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OLFACTORY SYSTEM IN *DROSOPHILA*

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Abstract. Smell is an ancient sensory system presented virtually in organisms from bacteria to humans. In *Drosophila* odors elicit a variety of behavioral responses in relatively simple but sensitive olfactory system. An increasing number of mutants have been found to be defective in olfactory function. Genetic and molecular analysis of the olfactory system of the fruit fly have identified many molecular components, and have revealed some principles of its function and organization.

Keywords: olfaction, *Drosophila*, olfactory receptors, olfactory sensory neurons.

1. INTRODUCTION

Insect olfactory systems vary enormously. Some species possess highly specialized olfactory systems, possibly limited to few odorants with innate significance, such as the carrot psyllid with only 50 receptor cells of possibly only four types [22]. Oligolectic pollinators (some moth or bee species that live on nectar of very few flower species) find their preferred flower based on a few key compounds. Some jewel beetles (e.g., *Melanophila acuminata*) fly long distances to find burning forests, where they deposit their eggs in burnt trunks. The highest sensitivity described so far is that of male moths following the sexual pheromone trail of female conspecifics, often at a distance of several miles. At the other end of the spectrum, some insect species have widely specialized olfactory systems. Honeybees visit whatever flower will deliver nectar: their olfactory system can learn hundreds of odors and distinguish them [11, 13]. Fruit flies are sensitive to an extraordinary variety of odorants. Airborne molecules drive a number of behavior reactions, including attraction and repulsion [5].

Drosophila melanogaster offers several advantages as an organism which may help to address some of these problems [33]. The first advantage is that its olfactory system is relatively simple, containing only 103 neurons. The second advantage is a significant number of genetic and molecular tools that can be conveniently applied to explore the system. The third, and perhaps most compelling, the olfactory response can be easily quantified *in vivo* using either physiological, or behavioral measures. Recent progress and difficulties in the study of vertebrate olfaction have stimulated new interest to *Drosophila* as a model system to study olfaction [5].

2. OLFACTORY RECEPTORS AND RECEPTOR CELLS

In vertebrates, olfactory receptors (ORs) were first identified in 1991 as a very large family of related genes encoding members of the G protein-coupled receptor (GPCR) superfamily, which couples ligand binding to production of cAMP to induce downstream signaling [4]. During the 1990s, efforts by multiple investigators to find homologues of vertebrate ORs in insect genomes failed. In 1999, three groups used a combination of difference cloning [37] and mining of genome databases for multi-transmembrane domain proteins [7, 14, 37] to identify candidate *Drosophila* ORs. There are 62 ORs, encoded by a family of 60 genes through alternative splicing [28]. The fly OR genes encode a highly divergent family of membrane-associated proteins that are selectively expressed in *Drosophila* olfactory sensory neurons (OSNs) [7, 14, 37].

The carbon dioxide receptor (CO₂) of *Drosophila* is a special case: the heteromer consists of dGR21a and dGR63a, with no need for Orco. While CO₂ is clearly an odor for a fly, and not a taste, the receptors are within the gustatory clade of the gene tree. The CO₂ receptor is more related to other gustatory receptors than to the olfactory receptors [12].

A second family of olfactory receptor genes has been called IRs (ionotropic receptors). These receptors are similar in structure to ionotropic glutamate receptors (iGluRs), but have lost the binding site for glutamate. *Drosophila* has 11 neuron types expressing IRs in four coeloconic sensillum types. Each receptor cell expresses at least a pair of IRs, generally one specific IR and either one or both of IR8a and IR25a, suggesting that they work as receptor complexes, most likely as odorant-activated ion channels [2]. ORs and IRs are not coexpressed. IR response profiles to odorants are similarly broad as ORs, and some odorants elicit activity both in IRs and in ORs, though for other odorants responses have only been found in one or other family [12].

In addition to IRs and ORs, some other protein families play important roles in olfaction. These are olfactory binding proteins (OBPs) and sensory neuron membrane proteins (SNMP). OBPs are produced by the accessory cells in the sensillum and released into the lymph at high concentrations. They perhaps serve several functions which are still debated, but helping the transport of lipophilic odorants across the aqueous lymph is uncontroversial [21]. A special family of OBPs is represented by the PBP (pheromone binding proteins). SNMPs are located in the membrane of receptor cells, and might belong to the receptor complex, participating in transduction of olfactory signal. SNMPs belong to the CD36 protein family, which have two transmembrane domains, and large extracellular binding domains. These proteins typically bind and transport cholesterol, fatty acids, and other hydrophobic molecules [12].

3. THE OLFACTORY SYSTEM

Drosophila detects odors through the two olfactory sensory organs on the head, the antenna and maxillary palp (Fig. 1.). They are covered with a large number of sensory hairs, called sensilla, which protect the underlying OSNs that are specialized to detect odors. Olfactory sensilla can be distinguished morphologically from thermo- and hygro-sensitive sensilla by the presence of a large number of small pores that perforate the shaft of the sensillum and which are believed to allow access to odors. A total number of about 410 olfactory sensilla cover the antenna, the maxillary palp has about 60 olfactory sensilla. These hairs can be divided into three distinct morphological and functional classes: club-shaped basiconic sensilla, long and pointed trichoid sensilla and short, peg-shaped coeloconic sensilla [23].

Morphological and functional distinctions subdivide both basiconic and trichoid sensilla into additional subclasses. They differ by the size and density of odor pores, the number of neurons housed in each sensillum and their distribution on the antenna [6, 8, 31, 32, 34]. The different sensilla types are distributed in a highly stereotyped fashion over the surface of the antenna. Large basiconic sensilla are

clustered at the medial face of the antenna, while the three types of trichoid sensilla are arranged in diagonal bands across the lateral face of the antenna [23].

Coeloconic sensilla are interspersed with other sensilla types, but are concentrated at the central face of the antenna. The relative position of these sensilla is well conserved as are the number of neurons innervating a given sensillum. Trichoid sensilla (T1, T2 and T3) contain one, two, or three OSNs, respectively [23]. Basiconic sensilla house contain two neurons, there are several cases of four neurons per basiconic sensillum [32, 34], coeloconic sensilla – two or three neurons. The third segment of the antenna is marked by a reproducibly ordered array of olfactory sensilla that house defined and stereotyped numbers of OSNs [16, 17, 26, 29].

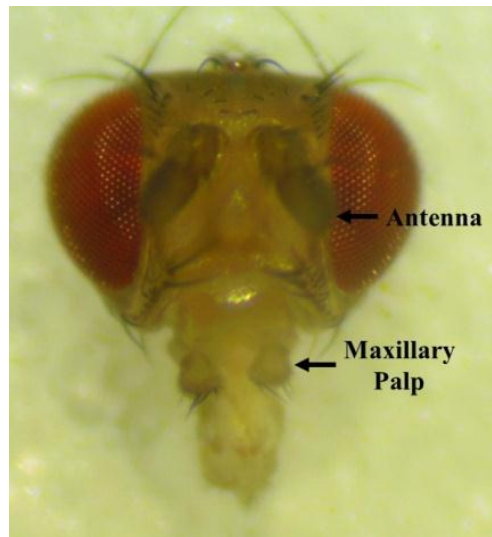


Fig. 1. Olfactory organs – the antennae and maxillary palps.

The maxillary palp is relatively simple olfactory organ, containing few OSNs housed in a small number of basiconic sensilla. About sixty basiconic sensilla each housing two OSNs can be found in this organ. Shanbhag et al. [32] used electron microscopic analysis of OSN terminal dendrite branching in the maxillary palp to further subdivide palp sensilla into three subtypes, PB-I, PB-II, and PB-III [31]. PB-I OSNs contain highly branched terminal dendrites, while PB-II OSNs are characterized by ribbon-shaped dendrites. PB-III OSNs are rarer on the palp and have an unusual thick, hollow dendritic segment. [12].

The antennal lobe (AL) is the deutocerebral neuropil of insects. AL receives the input from the olfactory sensory neurons on the antenna. Functionally, AL shares some similarities with the olfactory bulb in vertebrates.

In insects, the olfactory pathway starts at the antennae (Fig. 2.) (though in some insects like *Drosophila* there are olfactory sensory neurons in other parts of the body) from where the sensory neurons carry the information about the odorant molecules impinging on the antenna to the antennal lobe [18].

The antennal lobe is consist of tightly packed neuropils, called glomeruli, where the sensory neurons interact with the two other views of neurons, the projection neurons and the local neurons. In the *Drosophila* antennal lobe there are 43 glomeruli; in *Aedes aegypti* there are 32; locusts and social wasps may have over 1000 [18]. The projection neurons project to higher brain centers such as the mushroom body and the lateral horn [35]. The local neurons, have their neurites restricted to the antennal lobe. In fruit fly, each olfactory sensory neuron generally expresses a single olfactory receptor gene [38] and the neurons expressing a given gene all transmit information to one or two spatially invariant glomeruli in the antennal lobe [20]. Each projection neuron receives information from a single glomerulus. The interaction among the olfactory receptor neurons, local neurons and projection

neurons reformats the information received from the sensory neurons into a spatiotemporal code before it is sent to higher brain centers [23].

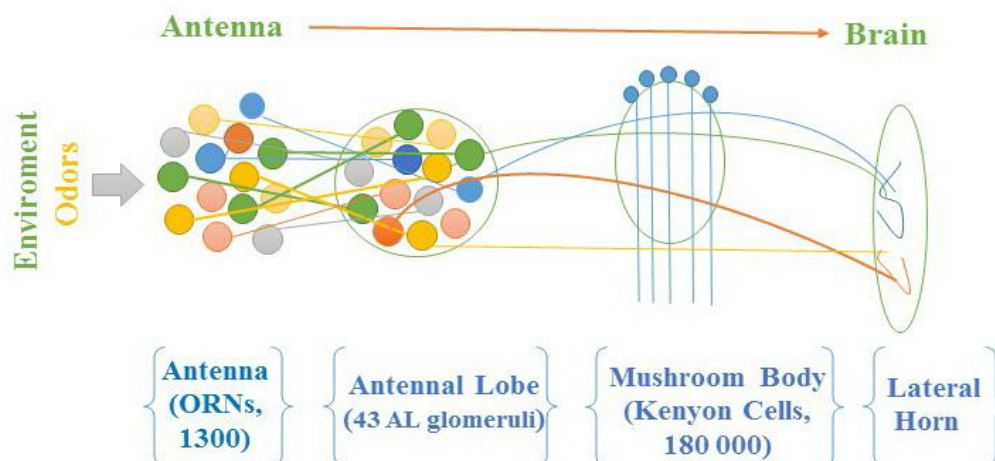


Fig. 2. Scheme *Drosophila* olfactory system. The olfactory receptor neurons (ORNs) are located in the antenna (1200 ORNs) and maxillary palp (120 ORNs). Each ORN that expresses the same odorant receptor targets its axons to a defined locus in the antennal lobe (olfactory bulb) called a glomerulus. In *Drosophila*, there are about 54 glomeruli. The antennal lobe is the first processing center for olfactory information. Olfactory information is then relayed by the projection neurons (PNs), which send their axons to synapse with the mushroom bodies and also the lateral horn (redrawn from [12, 23]).

The axons of antennal lobe projection neurons project to the protocerebrum, and here to the mushroom bodies and the lateral protocerebrum. The mushroom bodies have been studied in details, for two reasons. The first reason is that they were shown to be important for learning and memory. The second, they have a highly structured and geometrically regular architecture: their intrinsic cells, the Kenyon cells, form dense bundles of very fine axons that bifurcate to form the mushroom body peduncle and lobes. The cell bodies are arranged in a circular manner, forming a cuplike structure, and the axon bundles are like stalks of a mushroom. In most insects, Kenyon cells are the largest cell population in the brain: 180 000 in each hemilobe of the bee correspond to more than a third of all bee brain cells. In most insect species, mushroom bodies get olfactory and visual input, allowing multimodal processing and memory [12].

The lateral protocerebrum is an important part of the brain olfactory circuitry: premotor neurons receive input in the lateral protocerebrum, thus input from the antennal lobe to premotor neurons in the lateral protocerebrum is the fastest connection from a stimulus to a behavioral response. [12].

4. ODORANT RECEPTOR GENE EXPRESSION

Drosophila OR genes encode a highly divergent family of membrane-associated proteins that are selectively expressed in *Drosophila* OSNs [7, 14, 37]. These proteins contain seven transmembrane domains, but do not possess obvious homology to vertebrate ORs or the GPCR superfamily [1, 37, 39]. Different members of the fly OR family show considerably less homology to each other than most vertebrate ORs [28]. Detailed information about the expression of each *Drosophila* OR gene is now available. Recent studies have shown that there is a segregation of gene expression between the two major appendages: ORs expressed in the antenna are not expressed in the maxillary palp and vice versa. Examination of a group of 57 fly ORs confirmed this initial impression of segregation in OR repertoire between antenna and palp. These appendages express non-overlapping subsets of 32 and 7 OR genes, respectively (Table 1) [38]. OR gene expression with transgenic reporter techniques bring the total number of antennal-specific genes to 40 and maxillary palp-specific genes to seven [8, 10].

Each OR gene is expressed in a small subset of the OSNs in either olfactory organ, which varies from two to 50 OSNs per OR. The relative position and number of OR-expressing OSNs is bilaterally symmetric in the two appendages and highly stereotyped between individual flies. Early reports discussed the existence of “zones” of OR gene expression, reminiscent of the zones of OR gene expression on the olfactory turbinates of the rodent [27, 36]. Careful examination of the relationship between OR gene expression and sensilla type has revealed that there is a nearly perfect correlation between the expression of OR genes and subsets of morphologically distinct basiconic, trichoid and coeloconic sensilla [3, 8]. Thus, the same developmental pathways that specify the morphology of the sensilla must also dictate the numbers and functional properties of the OSNs and the specific ORs they express [23].

Antena					Maxillary palp
Basiconic sensilla		Trichoid sensilla	Coeloconic Sensilla		Basiconic sensilla
Food odors	CO ₂	Pheromones	Food odors	Water vapor	Food odors
OrX/GR83b	Gr21a/Gr63a	OrX/Gr83b	Or35a/Gr83b	Unknown receptor	OrX/Gr83b
Or7a Or56a	Gr21a	Or2a	Or35a	?	Or33c
Or9a Or59b	Gr63a	Or19a			Or42a
Or10a Or67a		Or19b			Or46aA
Gr10a Or67b		Or23a			Or59c
Or13a Or67c		Or43a			Or71a
Or22a Or69aA		Or47b			Or85d
Or22b Or69aB		Or65a			Or85e
Or33a Or82a		Or65c			
Or33b Or83c		Or67d			
Or42b Or85a		Or88a			
Or43b Or85b					
Or47a Or85f					
Or49a Or92a					
Or49b Or98a					
Or98b					

Tab. 1. Molecular organization of the *Drosophila* olfactory system. Gene expression of chemosensory receptors responding to different classes of ligands is indicated. Red – co-receptors (redrawn from [9, 10, 19, 24, 40]).

There are two unusual features of OR gene expression in *Drosophila* that installed this system, in which each OSN expresses only a single OR gene [30]. First, each *Drosophila* OSN expresses a broadly expressed member of the OR gene family called Or83b, which associates with ORs and is necessary for the proper ciliary targeting and function of all OR genes [1, 24, 25]. Second, a given OSN can co-express up to three conventional ORs mediating ligand selectivity along with the Or83b co-receptor [8, 10, 15].

5. CONCLUSIONS

The use of *Drosophila* to study olfaction has been growing rapidly. Expanded state of knowledge concerning gene expression and synaptic organization of the early olfactory system of the fly makes this a compelling system to address questions in odor coding. For instance, it is not yet clear in any species how and where odor concentration is encoded; how the brain solves odor mixture problems. Little is known about how the olfactory system processes odors to produce stereotyped behavioral outputs. The small size, genetic manipulability and availability of robust olfactory behavior paradigms for *Drosophila* olfaction strengthen the role of this small insect as a powerful genetic model system for the foreseeable future. It seems clear that genetics will continue to play an important role in following the path from odor to behavior in the fly.

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Нюхова система виявлена практично у всіх організмів від бактерій до людини. У представників роду *Drosophila* запахи викликають різні поведінкові реакції за участі відносно простої, але чутливої нюхової системи. Генетичні та молекулярні дослідження нюхової системи *Drosophila* дозволили ідентифікувати деякі компоненти, і виявили основні принципи її функціонування та організації.

Ключові слова: нюх, *Drosophila*, нюхові рецептори, нюхові сенсорні нейрони.