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On the ORigin of smell: odorant receptors in insects

R. Benton

Laboratory of Neurogenetics and Behavior, The Rockefeller University, 1230 York Avenue, Box 63, New York, New York 10021 (USA), Fax: +1 212 327 7238, e-mail: bentonr@mail.rockefeller.edu

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Abstract. Olfaction, the sense of smell, depends on large, divergent families of odorant receptors that detect odour stimuli in the nose and transform them into patterns of neuronal activity that are recognised in the brain. The olfactory circuits in mammals and insects display striking similarities in their sensory physiology and neuroanatomy, which has suggested that odours are perceived by a conserved mechanism. Here I review recent revelations of significant structural and functional differences between the Drosophila and mammalian odorant receptor proteins and discuss the implications for our understanding of the evolutionary and molecular biology of the insect odorant receptors.

Keywords. Olfaction, odorant receptor, signal transduction, GPCR, neuron, insect, mammal, evolution.

Olfaction: the basics

Olfaction is used by most animals to extract vital information from volatile chemicals in the environment, such as the presence of food or predators. Olfactory cues also control many social and sexual interactions between individuals of the same species, for example the delineation of territory through scent-marking by dogs or the stimulation of mating behaviours through sex pheromones in rodents [1, 2]. Even in humans, generally regarded as having a poor sense of smell, the nose is often first to alert us to potential danger through its detection of the fumes from a fire or the whiff of rotten milk.

The olfactory system is remarkable, as it can detect a huge diversity of distinct odours – numbering in the thousands – yet discriminate between them by forming a precise neural representation of individual stimuli that yields a vivid perception in the brain. A major breakthrough in our understanding of the molecular basis of this extraordinary feat of sensory perception came from the isolation of the mammalian odorant receptor (*OR*) genes by Linda Buck and Richard Axel [3], recognised by the 2004 Nobel Prize. These genes encode a family of G protein-coupled receptors (GPCRs), a class of transmembrane proteins characterised by the presence of seven membrane-spanning segments with an extracellular N terminus. OR proteins are exposed to odours on the ciliated endings of olfactory sensory neuron (OSN) dendrites in the olfactory epithelium of the nose. Upon odour binding, the receptors stimulate neuronal depolarisation via a G protein/cAMP-mediated signalling cascade, which is propagated to the synaptic termini located in the olfactory bulb in the brain [4].

OR gene families are enormous, comprising ~400 genes in humans and ~1200 in mice, and are highly divergent, with the encoded proteins displaying as little as 20% amino acid identity [5, 6]. Moreover, an individual OR is not dedicated to the recognition of a single odour but, rather, can be activated by multiple different chemical stimuli [7, 8]. Together, these properties provide a simple explanation for how so many different odour molecules can be detected.

How the brain *discriminates* between different odorants was illuminated by two key observations (Fig. 1). First, OSNs are likely to express only a single *OR* gene ([discussed in ref. 9]). Second, although the neurons expressing a given receptor are scattered throughout the olfactory epithelium, their axons converge into one or two

structures, called glomeruli, in the olfactory bulb [10]. Thus, neuronal activity in a given glomerulus reflects the stimulation of one specific type of OR in the nose. As an odour molecule can be recognised by multiple different receptors, it is thought to be the combination of activated glomeruli that defines the unique neuronal representation of an odour [11]. In addition to this spatial 'code', it is likely that higher brain centres interpret temporal features of glomerular activity, which may depend upon the differences in the dynamics of OSN stimulation by odours [12].

Odorant receptors in *Drosophila*: a model – or novel – organism?

Insects are an attractive system in which to study olfaction because they display a rich repertoire of olfactory-driven behaviours under the control of a nervous system that is much simpler than that of mammals. For example, the malaria mosquito, *Anopheles gambiae*, locates human hosts for blood feeding through its ability to detect multiple chemical cues emanating from our skin, including lactic acid and ammonia, and carbon dioxide in our breath [13]. In the hawkmoth, *Manduca sexta,* trace quantities of female pheromones are sufficient to induce the male to initiate stereotyped flight behaviours, often over several miles, in pursuit of its potential mate [14].

The anatomical and physiological properties of the insect olfactory system have been intensively studied in a wide range of species, including locusts, cockroaches, moths and honeybees [reviewed in refs. 15–19]. Despite this wealth of information, molecular analysis has lagged behind studies in mammals, as insect *OR* genes were only discovered in 1999 in the fruit fly, *Drosophila melanogaster* [20–23]. Nevertheless, the relatively small number of *Drosophila OR* genes, 62, has permitted rapid and comprehensive descriptions of *OR* gene expression in the antenna and maxillary palps (the 'noses' of *Drosophila*), the axonal projections of OSNs expressing specific ORs in the brain and the odour response profiles of individual receptor proteins [24–30]. These studies have revealed striking parallels with the organisation and physiological properties of the peripheral olfactory circuits in mammals: insect ORs recognise multiple odours, OSNs express one (or sometimes two) odour ligand-binding receptors and the axons of OSNs that express the same OR converge onto specific glomeruli in the antennal lobe, the insect equivalent of the olfactory bulb (Fig. 1).

Thus, superficially, *Drosophila* appears to have an ideal 'model' olfactory system, with a mammalian organisation in miniature, while offering powerful genetic tools to unravel the molecular mechanisms by which ORs encode odours in the neural circuitry of the brain [11, 31]. This view, however, has been challenged in the last few years by the revelation of fundamental differences in the functional properties and structural design of mouse and *Drosophila* OR proteins.

First, mammalian ORs appear to be multifunctional proteins that act not only in odour recognition in the sensory dendrites, but are also present in axons and have an essential role in guiding them to form specific glomeruli [10, 32–34]. Exactly how OR proteins participate in axon guidance remains to be defined, but genetic analysis suggests a model in which they promote OSN convergence in the olfactory bulb through homotypic interactions between neurons that express the same OR [35]. In contrast, there is no evidence that insect ORs play any role in guidance of OSN axons: the proteins are first detected only after axon termini have reached their target

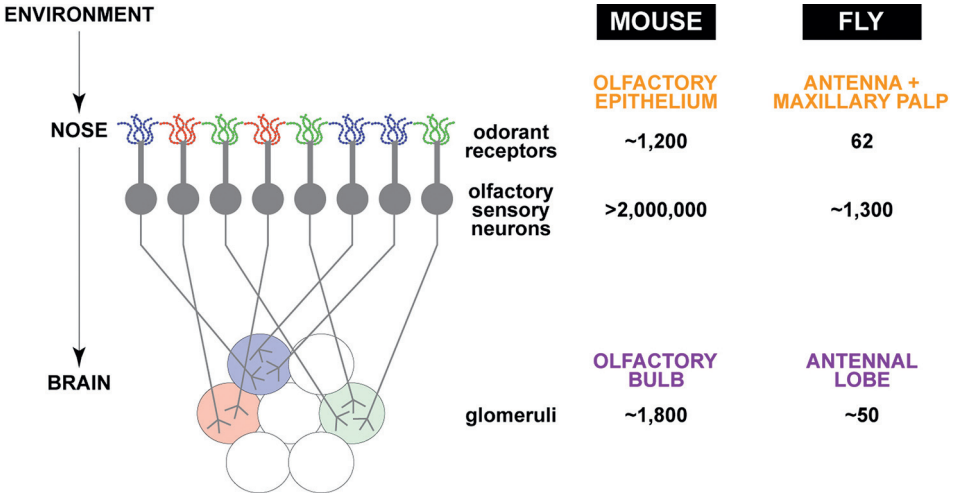

Figure 1. Similarities in the peripheral olfactory circuits in mammals and insects. In both mammals and insects, but at vastly different scales, olfactory sensory neurons (grey) in the nose generally express a single type of odorant receptor (red, blue, green) that localises to the sensory cilia. The axons of neurons expressing the same receptor converge into unique glomeruli in the brain.

glomeruli and concentrate almost exclusively in the sensory dendritic ending of these neurons [36]. Moreover, OSNs that lack expression of endogenous ORs and/or misexpress ectopic ORs display altered odour response properties but no defects in their wiring in the antennal lobe [37].

Mammalian ORs play a second function in olfactory system development, by participating in a negative feedback mechanism that ensures the singular expression of ORs in OSNs [38–40]. The precise nature and function of the feedback signal from ORs is unclear, but it is intriguing that mutations in components of the olfactory signal transduction cascade do not affect *OR* choice (or OSN axonal projections) as this implies that the receptor utilises distinct signalling pathways to fulfil these developmental functions [41]. In insects, almost nothing is known about *OR* gene choice, although considerable evidence rules out the existence of OR-dependent negative feedback: first, ten classes of OSN normally express two *OR* genes [24, 25, 29, 42]; second, ORs can be ectopically expressed in other OSNs without affecting endogenous OR expression, and, finally, mutant neurons that lack the endogenous OR do not activate the expression of a 'replacement' receptor [37, 43].

A third key difference in OR biology reflects the existence of an unusual member of the insect OR family known as OR83b. Unlike the 'conventional' odour ligand-binding ORs that are expressed in small subpopulations of OSNs, OR83b is co-expressed with these receptors in most, if not all, neurons [44, 45]. OR83b does not appear to function directly in odour recognition, however, but, rather, forms a complex with conventional ORs and is essential to escort them from the cell body to the sensory cilia [45–48]. This heteromeric receptor complex persists in the sensory compartment, raising the possibility that OR83b may also function as a co-receptor in olfactory signalling [46]. Consistent with this important general role in the olfactory system, OR83b is structurally and functionally conserved across diverse insect species [44, 49, 50]. There is no mammalian orthologue of OR83b, although mammalian OSNs do express a number of accessory factors, such as RTP1 and REEP, which are at least partly analogous to OR83b in their ability to assist membrane trafficking of mammalian ORs in cultured cells [51]. Unlike OR83b, these proteins are structurally unrelated to their OR 'cargo', bearing a single predicted transmembrane domain. A closer parallel with OR83b function may exist in the observation that the seven transmembrane domain β_2-adrenergic receptor (β_2-AR) associates with and promotes functional expression of some mouse ORs *in vitro* [52]. Like OR83b, β_2-AR is expressed widely in OSNs, although *in vivo* demonstration of a role for this receptor in OR trafficking awaits genetic analysis.

Finally, recent work has challenged the widely accepted notion that insect ORs are members of the same protein family as mammalian GPCR family ORs. *Drosophila* ORs were identified by bioinformatic strategies, and although computational analysis predicts that these proteins contain seven transmembrane domains, these proteins share no obvious primary sequence similarity to either mammalian ORs or any other known GPCR. Recent bioinformatic and experimental investigations have revealed that the membrane topology of *Drosophila* ORs is in fact distinct from GPCRs, with the N terminus of these receptors located intracellularly (Fig. 2) [46, 53]. These surprising observations indicate that insect ORs define a completely novel family of transmembrane receptors, setting them apart from not only mammalian ORs but all known chemosensory receptors in vertebrates and nematodes [54].

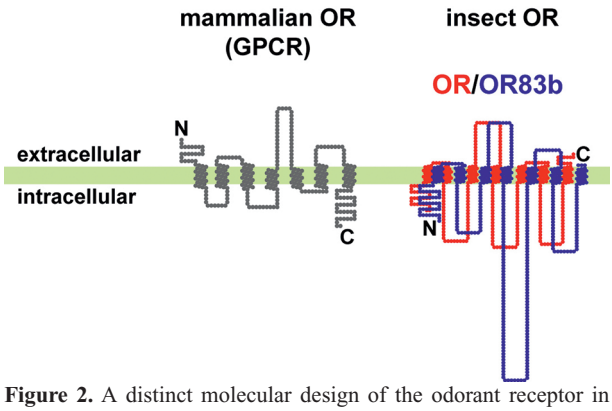

Figure 2. A distinct molecular design of the odorant receptor in mammals and insects. Mammalian ORs are members of the GPCR superfamily, while insect ORs define a topologically distinct family of transmembrane proteins. Furthermore, the insect OR comprises a heteromeric complex of a conventional odour ligand-binding receptor (red) and the broadly expressed receptor OR83b (blue), which functions in OR transport to the sensory cilia. Snake plots were adapted from Residue-based Diagram editor outputs obtained from the GPCR database [84].

What next for insect ORs?

The significant structural and functional distinctions between insect and mammalian ORs raise numerous questions about the molecular and evolutionary biology of the insect olfactory system.

The molecular biology of insect ORs: basic questions and global applications

The definition of insect ORs as a novel family of transmembrane proteins immediately prompts the question of how they transform odour binding into neuronal depolarisation. In contrast to mammalian ORs, in which odour ligand-receptor interactions are thought to occur in a similar location to the ligand-binding pocket of other GPCRs [55, 56], nothing is known about how insect ORs

interact with odour molecules. However, the continuously growing data available on the protein sequences of insects ORs and their cognate ligands in *Drosophila*, *A. gambiae* and the silkmoth *Bombyx mori* [27, 47, 57] provides the essential knowledge to begin to address this fascinating problem of molecular recognition. For example, comparative sequence analysis of insect ORs could permit identification of hypervariable residues that might be expected to contribute to ligand specificity. Ultimately, it seems likely that brute force functional analysis of mutant and chimeric versions of receptors whose odour response profiles are well defined will be required to understand how receptors interact with specific ligands.

A second outstanding issue is the nature of the signal transduction cascade downstream of insect ORs. The long-held view that these receptors are members of the GPCR superfamily has led to directed investigation into the role of G protein signalling in insects OSNs. Several G alpha subunits, in particular $G_{\alpha}q$, are indeed expressed in insect antennae, although they are not specifically enriched in the ciliated dendrite of OSNs [58–60]. Early pharmacological studies revealed that inhibition of G proteins in locust and cockroach antennal homogenates can impair odour-evoked increases in inositol 1,4,5-trisphosphate (a potential, but unproven, olfactory second messenger) [61, 62], while treatment of moth OSNs with a G protein activator can mimic odour-evoked neuronal depolarisation [60]. Reduction of $G_{\alpha}q$ levels in *Drosophila* OSNs by RNA interference produces defective behavioural responses to some, but not all, odour stimuli, although it is unknown whether this is due to a defect in primary olfactory signal transduction [63]. *In vitro* studies have shown that expression of OR/OR83b in cultured mammalian cells or *Xenopus* oocytes can confer odour-induced signalling with or without co-expression of exogenous insect $G_{\alpha}q$ proteins [47, 48, 64, 65]. This indicates that the receptors can couple to endogenous signal transduction cascades in these heterologous systems, but the identity of these pathways and their significance for the signalling mechanism in insect OSNs remains unclear. Thus, while these data are suggestive, definitive genetic and biochemical evidence demonstrating that insect ORs couple directly to G proteins to promote neuronal depolarisation in response to odour stimulation is lacking.

Determining conclusively if and how insect ORs use G proteins in olfactory signalling will be fascinating, because this could either demonstrate a remarkable case of convergent evolution, in which two distinct families of seven transmembrane domain receptors utilise the same intracellular signalling cascade, or reveal a novel mechanism of sensory transduction. With respect to the second of these possibilities, it is noteworthy that an unrelated family of seven transmembrane domain proteins, defined by the mammalian adiponectin receptors AdipoR1 and AdipoR2, have the same predicted topology as insect ORs, and these do not appear to couple to G proteins [66, 67].

The unique molecular nature of insect ORs also provides opportunities for the development of novel insect repellents. Insects pose enormous problems for humans in their roles as vectors of disease, such as the malaria mosquito, and as agricultural pests, such as the desert locust, *Schistocerca gregaria*, whose billion-animal swarms can destroy hundreds of square kilometres of crops in a day [68]. The reliance of insect behaviours on olfactory cues makes this sensory modality an attractive target for chemical intervention, and this is how the most commonly used insect repellent, N,N-diethyl-m-toluamide (DEET), is believed to work [69]. DEET, however, is only partially effective at discouraging biting insects and is toxic to humans, but, in the past 50 years, little progress has been made in identifying more effective alternatives. Because the OR/OR83b heteromer is unique to insects, this protein complex represents an ideal target for the development of specific chemical inhibitors or modulators, which could ultimately be used in the field to control the damaging olfactory-mediated behaviours of insects.

The evolutionary biology of insect ORs: from where did they come and where are they going?

The evolutionary origin of the insect ORs is completely mysterious: they bear no obvious homology to other eukaryotic proteins (apart from weak similarity to a small family of *Caenorhabditis elegans* proteins whose function is unclear [23, 70]) and it remains unknown whether they share a common ancestor with GPCRs or a completely different type of transmembrane protein. Together with the functional differences insect and mammalian ORs exhibit in olfactory system development, this suggests that the peripheral circuitry in these organisms evolved independently. This could imply that their notable anatomical and physiological parallels reflect a striking case of convergent evolution, reflecting perhaps an inevitable property of a sensory system that has evolved to detect and discriminate a large number of chemical stimuli [31]. Alternatively, the insect and mammalian olfactory systems could have evolved from a common origin and undergone dramatic divergence in the molecular mechanisms by which they detect odours. Notable in this context is that even within mammals, an accessory (or vomeronasal) olfactory system – which is principally involved in pheromone detection [71] – expresses two different chemosensory receptor gene families, the *V1R*s and *V2R*s [72–75]. While these genes also encode GPCRs, the V1Rs and V2Rs appear to be evolutionarily distinct from mammalian ORs (and from each other) [76].

The insect olfactory system is clearly still evolving. Insects number at least two million species and occupy al-

most every imaginable ecological niche, and this may be at least partly attributed to their adaptable olfactory preferences. Such preferences can evolve very rapidly, as seen in the apple maggot fly, *Rhagoletis pomonella*, which has shifted host plant from hawthorn to apple over the past ~150 years [77]. Whether these behavioural changes are due to alterations in peripheral olfactory detection capabilities or to the processing of this sensory information in the brain remains, however, unclear [78, 79]. A second striking example occurs within the *D. melanogaster* species complex: the island-endemic *Drosophila sechellia* lays eggs exclusively on the fruit of the shrub *Morinda citrifolia*, while this fruit is toxic and avoided by sibling *Drosophila* species [80]. Morinda fruit volatiles include both acids (e.g. hexanoic acid) and esters (e.g. methyl hexanoate) and *D. sechellia* is behaviourally more attracted than *D. melanogaster* to these compounds. Moreover, extensive electrophysiological analysis of *D. sechellia* antennae has revealed increases in both the number and sensitivity of methyl hexanoate-responsive OSNs, which express the orthologue of *D. melanogaster* OR22a [81, 82]. Although such peripheral differences may not fully account for the behavioural shifts, it will be exciting to determine whether the increased sensitivity of these neurons is due to an increased expression level of this receptor or to amino acid polymorphisms that exist between the *D. sechellia* and *D. melanogaster* OR22a sequences.

Beyond this example, the hypothesis that the evolution of a divergent OR repertoire is at least partly accountable for the olfactory adaptability of insects is highly appealing. The availability of a growing number of sequenced insect genomes now provides an excellent opportunity to examine the relationship between the genetic variation in *OR*s and the olfactory preferences of ecologically distinct species. For example, phylogenetic comparison of the OR families of *D. melanogaster* and *A. gambiae* reveals that while they are similar in overall size (62 versus 79 ORs), many of the ORs in one species do not have an obvious orthologue in the other [83]. It is attractive to suggest that these species-specific receptor proteins recognise the odour ligands that define host preference, that is, fermenting fruits for *D. melanogaster* and human body odours for *A. gambiae*. Indeed, expression of one of the mosquito-specific ORs, AgOR1, in the *Drosophila* antenna, was found to confer electrophysiological responses to a component of human sweat [57].

Finally, although variation in the OR repertoire may simply reflect adaptations of different species to their ecological niche, OR evolution might have *causal* effects on speciation. It is tempting to speculate that a mutation in an OR that altered its odour recognition properties might be sufficient to promote the shift in olfactory preference of an individual for a geographically isolated food source which could provide the initial driving force towards the evolution of a new species.

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