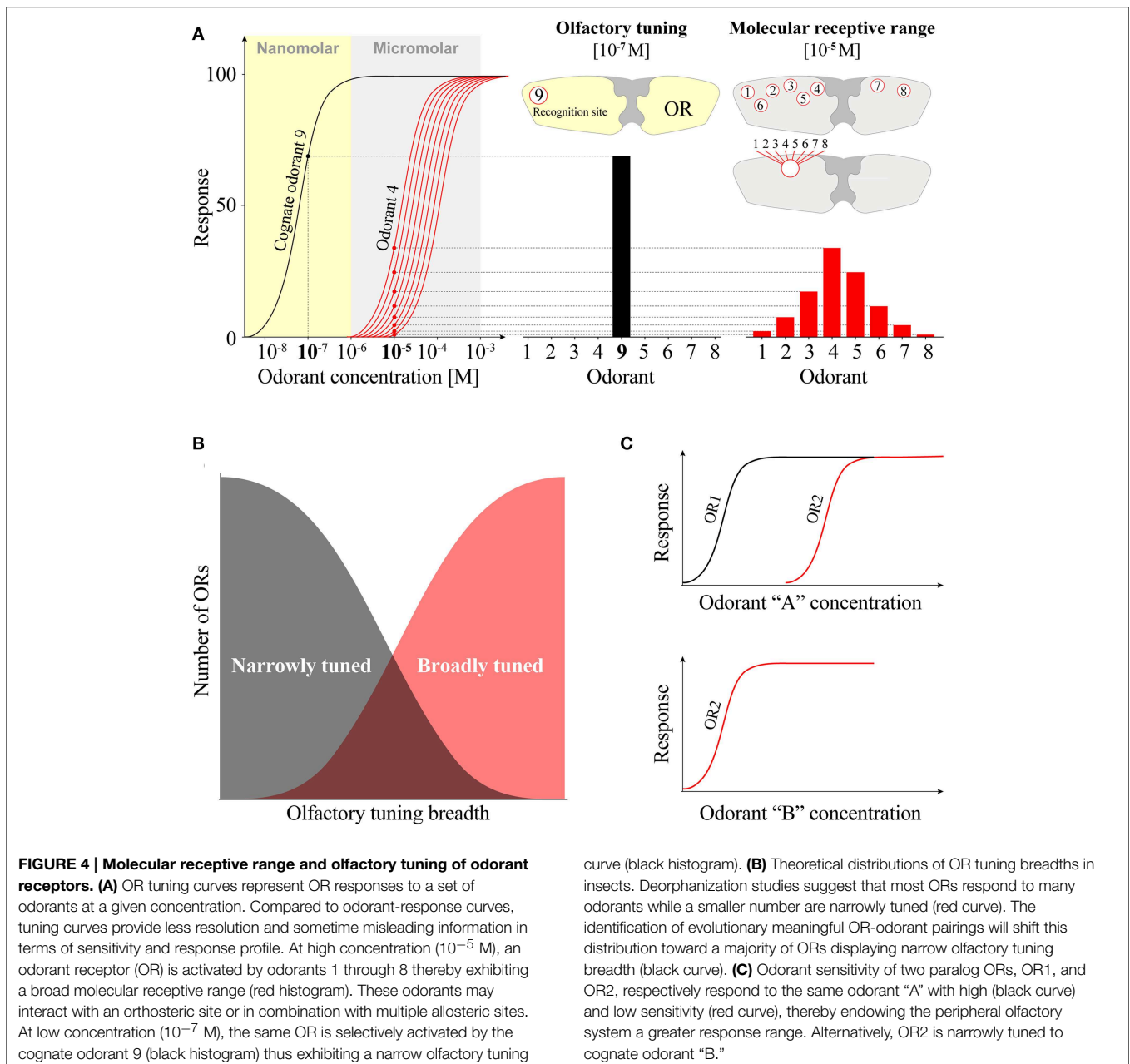
The second limitation concerns the use of very high concentrations of odorants that may produce "hits;" i.e., VOCs that activate ORs with low probability of having any adaptive value (Dobritsa et al., 2003; Carey et al., 2010; Wang et al., 2010; Ray et al., 2014). This conundrum may not be of concern if the goal of the study is simply to identify chemicals that activate ORs and can be used at high concentrations for studies of channel properties or perhaps to identify drugs that alter insect behavior. Indeed the OR screens that have been carried out to date have been extremely valuable in elucidating the mechanisms of OR function and in providing a broader framework from which we can continue to refine our understanding of insect olfaction (reviewed in Suh et al., 2014). However, if a hypothesis-driven study depends on understanding ORs in the context of chemical ecology, random screening is unlikely to produce meaningful information. There are excellent examples of insect ORs that can be activated by numerous VOCs at low millimolar/high micromolar concentrations, which would tend to support the conclusion those receptors are broadly tuned (Hallem and Carlson, 2004, 2006; Kreher et al., 2008; Carey et al., 2010; Wang et al., 2010). However, some receptors in the same screens appear to be much more narrowly tuned when the VOC concentrations are reduced to low micromolar/high nanomolar range. This would be expected for a natural receptor-ligand pairing. For example, AgORs 2 and 10 exhibit broad molecular receptivity at high VOC concentrations but are in fact highly sensitive and narrowly tuned to their apparent cognate ligands, indole and skatole, respectively (Wang et al., 2010). Moreover, follow up studies with these receptors have validated the conclusions that these receptors display highly selective responses with low EC_{50}-values (Bohbot et al., 2011). These examples are analogous to a mammalian LGIC, the NMDA receptor, where glutamate is the endogenous ligand with high potency ($EC_{50} = 640$ nM), while a synthetic competitive agonist for which the receptor is named after exhibits a potency that is over an order of magnitude less potent ($EC_{50} = 12,000$ nM) (Nakanishi et al., 1992) (**Figure 2B**). In addition, the full activation of NMDA receptors *in vivo* requires the binding of glycine to an allosteric site and has a potency of 77 nM. These examples illustrate two mechanisms by which LGICs may display apparent broad molecular receptivity range: (i) high concentrations of non-meaningful orthosteric ligands and (ii) contributions of topographically distinct (allosteric) sites (**Figure 4A**). Interestingly, most studies of insect PRs in heterologous systems have not suffered from these kinds of biases, probably because their likely cognate ligands were previously known in pheromone blends (Nakagawa et al., 2005; Wanner et al., 2007, 2010; Mitsuno et al., 2008; Wang et al., 2011; Leary et al., 2012; Liu et al., 2013; Sun et al., 2013). One test of our theory on this topic would be to "screen" PRs with high concentrations of VOC libraries like the ones described in previous studies. We speculate that numerous chemicals would activate PRs in that situation, leading to the false perception that PRs are "generalists." Taken together, the limitations described here prescribe caution when interpreting the results of heterologous expression data and OR function. The dogma that many ORs are functional "generalists" has often been based on limited VOC libraries administered at high concentrations in heterologous systems. This problem is also encountered when screening ORNs *in vivo* using high doses (up to 10^{-2} dilutions) of odorants (Hallem et al., 2004; Hallem and Carlson, 2006; Carey et al., 2010). An important question to ask is whether one would expect to obtain such a high percentage of positive OR activating odorants among such small numbers of VOCs? In our opinion this seems unlikely and would lead us to conclude that such receptors in natural settings would be prone to activation by a potentially huge number of odorants that would render them quite useless at encoding meaningful information.

Conclusions

The physiological, pharmacological, evolutionary, and experimental arguments presented in this communication are part speculative and part empirical. Altogether, they support an idea that both ORs and NRs are specialized in the detection of evolutionary meaningful chemical cues and their distinction may remain based on their ecological context rather than on their pharmacological properties. To demonstrate this, we have provided evidence that (i) narrow tuning is not the exclusive attribute of PRs, (ii) GLVs and other non-pheromonal VOCs specifically activate nPRs, (iii) ORs are subjected to powerful selective pressures, and (iv) high concentrations of odorant stimuli cause broad non-specific OR responses.

Are ORs distributed on a continuum of tuning breadths made of a small number of narrowly tuned PRs and a majority of general nPRs (**Figure 4B**)? According to our analysis, ORs exhibit both broad molecular receptivity and narrow olfactory tuning. Insect ORs may be narrowly tuned to behaviorally relevant odorants but this selectivity can be overcome by overloading the system with high concentrations of chemicals carrying no adaptive value. Therefore, we distinguish the notions of molecular receptive range and olfactory tuning based on their chemical and ecological merits.
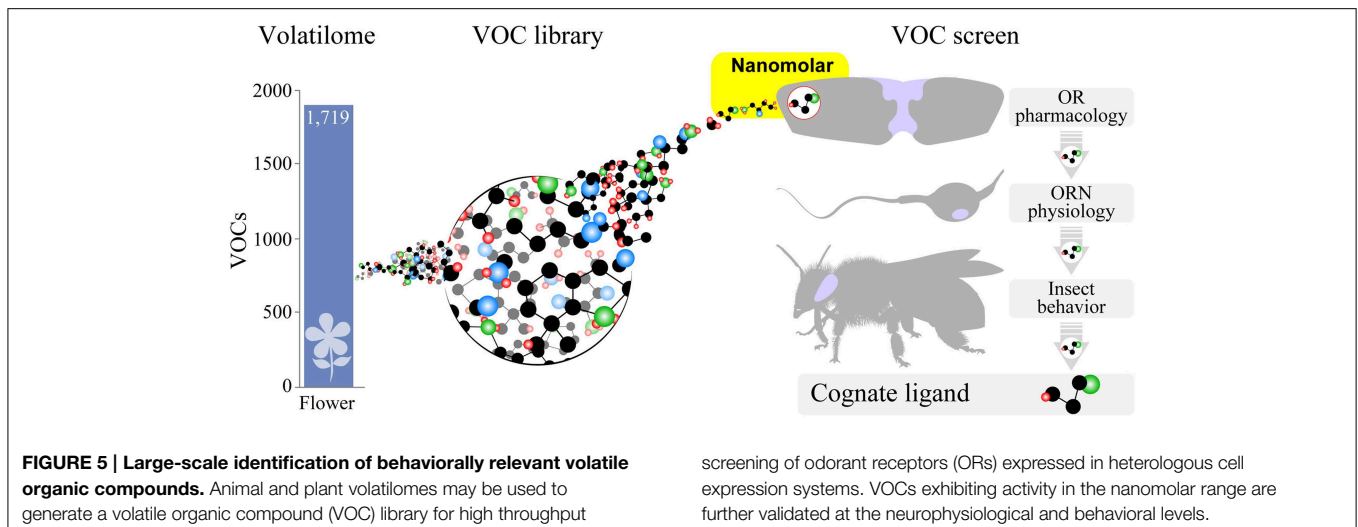
FIGURE 4 | Molecular receptive range and olfactory tuning of odorant receptors. (A) OR tuning curves represent OR responses to a set of odorants at a given concentration. Compared to odorant-response curves, tuning curves provide less resolution and sometime misleading information in terms of sensitivity and response profile. At high concentration (10^{-5} M), an odorant receptor (OR) is activated by odorants 1 through 8 thereby exhibiting a broad molecular receptive range (red histogram). These odorants may interact with an orthosteric site or in combination with multiple allosteric sites. At low concentration (10^{-7} M), the same OR is selectively activated by the cognate odorant 9 (black histogram) thus exhibiting a narrow olfactory tuning curve (black histogram). **(B)** Theoretical distributions of OR tuning breadths in insects. Deorphanization studies suggest that most ORs respond to many odorants while a smaller number are narrowly tuned (red curve). The identification of evolutionary meaningful OR-odorant pairings will shift this distribution toward a majority of ORs displaying narrow olfactory tuning breadth (black curve). **(C)** Odorant sensitivity of two paralog ORs, OR1, and OR2, respectively respond to the same odorant "A" with high (black curve) and low sensitivity (red curve), thereby endowing the peripheral olfactory system a greater response range. Alternatively, OR2 is narrowly tuned to cognate odorant "B."

While this hypothesis does not exclude the possibility that a few ORs exhibit broad olfactory tuning, it provides greater emphasis to the possibility that most ORs may be narrowly tuned (**Figure 4B**). Furthermore, we propose that nanomolar EC_{50}-values provide a criterion for insect OR deorphanization. If this is correct, the cognate ligands for *Bombyx mori* OR56 (Tanaka et al., 2009) and *Helicoverpa armigera* OR13 (Liu et al., 2013) remain to be identified (**Figure 2A**).

How do insects encode odorant intensity? It has been suggested that one of the mechanisms by which Drosophila ORs encode odorant intensity is by using paralogous OR pairs such as 42a and 42b, each detecting low and high amounts of the same odorant ligand (Kreher et al., 2008) (**Figure 4C**). This concept does not exclude the possibility that the response of an OR to high ligand concentrations rather indicates that the cognate ligand has yet to be identified. Such is the case with the mosquito indolergic receptors, OR2 and OR10, which respond to indole with high and low sensitivities, respectively (Bohbot et al., 2011). However, OR10 is narrowly tuned to skatole, a methylated analog of indole (Hughes et al., 2010; Bohbot and Dickens, 2012b). Perhaps more intriguing is the possibility that these two phenomena coexist. This is now a testable hypothesis using the aforementioned paralogous *Drosophila* ORs and looking for the cognate ligand of the low sensitivity receptor.

One of the greatest challenges in receptor neurobiology is the identification of OR-cognate odorant pairs. Based on our

FIGURE 5 | Large-scale identification of behaviorally relevant volatile organic compounds. Animal and plant volatilomes may be used to generate a volatile organic compound (VOC) library for high throughput screening of odorant receptors (ORs) expressed in heterologous cell expression systems. VOCs exhibiting activity in the nanomolar range are further validated at the neurophysiological and behavioral levels.

analysis, we conclude that ORs and NRs are specialized receptors that selectively detect low concentrations of cognate ligands in the context of chemical communication. However, the data presented here is only a snapshot of the insect OR family. Among a few species, only a handful of ORs have been deorphanized, i.e., for which a cognate ligand has been identified. Matching host or plant volatilomes to an OR repertoire will not be trivial but would offer a comprehensive strategy to identify additional OR-cognate odorant pairs. Many of these VOCs have been published (Knudsen et al., 2006; de Lacy Costello et al., 2014) and may be used to generate a VOC library to test the response of heterologously expressed ORs (**Figure 5**) (Bohbot et al., 2014). A similar strategy, using gas chromatography-coupled single sensillum recording, has been applied to discover that ORNs are narrowly tuned to GLVs (Binyameen et al., 2014; Suer, 2014). Both approaches may provide lead bioactive compounds for behavioral validation.

The notions of generalist and specialist receptors were first proposed in 1964 by Schneider et al. (Schneider, 1964) in the context of ORN activation and were later applied to ORs as their underlying molecular mechanisms. Earlier authors had reported that GLV-detecting ORNs were as specialized as sex pheromone receptor neurons (Kafka, 1987; Dickens, 1990; Anderson and Hansson, 1995). Anderson recognized that the use of a limited number of GLVs for physiological screens prevented the identification of behaviorally relevant odorant ligands. Likewise, we have underlined that current OR deorphanization efforts use between 80 and 100 odorants, which is at least three-fold lower than the number of VOCs insects encounter in nature. The pharmacological data currently available reveal that PRs and nPRs exhibit comparable functional specialization provided the correct cognate ligands are known (Hansson and Stensmyr, 2011; Bengtsson et al., 2014).

The combination of short lifespan and narrow behavioral complexity in insects may explain the evolution of a peripheral olfactory system tuned to a narrow range of odorants involved with reproduction, mate selection and food selection, which do not exclude the possibility of associative learning. Interestingly, mammals can learn to detect chemicals devoid of adaptive value with high sensitivity and specificity. However, the comparatively oversized olfactory epithelium of mammals combined with their sniffing behavior may compensate for the inherent lack of sensitivity of its OR repertoire. Indeed, detector dogs seem to only detect the most abundant volatile chemicals in the headspace of explosives (Harper et al., 2005). On the other hand, mammals may have developed a peripheral olfactory system with higher tolerance for a greater variety of odorants amenable to odorant-based associations (Leon and Johnson, 2003; Wilson and Stevenson, 2003). It is more likely that the complexity of the central nervous system account for most of the plasticity required for associative learning (Mandairon and Linster, 2008).

There is no expedient method to test the theory proposed herein. Indeed, the identification of cognate odorants is time consuming and resource intensive. Since the pioneering studies on the deorphanization of insect ORs (Hallem et al., 2004; Carey et al., 2010; Wang et al., 2010), targeted functional studies have identified cognate ligands for nPRs and it is reasonable to expect that more will be identified. What remains to be understood is indeed staggering and will have important consequences in the fields of olfactory coding, medicine, and agriculture.

Acknowledgments

Supplementary Material

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fevo.2015.00039/abstract

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