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REVIEW ARTICLE

Odor Detection in Insects: Volatile Codes

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Abstract Insect olfactory systems present models to study interactions between animal genomes and the environment. They have evolved for fast processing of specific odorant blends and for general chemical monitoring. Here, we review molecular and physiological mechanisms in the context of the ecology of chemical signals. Different classes of olfactory receptor neurons (ORNs) detect volatile chemicals with various degrees of specialization. Their sensitivities are determined by an insect-specific family of receptor genes along with other accessory proteins. Whereas moth pheromones are detected by highly specialized neurons, many insects share sensitivities to chemical signals from microbial processes and plant secondary metabolism. We promote a more integrated research approach that links molecular physiology of receptor neurons to the ecology of odorants.

Keywords Insects · Olfaction · Receptors · Pheromones · *Drosophila* · Lepidoptera · Behavior · Antenna · Odor binding proteins · Evolution · Sensillum · Odor plumes · Plant volatiles

Insect Olfaction: A Renewed Challenge

In their quest for locating important resources such as mates, nutrients, oviposition, and resting sites, insects rely on their chemical senses to a large extent. While gustatory neurons signal the quality of food or mates after contact, it is the information encoded by olfactory neurons that provides cues where to look in the first place. Equally important, and often overlooked, odors can indicate toxic or other life-threatening environments. Hence, olfactory receptor neurons (ORNs) play an important role in an insect's behavioral ecology.

The past 10 years has seen a tremendous increase in our understanding of insect olfaction. The discovery of odorant receptor (OR) genes in the vinegar fly *Drosophila melanogaster* (Clyne et al. 1999a; Vosshall et al. 1999) has affected profoundly the nature of our research and has made it an important model organism. However, the behavioral ecology of odor perception has been investigated largely in other species. In light of the recent sequencing of insect genomes, it is useful to review the wealth of information on insect olfactory ecology.

Insect species are numerous and can have very different life styles and feeding ecologies. They encounter a wide range of chemicals, as each species has its special interests. Though olfaction has been studied in many insect species, comprehensive data come mainly from a few groups. Cockroaches such as *Periplaneta americana* (Boeckh et al. 1987) are ground dwellers, predominantly feeding on decaying organic matter. Its sex pheromone is a unique sesquiterpene (periplanone B, Persoons et al. 1976). Several species of large moths (Hansson 1995) mainly from the Bombicoidea (*Bombyx mori*, *Manduca sexta*) and Noctuioidea (e.g., *Heliothis* spp.) traditionally have been investigated because of their powerful long-range sex pheromones. Many moths also deposit eggs on particular host plants with differing degrees of specialization. The vinegar fly, *D. melanogaster* (Vosshall and Stocker 2007), feeds and oviposits on a variety of decaying fruits. It does not have a close relation with a particular host-plant. The

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honeybee *Apis mellifera* (Galizia and Menzel 2000) is a social insect with an intricate pheromonal communication system. Asexual females collect pollen and nectar from flowers, and they have the ability to discriminate many odorants. Mosquitoes (Takken and Knols 1999) such as *Anopheles gambiae* and *Aedes aegypti* feed on various nectar sources, but females need a blood-meal to mature their eggs. *A. aegypti* is more a generalist feeder, while *A. gambiae* prefers to bite humans.

To respond to the environment with appropriate behaviors, insect peripheral olfactory systems need to do two things: (1) Provide fast and reliable information about specific cues that induce innate behaviors, and (2) Sample salient features of the broad chemical landscape that can be learned and guide adaptive behaviors. What are the features of insect olfactory receptors that do this, and how does our newfound knowledge of molecular and neural mechanisms contribute to our understanding of the ecology of volatile signals?

Insect Noses: Organization of the Peripheral Olfactory System

Although it is the brain that transforms a set of chemical stimuli comprised of individual odorants (chemicals) into distinct odors (sensations) and associates them with a behavioral response, the ORNs are the sensors that limit what can be detected. The scope and accuracy with which odors can be identified is determined by how many different sensors there are and how they are tuned to different chemicals. Insect ORNs are bipolar neurons that develop from epithelial cells (Keil 1997). In early development, a pre-sensillum cluster of cells is formed (Ray and Rodrigues 1995; Endo et al. 2007). Some of these will move basally and become neurons, but three remain apical and form the accessory cells that will wrap themselves around the neurons. While it is the neurons that process odor information, accessory cells provide the extracellular milieu that supports their function (Park et al. 2002). These cells form sensilla, small cuticular structures where the neuronal dendrites are bathed in a lumen filled with mucus-like sensillum lymph (Zacharuk 1985). Pores in the cuticle allow odorants to pass and dissolve in the lymph (Steinbrecht 1997).

The majority of olfactory sensilla can be found on the antennae, but smaller sets are also located on other head appendages such as maxillary (de Bruyne et al. 1999) or labial palps (Stange 1992; Kwon et al. 2006). Irrespective of their location, all ORNs project to the antennal lobe of the brain. Olfactory sensilla fall into two ultrastructural categories, double walled (dw) and single walled (sw; Altner and Prillinger 1980). While the former are usually small and uniformly peg shaped, the latter can take on many different shapes (Zacharuk 1985; Steinbrecht 1997). In moths, sw sensilla can be up to 100 μm long thick-walled pointed hairs (trichoid sensilla), but in most insects (e.g., *Drosophila*), they are much smaller. Trichoid sensilla typically house neurons with unbranched dendrites (Zacharuk 1985; Shanbhag et al. 2000). Hymenoptera (e.g., honeybees) and certain Coleoptera have pored plates (placoid sensilla), some with large numbers of branched dendrites (Behrend 1971; Esslen and Kaissling 1976; Nikonov and Leal 2002). Most insects have thin-walled short (5–30 μm) blunt hairs (basiconic sensilla, Shanbhag et al. 2000). It remains unclear why insect ORNs are housed in sensilla of different shapes and sizes or how sensillum structure affects olfactory function.

In *Drosophila*, genetic experiments have shown that dw and sw sensilla develop differently, using different neurogenic genes (Gupta and Rodrigues 1997; Goulding et al. 2000). The *atonal* gene determines dw sensilla, while the *amos* gene regulates sw sensilla. The different structural subcategories of sw sensilla are regulated by patterning genes such as *lozenge* (Gupta and Rodrigues 1998; Goulding et al. 2000). In insect sensilla, usually two to four ORNs with distinct neuronal identities are combined within a single sensillum type. (Boeck et al. 1987; de Bruyne et al. 1999, 2001). The *notch* gene is involved in determining the fate of neurons within a single sensillum by being expressed in only one of them, creating *notch*-on and *notch*-off neurons (Endo et al. 2007). Neuronal identity is further determined by the *acj6* gene (Clyne et al. 1999b).

The spatial organization of ORNs in the antennal epithelium is lost upon entry of their axons into the brain. It is tempting to think of the antennal lobe as a map of olfactory information. Are ORN axon projections sorted to glomeruli according to some sort of chemical logic (a chemotopic map), transforming the intermingled distribution of ORNs in the epithelium into a functional spatial arrangement in the brain? In mosquitoes and flies, there is evidence that the antennal lobe is organized in zones for the different structural categories of sensilla and the different appendages (Anton and Rospars 2004; Couto et al. 2005; Endo et al. 2007). Within these zones, ORNs that co-inhabit a single sensillum tend to be separated based on *notch* expression with *notch*-on glomeruli clustered together on one side and *notch*-off on the other (Endo et al. 2007). It remains to be seen if the main organizing principle of the axonal projections is based on odor chemistry or the neuron's place in structures of the periphery. However, *Drosophila* ORNs that have their odor responsiveness removed by a mutation show normal projection patterns (Dobritsa et al. 2003). Hence, neural identity i.e., an ORN's place in the system, is determined independent from (and probably before) its chemical response profile. This means

that evolution of gene regulation through transcription factors can potentially reorganize odor sensitivities in the periphery, thus causing evolutionary shifts in behavioral responses.

Molecular Codes: Olfactory Genes in Neurons

In vertebrate ORNs, odor receptor (OR) genes (Buck and Axel 1991) with seven prominent transmembrane domains have been shown to be G-protein coupled receptors that mediate odor sensitivity (Firestein 2001). A large family of genes with expression in ORNs has been discovered in *Drosophila* (Clyne et al. 1999a; Vosshall et al. 1999). Although hydropathy analysis of these insect OR proteins strongly suggests they have seven transmembrane domains, they are very different from the vertebrate ORs, and indeed from any other G-protein coupled receptors. Insect ORs lack many of the characteristics of other G-protein coupled receptors (Wistrand et al. 2006), and recent evidence suggests they may insert the other way around in the membrane (Benton et al. 2006). There is also no direct evidence that they activate a G-protein-mediated transduction cascade as vertebrate ORs clearly do. Furthermore, insect ORs require the co-expression of *Or83b*, a special member of this family that is needed for correct OR transport and function in vivo (Larsson et al. 2004). The Or83b gene is the only member of this family that is highly conserved in several insect species (Krieger et al. 2003; Jones et al. 2005), indicative of its fundamental role across insects. The most recent evidence suggests that insect ORs form ligand-gated channels (Sato et al. 2008; Wicher et al. 2008), that bypass a G-protein-coupled signal transduction cascade. This would enable rapid odor processing and corroborate earlier physiological investigations (Kaissling 1987; de Bruyne et al. 1999) that highlight the fast responses of insect ORNs.

It is clear that this insect-specific family determines the response properties of most insect ORNs. OR proteins are localized to ORN dendrites (Elmore and Smith 2001). Expression of an OR transgene in *Drosophila* ORNs (Störtkuhl and Kettler 2001) or in an in vitro cell system (Wetzel et al. 2001) induces odor responses and mutations that remove OR gene expression from ORNs cause loss of odor activity (Clyne et al. 1999b; Dobritsa et al. 2003). Dobritsa et al. (2003) also demonstrated that a single OR mediates activity for the full range of odorants that excite an ORN by expressing a *Drosophila* OR transgene in a neuron from which the indigenous gene has been removed. Hallem and Carlson (2006) have shown that this applies to most *Drosophila* ORs. Interestingly, these experiments also showed that OR genes normally expressed in sensilla with a grooved peg (dw) or trichoid (sw) structure are also functional in a basiconic (sw) sensillum. Receptor-swapping is also possible among different insect species. A mosquito receptor for 2-methylphenol and a moth sex pheromone receptor detect their ligands when expressed in a *Drosophila* ORN (Hallem et al. 2004; Syed et al. 2006).

OR genes form one of the largest gene families in insects, and because they guide behavioral interactions of insects with other organisms, the analysis of their molecular evolution is of considerable interest. OR sequences show low conservation except for relatively conserved intron/exon boundaries, which suggest they arose from a common ancestor (Robertson et al. 2003). Complete sets of OR genes have been sequenced from the genomes of *Drosophila* (Robertson et al. 2003, Guo and Kim 2007), the honeybee (*A. mellifera*, Robertson and Wanner 2006), and two mosquitoes (*A. gambiae* Hill et al. 2002, and *A. aegypti*, Bohbot et al. 2007) ,and their numbers are featured in Table 1. Some OR genes have also been sequenced from the moths *B. mori* (Sakurai et al. 2004, Wanner et al. 2007) and *Heliothis virescens* (Krieger et al. 2002, 2004). If we exclude the *Or83b* gene and its homologues, *D. mela-*

Table 1 Estimates of olfactory coding units in different insects

Insect species	Order	ORs[a]	ORNs[b]	Glomeruli[c]
Drosophila melanogaster	Diptera	61	44	50±2
Anopheles gambiae	Diptera	78	>27	60±2
Aedes aegypti	Diptera	100	>10	50±2
Bombyx mori	Lepidoptera	>48	>5	60±2
Heliothis virescens	Lepidoptera	>20	>20	64±2
Apis mellifera	Hymenoptera	162	n.a.	165±2
Periplaneta americana	Blattaria	n.a.	>25	125±2

[a] Predicted from the genome, excluding pseudogenes, incomplete sequences and Or83b orthologs, including multiple splice forms
[b] Identified electrophysiologically or by gene expression (in situ)
[c] Variability is due to sexual dimorphisms and/or individual variability

nogaster has 59 OR genes that encode 61 proteins (Robertson et al. 2003). The *A. gambiae* genome reveals 78 OR genes (Hill et al. 2002), while *A. aegypti* has at least 100 (Bohbot et al. 2007). The honeybee genome has 162 of them (Robertson and Wanner 2006). This tells us that the full complement of coding units in most insect systems is likely to be ca. 10× lower than that found in most mammals. This may reflect selection pressure trade-offs between detection sensitivity and discrimination ability.

The comparison of OR genes within a species (paralogs) or between species (orthologs) shows that the OR gene family has undergone rapid evolution. Some OR genes in *Drosophila* occur in small clusters of two to three genes, and many of these clusters suggest recent gene duplications (Robertson et al. 2003; Guo and Kim 2007; Nozawa and Nei 2007). Interestingly, in the honeybee, most genes occur in large arrays with one of them encompassing 60 genes, indicating recent rapid expansion of a few subfamilies that may be bee-specific (Robertson and Wanner 2006). Comparisons between the two mosquitoes, *A. aegypti* and *A. gambiae*, separated by ca. 150 m years of evolution, confirms this species-specific expansion of subgroups but also shows orthologous subgroups that are relatively conserved (Bohbot et al. 2007). It is likely that rapid evolution of OR sequences is driven by changes in chemical ecology of these species even if the exact relation between OR sequence and ligand binding remains obscure. These comparative analyses also demonstrate the need to study the variety in insect olfactory systems.

While OR sequences are highly varied, comparative analysis of closely related *Drosophila* species shows that structural integrity of OR proteins is evolutionarily preserved (Tunstall et al. 2007). However, positive selection appears to be acting on some of these genes, at specific sites and along certain lineages. In 12 more distantly related *Drosophila* species, gene numbers are conserved (Guo and Kim 2007; Nozawa and Nei 2007). Although sequences are variable, each gene still has a clear ortholog, which suggests that over a comparable evolutionary period, insect ORs may evolve more slowly than vertebrate ones. An electrophysiological comparison of nine closely related *Drosophila* species also suggests that ORN response properties are relatively conserved (Stensmyr et al. 2003). Out of eight classes studied, only one showed changes in three species. Dekker et al. (2006) show for one of these species, *Drosophila sechellia*, that these shifts are correlated with changes in behavior. *D. sechellia*, which unlike *D. melanogaster* specializes on one particular fruit, appears to have lost OR genes and to have fixed point-mutations in the remaining genes at a higher rate than *Drosophila simulans*, which has a feeding ecology similar to *D. melanogaster* (McBride 2007). Like the higher number of OR genes in *A. aegypti* compared to *A. gambiae*, this indicates that niche specialization may lead to a loss of ORN classes. Stensmyr et al. (2003) also noted a loss of one sensillum type in *D. sechellia.*

While the loss of a sensillum type is most likely due to changes in developmental genes, changes in the regulation of OR expression can potentially reshuffle the array. Specific regulatory elements were discovered upstream of OR genes that determine whether an OR is expressed in the antenna or palp and in which neuron class (Ray et al. 2007). While most ORNs express a single OR gene, there are several cases of two or three genes per cell. In some of these cases, the two genes arise from a recent duplication, and one of them is not functional (Dobritsa et al. 2003), but in another case, both genes contribute to the response spectrum of the ORN (Goldman et al. 2005). Ray et al. (2007) showed these genes share common neuron-specific regulatory elements.

Modifying the Code: Olfactory Genes in Accessory Cells

The sensillum lymph forms the watery transition medium between volatiles and ORN dendrites, and is a mucus-like liquid packed with proteins secreted by the accessory cells. Before OR genes were identified, a group of proteins was isolated from moth olfactory sensillum lymph and dubbed pheromone-binding proteins (PBP, Vogt and Riddiford 1981) or odorant-binding proteins (OBP, Vogt et al. 1991). In moths, PBPs form a distinct subfamily of OBPs associated with the different pheromone-sensitive sensilla. In general, these proteins are differentially expressed in the different olfactory sensillum types (Vogt et al. 1991). Unlike the membrane-bound ORs, they are small, secreted hydrophilic proteins that can be extremely abundant in the lymph (Pelosi et al. 2006). Vertebrate OBPs belong to the larger family of lipocalins, but insect OBPs again define an insect-specific family of proteins. There are 51 OBP-like sequences in the *Drosophila* genome (Galindo and Smith 2001). OBPs are characterized by six cystein residues that are thought to form three disulfide bonds and a hydrophobic pocket inside (Sandler et al. 2000). A second family dubbed chemosensory proteins (CSPs) contains somewhat smaller proteins that have four disulfide bonds (Angeli et al. 1999). Neither OBPs nor CSPs are olfactory-specific genes. Many OBPs are expressed in gustatory sensilla, and several OBPs and particularly CSPs occur elsewhere in the body (Galindo and Smith 2001; Shanbhag et al. 2001; Pelosi et al. 2006).

It is thought that PBPs help solubilize hydrophobic moth pheromone molecules in the sensillum lymph, and specific binding of moth pheromone components has been demon-

strated (Du and Prestwich 1995; Leal et al. 2005). However, the presence of a specific OBP is not necessary for odor response per se. *Drosophila*, *Bombyx*, and *Anopheles* OR genes are functional in a *Drosophila* ORN where they are in an environment that does not have the OBPs that normally surround them (Hallem and Carlson 2006; Syed et al. 2006). In addition, specific responses to pheromones and other odors can be obtained in vitro from OR genes expressed in cultures of transfected cells (Wetzel et al. 2001; Nakagawa et al. 2005; Grosse-Wilde et al. 2006; Kiely et al. 2007). In vitro experiments with OR-expressing cells indicate that PBPs solubilize pheromones and can improve specificity of the receptor (Grosse-Wilde et al. 2006).

There are 51 OBP-like sequences in the *Drosophila* genome, none of which are closely related to moth PBPs. However, one of them, *obp76a*, plays a crucial role in the perception of cis-vaccenyl acetate (Z11-18:Ac), a compound quite similar in structure to moth pheromone components. The *lush* mutation removes all *obp76a* from a subset of trichoid sensilla, and its ORNs become insensitive, a defect that can be rescued by adding recombinant OBP76a protein to the preparation (Xu et al. 2005). In *Drosophila*, this compound is thought to be an aggregation pheromone produced by males, passed on to females and then to the oviposition substrate (Bartelt et al. 1985).

OBP sequence similarities across insect species are low (Vogt 2005), with only some subfamilies present across insect orders, and most with radiations within a single insect order or family. For example, the PBPs appear to have adapted specifically to a role in lepidopteran pheromone communication (Vogt 2005). This suggests that, like ORs, OBPs are under strong adaptive selection. Another line of evidence highlights this potentially important role of OBPs in insect chemical ecology, but also demonstrates their function outside the olfactory system. The feeding preference of *D. sechellia* for the toxic noni fruit (*Morinda citrifolia*) is linked to the expression pattern of *Obp57d/e* in taste sensilla on the tarsi (Matsuo et al. 2007).

Finally, in addition to OBPs, accessory cells also secrete a variety of enzymes that are likely to play a role in removing active odorants from the dendritic membranes (Vogt and Riddiford 1981; Maïbèche-Coisne et al. 2004; Ishida and Leal 2005). It is still unclear which role all these specific proteins in the sensillar lymph play in modifying ORN responses. Most likely, their roles are varied. While some PBPs appear to enhance only ORN functions, obp76a (*lush*) appears crucial. Expression of OBPs also can bridge ORN classes. *Drosophila* obp76a is also expressed in another class of sensilla that do not contain Z11-18Ac-sensitive neurons where the *lush* mutation has a much less dramatic effect (Shanbhag et al. 2001; Xu et al. 2005).

Odor Responses across ORN Populations: A Broad Olfactory Net

How many different coding units does an insect need to encode relevant odors in its environment? The number of different OR genes is a reasonable estimate, but there is no strict one-to-one OR/ORN ratio. Some ORNs express two or three genes (Couto et al. 2005; Ray et al. 2007). Some ORNs do not express any of the known OR genes. The identification of all ORN classes physiologically is informative but is tedious (Table 1). Only in *Drosophila* are we close to achieving characterization of all ORN classes (de Bruyne et al. 1999, 2001; Yao et al. 2005; van der Goes van Naters and Carlson 2007), and Table 2 shows an overview of the response properties of most of its ORNs. The number of glomeruli in the antennal lobe can be indicative because, with few exceptions, ORNs of a single class converge on a single glomerulus (Hansson and Christensen 1999; Vosshal et al. 1999). Unfortunately, glomerulus borders are difficult to visualize in some insect species, and numbers can vary slightly among individuals. Nevertheless, the estimates for all three parameters in Table 1 show that for these model species, 50–60 units appears sufficient. The ca. 160 units for honeybees (genes and glomeruli) possibly indicate the high end.

Odor stimuli contain three elements of information: odor identity, odor intensity, and a temporal component, i.e., how these two vary in time. If an odor is a mixture, its identity can change if the composition changes. Studies in which a large number of odorants are tested on many ORNs invariably show that they can be classified into response types. Most ORN classes respond to a small subset of odorants, and several odorants evoke responses from a few classes. The identity of many odors is, thus, encoded in the activity of different sets of ORN classes (Hildebrand and Shepherd 1997). However, this is dose-dependent. For instance, at relatively high doses, ethyl acetate excites three ORN classes in *Drosophila* (pb1A, ab1A, and ab2A in Table 2), but at different levels (de Bruyne et al. 1999, 2001). At lower doses, only ab1A will respond because the other two are less sensitive to this odorant but better tuned to related esters. Odor concentration in a single ORN class is usually encoded over a short range of concentrations. Dose–response curves typically show a sigmoid shape with the dynamic part covering two to three log steps (de Bruyne et al., 1999, 2001). Table 2 also outlines that each ORN class is tuned to a different odorant. What the table does not show is that there are some odorants that are promiscuous, exciting many ORNs, while others appear to be encoded by the activity of a single ORN class.

It is important to realize that the olfactory system's main function is to capture changes in an insect's odor environment and guide it toward resources or away from

Table 2 Organisation of peripheral olfactory system in Drosophila

ORN		OR	Glom.	Odorant	Sens.	Tuning
Sw sensilla (palpal basiconic)						
Pb1	A	42a	VM7d	Ethyl propionate	●●●	■■
	B	71a	VC2	4-Methylphenol	●●●	■
pb2	A	85e+33c	VC1	Fenchone	●●●	■■
	B	46aA+46aB	VA7l	4-Methylphenol	●●	■■
pb3	A	59c	VM7v	–	–	■
	B	85d	VA4	2-Heptanone	●●	■■
Sw sensilla (antennal basiconic)						
ab1	A	42b	DM1	Ethyl acetate	●●●	■■
	B	92a	VA2	3-Hydroxy-2-butanone	●●	■
	C	Gr21a	V	CO_2	●●●	■
	D	10a	DL1	Ethylbenzoate	●●●	■■
ab2	A	59b	DM4	Methyl acetate	●●●	■■
	B	85a+33b	DM5	Ethyl 3-hydroxybutanoate	●●●	■■
Ab3	A	22a+22b	DM2	Ethyl hexanoate	●●●	■■■
	B	85b	VM5d	6-Methyl-5-hepten-2-one	●●●	■■■
ab4	A	7a	DL5	E2-Hexenal	●●●	■■
	B	56a+33a	DA2	–	–	■
ab5	A	82a	VA6	Geranyl acetate	●●	■
	B	47a	DM3	Pentyl acetate	●●●	■■
ab6	A	?	?	1-Octen-3ol	●●●	■■■
	B	49b	VA5	2-Methylphenol	●●●	■
ab7	A	98a	VM5v	3-Octanol	●●	■■■
	B	67c	VC4	Ethyl lactate	●●●	■■■
ab8	A	43b	VM2	Ethyl butanoate	●●	■■■
	B	9a	VM3	2,3-Butanediol	●●●	■■■
ab9	A	69aA+69aB	D	?	?	?
	B	67b	VA3	?	?	?
Ab10	A	49a+85f	DL4	Acetophenone	●	■■
	B	Or67a	DM6	2-Phenylethanol	●●●	■■■
Sw sensilla (antennal trichoid and intermediate)[a]						
ai1	A	?	VA7m	?	?	?
	B	13a	DC2	?	?	?
at1	A	67d	DA1	Z11-octadecenyl acetate	●	■
at2	A	23a	DC3	1-Pentanol	●	■
	B	83c	DA3	?	?	?
at3	A	2a	DA4m	Iso-pentyl actetate	●	■
	B	19a+19b	DC1	1-Octen-3-ol	●●	■■■
	C	43a	DA4l	Cyclohexanol	●●	■■
at4	A	47b	VA1v	– (m/f)	–	■
	B	65a+65b+65c	DL3	– (m)	–	■
	C	88a	VA1d	– (m/f)	–	■
Dw sensilla (antennal coeloconic)[b]						
ac1	A	?	?	Ammonia	●●	■
	B	?	?	(hygroreceptor)	–	–
ac2	A	?	?	1,4-Diaminobutane	●●●	■
	B	?	?	(Hygroreceptor)	–	–
ac3	A	?	?	Propanal	●●●	■■
	B	35a	VC3m	Z3-hexenol	●●●	■■■
ac4	A	?	?	Phenylacetaldehyde	●	■
	B	?	?	–	–	■
	C	?	?	–	–	■

ORN Olfactory receptor neuron name, *OR* olfactory receptor genes expressed, *glom* glomerulus innervated, *odor* odorant known to evoke highest response, – no odorant evokes responses >50 spikes/s, *sens* sensitivity to a standard dose of that odorant in spikes/s (– < 50 ●, < 115, ●● < 180 ●●● >180), tuning, tuning width as % of odorants tested that evoke >50 Spikes/s (■ < 6%, ■■ < 22%, ■■■ > 22%).

[a] The three ORNs in at4 sensilla respond to extracts of male (m) and/or female (f) flies. [b] Most ORNs in dw sensilla do not express an OR; hence, projections to the antennal lobe have not been traced to individual glomeruli. Question marks indicate information not available. Data from de Bruyne et al. 1999, 2001; Goldman et al. 2005; Couto et al. 2005; Hallem and Carlson 2006; Endo et al. 2007; and de Bruyne and Faucher unpublished

danger. It has evolved the ability to detect key odorants with high sensitivity and to perceive a wide variety of general odorants at higher doses. Table 2 shows that *Drosophila* is particularly sensitive to a variety of esters, most likely because these are often generated by fermentation processes. Many phytophagous species have a variety of ORNs to detect terpenoids (Bruce et al. 2005), which seem to be underrepresented in the *Drosophila* ORN responses (de Bruyne et al. 2001; Hallem and Carlson 2006). The cockroach *P. americana* has several ORN classes that detect small alcohols and fatty acids (Sass 1976; Boeckh et al. 1987). While many odorants evoke responses from ORNs across insect species with very different ecologies, the part of the chemical world covered by an insect's repertoire of ORNs is likely to reflect the evolutionary history of the taxa.

In discussing tuning width of ORNs, it is important to realize that statements in the literature are rarely quantitative and depend highly on the breadth and variety of the set of odorants tested. Nevertheless, the ability of different OR proteins to bind a range of different odorant molecules varies widely (Hallem and Carlson 2006). In general, most ORNs of insects exhibit a higher degree of odorant specificity than previously thought, when they were labeled as broadly tuned odorant generalists as opposed to the more specialist receptors for pheromones (Hildebrand and Shepherd 1997). Work on heliothine moths has shown that many ORN classes respond to a relatively narrow set of plant odorants (Stranden et al. 2003; Rostelien et al. 2005). A similar reassessment of the tuning width of insect ORNs has resulted from studies performed on two species of beetle (Stensmyr et al. 2001).

ORNs that are tuned to components of insect sex pheromones exhibit some of the highest degrees of odorant specificity. Extremely specific responses to small differences in carbon chain length, functional group, or double bond positions are well known from different moth species. For instance, in *Heliothis* species, male ORNs are tuned to only one compound out of a series of closely related structural analogs (cf. Berg and Mustaparta 1995; Cossé et al. 1998; Baker et al. 2006). Highly specific responses also have been shown to stereoisomers of beetle pheromones, with one species responding only to the *R*-isomer while the other picks out the *S*-isomer (Wojtasek et al. 1998). Nevertheless, cross-reactivity does occur. A *Heliothis subflexa* ORN that mediates male attraction responds to a component of its own pheromone as well as to a different compound emitted by *H. virescens* (Baker et al. 2006), as does an ORN type in a species of *Yponomeuta* (Löfstedt et al. 1990). Genetic experiments on hybrids between the heliothine species suggest that such instances may be due to two related ORs being expressed in a single ORN (Baker et al. 2006).

Flexible Noses: Life Stages and Induced Genes

An insect's genome needs to encode several noses, each adapted to different ecological needs. The peripheral olfactory system of larval and nymphal stages can be quite different from that of the adult. Hemimetabolous insects add more olfactory sensilla with each molt, and the adult in particular has ORN classes that do not occur in earlier stages (Zacharuk and Shields 1991). In holometabolous insects, larvae often have a small set of ORNs with each individual one having different properties (Roessingh et al. 2007; Zacharuk and Shields 1991). *Drosophila* larvae have a single sensillum with 21 ORNs, each expressing a different OR gene (Fishilevich et al. 2005; Kreher et al. 2005). Some of these genes are expressed only there, while others occur in both larval and adult ORNs (Couto et al. 2005; Fishilevich et al. 2005) indicating that there is only partial overlap between the coding properties of adult and larval systems. Mosquito larvae, although aquatic, show a similar partial overlap in OR expression (Bohbot et al. 2007). Together with earlier electrophysiological analysis of olfactory perception in air and water (Behrend 1971), these results also indicate that odor detection needs no major adjustments upon transition from water to air.

Adult olfactory systems often differ between males and females. In moths, males often have larger antennae that carry many thousands of the same two or three classes of sex pheromone receptors. This presumably increases the chance of detecting the weak signal emitted by females. In females, specific ORN classes tuned to plant compounds appear to replace pheromone-sensitive ones (Heinbockel and Kaissling 1996, King et al. 2000). Consequently, small sets of moth OR genes show female-specific expression (Wanner et al. 2007). Finally, adult ORNs can adapt their response properties to external or internal conditions. Mosquito ORNs sensitive to the host chemical lactic acid are tuned down after a blood meal when females stop orienting to host odors and turn to oviposition stimuli instead (Davis 1984). Expression of an OR gene for 2-methyl phenol, a urine-related compound, is also turned off (Hallem et al. 2004). Ray et al. (2007) also provide some evidence that transcription factors can alter OR gene expression levels in adult flies.

Tuning to Mates: Pheromone Receptors

Recent rapid expansion of knowledge in insect olfaction owes a great deal to the study of pheromone perception, particularly of sex pheromones in moths. Moth sex pheromones epitomize the development of an exquisitely sensitive and fine-tuned communication system. The majority of moth sex pheromones are blends of 10- to 18-

carbon-long unbranched primary alcohols, acetates, or aldehydes, emitted from the female pheromone gland. They are synthesized from C_{16} or C_{18} fatty acids, and initial species-specific structural differences are imparted by one or more desaturases that place double bonds at specific locations (Roelofs and Rooney 2003). Species specificity is achieved even if females emit only simple blends of two or sometimes three pheromone components in precise ratios (along with other behaviorally inert compounds). Conspecific males fly upwind only to blends that closely approximate this ratio. Further specificity is imparted by males' cessation of upwind flight if the blend contains these components in the correct ratio but also contains an additional odd component emitted by heterospecific females as part of their pheromone blend (Vickers and Baker 1997, Quero et al. 2001).

Lepidopteran pheromone molecules are detected by ORNs in extra-long trichoid (sw) sensilla on the antennae of male moths (Kaissling 1987; Steinbrecht 1999). Usually there are two, but sometimes three, ORNs that co-compartmentalize within one sensillum. Pheromone-sensitive neurons are exclusive to males in most species and occur in large numbers (up to 40,000). Neurons that detect components of a pheromone blend can be together in a single sensillum (e.g., in Tortricidae and Crambidae) or in different sensilla (in Noctuidae). Their axons invariably converge on a small set of closely apposed enlarged glomeruli (macroglomerular complex or MGC) in the antennal lobe near the entrance of the antennal nerve (Hansson and Christensen 1999).

The genes that encode sex-pheromone-component-sensitive ORs of *B. mori* have been characterized and shown to respond to the two pheromone components bombykol and bombykal (Sakurai et al. 2004; Krieger et al. 2005; Nakagawa et al. 2005; Syed et al. 2006). These receptors are expressed in adjacent neurons in a single trichoid sensillum. In *H. virescens*, six related ORs have been characterized, and three were shown to respond to its pheromone components (Krieger et al. 2002, 2004; Grosse-Wilde et al. 2007). One of these is expressed in the most abundant type of trichoid sensillum, while the other two co-inhabit another type. It is likely that the choice of an OR for placement in a moth-pheromone-sensitive ORN relies upon the same genetic processes as described for *Drosophila* ORs for general odorants (Ray et al. 2007).

Flying to an Odor Source: The Need for Speed

Detailed studies of flying moths orienting to sex pheromones have taught us how insects move towards an odor source (see contribution by Cardé and Willis this issue). The activation properties of insect ORNs are in tune with the physical properties of odor strands and pockets of clean air that occur in natural odor plumes due to turbulence (Vickers et al. 2001). It is the intermittency of stimulation from the plume strands that promotes sustained upwind flight (Kennedy et al. 1981; Baker et al. 1985). Such sustained attraction and source location is based on reiterative upwind surges in response to odor strands, interrupted by cross-wind casting reversals in response to clean air pockets (Mafra-Neto and Cardé 1994; Vickers and Baker 1994; but see Justus and Cardé 2002). In-flight attraction and source contact is optimal when males discriminate the "correct" pheromone blend sufficiently rapidly in each of the pheromone plume strands they contact. Hence, the performance of insect ORNs is optimized for fast, repetitive responses to ensure accurate odor identification of strands arriving irregularly, often in intervals of less than 100 ms, but also with gaps between strands of more than ten times that (Baker and Haynes 1989; Vickers et al. 2001).

The optimal ratio of components in the blend most closely approximates the natural ratio emitted by females. Linn et al. (1986) convincingly demonstrated for several species that for such optimal blends, male behavioral thresholds are lower, and more males initiate upwind flight and from greater distances than for partial blend compositions or suboptimal ratios. Moreover, in heliothine moths blend-quality evaluations take place on a strand-by-strand basis, within fractions of a second. If components of another species' blend are perceived in a strand, male upwind flight is instantly affected. Strands of the optimal blend tainted with small amounts of such heterospecific antagonist are poorer at eliciting long upwind surges than strands of the untainted blend (Vickers and Baker 1997, Quero et al. 2001). ORNs tuned to such heterospecific pheromone component are often slightly more broadly tuned than are ORNs sensitive to a moth's own pheromone components. They may have evolved to detect pheromone components of several sympatric species, turning off attraction to plume strands that contain an alien compound and preventing orientation toward females of the wrong species (Cossé et al. 1998; Linn et al. 2007). To do this, most moths have ORNs co-compartmentalized in the same sensillum, one for these important heterospecific compounds and another for one of their own pheromone components (Cossé et al. 1998).

Why are insect ORNs generally combined in sensilla in stereotypic combinations? In *Drosophila*, the same two classes of ORNs are always found together in a single sensillum (de Bruyne et al 1999, 2001). The special ecology of moth pheromone systems suggests an answer. Blend compositions of pheromone compounds in individual plume strands that are traversed at high speed need to be assessed in split-second behavioral choices (Vickers and

Baker 1997; Quero et al. 2001). Stereotypical pairing of ORNs in the same sensillum is likely to ensure a high fidelity of on-site ratio reporting, regardless of differences in odor flux, and with the optimal spatio-temporal precision (Todd and Baker 1999). It has been shown that moths can discriminate between two overlapping pheromone plumes, due to incomplete mixing of their intertwined strands (Witzgall and Priesner 1991; Liu and Haynes 1992). Baker et al. (1998) estimated the temporal precision of blend ratio assessment to be close to 1 ms.

Nikonov and Leal (2002) suggested that in the Japanese scarab beetle *Popillia japonica* pheromone components are not only detected in parallel but processed within single sensilla before integration in the antennal lobe. On-site, within-sensillum mixture interactions can alter ORNs' reporting capabilities. However, it remains to be seen whether such cases constitute interactions within a neuron or between neurons. On-site mixture interactions were also demonstrated in *Helicoverpa zea* sensilla, in which the major pheromone component mixed with certain plant volatiles elicited greater ORN activity with a more tonic response profile than the pheromone component alone (Ochieng and Baker 2002).

It is just as crucial for the insect to respond quickly to the pockets of clean air between pheromone strands as it is to respond to the strand itself because the upwind, clean-wind direction of a large gap due to large-scale turbulence (wind-swing) does not lead to the source; a fast switch from upwind flight to cross-wind casting flight is required to increase the chance of recontacting odor-laden air (David et al. 1982). The latency of behavioral response to both the onset (upwind surges) and loss of pheromone pulses (cross-wind casting reversals) can be as fast as 0.15 s (Baker and Haynes 1987), but is usually between 0.3 and 0.6 s (Baker and Vogt 1988; Vickers and Baker 1996). This response speed does not seem specific to pheromones, as the latency to casting flight from upwind flight after the loss of host-odor is only slightly lower (0.7 s) in female moths (Haynes and Baker 1989).

Integrating Odor Identity and Intensity in Time

The biochemical, neurophysiological, and molecular processes that contribute to the propagation of behavior after contact with airborne pheromone strands all emphasize high-speed processing related to temporal resolution of the strand encounters. The frequency of action potentials in response to each pulse is significantly reduced under rapid stimulation and would make concentration coding less accurate. However, there is no evidence thus far that male moths need, or use, strand concentration information to locate females. This explains why such ORN adaptation nevertheless retains a high fidelity of reporting the temporal aspects of strand presence or absence, while losing the ability to report absolute concentration (Kaissling 1987).

There is some evidence that the speed of both ON- and OFF-responses in pheromone-sensitive ORNs is influenced by perireceptor proteins such as PBPs and pheromone degrading enzymes (PDEs; Syed et al. 2006). Some experiments indicate that binding dynamics of PBPs can be within the order of ms (Leal et al. 2005). The half-life of pheromone component molecules of *Anthaerea polyphemus* in the presence of an *A. polyphemus* PDE has been calculated to be less than 15 ms (Ishida and Leal 2005).

Pheromone-sensitive ORNs display a wide variation in the phasic or more tonic elements of their responses to pheromone components (Kaissling 1987; Berg and Mustaparta 1995). Phasic ORNs are fast to disadapt and are able to track (respond to) strands of pheromone in natural plumes (Vickers et al. 2001; Justus et al. 2005); they can also reliably respond to mechanically generated pulses at 10 Hz (Almaas et al. 1991) or even more than twice that (Bau et al. 2002). More tonically firing neurons (often called phasic–tonic) are usually slower to return to baseline firing levels, sometimes taking minutes or tens of minutes to do so (Rumbo and Kaissling 1989).

The frequency of action potentials in response to each short pulse of pheromone, normally correlated with the flux intensity of pheromone contained in each pulse, is significantly reduced under rapid stimulation, but the fidelity of response with regard to the temporal presence (pheromone strand) or absence (clean-air pocket) of the pheromone is accurate. Differential adaptation of ORNs specific to one pheromone component in each blend-strand without concomitant adaptation of ORNs specific to other components can result in a shift in ORN firing ratios being received by higher-order neurons and being registered through combinatorial coding as an off-blend, resulting in a reduction in attraction (Hansson and Baker 1991).

In *Drosophila*, there is also wide variation among different classes of ORNs for non-pheromonal odorants in the temporal response characteristics, particularly in the waning of responses after stimulus offset. Within a single ORN class, some odorants evoke responses that are cut off immediately with stimulus removal, while other odorants evoke long-lasting stimulation. This variation is independent of odorant chemistry and concentration. Instead, these temporal characteristics are dictated by the way a specific odorant interacts with a specific OR, as evidenced by experiments in which a single ORN type was transfected with many different ORs (Hallem and Carlson 2006). This variability can affect the temporal accuracy of reporting odor strands, but it may also enrich the coding possibilities for odor discrimination (de Bruyne et al. 2001).

ORNs detecting moth pheromones are the best studied examples with respect to temporal coding of natural odor stimuli. However, many insect species employ pheromones whose components can be chemically diverse, and their perception relies on a wide variety of ORN types. Many insect pheromones are related to plant volatiles, e.g., terpenoids such as periplanone (related to germacranes) in cockroaches (Persoons et al. 1976). Pheromone-sensitive neurons also reside in non-trichoid types of sensilla. For instance, in scarab beetles, placoid sensilla house the pheromone-sensitive receptor neurons (Nikonov and Leal 2002). Because of strong selective pressures, highly specialized olfactory subsystems have evolved from different elements of the general olfactory system in different insect groups, but fundamental mechanisms of olfactory coding appear to be conserved (Christensen and Hildebrand 2002)

Evolution of Pheromone Communication Systems

The evolution of pheromone communication systems is thought to be driven by changes in the biosynthesis of pheromone components that are tracked by shifts in male olfactory response (Phelan 1997; Roelofs et al. 2002). Major shifts in moth pheromone blends have been hypothesized to occur when previously unexpressed desaturase genes become expressed in the pheromone glands of some females in a population (Roelofs et al. 2002). If a few males in the population are able to respond to both the new and old blend, a new population of males and females communicating with this new blend would be formed through a process of asymmetric tracking over generations by males (Phelan 1997; Roelofs et al. 2002).

For this to occur, tuning properties of pheromone-sensitive ORNs would have to show a degree of variability for selection to act upon. It has been shown that the numbers of ORNs tuned to different pheromone components vary among European and West-Asian populations of the noctuid moth *A. segetum*, correlating with the differences in female blend ratios and male behavioral response occurring in different geographic regions (Hansson et al. 1990). Response profiles of pheromone-sensitive ORNs in hybrids between closely related heliothine species also reveal underlying genetic variability by the occurrence of new response types in addition to pure parental types (Baker et al. 2006). In some *Yponomeuta* species that use unusual pheromone components compared to their congeners, the male pheromone-sensitive ORNs display a correspondingly enlarged response spectrum (Löfstedt et al. 1990). Such cases may perhaps be due to a novel OR specific for the unusual component being co-expressed on these ORNs, or else a singly expressed, more broadly tuned OR for a wider range of compounds (Domingue et al. 2007).

The Stuff of Life: Volatiles from Primary Metabolism and Microbial Decay

While pheromones are usually produced from specific glands as secondary metabolites, many odorants that insects respond to are generated from primary metabolism of their hosts or associated microorganisms. Detection of some of these odors appears widespread across insect species.

Carbon dioxide (CO_2) is an atmospheric gas, omnipresent, and at relatively high concentrations for an odor. Insects can sense fluctuations around ambient concentrations (ca. 0.03%). Sensory neurons for its detection show modified dendrites with increased membrane surface and have been found in antenna of honeybees, ants, tsetse flies, fruit flies (Tephritidae), and *Drosophila* (Stange and Stowe 1999). In other insects, CO_2-sensitive neurons are found on the maxillary palps (mosquitoes, Grant et al. 1995) or labial palps (moths, Stange 1992). Larval stages of moths (Roessingh et al. 2007) and *Drosophila* (Faucher et al. 2006) also possess such neurons, but interestingly in both cases, they appear less sensitive than in the adults. In *Drosophila,* CO_2-sensitive neurons are found in basiconic sensilla on the antenna (de Bruyne et al. 2001; Suh et al. 2004). Although they are housed together with other ORNs, they do not express the *Or83b* gene nor any other member of the OR family. Instead, two members from the gustatory receptor (GR) family, *Gr21a* and *Gr63a*, mediate the response to CO_2 (Jones et al. 2007; Kwon et al. 2007). Orthologs of these genes have been found in the genomes of mosquitoes, moths, and beetles (Kwon et al. 2007) but not in the honeybee (Robertson and Wanner 2006). In the honeybee and other hymenopterans, CO_2-sensitive neurons are found in a different category of sensilla in deep pits on the antenna. The neurons appear less sensitive (Lacher 1964; Kleineidam et al. 2000), and may well use a different mechanism for CO_2 detection.

While CO_2 is produced in large amounts by all living cells, alkyl amines are metabolic by-products commonly formed from protein degradation. Attraction to ammonia and small amines is common in hematophagic insects such as flesh flies and mosquitoes, but other insects, such as fruit flies (Tephritidae), also need protein-rich food sources to complete egg maturation. In Carribean fruit flies, antennal responses to ammonia peak with deposition of yolk-proteins (Kendra et al. 2005). ORNs sensitive to amines commonly occur in dw sensilla. They are found in hematophagous insects such as mosquitoes (Meijerink et al. 2001) and triatiomine bugs (Taneja and Guerin 1995), but also in *Periplaneta* (Sass 1976) and even *Drosophila*. In

Drosophila, one ORN class responds to ammonia and another to 1,4-diaminobutane (putrescine; Yao et al. 2005). Like CO_2, amine perception might employ a different molecular mechanism. Neither of these neurons are known to express an identified OR gene (Table 2).

A well-documented attractant for hematophagous insects is 1-octen-3-ol. It was first associated with cattle breath, attracting tsetse flies (*Glossina* spp.). These flies bear at least two ORN classes on their antennae that detect it (van der Goes van Naters et al. 1996). Mosquitoes possess receptors on antennae and maxillary palps (Syed and Leal 2007). Actually, 1-octen-3-ol is a typical fungal odor and has been shown to signal fungal/mold infestations at oviposition sites (Steiner et al. 2007). In *Drosophila*, several ORN classes show high sensitivity to this odorant (de Bruyne et al. 2001). This volatile appears to excite different ORN classes in many insect species, and ORNs that detect it tend to be broadly tuned.

Plant Secondary Metabolites

The group of insects that are most studied because they interfere with our agriculture are those species that feed on plants. Phytophagous species make up a large proportion, and insect–plant interactions have driven evolution of sophisticated host location and defense mechanisms in insects and plants. Plants produce the largest variety of odorants as evidenced by our own use of these in spices and perfumes. The largest class of volatiles produced by plants in dazzling variety of mixtures is that of the terpenoids, all produced by well-known biosynthetic pathways. There are many ORN classes described for terpenoids, predominantly in moths. Receptors for commonly occurring terpenoids such as linalool, geraniol, β-ocimene, and β-caryophyllene are found in several moth species (Anderson et al. 1995; Shields and Hildebrand 2001; Rostelien et al. 2005). Terpenoid-sensitive ORNs are often housed in trichoid sensilla, and many of them are narrowly tuned. This highlights an important limitation in our understanding of insect olfaction. Many ORN types are not studied simply because they are tuned to natural compounds we have not tested. In several *Heliothis* species, an abundant type of ORN is tuned to the sesquiterpene germacrene-D, which occurs in many plants but cannot be obtained commercially (Stranden et al. 2003). It is likely though that narrowly tuned ORNs are also involved in a combinatorial type of coding. For instance, in the hawkmoth *M. sexta*, two narrowly tuned ORNs were found to respond to E-nerolidol and farnesol, respectively, but both also showed responses to the more common geraniol (Shields and Hildebrand 2001). Interestingly, a few of the many ORN classes described for *Drosophila* are activated by terpenoids. Instead, terpenoids such as linalool appear to inhibit some ORNs (de Bruyne et al. 2001), a phenomenon that may be more general (Kaissling et al. 1989).

The second largest group of plant volatiles, phenylpropanoids, contains an aromatic ring derived from the amino acid phenylalanine. They may serve to protect plant tissues from microorganisms. Methylsalicylate is a product of a signaling cascade that follows tissue damage (Turlings and Ton 2006) and has been shown to excite insect ORNs (de Bruyne et al. 2001; Shields and Hildebrand 2001). Methyleugenol is a powerful attractant for tephritid flies (Raghu 2004), although it is still not fully understood why. It has been suggested to be a case of pharmocophagy, where male flies pick up methyleugenol and use it to attract females.

The third group consists of derivatives of various fatty acids and contains mainly short-chain aldehydes, alcohols, and esters. E2-hexenal is a green leaf volatile (GLV), one of several compounds typically released after damage to leaves (Visser 1986) but also found in many flower and fruit odors. Highly sensitive ORNs to GLVs have been found in moths (Anderson et al. 1995) beetles (Visser 1986; Blight et al. 1995; Hansson et al. 1999) and flies (Guerin et al. 1983; de Bruyne et al. 2001).

Many insects have developed specific interactions with a select group of host plants. In theory, they could use host-specific cues to locate them. While some insects certainly use specific chemicals that identify distinct plant taxa, e.g., isothiocyanates for cruciferous plants (Blight et al. 1995), host finding more often involves mixtures of odorants that are common to many plants (Fraser et al. 2003). It seems that most phytophagous species home in on specific ratios among components (Visser 1986). As in moth pheromone systems, stereotypical pairings of ORNs in single sensilla may facilitate this, and it has been suggested that these are often excited by odorants from different biosynthetic pathways (Blight et al. 1995). Compared to pheromone systems, there are many more compounds in plant-produced mixtures, and their ratios are more variable. Here, selection probably favors robust adaptable systems rather than finely tuned specialized ones. Recent comparative work on phytophagous insect species with differing host specificities suggest that ORN response properties do not easily specialize. Closely related tephritid species show similar responses even if their behaviors towards plant odors differ considerably (Olsson et al. 2006). Studies on *Heliothis* (Rostelien et al. 2005) and *Drosophila* (Stensmyr et al. 2003) also suggest that insect noses maintain a generally broad sensitivity with small modifications. Finally, it should be borne in mind that insects probably detect many compounds or evaluate ratios between them that are indicative of non-hosts or toxic substrates.

Concluding Remarks

These are exciting times in the study of insect olfactory ecology. The convergence of genomic, physiological, and ecological data will elucidate the physiological and genetic basis of odor-mediated behaviors. The availability of newly sequenced insect genomes will shed new light on how olfactory systems evolve to fit different ecological niches. Despite the millions of years of evolution and the different chemical ecologies that separate some of the insects we have discussed, they share common sensitivities. It will be interesting to see how much of this is homology and how much is convergent evolution.

The selectivity of the olfactory system depends on processes between odor molecules and OR proteins, but how odors activate ORs is still largely unclear. Because insect ORs have evolved independently from those in vertebrates, solving this mystery in both groups may teach us what fundamental biochemical interactions link chemical ecology with molecular evolution. The visual system can provide useful analogies. Compound eyes are relatively uniform in shape and composition across insect orders. Olfactory epithelia have many ORN classes, enveloped in different sensory structures that express OR genes and some GR genes. They may employ other mechanisms as well. We know which physical properties of light dictate the uniformity of eyes and what drives variability in opsin genes, but we know little about how the chemical environment molds olfactory genomes of insects.

Photoreceptor cells are accompanied by cells that generate lenses, and absorption spectra of receptors are modified by screening pigments. By analogy, we should keep our minds open for modifications in vivo to the response properties of ORs. For instance, because OBPs and PBPs are insect-specific, extracellular, and highly expressed, they provide good targets for novel strategies in pest control. Although we have learned much about the unifying principles of olfactory function, we still have much to understand about the ecological mechanisms that drive the amazing variety in form and function of insect noses.

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