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REVIEW

Access to the odor world: olfactory receptors and their role for signal transduction in insects

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Abstract The sense of smell enables insects to recognize and discriminate a broad range of volatile chemicals in their environment originating from prey, host plants and conspecifics. These olfactory cues are received by olfactory sensory neurons (OSNs) that relay information about food sources, oviposition sites and mates to the brain and thus elicit distinct odor-evoked behaviors. Research over the last decades has greatly advanced our knowledge concerning the molecular basis underlying the reception of odorous compounds and the mechanisms of signal transduction in OSNs. The emerging picture clearly indicates that OSNs of insects recognize odorants and pheromones by means of ligand-binding membrane proteins encoded by large and diverse families of receptor genes. In contrast, the mechanisms of the chemo-electrical transduction process are not fully understood; the present status suggests a contribution of ionotropic as well as metabotropic mechanisms. In this review, we will summarize current knowledge on the peripheral mechanisms of odor sensing in insects focusing on olfactory receptors and their specific role in the recognition and transduction of odorant and pheromone signals by OSNs.

Keywords Chemosensation · Gustatory receptor (GR) · Ionotropic receptor (IR) · Odorant receptor (OR) · Olfaction · Pheromone receptor (PR)

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Introduction

The reception of chemical cues in the environment is essential for the survival of almost all organisms. Accordingly, most terrestrial animals have evolved olfactory systems with remarkable sensitivities and discriminatory power to detect relevant volatile chemical compounds and olfaction largely controls their behavior. Insects rely on a powerful sense of smell to locate habitats and food sources, to identify mating partners and oviposition sites or to escape predators [1–3]. They may encounter chemical signals emitted from conspecifics, including sex pheromones, alarm pheromones or aggregation pheromones [4, 5] but also exploit chemical compounds emitted from very different organisms, such as predators and host plants [2, 6] (Fig. 1). The various chemical compounds are received by OSNs housed within sensilla, hair-like structures that extend from the insect cuticular surface on olfactory appendages [7], most notably the antennae and maxillary palps. Odorous molecules are thought to diffuse through pores in the sensilla walls, entering the sensillum lymph where they interact with odorant-binding proteins (OBPs) and are transferred through the aqueous medium towards the dendrites of OSNs [8, 9]. Adequate compounds induce a depolarization of responsive neurons leading to action potentials that can be monitored by single sensillum recordings [10–12]. The electrically encoded information is conveyed to the antennal lobe and the neuronal signal is finally decoded by the insect brain.

The recognition of odorants and the process of chemo-electrical transduction are mediated by specific receptors; these integral membrane proteins reside in the dendrites of the sensory neurons. To some extent, the role of receptors for odorants resembles that of receptors for neurotransmitters, converting chemicals signals into electrical inputs and thus achieving a flow of information. However, there are some

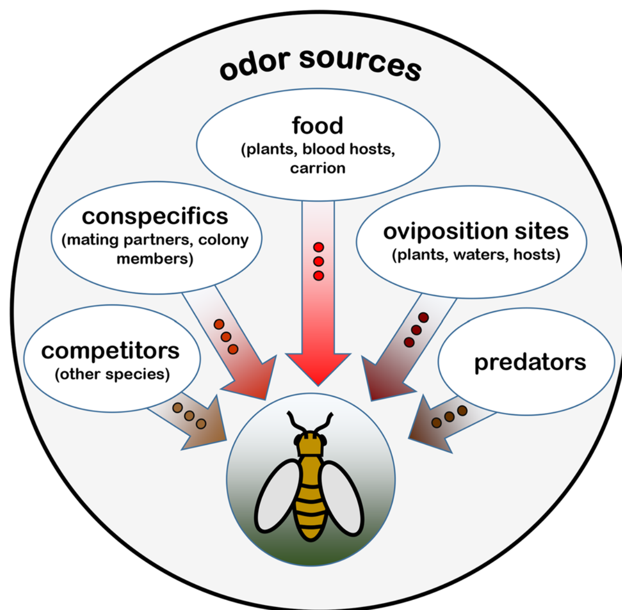

Fig. 1 Insect odor world. In their natural surroundings, insects encounter an almost unlimited number of chemical substances. This includes pheromones emitted from conspecifics and used for intraspecific communication as well as semiochemicals released by food sources, oviposition sites, predators or competitors. The insect olfactory system allows sensitive detection and precise discrimination of the relevant cues from the odor world

differences between olfactory receptors and neurotransmitter receptors. Synaptic receptors are exposed to millimolar concentrations of water-soluble neurotransmitters released in the synaptic cleft where the chemical content is regulated by the local cellular environment [13]. In contrast, olfactory receptors face a far more complex environment and the probability that a cognate odorant reaches an appropriate receptor is supposed to be orders of magnitude lower than an interaction of a neurotransmitter with its receptor at the synapse. Thus, the receptors for odorants are likely to be more sensitive towards their cognate ligands than transmitter receptors [14].

For recognition of olfactory signals, insects use several families of receptor proteins, including odorant receptors (ORs), ionotropic receptors (IRs) and carbon dioxide-sensing gustatory receptors (GRs) [15, 16]. Among these, ORs have been most extensively studied. Initially, insect ORs were thought to be seven-transmembrane domain (7-TMD) proteins acting as G protein-coupled receptors (GPCRs) [17, 18], similar to their counterparts in vertebrates and nematodes. However, they lack sequence similarity with GPCRs and have an inverted topology with an intracellular N-terminus and an extracellular C-terminus [19–21]. It is now generally accepted that insect ORs form a greatly expanded phylogenetic lineage derived from insect GRs [22, 23] and that they are not related to GPCRs [19]. Accordingly, it seems unlikely that insect ORs transduce chemical signals via G proteins but rather form heteromeric complexes with an OR co-receptor (Orco) that operate as non-selective cation channels [24, 25]. Yet, the results of several studies, most notably on moths, indicate the involvement of G protein-mediated activation of second messenger cascades in OR-expressing OSNs and it remains presently elusive whether all insects have adopted solely ionotropic mechanism for responding to olfactory signals from the environment or whether in some species, metabotropic processes may be used.

OR subtypes are expressed in spatially restricted subpopulations of antennal neurons [17, 26]. Functional studies have indicated that insects ORs respond to multiple ligands and that a distinct compound can activate multiple ORs [27]. Such overlapping response spectra are expected to allow discrimination of a larger number of odorants than predicted from the repertoire of ORs [28] and are the basis for combinatorial coding of the odor environment [29, 30]. However, this view has probably to be extended based on recent studies indicating that certain odorants carrying vital environmental information are recognized by highly specific receptors leading to the activation of dedicated neuronal pathways [31, 32]. For the recognition of sex pheromones, such labeled line pathways are well proven [33–35]. They are initiated by ligand-induced activation of specific pheromone receptors (PRs) that represent a subset of the OR superfamily and require a CD36 homolog called "sensory neuron membrane protein" (SNMP) for proper function [36, 37].

In this review, we highlight the discovery of the insect olfactory receptors and describe the recent advances in the identification of receptor candidates in numerous insect species that opened the avenue for comparative and phylogenetic studies. Furthermore, we outline experimental approaches that made it possible to explore the ligand specificity of distinct receptor types and to elucidate the unique roles of the proteins in the primary events of the chemo-electrical transduction process. Finally, we summarize and discuss current knowledge suggesting ionotropic and metabotropic mechanisms of olfactory signal transduction in insect OSNs.

Olfactory receptors

Olfactory receptors in the dendritic membrane of OSNs are responsible for the detection and discrimination of odors. In insects, the receptors for airborne volatiles belong to three classes of chemoreceptor proteins (Fig. 2). In most species, the ability to detect a vast array of chemically diverse odorants (including pheromones) is based on a class of 7-TMD proteins, the odorant receptors (ORs) [17, 26, 27, 34, 38, 39]. Insects express divergent families of OR genes, albeit

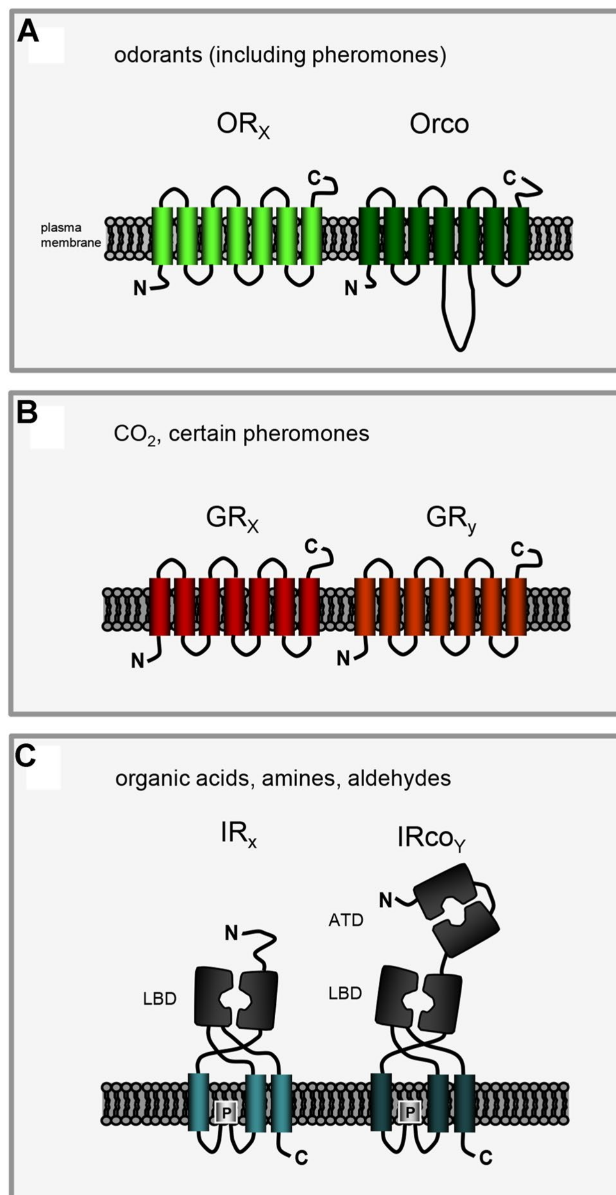

Fig. 2 Classes of insect olfactory receptors. **a** ORs are heteromers composed of a variable odorant-binding OR protein (OR$_X$) and an ubiquitous OR co-receptor (Orco). Both proteins are 7-TMD proteins with the N-terminus inside and the C-terminus outside the cell. The OR$_X$ subunit can bind to "general" odorants (including odorants originating from food plants, oviposition sites or predators) as well as to pheromonal substances. **b** The GR family includes receptors for CO_2 or given pheromones. The functional receptor is supposed to be a heteromer of GR$_X$ and GR$_Y$ subunits that display a structure and membrane topology similar to OR proteins. **c** IRs are composed of ligand-binding receptor proteins (IR$_X$) and co-receptor proteins [IRco$_Y$ (IR8a and/or IR25a)]. The functional IR can be a heterotetramer composed of two IR$_X$ and two IRco$_Y$ subunits. Alternatively, the complex can contain three different IR$_X$ proteins. Both IR$_X$ and IRco$_Y$ proteins possess three membrane-spanning domains, a pore region (P) and an extracellular region representing the ligand-binding domain (LBD). In addition, the IRco$_Y$ proteins possess an extended amino-terminal domain (ATD)

the number of different receptors in a given species varies between insect groups, ranging from 10 to several hundred ORs (reviewed in [40]). The second class of olfactory receptors are the so-called ionotropic receptors (IRs) that are related to ionotropic glutamate receptors (iGluRs) and seem to be predominantly tuned to certain short-chain organic acids, amines and aldehydes [41]. Typically, only a relatively low number of different IR types (10–20) are expressed in the olfactory system of an insect [42, 43]. The third class of olfactory receptors comprises another small group of 7-TMD proteins that are assigned to the GR family of insects based on sequence homology. These "olfactory" GR types mediate the detection of carbon dioxide in various insects [44–46] and of certain pheromones in *Drosophila melanogaster* [35].

Odorant receptors (ORs)

Discovery of ORs, OR repertoires and evolution

The discovery of insect ORs has proven as a particular challenging task. The first OR-encoding genes were identified in rat [47] following a strategy based on the finding that chemosensory signal transduction in OSNs of vertebrates is mediated by a G protein-mediated second messenger cascade activated by binding of odorants to a G protein-coupled receptor (GPCR). Since GPCRs are characterized by seven transmembrane domains, Buck and Axel screened for genes encoding 7-TMD proteins and found a large number of diverse GPCRs that were specifically expressed in the olfactory epithelium. Subsequently, homology-based cloning strategies led to the identification of gene families encoding ORs in other vertebrate species, including humans [48], fish [49] and birds [50]. However, all attempts using vertebrate or reported *Caenorhabditis elegans* olfactory receptor sequences [51] in homology-based approaches to identify insect ORs failed. A breakthrough was achieved when the first insect genome was sequenced. During the late 1990s, genomic sequences of *D. melanogaster* became available [52] and allowed strategies to search for genes encoding 7-TMD proteins expressed in the olfactory system of the fly. This search led to various candidate OR genes that were expressed in distinct non-overlapping subsets of OSNs in the antennae or the maxillary palps [17, 18, 26, 53]. Finally, the entire OR repertoire of *D. melanogaster* that comprises 60 functional OR genes encoding 62 diverse OR proteins was unraveled [23]. Functional analysis of candidate *Drosophila* ORs in heterologous expression systems (see below) has proven their identity as receptors for odorants and demonstrated that the OR repertoire of an insect is the molecular basis for the detection and discrimination of a large number of different odorant molecules [27, 54].

When genomic sequences of the malaria mosquito *Anopheles gambiae* were deciphered, homology searches

with the *Drosophila* sequences and application of the quasi periodic feature classifier (QFC) algorithm [55] identified 79 divergent ORs in the malaria mosquito [56, 57]. Comparison of the OR families from *D. melanogaster* and *A. gambiae* revealed high sequence diversity among their ORs (even though both species belong to the order Diptera) and specific expansions of OR subfamilies in each dipteran lineage [57]. Together, these findings appear to reflect the evolutionary adaption of the OR repertoires to the chemo-ecological needs of each species. Remarkably, one unequivocally orthologous pair of *Drosophila* and Anopheles OR genes (AgamGPRor7 and DmelOr83b) was found [57]. The encoded "OR" proteins later were uncovered as a unique odorant receptor co-receptor, renamed as Orco [58, 59]. Orco is characteristic for each OR-expressing OSN and highly conserved across insect species and orders. Besides Diptera, Orco homologous have been identified in various insect orders including Lepidoptera, Coleoptera, Hymenoptera, Hemiptera, Orthoptera, and Zygentoma [22, 60–64]. Functionally, Orco is required for membrane targeting of canonical ORs [58]; moreover, it appears to heteromerize with other ORs, forming odorant-gated ion channels (see below) [24, 25, 65, 66].

Over the past 15 years, rapid progress in sequencing technologies and bioinformatics tools together with a drastic reduction of the cost for sequencing have made it possible to search for OR gene families in the genomes of numerous insect species (for review see [40]). In addition to genomics, transcriptomics of insect olfactory tissues proved to be an applicable method for OR identification. By now, exploration of genomic data and cDNA sequences (derived from mRNA sequencing) by approaches similar to that used in *D. melanogaster* and *A. gambiae* or by application of known OR sequences as queries in BLAST searches have identified thousands of candidate insect OR gene sequences. Complete or partial repertoires of OR genes have been reported for a variety of species from many insect orders including Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera and Phthiraptera. Between insect species, the numbers of OR genes vary considerably. For example, the genomes of the ants *Harpegnathos saltator* and *Camponotus floridanus* comprise about 350 OR genes [67], whereas the fruit fly *D. melanogaster* has 62 OR genes [23, 68] and only 10 OR genes are found in the genome of the body louse *Pediculus humanus* [69]. Comparative phylogenetic analyses of the OR repertoire of insects have provided valuable information on the evolutionary origin of OR families and their expansion in insect lineages [22, 70, 71]. However, it is an open question whether the size of the OR gene family in an insect is linked to the complexity of its chemical environment. In some species, such a linkage appears reasonable. For instance, the honey bee (*Apis melifera*) comprises a repertoire of 163 ORs for odorant detection and lives in a complex world of floral, kin and pheromonal odors [72]. In other cases, a link seems not immediately obvious, e.g., this applies to the red flour beetle *Tribolium castaneum* that lives in an unnatural niche, the grain stores, and has 256 functional OR genes of which 129 are expressed in the adult antenna [73, 74]. As the beetle can survive for generation within its food source, one might ask why the beetle needs so many ORs. However, it is conceivable that the existing OR repertoire may still reflect a situation prior to the development of agriculture.

In contrast to IRs and GRs that appeared much earlier in evolution and are present in a diversity of organisms across the Protostomia [42, 75], ORs are restricted to insects [22, 76]. In comprehensive analyses of basal and primarily wingless insects, no ORs or only Orco-related proteins were found in the bristletails *Lepismachilis y-signata* and *Thermobia domestica,* respectively. Based on these findings, it has been suggested that ORs evolved later in insect evolution (presumably with the development of flight) and that Orco already was present before the phylogenetic appearance of other ORs [22]. In the coming years, endeavors like the i5 K initiative [77] that has set out to sequence 5000 Arthropod genomes including hundreds of nominated hexapods will give access to a large variety of further insect genomes. This wealth of information will allow more detailed insight into the evolution and functional implications of insect ORs.

Expression of OR repertoires

Nowadays, genome sequencing and bioinformatics technologies allow to analyze the OR gene repertoire of an insect in rather short time. The number of predicted OR-encoding genes in the genome may help to get first insight into the olfactory capacity of an insect. However, one has to be aware that the number of ORs actually expressed in a given insect species may vary considerably between sexes, distinct developmental stages or different olfactory tissues (i.e., antennae or maxillary palps). For example, in *D. melanogaster*, 60 functional OR genes are found in the genome [23] of which 43 are expressed in adult olfactory organs (antennae, maxillary palps) and 23 in the larval stage [78–80]. Similarly, from the 66 OR-encoding genes of *Bombyx mori*, 24 are expressed in larvae and 35 in adult antennae; for 25 OR genes, no transcripts could be verified by RT-PCR in adults or larval stages [81, 82].

Within an olfactory organ, distinct OR subtypes are expressed in different numbers of cells. On the antenna of adult *Drosophila*, the various OR subtypes are expressed in subsets of 2–50 cells of the altogether 1200 OSNs [17, 26, 80]. Similarly, in the female antenna of *A. gambiae*, distinct ORs are expressed in subsets of 10–75 cells of the 1500–1600 OSNs [83, 84]. It is unknown whether the expression of a certain OR subtype in a larger number of

OSNs is correlated with a high relevance of the detected odorants for an animal. Yet, for sex pheromone detection in moths, such a correlation is obvious. For example, in male *B. mori*, the OR type BmOR1 binds the major component (bombykol) of the female-released sex pheromone and it is expressed in a sex-specific manner in an extremely large subset of OSNs on the male antenna. Likewise, the male antenna contains a similar high number of OSNs expressing the receptor BmOR3 that is activated by the second female sex pheromone compound (bombykal) [85–87]. Sex-specific expression has been observed for several other moth OR types. In analogy to BmOR1 and BmOR3, these receptors are also considered as pheromone receptors (PRs) [86–93]. However, this notion has to be taken with care as functional analyses of some male-specific ORs revealed responses to kairomones and plant volatiles [90, 94]. With respect to a sexually biased expression of insect ORs, several receptor types are predominantly expressed in female individuals [95]. Such ORs are supposed to be important for host finding in blood-feeding female mosquitos [56, 96, 97], for locating appropriate oviposition sites or for detection of male-produced courtship pheromones [82, 89, 98–100].

Each OSN usually expresses one ligand-specific OR type [27, 79, 95]; a feature shared with vertebrate OSNs [101–104]. However, remarkable exceptions from this general "one-OR/one-OSN" rule have been found. For example, in the fly *D. melanogaster*, six of the 36 OSN classes in the antenna and maxillary palp co-express 2–3 different OR types [79, 105–107]. In the European corn borer moth *Ostrinia nubilalis*, certain classes of OSNs with up to five receptor types have been described [108] and four or six different ORs genes are co-expressed in two classes of OSNs in the antenna of the mosquito *A. gambiae* [83]. These examples suggest that the co-expression of multiple OR types in a single OSN may be more widespread among insects than previously thought. Functionally, the co-expression of several ORs apparently broadens the tuning of an OSN [30]. In line with this notion, the OSNs in *Ostrinia nubilalis* that co-express five ORs respond broadly to several antagonistic pheromone compounds. This is thought to enable male moths to detect a wide range of heterospecific signals, thus preventing cross-attraction to pheromones of other species [108]. Similarly, the ligand spectra of three of the six co-expressed *A. gambiae* ORs only partly overlap indicating broad responsiveness of these OSNs to a panel of odorants, including volatiles released from humans [39, 109]. This led to the hypothesis that co-expression of the ORs in the OSNs allows a sensitive detection of complex host odors and thus may direct the attention of female mosquitoes toward a relevant odor source and initiate host-seeking behavior [83].

It is largely unknown how the expression of multiple OR genes in the same cell is controlled. In *A. gambia*, the genes of the six co-expressed ORs are arranged as a cluster within the genome [83]. Similarly, the genes encoding some of the co-expressed *Drosophila* ORs are clustered [23]. Analyses of the RNA transcribed from clustered OR genes discovered polycistronic RNA suggesting that the encoded OR proteins are translated from the same primary transcript [83, 107]. Polycistronic RNA and co-expression have also been reported for six clustered GR genes encoding sugar receptors of *D. melanogaster* [110, 111], indicating a common principle underlying the co-expression of several chemoreceptor types in olfactory and gustatory neurons. A clustered organization of OR genes varies among insect species and may be linked to the number of OR genes. Whereas most of the 60 functional *Drosophila* OR genes are dispersed [23], many of the 131 OR genes of the yellow fever mosquito *Aedes aegypti* are paired in tandem arrays, triplets and large clusters of up to 11 genes [97]. Worth mentioning, part of the 160 OR genes in the honey bee *Apis mellifera* are arranged in large tandem arrays with up to 60 OR genes [72].

Methods used for functional characterization of ORs

While the identification of OR gene sequences was greatly facilitated during the last decades, the identification of ligands for the encoded proteins significantly lagged behind, leaving most ORs as orphan receptors. Only for a few species, including the dipterans *D. melanogaster* [27, 54, 78, 112] and *A. gambiae* [39, 109] as well as the moth *Spodoptera littoralis* [71], a significant portion of the OR repertoires has been deorphanized. Otherwise, ORs for distinct pheromones as well as certain non-pheromone odorants have been characterized in a number of lepidopteran species, such as *B. mori* [86, 113, 114], *Heliothis virescens* [90, 115], *Ostrinia nubilalis* [116], *Spodoptera littoralis* [117] and *Cydia pomonella* [94, 118, 119]. For deorphanization of insect OR proteins, several heterologous in vitro and in vivo expression systems have been successfully established (Fig. 3); more precisely, cultured cell lines and *Xenopus* oocytes as in vitro expression system and the "empty neuron system" of *Drosophila* for functional in vivo analyses [95, 120, 121]. The expression systems significantly differ in design, time requirement, convenience of handling and the opportunity for high throughput analyses.

OR expression and characterization in cell lines is rather straightforward. Successful characterization of insect ORs has been performed using the human embryonic kidney cell line 293 (HEK293 cells) [24, 25, 90, 114, 122–126], mammalian HeLa cells [24], *D. melanogaste*r Schneider 2 cells (S2 cells, derived from embryos) [20, 21] as well as cell lines derived from the ovaries of *Spodoptera frugiperda* (Sf9 cells) [20, 127–130], *Trichoplusia ni* (High five cells) [131, 132] and *B. mori* (Bm5 cells) [132].

For analysis of receptor function, cell lines are most often transfected with expression vectors driving transient

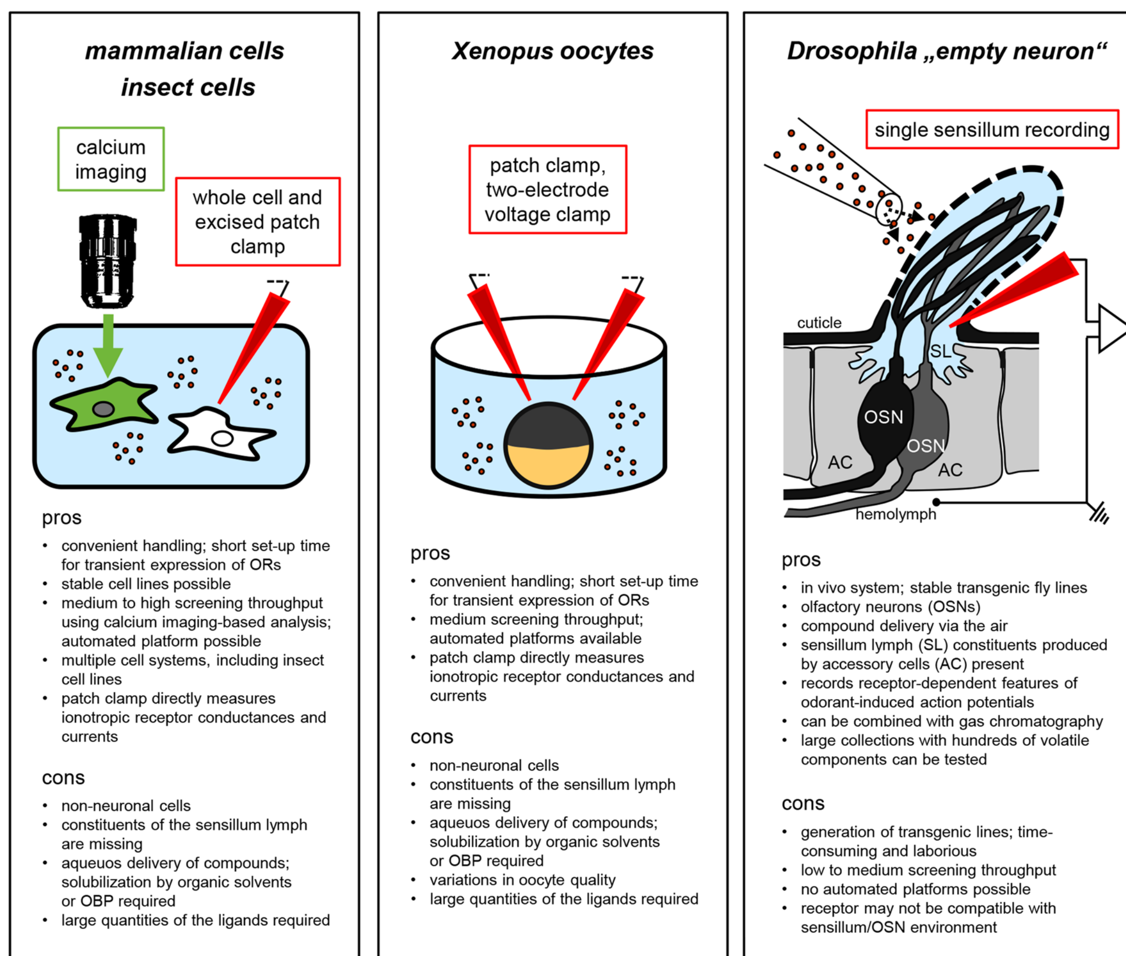

Fig. 3 Expression systems and methods used for the functional characterization of ORs. Schematic representation of in vitro approaches (mammalian or insect cell lines as well as *Xenopus* oocytes) and in vivo experiments (*Drosophila* "empty neuron system") to characterize heterologously expressed insect ORs. The advantages and disadvantages of each method are given

expressing of a distinct OR and Orco [24, 25]. The presence of Orco can significantly increase the sensitivity of the detection system [86]. In Sf9 cells, heterologous expression of Orco appears to be dispensable as this cell line expresses an endogenous version of the protein [20, 128]. Alternatively, cell lines are stably transformed by integrating an OR and Orco expression cassette in the genome [65, 124, 125] allowing an inducible or constitutive OR expression and offering the possibility of high throughput ligand screening. However, functional characterization of moth PRs was also possible in stably transformed HEK293 cells lacking Orco but expressing the G protein subunit $G_{\alpha15}$. This allowed coupling of OR activation to an intracellular signaling cascade leading to a calcium signal [90, 114, 122]. Generally, functional characterization of ORs in expressing cells involves stimulating with odorants solubilized by means of organic solvents (DMSO, methanol) or OBPs. The odorant-induced excitation of cells is analyzed by calcium imaging or patch clamp techniques. Using OBPs for ligand solubilization has revealed enhanced and more specific calcium responses of OR-expressing HEK cells [90], supporting the notion that OBPs contribute to the sensitivity and specificity of odorant detection.

Xenopus oocytes are convenient, robust and have been widely used as in vitro system for the expression and characterization of insect ORs [39, 124], including PRs [86, 133–136]. To analyze a given OR type, the respective RNA is micro-injected together with RNA for Orco into a frog egg that produces the two proteins and inserts them into the cell membrane. After a few days, the responsiveness of the oocyte to odorants can be assessed by two electrode voltage clamp technique in an aqueous bath solution. For stimulation, the hydrophobic odorants are usually solubilized in DMSO and applied to the liquid medium; so far, only in few cases, OBPs have been used for solubilization [134, 137]. In studies applying odorant/OBP solutions for OR stimulation, distinct binding proteins enhanced the sensitivity of OR-expressing oocytes and improved the specificity of the

response to given pheromonal components. In the *Xenopus* egg-based approach, each oocyte has to be injected prior to analysis and the odorant responsiveness of only one injected oocyte at a time can be analyzed, hindering high throughput assessment of large arrays of ORs and odorants. To overcome these problems, sophisticated semi- or fully-automated systems for oocyte injection have been developed [138–140]. Application of these advanced technologies will facilitate the deorphanization of ORs and thus contribute to the functional characterization of insect OR repertoires [141, 142].

In comparison to cell lines and frog oocytes, the characterization of ORs using *Drosophila* as in vivo expression system is more time-consuming and sophisticated; transgenic flies have to be generated and analysis of ORs is done through monitoring the response of OR-expressing OSNs by single sensillum recordings [143]. Thus, using the fly expression system may not be considered as first choice for OR analysis, in particular when planning high throughput OR deorphanization. However, large repertoire studies have been performed for *Drosophila* ORs, Anopheles ORs and most recently for ORs of S*podoptera littoralis* [27, 71, 109]. In summary, in vitro approaches are considered more practical in functional screenings of large numbers of ORs, while the in vivo transgenic *Drosophila* system is generally more accurate [120]. In this regard, for certain insect ORs, heterologous expression in OSNs of *Drosophila* could be a key to success as the insect in vivo system may provide the appropriate physiological environment and correct upstream processing machinery needed for proper OR reactivity. For heterologous expression of ORs, two different olfactory sensilla types of *D. melanogaste*r (sensilla basiconica and sensilla trichodea) providing different environments have been established. The elegant and most often used *Drosophila* "empty neuron" expression system is based on a mutant fly in which the endogenous receptor genes of one of the OSN classes in a sensillum basiconicum on the antenna (ab3A) are deleted [95]. Expression of a candidate OR in the "empty neuron" is achieved via the well established GAL4/UAS-system employing the promoter of the endogenous receptor gene (Or22a) to drive expression of Gal4 and subsequently of the UAS-controlled OR gene of interest. The reliability of combining the *Drosophila* "empty neuron" system with single sensillum recordings to determine the response properties of candidate ORs has been validated not only for receptors from *D. melanogaster* [27, 54, 78, 95] but also for ORs of the mosquito *A. gambiae* [109, 144] and moths [71].

The notion that the sensillum basiconica-based "empty neuron" system may not be suitable for ORs endogenously expressed in trichoid sensilla, such as certain lepidopteran and *Drosophila* PRs, motivated the development of the *Drosophila* T1 trichoid sensillum-based expression system [145]. The T1 sensillum endogenously contains a neuron that detects the pheromone cis-vaccenyl acetate via the receptor OR67d [145], thus providing an OSN environment tuned to pheromone detection. This includes expression of SNMP1 that is supposed to be required for the appropriate functioning of insect PRs [36, 146] and maybe for some other ORs, such as the *Drosophila* farnesol receptor Or83c [147]. The T1-system was shown convenient for the expression of moth PRs [117, 145] and recently for characterization of a broadly tuned OR from *Locusta migratoria* [148]. Similar to the sensillum basiconica-based "empty neuron" system, a Gal4/UAS-based procedure is used to replace the endogenous Or67d receptor by the OR of interest and receptor analysis is performed by single sensillum recordings.

In conclusion, oocyte-, cell culture- and *Drosophila*-based expression systems have been proven valuable tools for determining the ligand specificities of ORs. However, given the modern possibilities to rapidly determine the OR gene repertoires of insect species and the often very high numbers of ORs expressed, for the future, the development of fast, easy and convenient functional expression systems for receptor deorphanization will be a challenge. Most recently, application of novel techniques for genome editing (such as CRISPR/Cas9 and TALENs) as well as RNAi experiments have provided promising approaches to characterize Orco and other ORs [85, 149–158]. Future developments of these methods may provide a platform for deorphanization of large OR repertoires within short time, thus elucidating their functional relevance for the insect olfactory system.

Ligand specificities of ORs

As mentioned above, for some insect species, the ligand specificities of a larger OR repertoire have been explored using the *Drosophila* "empty neuron system" and *Xenopus* oocytes [71, 109, 159]. As result of these pioneering studies, a picture emerges indicating a wide range of OR specificities. Although some ORs appeared to be narrowly tuned, most ORs were activated by multiple ligands, i.e., they are broadly tuned. In addition, most ligands activated several receptor types. Together, the results suggest that combinatorial coding is the primary coding principle in the insect olfactory system. While the available data supported the view that some ORs are narrowly and others are broadly tuned, several experimental factors may affect the assessment of the tuning width of receptors (for review see [30]). A critical parameter is the number and selection of odorants tested in the functional assay and how this panel covers the range of chemicals recognized by the receptor. If the test panel is small and contains a given ligand but few or no chemically related compounds, the OR may appear to be very narrowly tuned. Accordingly, if the test panel is large and comprises a large number of substances related to a primary ligand, the receptor may appear more broadly tuned. Likewise, the

applied stimulus concentration is critical when assessing the response specificities of ORs. Since in functional studies odorant dilutions of 1:100 up to 1:10,000 are typically used, the question arises how these stimulus concentrations reflect the natural odor concentrations an insect encounters. Thus, one has to keep in mind that at least for some of the ORs, a classification as "broadly tuned" based on functional analysis in heterologous systems could be the misleading result of experimentally applied unnatural odorant concentrations.

Within the OR repertoire of an insect, a subset of narrowly tuned OR types is dedicated to the detection of pheromone components. First PRs were identified in two lepidopteran species, the tobacco budworm *H. virescens* [88, 90] and the silkmoth *B. mori* [86, 87, 113]. In these moths, the females release a blend of sex pheromone components for mate attraction that are detected by specialized pheromone-responsive OSNs on the male antenna [160–162]. Consequently, in search of PRs, antennal cDNA libraries and available genome sequences were screened for OR genes that were specifically expressed in OSNs of males. Using differential and homology-based screening methods in combination with bioinformatics approaches, in each species a small group of related genes was identified that were preferentially expressed in male antennae. Functional analysis of Heliothis candidate PRs in heterologous systems proved the receptor types HR13 (HvirOR13) and HR6 (HvirOR6) as receptors for the major (Z11-hexadecenal) and a minor (Z9-tetradecenal) sex pheromone component, respectively [90, 115]. Similarly, BmorOR1 and BmorOR3 were demonstrated as receptors for bombykol (E10, Z12-hexadecadienol) and bombykal (E10, Z12-hexadecadienal), the major and minor constituents of the *B. mori* sex pheromone [86, 113, 114]. Sequence comparison of PRs from *H. virescens* and *B. mori* uncovered a striking similarity between PR proteins [87]. This observation allowed homology-based strategies to identify candidate PRs of several lepidopteran species [89, 163–165]. High sequence similarity between PRs appears to be characteristic for Lepidoptera. In all moth and butterfly species analyzed so far, PRs were found to be highly related across insect species and form a separate and rather conserved group [70, 82, 88, 100, 165–168]. This remarkable conservation of the PRs indicates a high negative evolutionary selection pressure on the lepidopteran PR proteins. It also may reflect the chemical similarity of their pheromone ligands that are often long-chain unsaturated acetates, alcohols, aldehydes and polyenic hydrocarbons [169].

Beyond many lepidopteran species, ORs that are tuned to pheromone components have been identified only in few species from other insect orders. In the honey bee (Hymenoptera), the receptor type Or11 was reported as receptor for the queen pheromone substance 9-oxo-2-decenoic acid [170]. In *D. melanogaster* (Diptera), cis-vaccenyl acetate (cVA) that is produced by male flies and acts as anti-aphrodisiac pheromone and in aggression behavior [171–173] is recognized by both sexes through two receptors, Or67d and Or65a [145, 172, 174, 175]; yet, recent results indicate that Or65abc-expressing neurons are unresponsive to cVA [176]. Another *Drosophila* pheromone, 9-tricosene, that is deposited by males upon stimulation by food odors and acts as aggregation pheromone and oviposition guidance cue for females is detected via Or7a [177]. In addition, *Drosophila* Or88a and Or47b have been reported as receptors for fatty acid methyl esters mediating copulation and attraction [178]. However, their roles as PRs have been called into question since activation of Or88a and Or47b neurons were found to have little direct impact on courtship behaviors and both OSN classes are sensitive to a diverse array of fly and non-fly odors [176]. Most recently, PRs for components of the aggregation pheromone used by the common bed bug, *Cimex lectularius* (Hemiptera), were described. Interestingly, in this species, distinct components of the aggregation pheromone activate multiple ORs with various tuning properties [136] indicating a coding principle different from the one used for moth sex pheromones where distinct narrowly tuned PRs are employed for the detection of the components of the blend.

With respect to assigning specific functions to OR types based on ligand spectra determined in heterologous expression systems, a further point should be considered: analyses of OR function are generally conducted in the absence of OBPs that are present in the sensillum lymph surrounding the dendrite of OR-expressing neurons under natural conditions. Although the functional relevance of OBPs is not entirely clear [179], within a sensillum, distinct subsets of OBPs are supposed to mediate the transport of ligands through the sensillum lymph towards the respective ORs of the OSNs [8, 180, 181]. Competitive binding studies on various OBPs from moth and mosquito [182, 183] as well as RNA interference assays dissecting the function of 17 *Drosophila* OBPs [184] have demonstrated that different OBP types are tuned to defined, partly overlapping sets of ligands. Moreover, functional studies employing pheromone-binding proteins (PBPs) instead of DMSO to solubilize pheromonal ligands indicate that the response of receptor-expressing *Xenopus* oocytes or HEK cells is more specific [90, 122, 134, 137]. Hence, current data indicate that OBPs (including PBPs) as well as ORs contribute to the specificity of an odorant detection system and suggest that under natural conditions OBPs may operate as a pre-filter enabling only given compounds to reach the ORs. In light of these findings, the ligand spectra determined for ORs in heterologous systems in the absence of OBPs may include odorants that the respective ORs in the membrane of OSNs in olfactory sensilla would never encounter.

OR membrane topology and formation of heteromeric complexes with Orco

While insect and vertebrate ORs share 7-TMDs, no sequence similarities and evolutionary relationship between insect ORs and known GPCRs exist [19, 185]. Furthermore, detailed analyses revealed an inverse membrane topology of insect ORs compared to vertebrate ORs with intracellular N-termini and extracellular C-termini [19–21, 132, 186, 187]. These findings challenged the concept that insect ORs belong to the superfamily of GPCR proteins and initiate G protein-mediated signaling processes in OSNs. Substantial progress in better understanding the structure and functionality of insect olfactory receptors has been made by the observation that the OR repertoire comprises an unusual member (initially designated as OR83b in *Drosophila* or R2 in moths) named odorant receptor co-receptor (Orco) [59]). Orco shares only low sequence identity with other insect ORs, but is highly conserved across insects species of the same or different orders (up to ~ 95% sequence identity) [57, 60–62, 188]. In contrast to canonical ORs, Orco is not expressed in a distinct subset of OSNs but in most if not in all OR-expressing OSNs [26, 58, 60–62, 86]. However, Orco is absent from OSNs expressing other types of chemosensory receptor proteins, i.e., IRs and GRs (see below) [58, 117]. Consequently, Orco is considered as a marker for OR-expressing OSNs in insects and these OSNs usually express one or few distinct OR types (designated as canonical or classical ORs) along with Orco [27, 79, 86, 95].

Alike canonical ORs, Orco reveals an inverted membrane topology compared to GPCRs with an intracellular N-terminus and an extracellular C-terminus [19, 21, 189]. More importantly, ORs and Orco appear to physically interact via intracellular domains and form heteromers of yet unknown stoichiometry [19, 66, 190]. This finding has led to the notion that Orco serves as a ubiquitous co-receptor for ORs and that a functional receptor generally comprises a canonical OR type associated with Orco. In OR/Orco heteromers, the canonical OR type binds odorants and determines the ligand specificity while Orco is apparently not involved in ligand binding [24, 25, 65, 66, 86, 125, 141, 191, 192]. Yet, responses to a broad range of odorants are markedly impaired in Orco-deficient flies as well as in flies with reduced Orco expression due to RNA interference [58, 66]. Olfactory deficits in Orco-deficient flies can be rescued by the expression of Orco orthologues from other insect species (moths or mosquitoes), suggesting a substantial functional conservation and relevance of Orco [61]. In fact, Orco is of considerable importance for OR trafficking to dendritic membranes of OSNs [19, 58]. Consistent with its role in membrane targeting of ORs, OR-mediated odorant-induced responses in heterologous systems were markedly enhanced upon co-expression of Orco [20, 86]. The Orco-dependent trafficking of ORs to OSN dendrites seems to be modulated by calmodulin (CaM) [193] since binding of CaM to the respective binding site of Orco contributes to the sensitization of insect OSNs upon repeated or extended stimulation with odorants [193–195].

OR/Orco complexes operate as ligand-gated ion channels: implications for OR-mediated signaling

Analyses of Orco in heterologous expression systems have disclosed that in the absence of ORs, Orco can form a non-specific, spontaneously opening cation channel permeable for Ca^{2+} (and to a lower extent also for Na^{+} and K^{+}) [25, 125, 196]. Consistently, the spontaneous electrical activity of OSNs was attenuated in Orco-deficient flies [36, 58, 197]. These findings have led to speculations that Orco provides a dominant leak or pacemaker current triggering spontaneous activity (reviewed by [198]). This notion is supported by the observation that the Orco-specific agonist VUAA1 increases the spontaneous (odorant-independent) spike frequency of OSNs [125, 196]. However, the spontaneous activity patterns of OSNs were also found to be dependent on the co-expressed canonical OR type [27, 95], suggesting that both Orco and the associated OR(s) determine the spontaneous firing rate of an OSN.

In view of the finding that Orco can form an ion channel and a heteromeric complex with the ligand-binding canonical OR types, various cell lines and *Xenopus* oocytes were employed to co-express Orco and canonical OR types to decipher chemosensory transduction mechanisms. In these studies, short odorant pulses elicited a very fast and transient electrical response of the cells, indicating that the heteromeric OR/Orco complexes operate as odorant-activated ionotropic receptors, i.e., as ligand-gated ion channels [20, 24, 25]. Subsequent studies revealed that in OR/Orco complexes, the ligand-binding OR subunit and Orco contribute to the permeability of the ion channel, suggesting that both proteins participate in the formation of the ion pore [12, 24, 192]. Since neither the sequences of Orco nor of any of the ligand-binding OR types display homology to known pore domains in other ion channels, insect OR/Orco complexes appear to be endowed with a novel structural domain for ion permeability and selectivity [12, 199].

Odorant-induced transduction processes in OSNs: ionotropic versus G protein-mediated signaling

Although considerable evidence has been accumulated that OR/Orco complexes function as ionotropic receptors, the mechanisms underlying signal transduction in insect OSNs are still a matter of controversial discussion. The concept that signal transduction via OR/Orco complexes is exclusively ionotropic (Fig. 4a) was supported by experiments

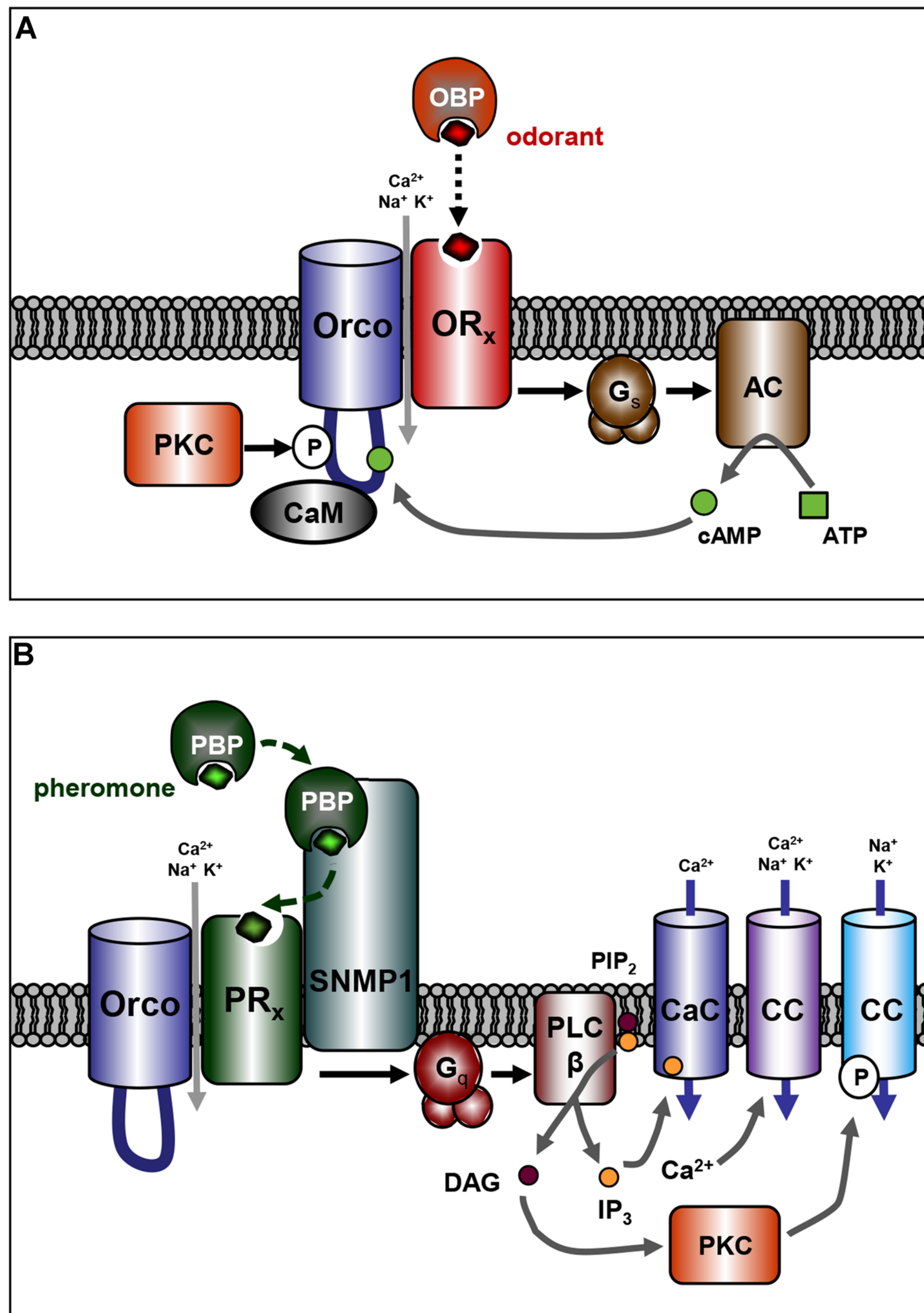

Fig. 4 Olfactory signal transduction in insects. **a** For receiving "general" odorants (e.g., food odors), OBPs transfer odorants to a specific OR (OR_X) that forms a ligand-activated receptor/ion channel complex with the OR co-receptor (Orco). Binding of an odorant to the OR_X subunit elicits opening of the non-selective cation channel and activates an ionotropic current. In parallel, the variable OR_X subunit activates a G protein ($G\alpha_s$), leading to increased production of the second messenger cAMP via enhancing the activity of adenylyl cyclase (AC). The secondary messenger substance opens the heteromeric OR_X/Orco channel or may activate homomeric Orco channels (not shown). The activity of Orco is also regulated by calmodulin (CaM) or phosphorylation through protein kinase C (PKC). **b** In pheromone-responsive OSNs, SNMP1 acts as co-receptor. SNMP1 is supposed to dock ligand-loaded PBPs near a given PR (PR_X) and/or is involved in the release of the pheromone to the PR. The PR_X/Orco channel complex opens following binding of the pheromone to the PR_X subunit leading to an influx of cations into the cell. Alternatively or in parallel, pheromone binding to the PR_X may activate a G protein-mediated ($G\alpha_q$) pathway in pheromone-sensitive cells. Pheromone-induced activation of Gq protein leads to an increased activity of phospholipase C (PLCβ) that converts phosphoinositol-(4,5)-biphosphate (PIP_2) into inositol trisphosphate (IP_3) and diacylglycerol (DAG). The increase in IP_3 opens a calcium-selective ion channel (CaC) in the plasma membrane. The rise of Ca^{2+} in turn opens Ca^{2+}-activated cation channels (CC). Increase in Ca^{2+} and DAG stimulates PKC activity, leading to activation of cation channels

with OR/Orco-expressing cell lines in which inhibitors of G proteins (such as the guanosine diphosphate analogue GDP-β S) failed to block odorant-induced responses [20, 24]. Furthermore, in single sensillum recordings from *Drosophila* mutants deficient of G protein alpha subunits, no change in odor sensitivity was observed, indicating that G proteins are not required to elicit odorant-evoked responses [200]. In marked contrast to these observations, a number of studies reported that odorant-induced responses in insects were substantially affected by appropriate inhibitors or activators of G protein signaling cascades. Consistently, it was found that various elements of G protein-mediated transduction pathways (including G proteins) are expressed in OSNs of insect antennae [201–209]. In addition, in OSNs as well as in HEK cells expressing ORs or OR/Orco, odorants induced the formation of the second messenger cyclic adenosine monophosphate (cAMP) and an activation of cAMP-gated channels. These responses were dependent on the G protein subunit $G\alpha_s$ or were significantly enhanced by $G\alpha_s$ [25, 197, 210]. Noteworthy, odorants also evoked G protein-mediated responses in HEK cells that expressed only ligand-binding ORs but lacked Orco suggesting that the ligand-binding ORs are sufficient to activate G proteins [87, 90, 122]. Therefore, although insect ORs are structurally distinct from typical GPCRs, they seem to be capable of operating as GPCRs [25, 197].

In insect OSNs, enhanced cAMP synthesis was found to cause increased spike rates [197]. Although the reason is unclear, it is interesting to note that Wicher and co-workers [25] observed that in heterologous systems, elevated cAMP concentrations led to an activation of Orco and OR/Orco complexes. The cAMP-evoked activation of Orco was not dependent on protein kinase A (PKA) [25] but seemed to rely on protein kinase C (PKC) and phospholipase C (PLC) signaling. Inhibition of PKC or PLC caused a reduced sensitivity of Orco to cAMP while stimulation of PKC led to an activation of Orco even in the absence of cAMP. Consistently, elimination of PKC phosphorylation sites in the Orco protein largely reduced its sensitivity to cAMP. Compatible with these observations, agents and mutations affecting PLC and/or PKC alter odorant-evoked responses of OSNs [211, 212]. Collectively, these findings suggest that both a $G\alpha_s$/cAMP pathway as well as a PLC/PKC pathway are involved in the olfactory signaling process and support the concept that activation of ORs by odorants initiates metabotropic reaction cascades (reviewed by [198, 213]).

At this point, it appears that insect ORs can play different and complex functional roles. This includes: (1) the OR/Orco complex can operate as odorant-activated ionotropic channel. (2) In the OR/Orco complex, the ligand-binding ORs can function as non-classical GPCRs activating G proteins leading to an increased cAMP concentration and/or enhanced PKC activity. (3) Orco can serve as a cAMP-activated channel whose activity is regulated by PLC/PKC signaling.

At the moment, it is difficult to reconcile the findings that either favor ionotropic or metabotropic signaling. However, it is conceivable that both aspects are relevant for insect OSNs as initially suggested by Wicher and co-workers [25] who noted that in a heterologous system the odorant-induced responses were composed of a rapid ionotropic and a slower but more sensitive metabotropic component; the latter was based on G protein signaling. Consequently, a model for a dual activation of OR signaling has been proposed [25, 213]. According to this model, an odorant-induced activation of ORs results in a fast ionotropic response followed by a slower but more sensitive metabotropic reaction that leads to a regulation of the cation channel formed by OR/Orco complexes (Fig. 4a). Thus, the ionotropic mechanism provides a direct and rapid response while the metabotropic signaling process might boost low signals due to signal amplification. Such interplay of the two mechanisms would allow signal detection over a broad range of odorant concentrations [214]. The ability to detect odors rapidly and with high sensitivity might be particularly important for flying insects since both speed and sensitivity are presumably crucial for tracking turbulent odor plumes encountered by insects during flight. Therefore, the complex ionotropic and metabotropic OR-mediated signaling mechanisms in insect OSNs might represent special adaptations to the evolution of ORs and flight in insects. This notion is in line with the finding that primitive and primarily wingless insects lack ORs or have only Orco-related genes [22]. By contrast, the other large group of insect olfactory receptors, the IRs, is not only found in insects but exists throughout the protostomes [22, 75]. Moreover, signaling through IRs is supposed to be solely ionotropic (as discussed below) and neurons expressing IRs are less sensitive [214, 215].

Pheromone signaling in moths: independent of Orco?

A high sensitivity of OSNs is supposed to be particularly important for the detection of pheromonal compounds in moth species since the males are capable of locating sex pheromone-releasing female conspecifics over long distances [216, 217]. According to the observation that Orco is co-expressed with PRs in OSNs of sex pheromone-responsive trichoid sensilla of *D. melanogaster* [174] and male moths [86, 89, 133], it has been proposed that the transduction of pheromone signals in insects is also generally based on a heteromeric complex of a ligand-binding PR and Orco. However, recent studies on the hawkmoth *Manduca sexta* found no evidence for a PR/Orco-based ionotropic pheromone transduction and negated a role of Orco in the primary transduction events [196, 218]. Instead, an alternative role of Orco in moth pheromone-responsive OSNs

was suggested. In tip recordings from intact sensilla, activation of Orco by the agonistic substance VUAA1 elicited an increased spontaneous activity that was reduced by the Orco antagonist OLC15. In addition, Orco from *M. sexta* expressed in a heterologous system formed a spontaneously active cation channel. Based on these findings, it has been proposed that in moth pheromone-sensitive OSNs, Orco serves as a voltage-gated and apparently second messenger-gated pacemaker channel, controlling the membrane potential and thus the threshold and kinetics of the pheromone responses. These findings in *M. sexta* also fueled the concept that in moth sex pheromone signaling, the transduction via receptors may be exclusively metabotropic [196, 198, 218]. In this regard, it is noteworthy that early studies have shown that pheromone stimulation of antennal tissues elicited the formation of the second messenger inositol 1,4,5-trisphosphate (IP_3) [219–221]. These observations were extended by studies reporting at least three electrical currents in cultured moth OSNs upon exposure to pheromones. A first and very rapid Ca^{2+} current that declines within several milliseconds. A second and slower current that is dependent on extracellular Ca^{2+} and declines within less than 3 s. And finally, a third and sustained inward current that lasts over several seconds and is Ca^{2+}-independent. Interestingly, perfusion of cultured moth OSNs with IP_3 elicits a similar sequence of inward currents that strongly resemble pheromone-evoked currents [222–224]. Although the molecular elements underlying the pheromone-evoked currents are still largely unclear, the similarities between the pheromone- and IP_3-induced currents in OSNs indicate that pheromones activate a metabotropic signaling pathway mediated by PLC type β, leading to an enhanced formation of IP_3 and diacylglycerol (DAG) via hydrolysis of the membrane lipid phosphatidyl inositol-bisphosphate (PIP_2). Consequently, activation of IP_3-gated Ca^{2+} channels in the membrane of OSNs leads to a rise in Ca^{2+} that rapidly opens Ca^{2+}-activated cation channels. In addition, the diacylglycerol might enhance PKC activity, thus eliciting the third pheromone-evoked inward current that is supposed to rely on a PKC-activated cation channel [198, 217] (Fig. 4b).

It is a matter of debate whether a metabotropic pheromone transduction process that includes a series of enzyme-catalyzed reactions is fast enough for the required physiological responses. Depending on their flapping frequency, flying insects sample odorants/pheromones approximately every 30 ms [225–227]; reviewed by [198]. In this context, it is interesting to note that the process of insect phototransduction is based on a PLC-mediated cascade and photoreceptors nevertheless have a high temporal resolution following light stimuli with up to 300 Hz [228, 229]. The high speed of phototransduction is supposed to rely on the clustering of the relevant signaling elements in multiprotein complexes called transducisomes or signalosomes (reviewed by [230]). Although it is unknown whether signaling proteins in the dendritic processes of insect OSNs are arranged in multiprotein complexes, the example of phototransduction indicates that a G protein-mediated reaction cascade could be indeed fast enough for a rapid response to pheromone signals.

Perireceptor events in olfactory signaling: relevance of OBPs and SNMP1

To reach the receptor proteins in the dendritic membranes of insect OSNs and to trigger signal transduction processes, odorants first have to overcome the aqueous sensillum lymph. A wealth of studies support the notion that small (13–17 kDa) and water-soluble odorant-binding proteins (OBPs) solubilize the often hydrophobic odorant molecules in the lymph after they have entered the sensillum through cuticular pores and mediate their transfer to ORs [8, 9, 180, 231–234] (Fig. 4a). The number of different OBPs considerably varies between insect species. Some species possess up to several dozens of OBP-encoding genes many of which are expressed in the antenna [234–237]. This diversity of OBPs supports the view that OBPs are not just simple general solubilizers and transporters for odorants but in addition make a decisive contribution to odorant recognition and may interact with distinct ORs [8, 180, 184]. Yet, some OBP types are apparently not required for odorant transport but seem to play a role in buffering changes in the odor environment [179]. Consistent with the role of OBPs as odorant transporters and interaction partners of ORs, ligand specificity and an interplay with distinct receptor types has been documented for the so-called pheromone-binding proteins (PBPs) [90, 114, 122] that comprise a subfamily of OBPs binding and transporting pheromone molecules to PRs (Fig. 4b). In contrast to the ligand specificity observed for some PBPs, distinct OBP types in a given species can have overlapping and rather broad ligand spectra [184, 238]. Thus, a single OBP may be involved in the detection of several compounds while different OBPs may contribute to the recognition of a given odorant, a scenario corroborated by results of a comprehensive analysis of 17 *Drosophila* OBPs [184].

Although OBPs are generally supposed to serve as passive carrier proteins transporting odorants through the lymph to appropriate ORs [239–241], some studies suggest that OR activation may depend on the OBP/ligand complex [242–244]. For pheromone detection in *Drosophila*, it has been reported that the ligand for activation of the receptor OR67d is not the free pheromone cis-vaccenyl acetate (cVA) but a PBP called LUSH [245] that is ''conformationally activated'' upon binding of cVA [242, 246]. However, this mode of OR activation was called into question by a recent study demonstrating that OR67d can be activated directly by cVA in the absence of LUSH [247].

For sensitive pheromone signaling in insects, besides PBPs and PRs, the "sensory neuron membrane protein 1" (SNMP1), seems to be required [36, 146, 248] (Fig. 4b). SNMP1 comprises two transmembrane domains and shares this structural feature and some sequence identity with the mammalian CD36 scavenger receptor family [249, 250]. Interestingly, recent studies found that CD36 is also expressed in subpopulations of murine OSNs and may be involved in odorant detection in mammals [251–253]. In insects, SNMP1 is co-expressed with PRs in pheromone-responsive OSNs [36, 254–258] and located in close proximity to the receptor in the membrane [36, 131]. PR-expressing OSNs require SNMP1 for proper function [36] and the protein appears to contribute to the remarkable sensitivity of pheromone detection. Moreover, it is required for rapid activation and termination of pheromone-induced activity [248, 255]. Since its discovery, SNMP1 has been proposed to function as a co-receptor that may help to unload the pheromone from the binding protein or to pass the signal molecules to the PR [9, 249, 259]. Although the precise functional relevance of SNMP1 for pheromone signaling is still unclear, a most recent study provided first evidence that SNMP1 indeed might funnel hydrophobic pheromones from the extracellular fluid to PRs in the cell membrane [37].

In addition to SNMP1, a second SNMP has been reported. Similar to SNMP1, the "sensory neuron membrane protein 2" (SNMP2) belongs to the CD36 family [260], is abundant in pheromone-sensitive sensilla of moth and is also expressed in other chemosensory organs, including the proboscis and the maxillary palps [261]. In the antenna, SNMP1 is specifically localized in the dendritic membrane of antennal OSNs [249, 262] whereas SNMP2 is expressed in supporting cells of sensilla [254, 257], suggesting a differential function of these two proteins. While SNMP1 is supposed to play a role in pheromone detection, SNMP2 has been proposed to be involved in the clearance of the sensillum lymph [254].

Gustatory receptors (GRs) as receptors for CO_2 and pheromones

In insects, gustatory cues are detected via specialized sensory neurons residing in taste sensilla on the mouthparts, tarsi, wings and ovipositors [263]. For the detection of sweet and bitter tasting substances, gustatory neurons are equipped with GRs [264]. In *D. melanogaster*, the GR family comprises 68 members; at least four GRs were found to be expressed in given OSNs of the antenna [265, 266], including GR types GR21a and GR63a that are required for responsiveness to CO_2 [44, 267]. While CO_2 is a stress signal in flies, it is also used to find fermenting food [268–270]. Moreover, blood-feeding female mosquitoes locate hosts via plumes of exhaled CO_2. Accordingly, orthologues of *Drosophila* CO_2 receptors have been identified in mosquito OSNs from capitate peg sensilla on the maxillary palps; these receptors were named Gr1, Gr2 and Gr3 in *Aedes aegypti* or Gr22, Gr23 and Gr24 in *A. gambiae*, respectively [45, 46]. Somewhat surprisingly, recent analyses revealed that mosquito CO_2 receptors also respond to an array of odorants from human skin, indicating that the relevant OSNs on the maxillary palps can react to both CO_2 and skin odors, thus informing the mosquitoes about the proximity of human hosts [271, 272]. Beyond flies and mosquitoes, carbon dioxide receptor-encoding genes have been reported for a number of other insects including moth and beetle species [273–275] in which CO_2 is an important volatile cue attracting adults or larvae to appropriate food sources.

In addition to a role of GR types in sensing CO_2, studies using *Drosophila* flies indicate that some GRs are involved in pheromone detection and are required for sexual behaviors [35, 276]. For example, Gr39a is supposed to be involved in the reception of a female pheromone. Knockdown of this receptor type led to reduced courtship behavior in males [277]. Similarly, the detection of courtship-inhibiting cuticular hydrocarbons present on the cuticle surface of both males and females is mediated by Gr32a [278], probably together with Gr33a [279]. Moreover, the receptor type Gr68a in males seems to be activated by female pheromones [280]. Gr68a may also be activated by male-produced compounds inhibiting the courtship behavior of males and serve as receptor for an anti-aphrodisiac agent [175, 281, 282]. Importantly, although such GR types are involved in the detection of pheromones that are usually considered as olfactory signals, they are expressed in gustatory cells of the labellum and the legs/tarsi [278–280, 283].

The membrane topology of insect GRs is unclear. A recent study using two different GR types from *B. mori* has provided first evidence that insect GRs—similar to insect ORs—have an inverted topology relative to GPCRs, i.e., they are endowed with an intracellular N-terminus, an extracellular C-terminus and an odd number of transmembrane spans [284] (Fig. 2). Also the molecular mechanisms underlying the GR-mediated transduction of chemical cues (including CO_2 and pheromones) in chemosensory neurons are largely elusive [276]. Studies on GR-mediated responses to sugars in *Drosophila* gustatory neurons revealed a requirement for G protein signaling [285]. By contrast, analyses of the *B. mori* receptor Gr-9 in Xenopus oocytes provided evidence that BmGr-9 is a ligand-gated ion channel activated by fructose [286]. Thus, distinct GR proteins may signal through different (metabotropic or ionotropic) mechanisms.

Ionotropic receptors (IRs)

Besides ORs and GRs, a third class of chemosensory receptors in the olfactory system of insects was recently

discovered, the ionotropic receptors (IRs) [41]. While the expression of ORs and olfactory GRs seems to be restricted (with few exceptions) to sensilla trichodea and sensilla basiconica, electrophysiological recordings have demonstrated that also OSNs in antennal sensilla coeloconica respond to chemical stimuli, primarily to organic acids and amines [287]. In search for the receptor types rendering these cells responsive to chemical compounds, a number of genes expressed in the antennae of *D. melanogaste*r that encode proteins related to ionotropic glutamate receptors (iGluRs) were identified and annotated as ionotropic receptors (IRs) [41, 288]. Subsequently, IR expression in the antenna has been reported for numerous insect species, such as Drosophilids, mosquitoes and locusts [75, 289, 290].

According to predictions based on amino acid sequences, IRs and iGluRs share similar molecular structures including an extracellular N-terminus, a cytoplasmic C-terminus as well as a bipartite ligand-binding domain and an ion channel domain [291]. The stretch of amino acids forming the ion channel domain is the most conserved region between IRs and iGluRs, indicating that IRs function as ion channels. By contrast, the predicted ligand-binding sites are more variable among the IRs and substantially differ from those of the iGluRs. These observations have led to the notion that glutamate is unlikely to be the cognate ligand for the IRs and opened the door for the concept that the diverse IR subtypes may be activated by a variety of different chemicals [41].

Comparative genomic analyses of the IR repertoire across various animal groups revealed that contrary to the insect-specific ORs, IRs are found throughout the protostomes, including nematodes, arthropods, molluscs and annelids. Yet, in contrast to the related iGluRs, they are absent from Deuterostomia. It has, therefore, been proposed that IRs may have evolved from iGluRs in ancient protostomes [22, 75].

The IR repertoire of an insect has been analyzed best in *D. melanogaster* in which the IR-encoding gene family comprises 66 members [75]. Overall, the amino acid sequence identity between *Drosophila* IRs ranges from 10–70%, suggesting functional diversity [41]. Based on amino acid sequences and divergent expression patterns, the IR family can be divided in three subgroups. The first subgroup encompasses 16 IR types; most of them are specifically expressed in the antenna (and in few cases also in the proboscis) but are absent from other tissues. Consequently, these IR types have been termed "antennal IRs" or "olfactory IRs" [41, 42, 75]. Contrary to ORs, where only Orco is substantially conserved between different species, for many *Drosophila* "antennal IRs", clear orthologues exist in numerous insects [2, 22, 72, 75]. Since these orthologues are also expressed in antennae, the antenna-specific expression of "antennal IRs" seems to be evolutionary conserved among insects. Moreover, a few orthologues of insect "antennal IRs" have been also detected in other protostomes. Some orthologues of insect "antennal IRs" in other Protostomia, such as snails and lobsters, are expressed in olfactory organs as well [75]. Analyzing the expression pattern of "antennal IRs" in several insect species revealed that these receptors are expressed in OSNs located in sensilla coeloconica. They are mostly absent from basiconic and trichoid sensilla as well as from OSNs expressing Orco [41, 290] and each "antennal IR" type seems to be expressed in only a smaller subset of the coeloconic OSNs. However, some IR-positive OSNs express more than one "antennal IR" type [41]. Interestingly, some of the "antennal IRs" appear to function also in other sensory systems. In adult *Drosophila* flies, IR93a and IR40a are co-expressed in neurons of the third antennal segment surrounding the so-called sacculus of the antenna [41] and serve as dry-activated hygrosensors [292]. Together with another "antennal IR" type (IR21a), IR93a is also expressed in thermosensory dorsal organ cool cells in the head of *D. melanogaster* larvae and functions as a thermoreceptor [292, 293].

While the "antennal IRs" account for only a smaller fraction of the IR repertoire in insects and other protostomes, the majority of the IRs belong to the second IR subgroup that has been largely expanded in dipterans, encompassing 48 genes (including 9 pseudogenes) in *D. melanogaster* [75]. Yet, analyzing the genomes of diverse *Drosophila* and mosquito species, no obvious orthologous relationships were found for these IR types. Thus, these receptors are largely species-specific forming a number of species-specific clades in phylogenetic trees. In addition, they share low amino acid sequence identity (as little as 8.5%) with other IRs in either the same or different species. Therefore, these IRs have been termed "divergent IRs" [75]. In marked contrast to the "antennal IRs", "divergent IRs" appear to be absent from antennae [41]. However, expression of some "divergent IRs" in insects has been reported for gustatory organs (e.g., the labellum), indicating a role for gustation [75].

Besides "antennal IRs" and "divergent IRs", a third IR group comprises the receptors IR25a and IR8a that are conserved among protostomes (IR25a) and insects (IR8a). In contrast to other insect IRs, IR25a and IR8a have retained an amino-terminal domain characteristic for iGluRs [41, 75]. IR25a and IR8a are broadly expressed in coeloconic OSNs of the antenna along with "antennal IRs". Although it cannot be excluded that IR25a and IR8a bind to given chemical ligands, their co-expression with "antennal IR" types suggests that they might acts as co-receptors, analogous to the heteromeric assembly of ligand-specific ORs with Orco [41, 75] (Fig. 2). This concept was supported by the observation that null mutations of IR8a and IR25a abolished odorant-evoked responses of IR-expressing OSNs in flies. Moreover, heterologous expression experiments with IR84 and IR8a demonstrated that co-expression of IR8a was required for responsiveness to the IR84 ligand phenylacetaldehyde [288]. Reconstitution of functional IRs in Xenopus oocytes

has allowed stoichiometric analysis of IR complexes in the plasma membrane. These studies indicated that the ligand-specific receptor and the co-receptor in fact form heterotetrameric complexes [42, 288]. Thus, the molecular architecture of functional IRs comprising a specific ligand-detecting IR and a co-receptor (IR25a or IR8a) is reminiscent of the OR/Orco complex [288, 294]. It is still elusive how the IR/co-receptor complex may recognize the ligand; however, recent results are consistent with a mechanism of ligand binding similar to that described for iGluRs [42].

The finding that clear orthologues for many "antennal IRs" exist in most or even all insect species suggests that the ligands for IRs are in general essential chemosensory cues for insects [42]. Determining the odor response profiles of IR-expressing coeloconic OSNs by electrophysiological recordings identified multiple agonists, the vast majority belonging to two chemical classes, amines and carboxylic acids [287, 295]. The ligand spectrum of IR-expressing OSNs appears to be largely complementary to that of OR-expressing OSNs, suggesting that insects are endowed with two distinct but complementary olfactory subsystems encompassing OSNs that either express IRs or ORs [295]. More recent studies imply that some IR subtypes are involved in the detection of food-derived odors [296] whereas others are tuned to polyamines, which seems to be important for the development of offspring [297].

Concluding remarks

The progress towards an understanding of how insects perceive the composition of chemical compounds in their surrounding is remarkable. On the level of odor recognition and discrimination, we now know that insects have co-opted two main receptor families, the IRs and the ORs. The later turned out to be a unique family of 7-TMD proteins that are not classical GPCRs but rather have an inverse membrane topology. They form heteromeric complexes composed of a ligand-tuning receptor subunit together with the well-conserved co-receptor subunit Orco and are supposed to mediate ionotropic and metabotropic signaling processes. However, many questions remain to be answered, including the molecular features of the receptor protein that determine the ligand specificity and the molecular processes that contribute to the striking sensitivity of the insect olfactory system. In this context, novel groundbreaking gene editing techniques (such as CRISPR/Cas9) might help to unravel the functional relevance(s) as well as the molecular properties of ORs and other proteins involved in olfactory signaling. Looking ahead, the molecular principles of insect olfaction remain exciting areas for future research since more detailed insight into the mechanisms underlying the sensing of chemical signals will not only contribute to a better understanding of insect behavior and physiology but may also open new avenues for the development of more specific and sustainable approaches to control pest insects.

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References

1. Gadenne C, Barrozo RB, Anton S (2016) Plasticity in insect olfaction: to smell or not to smell? Ann Rev Entomol 61:317–333. doi:10.1146/annurev-ento-010715-023523
2. Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. Neuron 72(5):698–711. doi:10.1016/j.neuron.2011.11.003
3. Schneider D (1969) Insect olfaction: deciphering system for chemical messages. Science 163(3871):1031–1037. doi:10.1126/science.163.3871.1031
4. Wyatt TD (2014) Pheromones and animal behavior. Cambridge Univ Press, Cambridge
5. Schneider D (1992) 100 Years of pheromone research—an essay on lepidoptera. Naturwissenschaften 79(6):241–250. doi:10.1007/Bf01175388
6. Bentley MD, Day JF (1989) Chemical ecology and behavioral aspects of mosquito oviposition. Ann Rev Entomol 34:401–421. doi:10.1146/annurev.en.34.010189.002153
7. Steinbrecht RA (1997) Pore structures in insect olfactory sensilla: a review of data and concepts. Int J Insect Morphol Embryol 26(3–4):229–245. doi:10.1016/S0020-7322(97)00024-X
8. Leal WS (2013) Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu Rev Entomol 58:373–391. doi:10.1146/annurev-ento-120811-153635
9. Vogt RG (2003) Biochemical diversity of odor detection: OBPs, ODEs and SNMPs. In: Blomquist G, Vogt RG (eds) Insect pheromone biochemistry and molecular biology. The biosynthesis and detection of pheromones and plant volatiles. Elsevier Academic Press, London, pp 391–445
10. de Bruyne M, Clyne PJ, Carlson JR (1999) Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. J Neurosci 19(11):4520–4532
11. Stensmyr MC, Giordano E, Balloi A, Angioy AM, Hansson BS (2003) Novel natural ligands for *Drosophila* olfactory receptor neurones. J Exp Biol 206(Pt 4):715–724. doi:10.1242/jeb.00143
12. Nakagawa T, Pellegrino M, Sato K, Vosshall LB, Touhara K (2012) Amino Acid residues contributing to function of the heteromeric insect olfactory receptor complex. PLoS ONE 7(3):e32372. doi:10.1371/journal.pone.0032372
13. Clements JD (1996) Transmitter timecourse in the synaptic cleft: its role in central synaptic function. Trends Neurosci 19(5):163–171. doi:10.1016/S0166-2236(96)10024-2
14. Bohbot J, Pitts RJ (2015) The narrowing olfactory landscape of insect odorant receptors. Front Ecol Evol. doi:10.3389/fevo.2015.00039
15. Suh E, Bohbot J, Zwiebel LJ (2014) Peripheral olfactory signaling in insects. Curr Opin Insect Sci 6:86–92. doi:10.1016/j.cois.2014.10.006
16. Wicher D (2015) Olfactory signaling in insects. Prog Mol Biol Transl Sci 130:37–54. doi:10.1016/bs.pmbts.2014.11.002
17. Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR (1999) A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron 22(2):327–338. doi:10.1016/S0896-6273(00)81093-4

18. Gao Q, Chess A (1999) Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. Genomics 60(1):31–39. doi:10.1006/geno.1999.5894
19. Benton R, Sachse S, Michnick SW, Vosshall LB (2006) Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. PLoS Biol 4(2):e20. doi:10.1371/journal.pbio.0040020
20. Smart R, Kiely A, Beale M, Vargas E, Carraher C, Kralicek AV, Christie DL, Chen C, Newcomb RD, Warr CG (2008) *Drosophila* odorant receptors are novel seven transmembrane domain proteins that can signal independently of heterotrimeric G proteins. Insect Biochem Mol Biol 38(8):770–780. doi:10.1016/j.ibmb.2008.05.002
21. Lundin C, Kall L, Kreher SA, Kapp K, Sonnhammer EL, Carlson JR, Heijne G, Nilsson I (2007) Membrane topology of the *Drosophila* OR83b odorant receptor. FEBS Lett 581(29):5601–5604. doi:10.1016/j.febslet.2007.11.007
22. Missbach C, Dweck HK, Vogel H, Vilcinskas A, Stensmyr MC, Hansson BS, Grosse-Wilde E (2014) Evolution of insect olfactory receptors. Elife 3:e02115. doi:10.7554/eLife.02115
23. Robertson HM, Warr CG, Carlson JR (2003) Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc Natl Acad Sci USA 100:14537–14542. doi:10.1073/pnas.2335847100
24. Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K (2008) Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452(7190):1002–1006. doi:10.1038/nature06850
25. Wicher D, Schafer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS (2008) *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452(7190):1007–1011. doi:10.1038/nature06861
26. Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R (1999) A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 96(5):725–736. doi:10.1016/S0092-8674(00)80582-6
27. Hallem EA, Ho MG, Carlson JR (2004) The molecular basis of odor coding in the *Drosophila* antenna. Cell 117(7):965–979. doi:10.1016/j.cell.2004.05.012
28. Bushdid C, Magnasco MO, Vosshall LB, Keller A (2014) Humans can discriminate more than 1 trillion olfactory stimuli. Science 343:1370–1372. doi:10.1126/science.1249168
29. Touhara K, Vosshall LB (2009) Sensing odorants and pheromones with chemosensory receptors. Annu Rev Physiol 71:307–332. doi:10.1146/annurev.physiol.010908.163209
30. Andersson MN, Löfstedt C, Newcomb RD (2015) Insect olfaction and the evolution of receptor tuning. Front Ecol Evol 3(53):1–14. doi:10.3389/fevo.2015.00053
31. Stensmyr MC, Dweck HK, Farhan A, Ibba I, Strutz A, Mukunda L, Linz J, Grabe V, Steck K, Lavista-Llanos S, Wicher D, Sachse S, Knaden M, Becher PG, Seki Y, Hansson BS (2012) A conserved dedicated olfactory circuit for detecting harmful microbes in *Drosophila*. Cell 151(6):1345–1357. doi:10.1016/j.cell.2012.09.046
32. Dweck HK, Ebrahim SA, Kromann S, Bown D, Hillbur Y, Sachse S, Hansson BS, Stensmyr MC (2013) Olfactory preference for egg laying on citrus substrates in *Drosophila*. Curr Biol 23(24):2472–2480. doi:10.1016/j.cub.2013.10.047
33. Berg BG, Zhao XC, Wang G (2014) Processing of pheromone information in related species of heliothine moths. Insects 5(4):742–761. doi:10.3390/insects5040742
34. Zhang J, Walker WB, Wang G (2015) Pheromone reception in moths: from molecules to behaviors. Prog Mol Biol Transl Sci 130:109–128. doi:10.1016/bs.pmbts.2014.11.005
35. Kohl J, Huoviala P, Jefferis GS (2015) Pheromone processing in *Drosophila*. Curr Opin Neurobiol 34:149–157. doi:10.1016/j.conb.2015.06.009
36. Benton R, Vannice KS, Vosshall LB (2007) An essential role for a CD36-related receptor in pheromone detection in *Drosophila*. Nature 450(7167):289–293. doi:10.1038/nature06328
37. Gomez-Diaz C, Bargeton B, Abuin L, Bukar N, Reina JH, Bartoi T, Graf M, Ong H, Ulbrich MH, Masson JF, Benton R (2016) A CD36 ectodomain mediates insect pheromone detection via a putative tunnelling mechanism. Nat Commun 7:11866. doi:10.1038/ncomms11866
38. Jacquin-Joly E, Merlin C (2004) Insect olfactory receptors: contributions of molecular biology to chemical ecology. J Chem Ecol 30(12):2359–2397. doi:10.1007/s10886-004-7941-3
39. Wang G, Carey AF, Carlson JR, Zwiebel LJ (2010) Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. Proc Natl Acad Sci USA 107(9):4418–4423. doi:10.1073/pnas.0913392107
40. Montagne N, de Fouchier A, Newcomb RD, Jacquin-Joly E (2015) Advances in the identification and characterization of olfactory receptors in insects. Prog Mol Biol Transl Sci 130:55–80. doi:10.1016/bs.pmbts.2014.11.003
41. Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB (2009) Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136(1):149–162. doi:10.1016/j.cell.2008.12.001
42. Rytz R, Croset V, Benton R (2013) Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. Insect Biochem Mol Biol 43(9):888–897. doi:10.1016/j.ibmb.2013.02.007
43. Olivier V, Monsempes C, Francois MC, Poivet E, Jacquin-Joly E (2011) Candidate chemosensory ionotropic receptors in a Lepidoptera. Insect Mol Biol 20(2):189–199. doi:10.1111/j.1365-2583.2010.01057.x
44. Kwon JY, Dahanukar A, Weiss LA, Carlson JR (2007) The molecular basis of CO_2 reception in *Drosophila*. Proc Natl Acad Sci USA 104(9):3574–3578. doi:10.1073/pnas.0700079104
45. Robertson HM, Kent LB (2009) Evolution of the gene lineage encoding the carbon dioxide receptor in insects. J Insect Sci 9:19. doi:10.1673/031.009.1901
46. Lu T, Qiu YT, Wang G, Kwon JY, Rutzler M, Kwon HW, Pitts RJ, van Loon JJ, Takken W, Carlson JR, Zwiebel LJ (2007) Odor coding in the maxillary palp of the malaria vector mosquito *Anopheles gambiae*. Curr Biol 17(18):1533–1544. doi:10.1016/j.cub.2007.07.062
47. Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65(1):175–187. doi:10.1016/0092-8674(91)90418-X
48. Ben-Arie N, Lancet D, Taylor C, Khen M, Walker N, Ledbetter DH, Carrozzo R, Patel K, Sheer D, Lehrach H et al (1994) Olfactory receptor gene cluster on human chromosome 17: possible duplication of an ancestral receptor repertoire. Hum Mol Genet 3(2):229–235. doi:10.1093/hmg/3.2.229
49. Ngai J, Dowling MM, Buck L, Axel R, Chess A (1993) The family of genes encoding odorant receptors in the channel catfish. Cell 72(5):657–666. doi:10.1016/0092-8674(93)90395-7
50. Nef S, Allaman I, Fiumelli H, De Castro E, Nef P (1996) Olfaction in birds: differential embryonic expression of nine putative odorant receptor genes in the avian olfactory system. Mech Dev 55(1):65–77. doi:10.1016/0925-4773(95)00491-2
51. Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell 83(2):207–218. doi:10.1016/0092-8674(95)90162-0
52. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF,

George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YH, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos PV, Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Cherry JM, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Mays AD, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferriera S, Fleischmann W, Fosler C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Gorrell JH, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina NV, Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacleb JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RD, Scheeler F, Shen H, Shue BC, Siden-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirskas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, WoodageT Worley KC, Wu D, Yang S, Yao QA, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM, Venter JC (2000) The genome sequence of *Drosophila melanogaster*. Science 287(5461):2185–2195. doi:10.1126/science.287.5461.2185

53. Vosshall LB (2001) The molecular logic of olfaction in *Drosophila*. Chem Senses 26(2):207–213. doi:10.1093/chemse/26.2.207
54. Hallem EA, Carlson JR (2006) Coding of odors by a receptor repertoire. Cell 125(1):143–160. doi:10.1016/j.cell.2006.01.050
55. Kim J, Moriyama EN, Warr CG, Clyne PJ, Carlson JR (2000) Identification of novel multi-transmembrane proteins from genomic databases using quasi-periodic structural properties. Bioinformatics 16(9):767–775. doi:10.1093/bioinformatics/16.9.767
56. Fox AN, Pitts RJ, Robertson HM, Carlson JR, Zwiebel LJ (2001) Candidate odorant receptors from the malaria vector mosquito *Anopheles gambiae* and evidence of down-regulation in response to blood feeding. Proc Natl Acad Sci USA 98(25):14693–14697. doi:10.1073/pnas.261432998
57. Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Cravchik A, Collins FH, Robertson HM, Zwiebel LJ (2002) G protein-coupled receptors in *Anopheles gambiae*. Science 298(5591):176–178. doi:10.1126/science.1076196
58. Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB (2004) Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 43(5):703–714. doi:10.1016/j.neuron.2004.08.019
59. Vosshall LB, Hansson BS (2011) A unified nomenclature system for the insect olfactory coreceptor. Chem Senses 36(6):497–498. doi:10.1093/chemse/bjr022
60. Krieger J, Klink O, Mohl C, Raming K, Breer H (2003) A candidate olfactory receptor subtype highly conserved across different insect orders. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 189(7):519–526. doi:10.1007/s00359-003-0427-x
61. Jones WD, Nguyen TAT, Kloss B, Lee KJ, Vosshall LB (2005) Functional conservation of an insect odorant receptor gene across 250 million years of evolution. Curr Biol 15(4):R119–R121. doi:10.1016/j.cub.2005.02.007
62. Pitts RJ, Fox AN, Zwiebel LJ (2004) A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. Proc Natl Acad Sci USA 101(14):5058–5063. doi:10.1073/pnas.0308146101
63. Yang Y, Krieger J, Zhang L, Breer H (2012) The olfactory coreceptor Orco from the migratory locust (*Locusta migratoria*) and the desert locust (*Schistocerca gregaria*): identification and expression pattern. Int J Biol Sci 8(2):159–170. doi:10.7150/ijbs.8.159
64. Smadja C, Shi P, Butlin RK, Robertson HM (2009) Large gene family expansions and adaptive evolution for odorant and gustatory receptors in the pea aphid. Acyrthosiphon pisum. Mol Biol Evol 26(9):2073–2086. doi:10.1093/molbev/msp116
65. Pask GM, Jones PL, Rutzler M, Rinker DC, Zwiebel LJ (2011) Heteromeric anopheline odorant receptors exhibit distinct channel properties. PLoS ONE 6(12):e28774. doi:10.1371/journal.pone.0028774
66. Neuhaus EM, Gisselmann G, Zhang WY, Dooley R, Stortkuhl K, Hatt H (2005) Odorant receptor heterodimerization in the olfactory system of *Drosophila melanogaster*. Nat Neurosci 8(1):15–17. doi:10.1038/nn1371
67. Zhou X, Slone JD, Rokas A, Berger SL, Liebig J, Ray A, Reinberg D, Zwiebel LJ (2012) Phylogenetic and transcriptomic analysis of chemosensory receptors in a pair of divergent ant species reveals sex-specific signatures of odor coding. PLoS Genet 8(8):e1002930. doi:10.1371/journal.pgen.1002930
68. Nozawa M, Nei M (2007) Evolutionary dynamics of olfactory receptor genes in *Drosophila* species. Proc Natl Acad Sci USA 104(17):7122–7127. doi:10.1073/pnas.0702133104
69. Kirkness EF, Haas BJ, Sun W, Braig HR, Perotti MA, Clark JM, Lee SH, Robertson HM, Kennedy RC, Elhaik E, Gerlach D, Kriventseva EV, Elsik CG, Graur D, Hill CA, Veenstra JA, Walenz B, Tubio JM, Ribeiro JM, Rozas J, Johnston JS, Reese JT, Popadic A, Tojo M, Raoult D, Reed DL, Tomoyasu Y, Kraus E, Mittapalli O, Margam VM, Li HM, Meyer JM, Johnson RM, Romero-Severson J, Vanzee JP, Alvarez-Ponce D, Vieira FG, Aguade M, Guirao-Rico S, Anzola JM, Yoon KS, Strycharz JP, Unger MF, Christley S, Lobo NF, Seufferheld MJ, Wang N, Dasch GA, Struchiner CJ, Madey G, Hannick LI, Bidwell S, Joardar V, Caler E, Shao R, Barker SC, Cameron S, Bruggner RV, Regier A, Johnson J, Viswanathan L, Utterback TR, Sutton GG, Lawson D, Waterhouse RM, Venter JC, Strausberg RL, Berenbaum MR, Collins FH, Zdobnov EM, Pittendrigh BR (2010) Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proc Natl Acad Sci USA 107(27):12168–12173. doi:10.1073/pnas.1003379107
70. Koenig C, Hirsh A, Bucks S, Klinner C, Vogel H, Shukla A, Mansfield JH, Morton B, Hansson BS, Grosse-Wilde E (2015) A reference gene set for chemosensory receptor genes of *Manduca sexta*. Insect Biochem Mol Biol 66:51–63. doi:10.1016/j.ibmb.2015.09.007
71. de Fouchier A, Walker WB 3rd, Montagne N, Steiner C, Binyameen M, Schlyter F, Chertemps T, Maria A, Francois MC, Monsempes C, Anderson P, Hansson BS, Larsson MC, Jacquin-Joly E (2017) Functional evolution of Lepidoptera olfactory receptors revealed by deorphanization of a moth repertoire. Nat Commun 8:15709. doi:10.1038/ncomms15709
72. Robertson HM, Wanner KW (2006) The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. Genome Res 16(11):1395–1403. doi:10.1101/gr.5057506

73. Engsontia P, Sanderson AP, Cobb M, Walden KK, Robertson HM, Brown S (2008) The red flour beetle's large nose: an expanded odorant receptor gene family in *Tribolium castaneum*. Insect Biochem Mol Biol 38(4):387–397. doi:10.1016/j.ibmb.2007.10.005
74. Dippel S, Kollmann M, Oberhofer G, Montino A, Knoll C, Krala M, Rexer KH, Frank S, Kumpf R, Schachtner J, Wimmer EA (2016) Morphological and transcriptomic analysis of a beetle chemosensory system reveals a gnathal olfactory center. BMC Biol 14(1):90. doi:10.1186/s12915-016-0304-z
75. Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R (2010) Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. PLoS Genet 6(8):e1001064. doi:10.1371/journal.pgen.1001064
76. Penalva-Arana DC, Lynch M, Robertson HM (2009) The chemoreceptor genes of the waterflea *Daphnia pulex*: many Grs but no Ors. BMC Evol Biol 9:79. doi:10.1186/1471-2148-9-79
77. Evans JD, Brown SJ, Hackett KJ, Robinson G, Richards S, Lawson D, Elsik C, Coddington J, Edwards O, Emrich S, Gabaldon T, Goldsmith M, Hanes G, Misof B, Munoz-Torres M, Niehuis O, Papanicolaou A, Pfrender M, Poelchau M, Purcell-Miramontes M, Robertson HM, Ryder O, Tagu D, Torres T, Zdobnov E, Zhang GJ, Zhou X, Consortium iK (2013) The i5 K initiative: advancing arthropod genomics for knowledge, human health, agriculture, and the environment i5 K CONSORTIUM. J Heredity 104(5):595–600. doi:10.1093/jhered/est050
78. Kreher SA, Kwon JY, Carlson JR (2005) The molecular basis of odor coding in the *Drosophila* larva. Neuron 46(3):445–456. doi:10.1016/j.neuron.2005.04.007
79. Couto A, Alenius M, Dickson BJ (2005) Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. Curr Biol 15(17):1535–1547. doi:10.1016/j.cub.2005.07.034
80. Vosshall LB, Wong AM, Axel R (2000) An olfactory sensory map in the fly brain. Cell 102(2):147–159. doi:10.1016/S0092-8674(00)00021-0
81. Tanaka K, Uda Y, Ono Y, Nakagawa T, Suwa M, Yamaoka R, Touhara K (2009) Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. Curr Biol 19(11):881–890. doi:10.1016/j.cub.2009.04.035
82. Wanner KW, Anderson AR, Trowell SC, Theilmann DA, Robertson HM, Newcomb RD (2007) Female-biased expression of odourant receptor genes in the adult antennae of the silkworm, *Bombyx mori*. Insect Mol Biol 16(1):107–119. doi:10.1111/j.1365-2583.2007.00708.x
83. Karner T, Schneider I, Schultze A, Breer H, Krieger J (2015) Co-expression of six tightly clustered odorant receptor genes in the antenna of the malaria mosquito. Front Ecol Evol 3(26):1–8. doi:10.3389/fevo.2015.00026
84. Schymura D, Forstner M, Schultze A, Krober T, Swevers L, Iatrou K, Krieger J (2010) Antennal expression pattern of two olfactory receptors and an odorant binding protein implicated in host odor detection by the malaria vector *Anopheles gambiae*. Int J Biol Sci 6(7):614–626. doi:10.7150/ijbs.6.614
85. Sakurai T, Mitsuno H, Mikami A, Uchino K, Tabuchi M, Zhang F, Sezutsu H, Kanzaki R (2015) Targeted disruption of a single sex pheromone receptor gene completely abolishes in vivo pheromone response in the silkmoth. Sci Rep 5:11001. doi:10.1038/srep11001
86. Nakagawa T, Sakurai T, Nishioka T, Touhara K (2005) Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307(5715):1638–1642. doi:10.1126/science.1106267
87. Krieger J, Grosse-Wilde E, Gohl T, Breer H (2005) Candidate pheromone receptors of the silkmoth *Bombyx mori*. Eur J Neurosci 21(8):2167–2176. doi:10.1111/j.1460-9568.2005.04058.x
88. Krieger J, Grosse-Wilde E, Gohl T, Dewer YME, Raming K, Breer H (2004) Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). Proc Natl Acad Sci USA 101(32):11845–11850. doi:10.1073/pnas.0403052101
89. Grosse-Wilde E, Stieber R, Forstner M, Krieger J, Wicher D, Hansson BS (2010) Sex-specific odorant receptors of the tobacco hornworm *manduca sexta*. Front Cell Neurosci. doi:10.3389/fncel.2010.00022
90. Grosse-Wilde E, Gohl T, Bouche E, Breer H, Krieger J (2007) Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. Eur J Neurosci 25(8):2364–2373. doi:10.1111/j.1460-9568.2007.05512.x
91. Patch HM, Velarde RA, Walden KK, Robertson HM (2009) A candidate pheromone receptor and two odorant receptors of the hawkmoth *Manduca sexta*. Chem Senses 34(4):305–316. doi:10.1093/chemse/bjp002
92. Miura N, Nakagawa T, Tatsuki S, Touhara K, Ishikawa Y (2009) A male-specific odorant receptor conserved through the evolution of sex pheromones in *Ostrinia* moth species. Int J Biol Sci 5(4):319–330. doi:10.7150/ijbs.5.319
93. Miura N, Nakagawa T, Touhara K, Ishikawa Y (2010) Broadly and narrowly tuned odorant receptors are involved in female sex pheromone reception in Ostrinia moths. Insect Biochem Mol Biol 40(1):64–73. doi:10.1016/j.ibmb.2009.12.011
94. Bengtsson JM, Gonzalez F, Cattaneo AM, Montagne N, Walker WB, Bengtsson M, Anfora G, Ignell R, Jacquin-Joly E, Witzgall P (2014) A predicted sex pheromone receptor of codling moth *Cydia pomonella* detects the plant volatile pear ester. Front Ecol Evol 2(33):1–11. doi:10.3399/fevo.2014.00033
95. Dobritsa AA, Naters VDGV, Warr CG, Steinbrecht RA, Carlson JR (2003) Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron 37(5):827–841. doi:10.1016/S0896-6273(03)00094-1
96. Iatrou K, Biessmann H (2008) Sex-biased expression of odorant receptors in antennae and palps of the African malaria vector *Anopheles gambiae*. Insect Biochem Mol Biol 38(2):268–274. doi:10.1016/j.ibmb.2007.11.008
97. Bohbot J, Pitts RJ, Kwon HW, Rutzler M, Robertson HM, Zwiebel LJ (2007) Molecular characterization of the *Aedes aegypti* odorant receptor gene family. Insect Mol Biol 16(5):525–537. doi:10.1111/j.1365-2583.2007.00748.x
98. Liu Y, Gu S, Zhang Y, Guo Y, Wang G (2012) Candidate olfaction genes identified within the *Helicoverpa armigera* antennal transcriptome. PLoS ONE 7(10):e48260. doi:10.1371/journal.pone.0048260
99. An XK, Sun L, Liu HW, Liu DF, Ding YX, Li LM, Zhang YJ, Guo YY (2016) Identification and expression analysis of an olfactory receptor gene family in green plant bug *Apolygus lucorum* (Meyer-Dur). Sci Rep 6:37870. doi:10.1038/srep37870
100. Steinwender B, Thrimawithana AH, Crowhurst R, Newcomb RD (2016) Odorant receptors of the New Zealand endemic leafroller moth species *Planotortrix octo* and *P. excessana*. PLoS ONE 11(3):e0152147. doi:10.1371/journal.pone.0152147
101. Rodriguez I (2013) Singular expression of olfactory receptor genes. Cell 155(2):274–277. doi:10.1016/j.cell.2013.09.032
102. Malnic B, Hirono J, Sato T, Buck LB (1999) Combinatorial receptor codes for odors. Cell 96(5):713–723. doi:10.1016/S0092-8674(00)80581-4
103. Chess A, Simon I, Cedar H, Axel R (1994) Allelic inactivation regulates olfactory receptor gene expression. Cell 78(5):823–834. doi:10.1016/S0092-8674(94)90562-2
104. Monahan K, Lomvardas S (2015) Monoallelic expression of olfactory receptors. Ann Rev Cell Develop Biol 31:721–740. doi:10.1146/annurev-cellbio-100814-125308

105. Goldman AL, van Naters WV, Lessing D, Warr CG, Carlson JR (2005) Coexpression of two functional odor receptors in one neuron. Neuron 45(5):661–666. doi:10.1016/j.neuron.2005.01.025
106. Fishilevich E, Vosshall LB (2005) Genetic and functional subdivision of the *Drosophila* antennal lobe. Curr Biol 15(17):1548–1553. doi:10.1016/j.cub.2005.07.066
107. Ray A, van Naters WG, Shiraiwa T, Carlson JR (2007) Mechanisms of odor receptor gene choice in *Drosophila*. Neuron 53(3):353–369. doi:10.1016/j.neuron.2006.12.010
108. Koutroumpa FA, Kárpáti Z, Monsempes C, Hill SR, Hansson BS, Jacquin-Joly E, Krieger J, Dekker T (2014) Shifts in sensory neuron identity parallel differences in pheromone preference in the European corn borer. Front Ecol Evol. doi:10.3389/fevo.2014.00065
109. Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR (2010) Odorant reception in the malaria mosquito *Anopheles gambiae*. Nature 464(7285):66–71. doi:10.1038/nature08834
110. Slone J, Daniels J, Amrein H (2007) Sugar receptors in *Drosophila*. Curr Biol 17(20):1809–1816. doi:10.1016/j.cub.2007.09.027
111. Dahanukar A, Lei YT, Kwon JY, Carlson JR (2007) Two Gr genes underlie sugar reception in *Drosophila*. Neuron 56(3):503–516. doi:10.1016/j.neuron.2007.10.024
112. Mathew D, Martelli C, Kelley-Swift E, Brusalis C, Gershow M, Samuel AD, Emonet T, Carlson JR (2013) Functional diversity among sensory receptors in a *Drosophila* olfactory circuit. Proc Natl Acad Sci USA 110(23):2134–2143. doi:10.1073/pnas.1306976110
113. Sakurai T, Nakagawa T, Mitsuno H, Mori H, Endo Y, Tanoue S, Yasukochi Y, Touhara K, Nishioka T (2004) Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. Proc Natl Acad Sci USA 101(47):16653–16658. doi:10.1073/pnas.0407596101
114. Grosse-Wilde E, Svatos A, Krieger J (2006) A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. Chem Senses 31(6):547–555. doi:10.1093/chemse/bjj059
115. Wang G, Vasquez GM, Schal C, Zwiebel LJ, Gould F (2011) Functional characterization of pheromone receptors in the tobacco budworm *Heliothis virescens*. Insect Mol Biol 20(1):125–133. doi:10.1111/j.1365-2583.2010.01045.x
116. Wanner KW, Nichols AS, Allen JE, Bunger PL, Garczynski SF, Linn CE, Robertson HM, Luetje CW (2010) Sex pheromone receptor specificity in the European corn borer moth, *Ostrinia nubilalis*. PLoS ONE 5(1):e8685. doi:10.1371/journal.pone.0008685
117. Montagne N, Chertemps T, Brigaud I, Francois A, Francois MC, de Fouchier A, Lucas P, Larsson MC, Jacquin-Joly E (2012) Functional characterization of a sex pheromone receptor in the pest moth *Spodoptera littoralis* by heterologous expression in *Drosophila*. Eur J Neurosci 36(5):2588–2596. doi:10.1111/j.1460-9568.2012.08183.x
118. Cattaneo AM, Gonzalez F, Bengtsson JM, Corey EA, Jacquin-Joly E, Montagne N, Salvagnin U, Walker WB, Witzgall P, Anfora G, Bobkov YV (2017) Candidate pheromone receptors of codling moth *Cydia pomonella* respond to pheromones and kairomones. Sci Rep 7:41105. doi:10.1038/srep41105
119. Gonzalez F, Bengtsson JM, Walker WB, Sousa MFR, Cattaneo AM, Montagne N, de Fouchier A, Anfora G, Jacquin-Joly E, Witzgall P, Ignell R, Bengtsson M (2015) A conserved odorant receptor detects the same 1-indanone analogs in a tortricid and a noctuid moth. Front Ecol Evol 3(131):1–12. doi:10.3389/fevo.2015.00131
120. Wang B, Liu Y, He K, Wang G (2016) Comparison of research methods for functional characterization of insect olfactory receptors. Sci Rep 6:32806. doi:10.1038/srep32806
121. Gonzalez D, Witzgall P, Walker WB (2016) Protocol for heterologous expression of insect odourant receptors in *Drosophila*. Front Ecol Evol 4(24):1–5. doi:10.3389/fevo.2016.00024
122. Forstner M, Breer H, Krieger J (2009) A receptor and binding protein interplay in the detection of a distinct pheromone component in the silkmoth *Antheraea polyphemus*. Int J Biol Sci 5(7):745–757. doi:10.7150/ijbs.5.745
123. Pregitzer P, Schubert M, Breer H, Hansson BS, Sachse S, Krieger J (2012) Plant odorants interfere with detection of sex pheromone signals by male *Heliothis virescens*. Front Cell Neurosci 6:42. doi:10.3389/fncel.2012.00042
124. Bohbot JD, Jones PL, Wang G, Pitts RJ, Pask GM, Zwiebel LJ (2011) Conservation of indole responsive odorant receptors in mosquitoes reveals an ancient olfactory trait. Chem Senses 36(2):149–160. doi:10.1093/chemse/bjq105
125. Jones PL, Pask GM, Rinker DC, Zwiebel LJ (2011) Functional agonism of insect odorant receptor ion channels. Proc Natl Acad Sci USA 108(21):8821–8825. doi:10.1073/pnas.1102425108
126. Corcoran JA, Jordan MD, Carraher C, Newcomb RD (2014) A novel method to study insect olfactory receptor function using HEK293 cells. Insect Biochem Mol Biol 54:22–32. doi:10.1016/j.ibmb.2014.08.005
127. Jordan MD, Anderson A, Begum D, Carraher C, Authier A, Marshall SD, Kiely A, Gatehouse LN, Greenwood DR, Christie DL, Kralicek AV, Trowell SC, Newcomb RD (2009) Odorant receptors from the light brown apple moth (*Epiphyas postvittana*) recognize important volatile compounds produced by plants. Chem Senses 34(5):383–394. doi:10.1093/chemse/bjp010
128. Kiely A, Authier A, Kralicek AV, Warr CG, Newcomb RD (2007) Functional analysis of a *Drosophila melanogaster* olfactory receptor expressed in Sf9 cells. J Neurosci Methods 159(2):189–194. doi:10.1016/j.jneumeth.2006.07.005
129. Anderson AR, Wanner KW, Trowell SC, Warr CG, Jaquin-Joly E, Zagatti P, Robertson H, Newcomb RD (2009) Molecular basis of female-specific odorant responses in *Bombyx mori*. Insect Biochem Molec 39(3):189–197. doi:10.1016/j.ibmb.2008.11.002
130. Claudianos C, Lim J, Young M, Yan SZ, Cristino AS, Newcomb RD, Gunasekaran N, Reinhard J (2014) Odor memories regulate olfactory receptor expression in the sensory periphery. Eur J Neurosci 39(10):1642–1654. doi:10.1111/ejn.12539
131. German PF, van der Poel S, Carraher C, Kralicek AV, Newcomb RD (2013) Insights into subunit interactions within the insect olfactory receptor complex using FRET. Insect Biochem Mol Biol 43(2):138–145. doi:10.1016/j.ibmb.2012.11.002
132. Tsitoura P, Andronopoulou E, Tsikou D, Agalou A, Papakonstantinou MP, Kotzia GA, Labropoulou V, Swevers L, Georgoussi Z, Iatrou K (2010) Expression and membrane topology of *Anopheles gambiae* odorant receptors in lepidopteran insect cells. PLoS ONE. doi:10.1371/journal.pone.0015428
133. Zhang DD, Wang HL, Schultze A, Fross H, Francke W, Krieger J, Lofstedt C (2016) Receptor for detection of a Type II sex pheromone in the winter moth *Operophtera brumata*. Sci Rep 6:18576. doi:10.1038/srep18576
134. Sun M, Liu Y, Walker WB, Liu C, Lin K, Gu S, Zhang Y, Zhou J, Wang G (2013) Identification and characterization of pheromone receptors and interplay between receptors and pheromone binding proteins in the diamondback moth, *Plutella xyllostella*. PLoS ONE 8(4):e62098. doi:10.1371/journal.pone.0062098
135. Zhang J, Yan S, Liu Y, Jacquin-Joly E, Dong S, Wang G (2015) Identification and functional characterization of sex pheromone receptors in the common cutworm (*Spodoptera litura*). Chem Senses 40(1):7–16. doi:10.1093/chemse/bju052
136. Liu F, Xiong C, Liu N (2017) Chemoreception to aggregation pheromones in the common bed bug, *Cimex lectularius*. Insect Biochem Mol Biol 82:62–73. doi:10.1016/j.ibmb.2017.01.012

137. Chang H, Liu Y, Yang T, Pelosi P, Dong S, Wang G (2015) Pheromone binding proteins enhance the sensitivity of olfactory receptors to sex pheromones in *Chilo suppressalis*. Sci Rep 5:13093. doi:10.1038/srep13093
138. Papke RL, Stokes C (2010) Working with OpusXpress: methods for high volume oocyte experiments. Methods 51(1):121–133. doi:10.1016/j.ymeth.2010.01.012
139. Schnizler K, Kuster M, Methfessel C, Fejtl M (2003) The roboocyte: automated cDNA/mRNA injection and subsequent TEVC recording on Xenopus oocytes in 96-well microtiter plates. Receptors Channels 9(1):41–48. doi:10.3109/10606820308253
140. Leisgen C, Kuester M, Methfessel C (2007) The roboocyte: automated electrophysiology based on Xenopus oocytes. Methods Mol Biol 403:87–109. doi:10.1007/978-1-59745-529-9_6
141. Chen S, Luetje CW (2012) Identification of new agonists and antagonists of the insect odorant receptor co-receptor subunit. PLoS ONE 7(5):e36784. doi:10.1371/journal.pone.0036784
142. Chen S, Luetje CW (2014) Trace amines inhibit insect odorant receptor function through antagonism of the co-receptor subunit. F1000Res 3:84. doi:10.12688/f1000research.3825.1
143. Pellegrino M, Nakagawa T, Vosshall LB (2010) Single sensillum recordings in the insects *Drosophila melanogaster* and *Anopheles gambiae*. J Vis Exp 36:1–5. doi:10.3791/1725
144. Hallem EA, Nicole FA, Zwiebel LJ, Carlson JR (2004) Olfaction: mosquito receptor for human-sweat odorant. Nature 427(6971):212–213. doi:10.1038/427212a
145. Kurtovic A, Widmer A, Dickson BJ (2007) A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. Nature 446(7135):542–546. doi:10.1038/nature05672
146. Jin X, Ha TS, Smith DP (2008) SNMP is a signaling component required for pheromone sensitivity in *Drosophila*. Proc Natl Acad Sci USA 105(31):10996–11001. doi:10.1073/pnas.0803309105
147. Ronderos DS, Lin CC, Potter CJ, Smith DP (2014) Farnesol-detecting olfactory neurons in *Drosophila*. J Neurosci 34(11):3959–3968. doi:10.1523/JNEUROSCI.4582-13.2014
148. You Y, Smith DP, Lv M, Zhang L (2016) A broadly tuned odorant receptor in neurons of trichoid sensilla in locust, *Locusta migratoria*. Insect Biochem Mol Biol 79:66–72. doi:10.1016/j.ibmb.2016.10.008
149. DeGennaro M, McBride CS, Seeholzer L, Nakagawa T, Dennis EJ, Goldman C, Jasinskiene N, James AA, Vosshall LB (2013) *orco* mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. Nature 498(7455):487–491. doi:10.1038/nature12206
150. Yi X, Zhao H, Wang P, Hu M, Zhong G (2014) Bdor\Orco is important for oviposition-deterring behavior induced by both the volatile and non-volatile repellents in Bactrocera dorsalis (Diptera: Tephritidae). J Insect Physiol 65:51–56. doi:10.1016/j.jinsphys.2014.05.007
151. Zhou YL, Zhu XQ, Gu SH, Cui HH, Guo YY, Zhou JJ, Zhang YJ (2014) Silencing in *Apolygus lucorum* of the olfactory coreceptor Orco gene by RNA interference induces EAG response declining to two putative semiochemicals. J Insect Physiol 60:31–39. doi:10.1016/j.jinsphys.2013.10.006
152. Fan J, Zhang Y, Francis F, Cheng D, Sun J, Chen J (2015) Orco mediates olfactory behaviors and winged morph differentiation induced by alarm pheromone in the grain aphid, *Sitobion avenae*. Insect Biochem Mol Biol 64:16–24. doi:10.1016/j.ibmb.2015.07.006
153. Lin W, Yu Y, Zhou P, Zhang J, Dou L, Hao Q, Chen H, Zhu S (2015) Identification and knockdown of the olfactory receptor (OrCo) in Gypsy Moth, *Lymantria dispar*. Int J Biol Sci 11(7):772–780. doi:10.7150/ijbs.11898
154. Franco TA, Oliveira DS, Moreira MF, Leal WS, Melo AC (2016) Silencing the odorant receptor co-receptor RproOrco affects the physiology and behavior of the Chagas disease vector *Rhodnius prolixus*. Insect Biochem Mol Biol 69:82–90. doi:10.1016/j.ibmb.2015.02.012
155. Koutroumpa FA, Monsempes C, Francois MC, de Cian A, Royer C, Concordet JP, Jacquin-Joly E (2016) Heritable genome editing with CRISPR/Cas9 induces anosmia in a crop pest moth. Sci Rep 6:29620. doi:10.1038/srep29620
156. Li Y, Zhang J, Chen D, Yang P, Jiang F, Wang X, Kang L (2016) CRISPR/Cas9 in locusts: successful establishment of an olfactory deficiency line by targeting the mutagenesis of an odorant receptor co-receptor (Orco). Insect Biochem Mol Biol 79:27–35. doi:10.1016/j.ibmb.2016.10.003
157. Zhang R, Gao G, Chen H (2016) Silencing of the olfactory co-receptor gene in *Dendroctonus armandi* leads to EAG response declining to major host volatiles. Sci Rep 6:23136. doi:10.1038/srep23136
158. Liu Q, Liu W, Zeng B, Wang G, Hao D, Huang Y (2017) Deletion of the *Bombyx mori* odorant receptor co-receptor (BmOrco) impairs olfactory sensitivity in silkworms. Insect Biochem Mol Biol 86:58–67. doi:10.1016/j.ibmb.2017.05.007
159. Carey AF, Carlson JR (2011) Insect olfaction from model systems to disease control. Proc Natl Acad Sci USA 108(32):14849–14854. doi:10.1073/pnas.1103472108
160. Almaas TJ, Mustaparta H (1991) *Heliothis virescens*: response characteristics of receptor neurons in sensilla trichodea type 1 and type 2. J Chem Ecol 17(5):953–972. doi:10.1007/BF01395602
161. Baker TC, Ochieng SA, Cosse AA, Lee SG, Todd JL, Quero C, Vickers NJ (2004) A comparison of responses from olfactory receptor neurons of *Heliothis subflexa* and *Heliothis virescens* to components of their sex pheromone. J Comp Physiol A 190(2):155–165. doi:10.1007/s00359-003-0483-2
162. Kaissling K-E, Kasang G, Bestmann HJ, Stransky W, Vostrowsky O (1978) A new pheromone of the silkworm moth *Bombyx mori*. Naturwissenschaften 65:382–384. doi:10.1007/BF00439702
163. Mitsuno H, Sakurai T, Murai M, Yasuda T, Kugimiya S, Ozawa R, Toyohara H, Takabayashi J, Miyoshi H, Nishioka T (2008) Identification of receptors of main sex-pheromone components of three Lepidopteran species. Eur J Neurosci 28(5):893–902. doi:10.1111/j.1460-9568.2008.06429.x
164. Legeai F, Malpel S, Montagne N, Monsempes C, Cousserans F, Merlin C, Francois MC, Maibeche-Coisne M, Gavory F, Poulain J, Jacquin-Joly E (2011) An Expressed Sequence Tag collection from the male antennae of the Noctuid moth *Spodoptera littoralis*: a resource for olfactory and pheromone detection research. BMC Genomics 12:86. doi:10.1186/1471-2164-12-86
165. Bengtsson JM, Trona F, Montagne N, Anfora G, Ignell R, Witzgall P, Jacquin-Joly E (2012) Putative chemosensory receptors of the codling moth, *Cydia pomonella*, identified by antennal transcriptome analysis. PLoS ONE 7(2):e31620. doi:10.1371/journal.pone.0031620
166. Engsontia P, Sangket U, Chotigeat W, Satasook C (2014) Molecular evolution of the odorant and gustatory receptor genes in lepidopteran insects: implications for their adaptation and speciation. J Mol Evol 79(1–2):21–39. doi:10.1007/s00239-014-9633-0
167. Steinwender B, Thrimawithana AH, Crowhurst RN, Newcomb RD (2015) Pheromone receptor evolution in the cryptic leafroller species, *Ctenopseustis obliquana* and *C. herana*. J Mol Evol 80(1):42–56. doi:10.1007/s00239-014-9650-z
168. Dong J, Song Y, Li W, Shi J, Wang Z (2016) Identification of putative chemosensory receptor genes from the *Athetis dissimilis* antennal transcriptome. PLoS ONE 11(1):e0147768. doi:10.1371/journal.pone.0147768
169. Yew JY, Chung H (2015) Insect pheromones: an overview of function, form, and discovery. Prog Lipid Res 59:88–105. doi:10.1016/j.plipres.2015.06.001

170. Wanner KW, Nichols AS, Walden KK, Brockmann A, Luetje CW, Robertson HM (2007) A honey bee odorant receptor for the queen substance 9-oxo-2-decenoic acid. Proc Natl Acad Sci USA 104(36):14383–14388. doi:10.1073/pnas.0705459104
171. Wang L, Anderson DJ (2010) Identification of an aggression-promoting pheromone and its receptor neurons in *Drosophila*. Nature 463(7278):227–231. doi:10.1038/nature08678
172. Naters VDGV, Carlson JR (2007) Receptors and neurons for fly odors in *Drosophila*. Curr Biol 17(7):606–612. doi:10.1016/j.cub.2007.02.043
173. Liu W, Liang X, Gong J, Yang Z, Zhang YH, Zhang JX, Rao Y (2011) Social regulation of aggression by pheromonal activation of Or65a olfactory neurons in *Drosophila*. Nat Neurosci 14(7):896–902. doi:10.1038/nn.2836
174. Ha TS, Smith DP (2006) A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in *Drosophila*. J Neurosci 26(34):8727–8733. doi:10.1523/JNEUROSCI.0876-06.2006
175. Ejima A, Smith BP, Lucas C, Naters VDGV, Miller CJ, Carlson JR, Levine JD, Griffith LC (2007) Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. Curr Biol 17(7):599–605. doi:10.1016/j.cub.2007.01.053
176. Pitts S, Pelser E, Meeks J, Smith D (2016) Odorant responses and courtship behaviors influenced by at4 neurons in *Drosophila*. PLoS ONE 11(9):e0162761. doi:10.1371/journal.pone.0162761
177. Lin CC, Prokop-Prigge KA, Preti G, Potter CJ (2015) Food odors trigger *Drosophila* males to deposit a pheromone that guides aggregation and female oviposition decisions. Elife. doi:10.7554/eLife.08688
178. Dweck HKM, Ebrahim SAM, Thoma M, Mohamed AAM, Keesey IW, Trona F, Lavista-Llanos S, Svatos A, Sachse S, Knaden M, Hansson BS (2015) Pheromones mediating copulation and attraction in *Drosophila*. Proc Natl Acad Sci USA 112(21):2829–2835. doi:10.1073/pnas.1504527112
179. Larter NK, Sun JS, Carlson JR (2016) Organization and function of *Drosophila* odorant binding proteins. Elife. doi:10.7554/eLife.20242
180. Pelosi P, Zhou JJ, Ban LP, Calvello M (2006) Soluble proteins in insect chemical communication. Cell Mol Life Sci 63(14):1658–1676. doi:10.1007/s00018-005-5607-0
181. Schultze A, Pregitzer P, Walter MF, Woods DF, Marinotti O, Breer H, Krieger J (2013) The co-expression pattern of odorant binding proteins and olfactory receptors identify distinct trichoid sensilla on the antenna of the malaria mosquito *Anopheles gambiae*. PLoS ONE 8(7):e69412. doi:10.1371/journal.pone.0069412
182. Qiao H, He X, Schymura D, Ban L, Dani FR, Michelucci E, Caputo B, Torre AD, Iatrou K, Zhou JJ, Krieger J, Pelosi P (2010) Cooperative interactions between odorant-binding proteins of *Anopheles gambiae*. Cell Mol Life Sci 68:1799–1813. doi:10.1007/s00018-010-0539-8
183. Guo H, Huang LQ, Pelosi P, Wang CZ (2012) Three pheromone-binding proteins help segregation between two *Helicoverpa* species utilizing the same pheromone components. Insect Biochem Mol Biol 42(9):708–716. doi:10.1016/j.ibmb.2012.06.004
184. Swarup S, Williams TI, Anholt RR (2011) Functional dissection of Odorant binding protein genes in *Drosophila melanogaster*. Genes Brain Behav 10(6):648–657. doi:10.1111/j.1601-183X.2011.00704.x
185. Nordström KJ, Sallman Almen M, Edstam MM, Fredriksson R, Schioth HB (2011) Independent HHsearch, Needleman–Wunsch-based, and motif analyses reveal the overall hierarchy for most of the G protein-coupled receptor families. Mol Biol Evol 28(9):2471–2480. doi:10.1093/molbev/msr061
186. Wistrand M, Kall L, Sonnhammer EL (2006) A general model of G protein-coupled receptor sequences and its application to detect remote homologs. Protein Sci 15(3):509–521. doi:10.1110/ps.051745906
187. Guo S, Kim J (2010) Dissecting the molecular mechanism of *Drosophila* odorant receptors through activity modeling and comparative analysis. Proteins 78(2):381–399. doi:10.1002/prot.22556
188. Olafson PU (2013) Molecular characterization and immunolocalization of the olfactory co-receptor Orco from two blood-feeding muscid flies, the stable fly (*Stomoxys calcitrans, L.*) and the horn fly (*Haematobia irritans irritans, L.*). Insect Mol Biol 22(2):131–142. doi:10.1111/imb.12009
189. Hull JJ, Hoffmann EJ, Perera OP, Snodgrass GL (2012) Identification of the western tarnished plant bug (*Lygus hesperus*) olfactory co-receptor Orco: expression profile and confirmation of atypical membrane topology. Arch Insect Biochem Physiol 81(4):179–198. doi:10.1002/arch.21042
190. Miller R, Tu ZJ (2008) Odorant receptor c-terminal motifs in divergent insect species. J Insect Sci 8(53):1–10. doi:10.1673/031.008.5301
191. Elmore T, Ignell R, Carlson JR, Smith DP (2003) Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. J Neurosci 23(30):9906–9912
192. Nichols AS, Chen S, Luetje CW (2011) Subunit contributions to insect olfactory receptor function: channel block and odorant recognition. Chem Senses 36(9):781–790. doi:10.1093/chemse/bjr053
193. Bahk S, Jones WD (2016) Insect odorant receptor trafficking requires calmodulin. BMC Biol. doi:10.1186/s12915-016-0306-x
194. Mukunda L, Miazzi F, Kaltofen S, Hansson BS, Wicher D (2014) Calmodulin modulates insect odorant receptor function. Cell Calcium 55(4):191–199. doi:10.1016/j.ceca.2014.02.013
195. Mukunda L, Miazzi F, Sargsyan V, Hansson BS, Wicher D (2016) Calmodulin affects sensitization of *Drosophila melanogaster* odorant receptors. Front Cell Neurosci. doi:10.3389/fncel.2016.00028
196. Nolte A, Funk NW, Mukunda L, Gawalek P, Werckenthin A, Hansson BS, Wicher D, Stengl M (2013) In situ tip-recordings found no evidence for an Orco-based ionotropic mechanism of pheromone-transduction in *Manduca sexta*. PLoS ONE 8(5):e62648. doi:10.1371/journal.pone.0062648
197. Deng Y, Zhang W, Farhat K, Oberland S, Gisselmann G, Neuhaus EM (2011) The stimulatory Galpha(s) protein is involved in olfactory signal transduction in *Drosophila*. PLoS ONE 6(4):e18605. doi:10.1371/journal.pone.0018605
198. Stengl M, Funk NW (2013) The role of the coreceptor Orco in insect olfactory transduction. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 199(11):897–909. doi:10.1007/s00359-013-0837-3
199. Carraher C, Dalziel J, Jordan MD, Christie DL, Newcomb RD, Kralicek AV (2015) Towards an understanding of the structural basis for insect olfaction by odorant receptors. Insect Biochem Mol Biol 66:31–41. doi:10.1016/j.ibmb.2015.09.010
200. Yao CA, Carlson JR (2010) Role of G-proteins in odor-sensing and CO_2-sensing neurons in *Drosophila*. J Neurosci 30(13):4562–4572. doi:10.1523/JNEUROSCI.6357-09.2010
201. Talluri S, Bhatt A, Smith DP (1995) Identification of a *Drosophila* G protein alpha subunit (dGq alpha-3) expressed in chemosensory cells and central neurons. Proc Natl Acad Sci USA 92(25):11475–11479. doi:10.1073/pnas.92.25.11475
202. Laue M, Maida R, Redkozubov A (1997) G-protein activation, identification and immunolocalization in pheromone-sensitive sensilla trichodea of moths. Cell Tissue Res 288(1):149–158. doi:10.1007/s004410050802
203. Jacquin-Joly E, Francois MC, Burnet M, Lucas P, Bourrat F, Maida R (2002) Expression pattern in the

antennae of a newly isolated lepidopteran Gq protein alpha subunit cDNA. Eur J Biochem 269(8):2133–2142. doi:10.1046/j.1432-1033.2002.02863.x
204. Kalidas S, Smith DP (2002) Novel genomic cDNA hybrids produce effective RNA interference in adult *Drosophila*. Neuron 33(2):177–184. doi:10.1016/S0896-6273(02)00560-3
205. Miura N, Atsumi S, Tabunoki H, Sato R (2005) Expression and localization of three G protein alpha subunits, Go, Gq, and Gs, in adult antennae of the silkmoth (*Bombyx mori*). J Comp Neurol 485(2):143–152. doi:10.1002/cne.20488
206. Boto T, Gomez-Diaz C, Alcorta E (2010) Expression analysis of the 3 G-protein subunits, Gα, Gβ, and Gγ, in the olfactory receptor organs of adult *Drosophila melanogaster*. Chem Senses 35(3):183–193. doi:10.1093/chemse/bjp095
207. Rutzler M, Lu T, Zwiebel LJ (2006) Galpha encoding gene family of the malaria vector mosquito *Anopheles gambiae*: expression analysis and immunolocalization of AGαq and AGαo in female antenna. J Comp Neurol 499(4):533–545. doi:10.1002/cne.21083
208. Gomez-Diaz C, Martin F, Alcorta E (2004) The cAMP transduction cascade mediates olfactory reception in *Drosophila melanogaster*. Behav Genet 34(4):395–406. doi:10.1023/B:BEGE.0000023645.02710.fe
209. Gomez-Diaz C, Martin F, Alcorta E (2006) The inositol 1,4,5-triphosphate kinase1 gene affects olfactory reception in *Drosophila* melanogaster. Behav Genet 36(2):309–321. doi:10.1007/s10519-005-9031-x
210. Miazzi F, Hansson BS, Wicher D (2016) Odor-induced cAMP production in *Drosophila melanogaster* olfactory sensory neurons. J Exp Biol 219(12):1798–1803. doi:10.1242/jeb.137901
211. Sargsyan V, Getahun MN, Llanos SL, Olsson SB, Hansson BS, Wicher D (2011) Phosphorylation via PKC regulates the function of the *Drosophila* odorant co-receptor. Front Cell Neurosci. doi:10.3389/fncel.2011.00005
212. Getahun MN, Thoma M, Lavista-Llanos S, Keesey I, Fandino RA, Knaden M, Wicher D, Olsson SB, Hansson BS (2016) Intracellular regulation of the insect chemoreceptor complex impacts odour localization in flying insects. J Exp Biol 219(Pt 21):3428–3438. doi:10.1242/jeb.143396
213. Nakagawa T, Vosshall LB (2009) Controversy and consensus: noncanonical signaling mechanisms in the insect olfactory system. Curr Opin Neurobiol 19(3):284–292. doi:10.1016/j.conb.2009.07.015
214. Getahun MN, Olsson SB, Lavista-Llanos S, Hansson BS, Wicher D (2013) Insect odorant response sensitivity is tuned by metabotropically autoregulated olfactory receptors. PLoS ONE 8(3):e58889. doi:10.1371/journal.pone.0058889
215. Getahun MN, Wicher D, Hansson BS, Olsson SB (2012) Temporal response dynamics of *Drosophila* olfactory sensory neurons depends on receptor type and response polarity. Front Cell Neurosci 6:54. doi:10.3389/fncel.2012.00054
216. Carde RT, Willis MA (2008) Navigational strategies used by insects to find distant, wind-borne sources of odor. J Chem Ecol 34(7):854–866. doi:10.1007/s10886-008-9484-5
217. Stengl M (2010) Pheromone transduction in moths. Front Cell Neurosci 4:133. doi:10.3389/fncel.2010.00133
218. Nolte A, Gawalek P, Koerte S, Wei H, Schumann R, Werckenthin A, Krieger J, Stengl M (2016) No evidence for ionotropic pheromone transduction in the hawkmoth *Manduca sexta*. PLoS ONE 11(11):e0166060. doi:10.1371/journal.pone.0166060
219. Boekhoff I, Strotmann J, Raming K, Tareilus E, Breer H (1990) Odorant-sensitive phospholipase C in insect antennae. Cell Signal 2(1):49–56. doi:10.1016/0898-6568(90)90032-6
220. Breer H, Boekhoff I, Strotmann J, Raming K, Tareilus E (1990) Molecular elements of olfactory signal transduction in insect antennae. Nato ASI Series H 39:77–86. doi:10.1007/978-3-642-75127-1_5
221. Ziegelberger G, Vandenberg MJ, Kaissling KE, Klumpp S, Schultz JE (1990) Cyclic-GMP levels and guanylate-cyclase activity in pheromone-sensitive antennae of the silkmoths *Antheraea polyphemus* and *Bombyx mori*. J Neurosci 10(4):1217–1225
222. Stengl M, Zufall F, Hatt H, Hildebrand JG (1992) Olfactory receptor neurons from antennae of developing male *Manduca sexta* respond to components of the species-specific sex pheromone in vitro. J Neurosci 12(7):2523–2531
223. Stengl M (1994) Inositol-trisphosphate-dependent calcium currents precede cation currents in insect olfactory receptor neurons in vitro. J Comp Physiol A 174(2):187–194. doi:10.1007/BF00193785
224. Stengl M (1993) Intracellular-messenger-mediated cation channels in cultured olfactory receptor neurons. J Exp Biol 178:125–147
225. Ito I, Ong RC, Raman B, Stopfer M (2008) Olfactory learning and spike timing dependent plasticity. Commun Integr Biol 1(2):170–171. doi:10.4161/cib.1.2.7140
226. Justus KA, Carde RT, French AS (2005) Dynamic properties of antennal responses to pheromone in two moth species. J Neurophysiol 93(4):2233–2239. doi:10.1152/jn.00888.2004
227. Tripathy SJ, Peters OJ, Staudacher EM, Kalwar FR, Hatfield MN, Daly KC (2010) Odors pulsed at wing beat frequencies are tracked by primary olfactory networks and enhance odor detection. Front Cell Neurosci. doi:10.3389/neuro.03.001.2010
228. Autrum H (1950) Die Belichtungspotentiale und das Sehen der Insekten (Untersuchungen an Calliphora und Dixippus). Zeitschrift Fur Vergleichende Physiologie 32(3):176–227. doi:10.1007/Bf00344524
229. Tatler B, O'Carroll DC, Laughlin SB (2000) Temperature and the temporal resolving power of fly photoreceptors. J Comp Physiol A Sens Neural and Behav Physiol 186(4):399–407. doi:10.1007/s003590050439
230. Hardie RC, Raghu P (2001) Visual transduction in *Drosophila*. Nature 413(6852):186–193. doi:10.1038/35093002
231. Brito NF, Moreira MF, Melo AC (2016) A look inside odorant-binding proteins in insect chemoreception. J Insect Physiol 95:51–65. doi:10.1016/j.jinsphys.2016.09.008
232. Fan J, Francis F, Liu Y, Chen JL, Cheng DF (2011) An overview of odorant-binding protein functions in insect peripheral olfactory reception. Genet Mol Res 10(4):3056–3069. doi:10.4238/2011.December.8.2
233. Zhou JJ (2010) Odorant-binding proteins in insects. Vitam Horm 83:241–272. doi:10.1016/S0083-6729(10)83010-9
234. Vieira FG, Rozas J (2011) Comparative genomics of the odorant-binding and chemosensory protein gene families across the Arthropoda: origin and evolutionary history of the chemosensory system. Genome Biol Evol 3:476–490. doi:10.1093/gbe/evr033
235. Gong DP, Zhang HJ, Zhao P, Xia QY, Xiang ZH (2009) The odorant binding protein gene family from the genome of silkworm, *Bombyx mori*. BMC Genomics 10:332. doi:10.1186/1471-2164-10-332
236. Hekmat-Scafe DS, Scafe CR, McKinney AJ, Tanouye MA (2002) Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. Genome Res 12(9):1357–1369. doi:10.1101/gr.239402
237. Manoharan M, Ng Fuk CM, Vaitinadapoule A, Frumence E, Sowdhamini R, Offmann B (2013) Comparative genomics of odorant binding proteins in *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*. Genome Biol Evol 5(1):163–180. doi:10.1093/gbe/evs131
238. Qiao H, Xiaoli H, Schymura D, Ban L, Field L, Dani FR, Michelucci E, Caputo B, Della Torre A, Iatrou K, Krieger J, Zhou JJ, Pelosi P (2010) Cooparative interactions between odorant-binding proteins of *Anopheles gambiae*. Cell Mol Life Sci 68:1799–1813. doi:10.1007/s00018-010-0539-8

239. Sandler BH, Nikonova L, Leal WS, Clardy J (2000) Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein-bombykol complex. Chem Biol 7(2):143–151. doi:10.1016/S1074-5521(00)00078-8
240. Horst R, Damberger F, Luginbuhl P, Guntert P, Peng G, Nikonova L, Leal WS, Wuthrich K (2001) NMR structure reveals intramolecular regulation mechanism for pheromone binding and release. Proc Natl Acad Sci USA 98(25):14374–14379. doi:10.1073/pnas.251532998
241. Wojtasek H, Leal WS (1999) Conformational change in the pheromone-binding protein from *Bombyx mori* induced by pH and by interaction with membranes. J Biol Chem 274(43):30950–30956. doi:10.1074/jbc.274.43.30950
242. Laughlin JD, Ha TS, Jones DN, Smith DP (2008) Activation of pheromone-sensitive neurons is mediated by conformational activation of pheromone-binding protein. Cell 133(7):1255–1265. doi:10.1016/j.cell.2008.04.046
243. Pophof B (2004) Pheromone-binding proteins contribute to the activation of olfactory receptor neurons in the silkmoths *Antheraea polyphemus* and *Bombyx mori*. Chem Senses 29(2):117–125. doi:10.1093/chemse/bjh012
244. Pophof B (2002) Moth pheromone binding proteins contribute to the excitation of olfactory receptor cells. Naturwissenschaften 89(11):515–518. doi:10.1007/s00114-002-0364-5
245. Xu PX, Atkinson R, Jones DNM, Smith DP (2005) *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. Neuron 45(2):193–200. doi:10.1016/j.neuron.2004.12.031
246. Ronderos DS, Smith DP (2010) Activation of the T1 neuronal circuit is necessary and sufficient to induce sexually dimorphic mating behavior in *Drosophila melanogaster*. J Neurosci 30(7):2595–2599. doi:10.1523/Jneurosci.4819-09.2010
247. Gomez-Diaz C, Reina JH, Cambillau C, Benton R (2013) Ligands for pheromone-sensing neurons are not conformationally activated odorant binding proteins. PLoS Biol 11(4):e1001546. doi:10.1371/journal.pbio.1001546
248. Li Z, Ni JD, Huang J, Montell C (2014) Requirement for *Drosophila* SNMP1 for rapid activation and termination of pheromone-induced activity. PLoS Genet 10(9):e1004600. doi:10.1371/journal.pgen.1004600
249. Rogers ME, Sun M, Lerner MR, Vogt RG (1997) Snmp-1, a novel membrane protein of olfactory neurons of the silk moth *Antheraea polyphemus* with homology to the CD36 family of membrane proteins. J Biol Chem 272(23):14792–14799. doi:10.1074/jbc.272.23.14792
250. Nichols Z, Vogt RG (2008) The SNMP/CD36 gene family in Diptera, Hymenoptera and Coleoptera: *Drosophila melanogaster*, *D. pseudoobscura*, *Anopheles gambiae*, *Aedes aegypti*, *Apis mellifera*, and *Tribolium castaneum*. Insect Biochem Mol Biol 38(4):398–415. doi:10.1016/j.ibmb.2007.11.003
251. Oberland S, Ackelst T, Gaab S, Pelz T, Spehr J, Spehr M, Neuhaus EM (2015) CD36 is involved in oleic acid detection by the murine olfactory system. Front Cell Neurosci. doi:10.3389/fncel.2075.00366
252. Xavier AM, Ludwig RG, Nagai MH, de Almeida TJ, Watanabe HM, Hirata MY, Rosenstock TR, Papes F, Malnic B, Glezer I (2016) CD36 is expressed in a defined subpopulation of neurons in the olfactory epithelium. Sci Rep 6:25507. doi:10.1038/srep25507
253. Lee S, Eguchi A, Tsuzuki S, Matsumura S, Inoue K, Iwanaga T, Masuda D, Yamashita S, Fushiki T (2015) Expression of CD36 by olfactory receptor cells and its abundance on the epithelial surface in mice. PLoS ONE 10(7):e0133412. doi:10.1371/journal.pone.0133412
254. Forstner M, Gohl T, Gondesen I, Raming K, Breer H, Krieger J (2008) Differential expression of SNMP-1 and SNMP-2 proteins in pheromone-sensitive hairs of moths. Chem Senses 33(3):291–299. doi:10.1093/chemse/bjm087
255. Pregitzer P, Greschista M, Breer H, Krieger J (2014) The sensory neurone membrane protein SNMP1 contributes to the sensitivity of a pheromone detection system. Insect Mol Biol 23(6):733–742. doi:10.1111/imb.12119
256. Liu C, Zhang J, Liu Y, Wang G, Dong S (2014) Expression of SNMP1 and SNMP2 genes in antennal sensilla of *Spodoptera exigua* (Hubner). Arch Insect Biochem Physiol 85(2):114–126. doi:10.1002/arch.21150
257. Gu SH, Yang RN, Guo MB, Wang GR, Wu KM, Guo YY, Zhou JJ, Zhang YJ (2013) Molecular identification and differential expression of sensory neuron membrane proteins in the antennae of the black cutworm moth *Agrotis ipsilon*. J Insect Physiol 59(4):430–443. doi:10.1016/j.jinsphys.2013.02.003
258. Zhang J, Liu Y, Walker WB, Dong SL, Wang GR (2015) Identification and localization of two sensory neuron membrane proteins from *Spodoptera litura* (Lepidoptera: Noctuidae). Insect Sci 22(3):399–408. doi:10.1111/1744-7917.12131
259. Vogt RG, Miller NE, Litvack R, Fandino RA, Sparks J, Staples J, Friedman R, Dickens JC (2009) The insect SNMP gene family. Insect Biochem Mol Biol 39(7):448–456. doi:10.1016/j.ibmb.2009.03.007
260. Rogers ME, Krieger J, Vogt RG (2001) Antennal SNMPs (sensory neuron membrane proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. J Neurobiol 49(1):47–61. doi:10.1002/neu.1065
261. Huang X, Liu L, Fang Y, Feng J (2016) Expression of a sensory neuron membrane protein SNMP2 in olfactory sensilla of codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). J Econ Entomol 109(4):1907–1913. doi:10.1093/jee/tow098
262. Rogers ME, Steinbrecht RA, Vogt RG (2001) Expression of SNMP-1 in olfactory neurons and sensilla of male and female antennae of the silkmoth *Antheraea polyphemus*. Cell Tissue Res 303(3):433–446. doi:10.1007/s004410000305
263. Hallem EA, Dahanukar A, Carlson JR (2006) Insect odor and taste receptors. Annu Rev Entomol 51:113–135. doi:10.1146/annurev.ento.51.051705.113646
264. Montell C (2009) A taste of the *Drosophila* gustatory receptors. Curr Opin Neurobiol 19(4):345–353. doi:10.1016/j.conb.2009.07.001
265. Scott K, Brady R Jr, Cravchik A, Morozov P, Rzhetsky A, Zuker C, Axel R (2001) A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. Cell 104(5):661–673. doi:10.1016/S0092-8674(01)00263-X
266. Thorne N, Amrein H (2008) Atypical expression of *Drosophila* gustatory receptor genes in sensory and central neurons. J Comp Neurol 506(4):548–568. doi:10.1002/cne.21547
267. Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB (2007) Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. Nature 445(7123):86–90. doi:10.1038/nature05466
268. Suh GSB, Wong AM, Hergarden AC, Wang JW, Simon AF, Benzer S, Axel R, Anderson DJ (2004) A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. Nature 431(7010):854–859. doi:10.1038/nature02980
269. Suh GS, Ben-Tabou de Leon S, Tanimoto H, Fiala A, Benzer S, Anderson DJ (2007) Light activation of an innate olfactory avoidance response in *Drosophila*. Curr Biol 17(10):905–908. doi:10.1016/j.cub.2007.04.046
270. Faucher C, Forstreuter M, Hilker M, de Bruyne M (2006) Behavioral responses of *Drosophila* to biogenic levels of carbon dioxide depend on life-stage, sex and olfactory context. J Exp Biol 209(Pt 14):2739–2748. doi:10.1242/jeb.02297

271. Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A (2013) Targeting a dual detector of skin and CO_2 to modify mosquito host seeking. Cell 155(6):1365–1379. doi:10.1016/j.cell.2013.11.013
272. McMeniman CJ, Corfas RA, Matthews BJ, Ritchie SA, Vosshall LB (2014) Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans. Cell 156(5):1060–1071. doi:10.1016/j.cell.2013.12.044
273. Rodrigues TB, Moriyama EN, Wang H, Khajuria C, Siegfried BD (2016) Carbon dioxide receptor genes and their expression profile in *Diabrotica virgifera virgifera*. BMC Res Notes 9:18. doi:10.1186/s13104-015-1794-4
274. Ning C, Yang K, Xu M, Huang LQ, Wang CZ (2016) Functional validation of the carbon dioxide receptor in labial palps of *Helicoverpa armigera* moths. Insect Biochem Mol Biol 73:12–19. doi:10.1016/j.ibmb.2016.04.002
275. Xu W, Anderson A (2015) Carbon dioxide receptor genes in cotton bollworm *Helicoverpa armigera*. Naturwissenschaften 102(3–4):11. doi:10.1007/s00114-015-1260-0
276. Joseph RM, Carlson JR (2015) *Drosophila* chemoreceptors: a molecular interface between the chemical world and the brain. Trends Genet 31(12):683–695. doi:10.1016/j.tig.2015.09.005
277. Watanabe K, Toba G, Koganezawa M, Yamamoto D (2011) Gr39a, a highly diversified gustatory receptor in *Drosophila*, has a role in sexual behavior. Behav Genet 41(5):746–753. doi:10.1007/s10519-011-9461-6
278. Miyamoto T, Amrein H (2008) Suppression of male courtship by a *Drosophila* pheromone receptor. Nat Neurosci 11(8):874–876. doi:10.1038/nn.2161
279. Moon SJ, Lee Y, Jiao Y, Montell C (2009) A *Drosophila* gustatory receptor essential for aversive taste and inhibiting male-to-male courtship. Curr Biol 19(19):1623–1627. doi:10.1016/j.cub.2009.07.061
280. Bray S, Amrein H (2003) A putative *Drosophila* pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. Neuron 39(6):1019–1029. doi:10.1016/S0896-6273(03)00542-7
281. Toda H, Zhao X, Dickson BJ (2012) The *Drosophila* female aphrodisiac pheromone activates ppk23(+) sensory neurons to elicit male courtship behavior. Cell Rep 1(6):599–607. doi:10.1016/j.celrep.2012.05.007
282. Shankar S, Chua JY, Tan KJ, Calvert ME, Weng R, Ng WC, Mori K, Yew JY (2015) The neuropeptide tachykinin is essential for pheromone detection in a gustatory neural circuit. Elife 4:e06914. doi:10.7554/eLife.06914
283. Jeong YT, Shim J, Oh SR, Yoon HI, Kim CH, Moon SJ, Montell C (2013) An odorant-binding protein required for suppression of sweet taste by bitter chemicals. Neuron 79(4):725–737. doi:10.1016/j.neuron.2013.06.025
284. Zhang HJ, Anderson AR, Trowell SC, Luo AR, Xiang ZH, Xia QY (2011) Topological and functional characterization of an insect gustatory receptor. PLoS ONE 6(8):e24111. doi:10.1371/journal.pone.0024111
285. Liman ER, Zhang YV, Montell C (2014) Peripheral coding of taste. Neuron 81(5):984–1000. doi:10.1016/j.neuron.2014.02.022
286. Sato K, Tanaka K, Touhara K (2011) Sugar-regulated cation channel formed by an insect gustatory receptor. Proc Natl Acad Sci USA 108(28):11680–11685. doi:10.1073/pnas.1019622108
287. Yao CA, Ignell R, Carlson JR (2005) Chemosensory coding by neurons in the coeloconic sensilla of the *Drosophila antenna*. J Neurosci 25(37):8359–8367. doi:10.1523/JNEUROSCI.2432-05.2005
288. Abuin L, Bargeton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R (2011) Functional architecture of olfactory ionotropic glutamate receptors. Neuron 69(1):44–60. doi:10.1016/j.neuron.2010.11.042
289. Chen Q, Man Y, Li J, Pei D, Wu W (2017) Olfactory ionotropic receptors in mosquito *Aedes albopictus* (Diptera: Culicidae). J Med Entomol. doi:10.1093/jme/tjx063
290. Guo M, Krieger J, Grosse-Wilde E, Missbach C, Zhang L, Breer H (2013) Variant ionotropic receptors are expressed in olfactory sensory neurons of coeloconic sensilla on the antenna of the desert locust (*Schistocerca gregaria*). Int J Biol Sci 10(1):1–14. doi:10.7150/ijbs.7624
291. Mayer ML (2011) Emerging models of glutamate receptor ion channel structure and function. Structure 19(10):1370–1380. doi:10.1016/j.str.2011.08.009
292. Knecht ZA, Silbering AF, Ni L, Klein M, Budelli G, Bell R, Abuin L, Ferrer AJ, Samuel AD, Benton R, Garrity PA (2016) Distinct combinations of variant ionotropic glutamate receptors mediate thermosensation and hygrosensation in *Drosophila*. Elife. doi:10.7554/eLife.17879
293. Ni L, Klein M, Svec KV, Budelli G, Chang EC, Ferrer AJ, Benton R, Samuel AD, Garrity PA (2016) The Ionotropic Receptors IR21a and IR25a mediate cool sensing in *Drosophila*. Elife. doi:10.7554/eLife.13254
294. Ai M, Blais S, Park JY, Min S, Neubert TA, Suh GS (2013) Ionotropic glutamate receptors IR64a and IR8a form a functional odorant receptor complex in vivo in *Drosophila*. J Neurosci 33(26):10741–10749. doi:10.1523/JNEUROSCI.5419-12.2013
295. Silbering AF, Rytz R, Grosjean Y, Abuin L, Ramdya P, Jefferis GS, Benton R (2011) Complementary function and integrated wiring of the evolutionarily distinct *Drosophila* olfactory subsystems. J Neurosci 31(38):13357–13375. doi:10.1523/JNEUROSCI.2360-11.2011
296. Grosjean Y, Rytz R, Farine JP, Abuin L, Cortot J, Jefferis GS, Benton R (2011) An olfactory receptor for food-derived odours promotes male courtship in *Drosophila*. Nature 478(7368):236–240. doi:10.1038/nature10428
297. Hussain A, Zhang M, Ucpunar HK, Svensson T, Quillery E, Gompel N, Ignell R, Kadow ICG (2016) Ionotropic chemosensory receptors mediate the taste and smell of polyamines. PLoS Biol. doi:10.1371/journal.pbio.1002454