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INSECT ODOR AND TASTE RECEPTORS

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■ **Abstract** Insect odor and taste receptors are highly sensitive detectors of food, mates, and oviposition sites. Following the identification of the first insect odor and taste receptors in *Drosophila melanogaster*, these receptors were identified in a number of other insects, including the malaria vector mosquito *Anopheles gambiae*; the silk moth, *Bombyx mori*; and the tobacco budworm, *Heliothis virescens*. The chemical specificities of many of the *D. melanogaster* receptors, as well as a few of the *A. gambiae* and *B. mori* receptors, have now been determined either by analysis of deletion mutants or by ectopic expression in in vivo or heterologous expression systems. Here we discuss recent advances in our understanding of the molecular and cellular basis of odor and taste coding in insects.

INTRODUCTION

Olfactory and gustatory systems play crucial roles in insect survival and reproductive success, mediating responses to food, mates, and oviposition sites. Insects possess sensitive chemosensory systems that can detect and discriminate among a diverse array of chemicals. The ability to respond to these compounds is conferred by odor and taste receptors, which in both insects and mammals are seven-transmembrane-domain receptors encoded by highly diverse gene families. Recent progress in determining the chemical specificities and functional properties of these receptors has provided insight into the mechanisms underlying odor and taste coding in insects.

The fruit fly, *Drosophila melanogaster*, is a valuable model system for the study of insect olfaction and gustation, and much of our knowledge of the molecular basis of insect chemoreception comes from work on this fly. *D. melanogaster* exhibits robust responses to odors, is uniquely amenable to genetic manipulation, and possesses a chemosensory system that can be easily studied with both molecular and electrophysiological techniques. The first insect odor (11, 31, 138) and taste (10) receptor genes identified were those of *D. melanogaster*, and the availability of a completely sequenced genome has facilitated a comprehensive genomic analysis

of these receptor gene families (99). Functional studies of these receptors are now providing an integrated view of the molecular and cellular basis of insect chemoreception.

In the past few years, odor and taste receptors have also been identified in other insects, including the malaria vector mosquito *Anopheles gambiae* (27, 43); the tobacco budworm, *Heliothis virescens* (61a); and the silk moth, *Bombyx mori* (80, 102). Although a large-scale analysis of these receptors has not yet been undertaken, the first of these receptors has been functionally characterized either by ectopic expression in *D. melanogaster* (36, 51) or by heterologous expression in *Xenopus laevis* oocytes (80, 102). Odor and taste receptors from many other insect species are likely to be identified from genome-sequencing projects that are currently underway.

INSECT ODOR RECEPTORS

Functional Characterization of Olfactory Neurons

In many insects, olfactory receptor neurons (ORNs) are found in two bilaterally symmetrical pairs of olfactory organs, the antennae and the maxillary palps. The surfaces of the olfactory organs are covered with sensory hairs called sensilla, which contain the ORN dendrites. Despite considerable variability in the gross morphology of olfactory organs across species, the structure of the olfactory sensillum is generally well conserved and consists of a cuticular wall containing multiple pores through which odors can enter (101, 108). Olfactory sensilla typically contain the dendrites of between one and five ORNs (134). The axons of the ORNs project to functional processing units called glomeruli in the antennal lobes of the brain (42). In addition to ORNs, many insect olfactory organs also contain smaller numbers of mechanosensory, thermosensory, hygrosensory, and gustatory neurons (101).

In the *D. melanogaster* adult, each antenna has ∼1200 ORNs, and each maxillary palp has ∼120 ORNs (105, 108, 119). Each sensillum contains the dendrites of up to four ORNs (105, 108). The olfactory sensilla of the antenna can be subdivided into three major morphological types—basiconic, coeloconic, and trichoid—which differ in size, shape, and cuticular structure (105). The olfactory sensilla of the maxillary palp consist entirely of basiconic sensilla (105). The axons of both antennal and maxillary palp ORNs converge onto ∼43 glomeruli in the antennal lobe (62). Most ORNs project bilaterally to the antennal lobes, although a subset projects only ipsilaterally (95, 120).

Insect ORNs have been studied extensively by single-unit electrophysiology, which is an extracellular recording technique used to examine the responses of single ORNs to odors (53). Single-unit recordings from many insect species including moths, honey bees, mosquitoes, and flies have revealed that different ORNs respond to different odors and that they also differ in response properties such as

signaling mode (whether the response is excitatory or inhibitory) and response dynamics (2, 9, 14, 15, 39, 64, 73, 74, 86, 87, 109, 110, 117, 118, 132).

In *D. melanogaster*, the maxillary palp contains six different functional classes of ORNs, which are found in stereotyped combinations within three types of sensilla (14). The sensilla have been designated pb1 through pb3, and the ORNs have been designated pb1A, pb1B, pb2A, pb2B, pb3A, and pb3B. A detailed characterization of the antennal ORNs found in basiconic sensilla identified 18 different functional classes within eight types of sensilla, designated ab1 through ab8 (15, 21). The ab1 sensillum contains four ORNs, and the other sensilla each contain two ORNs. The antennal ORNs found in trichoid and coeloconic sensilla have not yet been characterized in detail (9, 37, 143).

D. melanogaster larvae have one pair of olfactory organs, which are called the dorsal organs. Each dorsal organ has 21 olfactory neurons, which extend dendrites into a single sensillum called the dome sensillum (88, 114, 119). Electrophysiological recordings from the dome sensillum have demonstrated the odor-responsiveness of these ORNs (58, 88). ORN axons project to small glomeruli in the larval antennal lobe (38, 94).

Identification and Genomic Organization of *Or* Genes

Odor receptor genes were discovered first in the rat in 1991 (7) and then in *Caenorhabditis elegans* four years later (130). Insect odor receptor genes were sought for many years without success but were finally identified in *D. melanogaster* in 1999 by both bioinformatic (11) and molecular (138) approaches. Both approaches led to the initial identification of members of the odor receptor (*Or*) gene family. A bioinformatic search of the completed *D. melanogaster* genome subsequently identified 60 *Or* genes that encode 62 Or proteins by alternative splicing (99).

The Or proteins are highly diverse, with many sharing only ~20% amino acid similarity. A comparison of *D. melanogaster* and mammalian odor receptor genes revealed that these gene families do not share sequence similarity. The *Or* genes are widely distributed throughout the genome, and many are found in small clusters of two or three genes (99). Genes within a cluster often share a higher degree of sequence similarity with each other than with the rest of the *Or* genes, suggesting that some of the ancestral *Or* genes may have undergone recent duplication events to give rise to clusters of *Or* genes. Analysis of patterns of intron gain and loss among *Or* genes suggests an ancient origin for this insect receptor gene family (99).

Expression of *Or* Genes

Functional and anatomical studies of insect and mammalian olfactory systems have revealed that olfactory systems contain heterogeneous populations of first- and second-order neurons, that the insect antennal lobe and mammalian olfactory bulb are subdivided into glomeruli, and that different glomeruli receive input from different subsets of ORNs (4, 33, 42, 49, 103). In the context of these studies, the

identification of *Or* genes and the analysis of their expression patterns provided a molecular explanation for many of the organizational principles of olfactory systems.

Researchers have examined the expression of individual *Or* genes using a number of different techniques, including in situ hybridization, immunohistochemistry, and reporter gene analysis. Reporter gene analysis has relied primarily on the *GAL4-UAS* system (5), in which regulatory sequences of an *Or* gene are used to drive expression of the yeast transcriptional activator GAL4 (*Or-GAL4*). GAL4 in turn drives expression of a reporter gene such as green fluorescent protein (GFP) specifically in the subpopulation of ORNs that expresses the particular *Or* gene.

These approaches have revealed that different *Or* genes are expressed in different subpopulations of ORNs and that each ORN expresses only one or a small number of *Or* genes (11, 18, 137, 138). In situ hybridization revealed that 32 *Or* genes are expressed in the antenna and 7 *Or* genes are expressed in the maxillary palp (137). Or protein is localized primarily to ORN dendrites, which extend into the olfactory sensilla and come into contact with odors present in the sensillum lymph (18, 22).

Analysis of ORN projections using *Or-GAL4* constructs to genetically label specific subpopulations of ORNs revealed that ORNs expressing the same *Or* gene converge onto one or a small number of common glomeruli in the antennal lobes (3, 32, 137). In mammals, the mechanism by which ORN axons converge onto their cognate glomeruli in the olfactory bulb is receptor dependent, involving homotypic interactions among axons of ORNs expressing the same odor receptor (25, 26). However, this does not appear to be the case in *D. melanogaster*, as ORNs that lack odor receptors and ORNs that ectopically express different receptors target the same glomeruli in the antennal lobe (18).

The basic principles underlying the molecular organization of olfactory systems appear to be conserved from insects to mammals. Most mammalian ORNs appear to express only one or a small number of odor receptors (71, 96), and ORNs expressing the same *Or* gene converge onto the same glomeruli in the olfactory bulb (78, 98, 133).

Functional Characterization of Odor Receptors

INITIAL IDENTIFICATION OF ODOR RECEPTOR LIGANDS Until recently, relatively little was known about the ligand specificity of individual odor receptors in any species, primarily because expression of odor receptors in heterologous systems is difficult. The first insect odor receptor to be functionally characterized was the *D. melanogaster* antennal receptor Or43a. Overexpression of Or43a in the antenna, as well as heterologous expression in *X. laevis* oocytes, identified the odorants cyclohexanone, cyclohexanol, benzaldehyde, and benzyl alcohol—all of which are found in fruits and other natural odor sources (129a)—as ligands for Or43a (122, 141). The antennal receptors Or22a and Or22b were subsequently characterized in detail by molecular, genetic, and electrophysiological analyses (18). Both receptors

are coexpressed in the ab3A antennal neuron. Or22a and Or22b are highly similar receptors that share 78% amino acid identity. The genes encoding these receptors are separated by an intergenic region of only ~650 base pairs and appear to have arisen through recent genome duplication. Analysis of deletion mutants lacking one or both of these receptors demonstrated that Or22a appears to account for the full odor response spectrum of the ab3A neuron, mediating strong responses to a number of fruit esters such as ethyl butyrate, ethyl hexanoate, and pentyl acetate. A function for Or22b has not yet been identified, as the ab3A neuron appears unresponsive to odors in flies that contain Or22b but not Or22a (18). Analysis of an Or43b deletion mutant subsequently identified Or43b as the receptor for the ab8A antennal neuron (21). Expression of Or47a in a mutant ab3A neuron lacking Or22a and Or22b identified it as the receptor for the ab5B neuron (18).

A RECEPTOR-TO-NEURON MAP FOR THE ANTENNA AND MAXILLARY PALP Nearly all of the odor receptors expressed in the antenna and maxillary palp have now been characterized, and many of these receptors have been mapped to the functional classes of neurons in which they are expressed. A large-scale analysis of the antennal receptors of *D. melanogaster* was accomplished using the deletion mutant lacking *Or22a* and *Or22b* as an in vivo expression system (18, 37). The absence of these *Or* genes results in a mutant ab3A antennal neuron—the "empty neuron"—that is unresponsive to odors. Individual receptors can be expressed specifically in the empty neuron with an *Or22a-GAL4* driver, and the resulting odor response profile conferred by each receptor can be measured electrophysiologically. This approach (37) was used to analyze 31 of 32 odor receptors expressed in the antenna in an in situ hybridization study (137). Twenty-four of 31 receptors conferred odor responses upon the empty neuron; 13 of these receptors showed an odor response profile corresponding to that of a previously characterized ORN, thus identifying the ORNs from which these receptors are derived. This analysis revealed that most, if not all, antennal ORNs express only one functional odor receptor (37).

A receptor-to-neuron map has also been established for the maxillary palp using in situ hybridization, as well as *Or-GAL4* constructs to drive expression of GFP or the cell death gene *reaper* (34). Five of six maxillary palp neuron classes express only one *Or* gene. Interestingly, one neuron class expresses two *Or* genes: The pb2A neuron expresses both *Or33c* and *Or85e*. The axons of the pb2A neurons project to a single glomerulus in the antennal lobe. *Or33c* and *Or85e* are expressed on different chromosomes and show only 16% amino acid identity. Functional analysis of these receptors by expression in the empty neuron revealed that, although both receptors are odor responsive, most of the odor response of the pb2A neuron is conferred by Or85e. A strong ligand for Or33c has not yet been identified, suggesting the possibility that Or33c is narrowly tuned to a specific ligand such as a pheromone. Interestingly, *Or85e* and *Or33c* are also coexpressed in the pb2A neuron in *D. pseudoobscura*, indicating that the coexpression of these two genes has been conserved for >45 million years of evolution and may therefore be behaviorally significant (34).

The receptor-to-neuron maps of the antenna and maxillary palp revealed that *Or* genes expressed in the same sensillum are not more closely related to each other than to the rest of the *Or* genes in terms of sequence similarity or genomic location (34, 37, 99). In addition, despite the nonrandom distribution of sensillar types on the surfaces of the olfactory organs, receptors with similar patterns of spatial distribution are not more closely related by sequence similarity or genomic location.

A comparison of the ligand specificities of individual odor receptors demonstrated that many odor receptors respond to common ligands, and thus one odor typically activates multiple receptors (34, 37, 139). This result helps explain why early behavioral screens for olfactory mutants failed to identify mutations in odor receptor genes, and why flies containing an engineered deletion of an odor receptor showed normal olfactory-mediated behavior (21). However, the inactivation of selected subsets of olfactory neurons induces behavioral defects (54, 90, 124), and the recent demonstration that overexpression of the antennal receptor Or43a in the antenna results in reduced behavioral avoidance of benzaldehyde demonstrates that misexpression of a single odor receptor can result in a behavioral phenotype (123).

THE ODOR RECEPTOR DETERMINES MULTIPLE ORN RESPONSE PROPERTIES The in vivo expression system provided by the empty neuron made it possible to examine the contribution of the odor receptor to the different neuronal response properties of ORNs. As in other insects, ORNs in *D. melanogaster* vary in their odor response spectra and also in other response properties such as spontaneous firing rate, signaling mode, and response dynamics (14, 15, 118). These diverse response properties of ORNs could be determined by the odor receptor or by the neuronal context.

Expression of receptors in the empty neuron revealed that in many if not all cases the odor receptor is the primary determinant of the odor response spectrum of the ORN: When receptors were analyzed in the empty neuron, the odor response spectrum conferred by a particular receptor often matched that of a wild-type ORN class (18, 37). A similar result was also obtained in a calcium imaging study of antennal lobe glomeruli, in which misexpression of Or43a in the ORNs that target the VA1lm glomerulus conferred upon VA1lm novel responses to ligands for Or43a (139). In addition, the odor receptor determined the spontaneous firing rate, signaling mode, and response dynamics of the ORN in which it is expressed (37). Thus the odor receptor determines multiple aspects of odor coding in *D. melanogaster* ORNs.

Larval Odor Receptors

Like adults, the *D. melanogaster* larvae express members of the *Or* family (58). Some *Or* genes are larval specific, and others are expressed in both the larva and the adult. Most larval ORNs appear to express only a single functional odor receptor, and most odor receptors are expressed in a single ORN (58). Larval odor receptors

vary in their odor specificities, breadth of tuning, response dynamics, and signaling mode (58).

ORN projection patterns in the larval antennal lobe (LAL) were also examined by using a number of *Or-GAL4* constructs to label the axons of different ORNs with GFP. Most if not all ORNs project to individual glomeruli in the LAL. An analysis of the larval specific receptors revealed that a spatially segregated map of odor information may exist in the brain: The ORNs that express odor receptors tuned to aromatic compounds project to the lateral periphery of the LAL, and the ORNs that express odor receptors tuned to aliphatic compounds project more centrally (58). A spatially segregated odor map has also been found in the mammalian olfactory bulb and cortex (28a, 50a, 101a, 146). The functional significance of this spatially segregated odor map is not yet clear. This spatial arrangement may facilitate the formation of lateral inhibitory connections between neighboring glomeruli that respond to similar odors, and these inhibitory connections may be important for narrowing the tuning profiles of individual glomeruli (101a). A more recent anatomical study further demonstrated that the 21 ORNs synapse onto 21 glomeruli in the LAL, and the projection neurons in these glomeruli in turn target ~28 glomerulus-like domains in the mushroom body (95a). The result is a simple olfactory circuit that is essentially lacking in cellular redundancy.

ANOPHELES GAMBIAE *A. gambiae* larvae were similarly found to express members of the *AgOr* gene family in an RT-PCR experiment, although a functional characterization of these receptors has not yet been performed (28). Odor receptors have not yet been identified in the larvae of other insect species.

The Noncanonical Odor Receptor Or83b

While most *Or* genes are expressed in small subpopulations of ORNs, the Or83b receptor is a notable exception. Or83b is expressed in ~70% of antennal ORNs and in all maxillary palp ORNs, where it is coexpressed with other *Or* genes (63, 85, 137). In the larva, Or83b is expressed in all 21 ORNs of the dome sensillum (63). In addition to its widespread expression in antennal neurons, the mosquito homolog of Or83b, AgOr7, is also expressed in a subset of chemosensory neurons on the labellum, suggesting that these neurons may have an olfactory function (92).

Unlike most other odor receptors, Or83b is highly conserved across insect species, including the mosquitoes *Anopheles gambiae* and *Aedes aegypti*; the blow fly *Calliphora erythrocephala*; the moths *Bombyx mori*, *Heliothis virescens*, *Antheraea pernyi*, and *Helicoverpa zea*; the mealworm *Tenebrio molitor*; the honey bee, *Apis mellifera*; and the Mediterranean fruit fly, *Ceratitis capitata* (51, 59, 61, 75, 92). Among these species, the highest conservation is between *D. melanogaster* Or83b and *A. gambiae* AgOr7, which share 76% amino acid identity despite ~100 million years of evolutionary divergence (92). The relatively high degree of sequence similarity among Or83b orthologs suggests an evolutionarily conserved function in insect olfaction.

A recent characterization of *D. melanogaster* Or83b mutants demonstrated that Or83b is involved in the localization of odor receptors to ORN dendrites: In the absence of Or83b, odor receptors localize primarily to ORN cell bodies rather than dendrites (63). Adult flies and larvae that lack Or83b or that show reduced levels of Or83b protein expression show severely reduced olfactory responses (63, 84). The mutant phenotype can be rescued by expression of Or83b homologs from the mosquito, moth, or Mediterranean fruit fly, thus confirming an evolutionarily conserved function for Or83b in insect olfaction (51).

The mechanism of Or83b function was further investigated using heterologous expression systems. In HEK293 cells, coexpression of Or83b increased the odor-responsiveness conferred by expression of Or22a or Or43a, without altering their ligand specificity (84). Bioluminescence resonance energy transfer analysis further revealed that Or83b forms heterodimers with both Or22a and Or43a (84). Similar results were subsequently obtained with heterologous expression of receptors in *X. laevis* oocytes: Neither Or47a nor Or83b alone showed odor responses, but coexpression of Or47a and Or83b elicited responses to two of the cognate ligands for Or47a, pentyl acetate and 2-heptanone (80). Thus Or83b facilitates the cell surface expression of odor receptors and forms heterodimers with odor receptors, although its precise mechanism of action is not yet known. Or83b and Or43a also form homodimers, although the functional significance of homodimer formation is also unclear (84). Interestingly, Or83b is expressed in most but not all olfactory neurons (63), suggesting that ORNs that lack Or83b may use a different mechanism for the cell surface expression of odor receptors. Or83b alone does not appear to confer odor-responsiveness, as a mutant ORN expressing only Or83b is unresponsive to all tested odors (21).

Odor Binding Proteins and Volatile Pheromone Perception

Insect odor binding proteins (OBPs) were first discovered in 1981 in the antenna of the moth *Antheraea polyphemus* (135) and have since been identified in a wide variety of insect species (65, 128). Some OBPs are expressed in pheromone-responsive sensilla, bind to pheromone molecules, and are therefore called pheromone binding proteins (PBPs) (42, 65, 66, 135). Diverse biochemical roles for OBPs and PBPs have been proposed, including the solubilization of odors in the sensillum lymph, the transport of odors through the lymph to the odor receptors, the removal of deleterious compounds from the lymph, and the deactivation of odors following receptor activation (52, 65, 66, 116, 136).

DROSOPHILA MELANOGASTER *D. melanogaster* has a family of 51 OBPs, whose members are expressed in different subsets of olfactory and gustatory neurons (30, 40, 41, 72, 91). Only one OBP mutant, *lush* (also called *obp76a*), has been reported (56). LUSH is expressed specifically in antennal trichoid sensilla, along with two other OBPs, OS-E and OS-F (107, 108a). A recent study of the *lush* mutant showed a role for *lush* in the detection of the male-specific pheromone component

11-*cis* vaccenyl acetate (VA) (143). In the absence of LUSH, trichoid neurons that normally respond to VA showed a complete loss of VA-responsiveness. In addition, behavioral analysis of the *lush* mutant revealed that mutant flies are significantly less attracted to traps containing male flies, providing evidence of a role for LUSH in social aggregation (143).

Mosquito Odor Receptors

The malaria vector mosquito *A. gambiae* has a family of 79 odor receptor genes that was identified on the basis of sequence similarity to the *Or* gene family (27, 43). As in *D. melanogaster*, odor receptors in *A. gambiae* are highly divergent in sequence. Whereas many *D. melanogaster Or* genes are found in genomic clusters of up to three genes, the *AgOr* genes are often found in larger clusters of up to nine genes. Expression of many of the *AgOr* genes in olfactory organs has been demonstrated by RT-PCR (27, 43). The successful identification of the *AgOr* genes on the basis of their sequence similarity to the *Or* genes demonstrates the feasibility of using *Or* gene sequences to identify odor receptor gene families from other insect species.

The first functional demonstration that *AgOr* genes encode odor receptors was achieved using the in vivo expression system provided by the *D. melanogaster* empty neuron (36). Two *AgOr* genes, *AgOr1* and *AgOr2*, were individually expressed in the empty neuron, and the odor response of the neuron was assayed by single-unit physiology. AgOr1 responded strongly to 4-methylphenol, a known component of human sweat (12), and AgOr2 responded to a different odor, 2-methylphenol (36). The fact that AgOr1 is female specific (27) and responds to a human-sweat odor suggests that it may play a role in the human host-seeking behavior of the female mosquito.

Moth Odor and Pheromone Receptors

Candidate odor and pheromone receptors from the heliothine moth *H. virescens* have also been identified, again on the basis of sequence similarity to *D. melanogaster* odor receptors (60, 61a). Expression of *H. virescens* odor receptor genes in the antenna was verified by RT-PCR and in situ hybridization. In many moth species, some of the trichoid neurons on the male antenna are, as demonstrated electrophysiologically, sensitive to species-specific sex pheromones, such as bombykol in the case of *B. mori* (53). Several of the *H. virescens* receptors are expressed specifically or preferentially in male trichoid sensilla and may therefore be pheromone receptors (60). Some *H. virescens* receptors show expression in gustatory organs, suggesting that some neurons on the moth proboscis may have an olfactory function (60).

A family of at least 30 candidate odor receptors was recently identified in the silk moth, *B. mori* (80, 102). Pheromone-sensitive sensilla on the silk moth antenna contain one ORN that is responsive to the sex pheromone bombykol and one ORN that is responsive to bombykal, a secondary pheromone component that

is released by the female pheromone gland along with bombykol (53). The *B. mori* odor receptor BmOR1 was identified as a male antenna-specific odor receptor and is expressed in the region of the antenna containing pheromone-sensitive trichoid sensilla (102). Heterologous expression of BmOR1 in *X. laevis* oocytes resulted in a small but specific odor response to bombykol, and ectopic expression of BmOR1 in the antenna of the female moth conferred electrophysiological responses to bombykol, thus identifying BmOR1 as a bombykol receptor (102). A second male-specific receptor, BmOR3, was subsequently identified as a bombykal receptor. In one study, the *B. mori* Or83b homolog BmOR2 was coexpressed with BmOR1 and enhanced the response of BmOR1 to bombykol (80). However, a second study found that BmOR2 is not coexpressed with BmOR1 (59). Thus the role of BmOr2 in the pheromone response of *B. mori* remains unclear.

INSECT TASTE RECEPTORS

Functional Characterization of Gustatory Neurons

Taste sensilla have been studied in a wide variety of insects, including flies, mosquitoes, honey bees, moths, locusts, leafhoppers, aphids, and butterflies (145). The basic characteristics of the taste sensillum are remarkably conserved—in general, a taste sensillum is a uniporous sensillum that has both chemosensory and mechanosensory cells. The sensilla on the mouthparts of dipterans such as *D. melanogaster*, *Phormia regina*, and *Calliphora erythrocephala* are perhaps the most extensively studied taste sensilla; these belong to two morphological types, the taste hairs or bristles, and the taste pegs. Both types of sensilla are found elsewhere on the body; taste hairs are also present on the surface of the tarsi, the wing margins, and the ovipositor in females, and taste pegs are found on the internal mouthparts. Typically, each taste hair is innervated by the single, unbranched dendrites of multiple chemosensory neurons as well as a single mechanosensory neuron (24, 81, 115). This is also the case for the taste pegs in larger flies (17, 93). In contrast, labellar taste pegs in *D. melanogaster* house only one chemosensory neuron and one mechanosensory neuron (24).

The gustatory receptor neurons (GRNs) of many insects have been analyzed by the tip-recording technique, which involves making contact with the pore at the tip of the sensillum with a solution containing an electrolyte as well as the taste stimulus (46, 79). Experiments with various taste stimuli revealed the presence of four types of neurons—a sugar-sensitive neuron (S), a water-sensitive neuron (W), a neuron sensitive to low concentrations of salts (L1), and a neuron sensitive to high concentrations of salts (L2) (16, 23, 47, 100, 106).

In larger flies such as the flesh fly *Boettcherisca peregrina*, S cells respond to at least five different types of sugars and sugar derivatives: pyranose, furanose, nucleotides, sugars with an aryl group, and sugars with an alkyl group (29, 111–113).

In *D. melanogaster*, S cells respond to pyranose, fructose, trehalose, and glycerol (57, 125, 127, 142); responses to amino acids and fatty acids have not been described. Quantitative differences in sugar sensitivity among S cells have been described (44, 76), but qualitative differences have not yet been found. In addition, some S cells also respond to low concentrations of salt (45).

The L2 cell was recently found to respond to bitter stimuli in addition to salt stimuli (45, 68, 69, 77, 89). In *D. melanogaster*, recordings of responses to bitter compounds show that there is some degree of functional heterogeneity among sensilla (77).

In both insects and mammals, salt reception is thought to function via amiloride-sensitive DEG/ENaC sodium channels (67). Two *D. melanogaster* DEG/ENaC genes, *Pickpocket11* and *Pickpocket19*, are expressed in taste sensilla, and disruption of these genes results in a diminished behavioral response to salt but not to sucrose (70).

Identification and Genomic Organization of *Gr* genes

A decade after the identification of the first odor receptors (7), taste receptors were identified in both mammals (1, 48) and *D. melanogaster* (10). The method that proved successful for the initial identification of members of the *D. melanogaster* gustatory receptor (*Gr*) gene family was the bioinformatic mining of the available genome (10). A thorough bioinformatic analysis of the completed genome subsequently extended this family to include 68 receptors encoded by 60 genes (99), comparable to the number in the *Or* family.

Gr proteins are extraordinarily divergent in sequence, sharing as little as 8% amino acid identity (10, 104). Phylogenetic analysis suggests that the *Gr* gene family is an ancient chemoreceptor family from which a branch of *Or* genes subsequently evolved (99, 104).

Like the *Or* genes, *Gr* genes are dispersed throughout the genome, and several are present in clusters of two to six genes (99). In some cases, mRNAs are predicted to be spliced to common 3′ exons (10, 19, 99, 104). For example, the *Gr39a* locus encodes four receptors, each of which has independent 5′ exons but shares the last three exons that encode the seventh transmembrane domain and the C-terminal part of the protein (10).

A family of 76 gustatory receptor genes (*AgGr* genes) was subsequently identified in *A. gambiae* (43). Comparison of the sequences of the *Gr* and *AgGr* genes confirms the ancient origins of the insect chemoreceptor superfamily but also indicates that species-specific expansions have occurred within some subfamilies (43). There are seven possible orthologous pairs of *A. gambiae* and *D. melanogaster* Gr receptors, some of which are relatively well conserved—for example, Gr21a and its mosquito ortholog AgGr22 share 68% identity. At least five of the *AgGr* loci are predicted to generate multiple alternatively spliced transcripts in a fashion similar to that of *Gr39a* (43).

EXPRESSION OF *Gr* GENES

Peripheral Organization of *Gr* Gene Expression

Gr gene expression in gustatory tissues was first analyzed by RT-PCR analysis (10) and subsequently by a combination of in situ hybridization and reporter gene expression techniques (6, 8, 19, 44, 104, 129, 140). In particular, the expression patterns of several genes have been analyzed with *Gr-GAL4* constructs to drive expression of reporter genes. These studies have shown that individual *Gr* genes are expressed in subsets of chemosensory neurons, in one or more of the gustatory organs. Some *Gr* genes are expressed only in a particular taste tissue, whereas others are widely expressed in multiple taste tissues. The number and distribution of the labellar sensilla that express particular *Gr* genes are also varied. Furthermore, some *Gr* genes are expressed in more than one morphological class of sensilla or in only a subset of the sensilla of one class, suggesting that there may be functional heterogeneity within and between morphological classes.

Expression of at least one *Gr* gene, *Gr5a*, has also been observed in taste pegs (129). A few genes, such as *Gr2a* and *Gr66a*, are expressed in larvae as well as in the adult (104). In addition, at least three *Gr* genes—*Gr10a*, *Gr21a*, and *Gr63a*—are expressed in the antenna, suggesting that some members of this gene family may have an olfactory function (104). *Gr21a*-positive neurons send projections to a glomerulus in the antennal lobe that is CO_2 responsive (104, 124), and disruption of these neurons blocks CO_2 avoidance (124). However, there is no direct evidence that Gr21a is in fact a CO_2 receptor.

Whereas most olfactory neurons express only one odor receptor, many gustatory neurons express multiple taste receptors. In mutant *D. melanogaster* that lack Gr5a, sugar neurons show a severely reduced trehalose response but a normal sucrose response, suggesting that Gr5a mediates the response to trehalose, and a second receptor coexpressed in these neurons mediates the response to sucrose (13). Experiments using *Gr-GAL4* transgenes have also demonstrated that at least some GRNs house multiple taste receptors. The *Gr* genes *Gr5a* and *Gr66a* define two nonoverlapping populations of GRNs in the labellum, and all other *Gr* genes examined in these experiments are expressed in subsets of *Gr66a*-positive neurons (129, 140). A caveat in this *Gr-GAL4* analysis is that in no case has the expression pattern been confirmed to represent that of the endogenous gene, and thus an important goal for the future is to confirm reporter gene expression by in situ hybridization or other independent techniques.

A Map of Taste Quality and Location in the Brain

Anatomical and cobalt filling studies have shown that GRNs from different peripheral tissues project to different regions of the suboesophageal ganglion (SOG) and tritocerebrum in the brain (20, 55, 82, 97, 121, 144). In addition, different populations of GRNs from the same tissue also have distinct projection patterns. Backfills of labellar GRNs have shown that their arborizations in the SOG are

varied, even though the SOG lacks glomerular organization like that in the antennal lobe (82, 121). Moreover, the activity-dependent staining of single neurons revealed that different GRNs within the same sensillum have different projection patterns (106).

More recent molecular studies have revealed that different classes of projections to the SOG correspond to different populations of neurons: The two GRN populations defined by either *Gr5a* or *Gr66a* expression project to nonoverlapping regions of the SOG (129, 140). Genetic ablation of these sets of neurons demonstrated that *Gr5a* defines a population of sugar-sensitive neurons and that *Gr66a* defines a population of bitter-sensitive neurons (129, 140). Projections from different peripheral tissues are also segregated in the brain, even when the neurons express the same receptor: Examination of transgenic *D. melanogaster* in which only *Gr32a*-positive neurons were labeled with GFP showed that such neurons in the labellum project to a medial region of the SOG, those in the internal mouthparts project to a more anterior region of the SOG, and those in the leg project through the thoracic ganglion and terminate in a region posterior to the SOG (140).

Functional Characterization of Taste Receptors

Gr5a AND THE TREHALOSE RESPONSE A ligand has been identified for only one insect taste receptor, the *Gr5a* receptor in *D. melanogaster*. Although the *Gr* genes were predicted to encode taste receptors because of their sequence, membership in a large family, and expression in taste tissue, it was imperative to determine whether they indeed functioned as taste receptors in vivo. Following the identification of the *Gr* genes as candidate taste receptors, the genomic locations of the *Gr* genes were compared with those of loci that had been implicated in taste function. In particular, a locus at position 5A on the X chromosome is involved in the behavioral response to the disaccharide trehalose. Naturally occurring polymorphisms at this locus conferred different levels of sensitivity to trehalose (125). Furthermore, changes in gene dosage also altered the sensitivity to this sugar (126).

The *Gr* gene *Gr5a* mapped to this region. Interestingly, all deletion mutations that affect the trehalose response uncovered the *Gr5a* gene (13, 131). These mutants have a dramatically lowered response to trehalose in both physiological and behavioral assays. The possibility that *Gr5a* encodes the trehalose receptor was tested directly by rescuing the mutant phenotype with a transgene containing a functional copy of *Gr5a*, or with a modified transgene in which *Gr5a* had been mutated by engineering a nonsense mutation in its coding region (13). Both physiological and behavioral assays showed that the trehalose sensitivity was restored only when a functional copy of *Gr5a* was present, demonstrating a role for *Gr5a* in the trehalose response of taste neurons.

These results are consistent with those of another study that examined the association of various alleles of *Gr5a* with trehalose sensitivity (131). The *Gr5a* gene was sequenced from multiple *D. melanogaster* strains, and a single substitution of threonine for alanine in the predicted second intracellular loop segregated with

a reduced sensitivity for trehalose (131). Analysis of the sequence variation and trehalose sensitivity in numerous lines from a natural population confirmed that this dimorphism correlates with trehalose sensitivity (50).

Direct evidence that *Gr5a* encodes a trehalose receptor was obtained by heterologous expression of the *Gr5a* cDNA in *D. melanogaster* Schneider2 cells. Trehalose response of transfected cells was measured by Ca^{2+} imaging (8). Cells transfected with *Gr5a*, but not with the vector alone, showed a transient increase in the level of intracellular Ca^{2+} in response to trehalose. Three characteristics of the response were examined in detail: its kinetics, dose dependence, and specificity. The response showed a steep dose dependence to concentrations of trehalose between 0.025 and 250 mM. Interestingly, *Gr5a* appears to be specific for trehalose; a number of other sugars tested did not elicit a response (8). The only other sugar receptor that has been identified is the mammalian sweet receptor, which functions as a heterodimer and is broadly tuned to several sugars, D-amino acids, and artificial sweeteners (83).

Gr68a AND PHEROMONE RECEPTION In *D. melanogaster*, a large body of behavioral evidence suggests that contact chemoreception plays a role in courtship behavior (35). The *Gr* gene *Gr68a* is an excellent candidate for a pheromone receptor that plays a role in this process (6). *Gr68a* is expressed specifically in a subset of taste neurons in male forelegs, and inactivation of *Gr68a*-positive neurons or reduction in Gr68a protein levels results in inefficient courtship behavior and reduced mating success. Thus *Gr68a* may be a receptor for a nonvolatile attractive pheromone transmitted from the female to the male during an early stage of courtship (6).

CONCLUSION

Insect chemoreceptors were identified first in *D. melanogaster* and have since been identified in a number of other insect species, including the moths *B. mori* and *H. virescens* and the malaria vector mosquito *A. gambiae*. It should now be possible to identify odor and taste receptors from many other insect species by screening for genes that have sequence similarity to *Or* and *Gr* genes. An important direction for future research will be the large-scale functional characterization of chemoreceptors from diverse insect species. Currently, little is known about the ligand specificities of insect taste receptors. A ligand for only one taste receptor has been identified: the *D. melanogaster* Gr5a receptor responds to the sugar trehalose. The response specificity of Gr5a was examined in detail by heterologous expression in *D. melanogaster* S2 cells, and in the future it may be possible to identify ligands for additional taste receptors using this expression system.

Significantly more is known about the ligand specificities of odor receptors. Ligands for most of the *D. melanogaster* odor receptors have been identified, as well as two *B. mori* and two *A. gambiae* receptors, either by expression in a

mutant *D. melanogaster* neuron or by heterologous expression in *X. laevis* oocytes. Both of these expression systems are likely to be effective in decoding the ligand specificities of many additional insect odor receptors. A functional characterization of odor receptors from disease vectors and agricultural pests may prove useful in the design of better insect traps and repellants. In addition, a comparison of the odor and taste receptor repertoires from many different insect species may provide important insight into chemosensory mechanisms of host adaptation and speciation in ecologically and evolutionarily diverse insect taxa. Finally, the convergence of molecular and cellular analyses of insect odor and taste receptors should soon provide a better understanding of how chemoreceptor neuron responses give rise to complex chemosensory behaviors.

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LITERATURE CITED

1. Adler E, Hoon M, Mueller K, Chandrashekar J, Ryba N, Zuker C. 2000. A novel family of mammalian taste receptors. *Cell* 100:693–702
2. Baker TC, Ochieng SA, Cosse AA, Lee SG, Todd JL, et al. 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: 155–65
3. Bhalerao S, Sen A, Stocker R, Rodrigues V. 2003. Olfactory neurons expressing identified receptor genes project to subsets of glomeruli within the antennal lobe of *Drosophila melanogaster*. *J. Neurobiol.* 54:577–92
4. Boeckh J, Kaissling K, Schneider D. 1965. Insect olfactory receptors. *Cold Spring Harb. Symp. Quant. Biol.* 30: 263–80
5. Brand A, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401–15
6. Bray S, Amrein H. 2003. A putative *Drosophila* pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. *Neuron* 39:1019–29
7. Buck L, Axel R. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65:175–87
8. Chyb S, Dahanukar A, Wickens A, Carlson JR. 2003. *Drosophila* Gr5a encodes a taste receptor tuned to trehalose. *Proc. Natl. Acad. Sci. USA* 100:14526–30
9. Clyne P, Grant A, McConnell R, Carlson. 1997. Odorant response of individual sensilla on the *Drosophila* antenna. *Invertebr. Neurosci.* 3:127–35
10. Clyne P, Warr C, Carlson J. 2000. Candidate taste receptors in *Drosophila*. *Science* 287:1830–34
11. Clyne PJ, Warr CG, Freeman MR,

Lessing D, Kim JH, Carlson JR. 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* 22: 327–38

12. Cork A, Park KC. 1996. Identification of electrophysiologically-active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat extracts. *Med. Vet. Entomol.* 10:269–76
13. Dahanukar A, Foster K, van der Goes van Naters W, Carlson JR. 2001. A Gr receptor is required for response to the sugar trehalose in taste neurons of *Drosophila*. *Nat. Neurosci.* 4:1182–86
14. de Bruyne M, Clyne PJ, Carlson JR. 1999. Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. *J. Neurosci.* 19:4520–32
15. de Bruyne M, Foster K, Carlson J. 2001. Odor coding in the *Drosophila* antenna. *Neuron* 30:537–52
16. Dethier V. 1976. *The Hungry Fly*. Cambridge, UK: Harvard Press
17. Dethier VG, Hanson FE. 1965. Taste papillae of the blowfly. *J. Cell. Physiol.* 65:93–99
18. Dobritsa AA, van der Goes van Naters W, Warr CG, Steinbrecht RA, Carlson JR. 2003. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron* 37:827–41
19. Dunipace L, Meister S, McNealy C, Amrein H. 2001. Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. *Curr. Biol.* 11:822–35
20. Edgecomb RS, Murdock LL. 1992. Central projections of axons from taste hairs on the labellum and tarsi of the blowfly, *Phormia regina* Meigen. *J. Comp. Neurol.* 315:431–44
21. 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.* 23:9906–12
22. Elmore T, Smith D. 2001. Putative *Drosophila* odor receptor OR43b localizes to dendrites of olfactory neurons. *Insect Biochem. Mol. Biol.* 31:791–98
23. Falk R, Atidia J. 1975. Mutation affecting taste perception in *Drosophila melanogaster*. *Nature* 254:325–26
24. Falk R, Bleiser-Avivi N, Atidia J. 1976. Labellar taste organs of *Drosophila melanogaster*. *J. Morphol.* 150:327–42
25. Feinstein P, Bozza T, Rodriguez I, Vassalli A, Mombaerts P. 2004. Axon guidance of mouse olfactory sensory neurons by odorant receptors and the β2 adrenergic receptor. *Cell* 117:833–46
26. Feinstein P, Mombaerts P. 2004. A contextual model for axonal sorting into glomeruli in the mouse olfactory system. *Cell* 117:817–31
27. Fox A, Pitts R, Robertson H, Carlson JR, Zwiebel L. 2001. Candidate odor receptors from the malaria vector mosquito, *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* 98:14693–97
28. Fox AN, Pitts RJ, Zwiebel LJ. 2002. A cluster of candidate odorant receptors from the malaria vector mosquito, *Anopheles gambiae*. *Chem. Senses* 27: 453–59

28a. Friedrich RW, Korsching SI. 1997. Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. *Neuron* 18: 737–52

29. Furuyama A, Koganezawa M, Shimada I. 1999. Multiple receptor sites for nucleotide reception in the labellar taste receptor cells of the fleshfly *Boettcherisca peregrina*. *J. Insect Physiol.* 45:249–55
30. Galindo K, Smith DP. 2001. A large family of divergent *Drosophila* odorant-binding proteins expressed in gustatory and olfactory sensilla. *Genetics* 159: 1059–72
31. Gao Q, Chess A. 1999. Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. *Genomics* 60:31–39

32. Gao Q, Yuan B, Chess A. 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nat. Neurosci.* 3:780–85
33. Gesteland RC, Lettvin JY, Pitts WH. 1965. Chemical transmission in the nose of the frog. *J. Physiol.* 181:525–59
34. Goldman AL, Van der Goes van Naters W, Lessing D, Warr CG, Carlson JR. 2005. Coexpression of two functional odor receptors in one neuron. *Neuron* 45: 661–66
35. Greenspan RJ, Ferveur JF. 2000. Courtship in *Drosophila*. *Annu. Rev. Genet.* 34:205–32
36. Hallem EA, Fox AN, Zwiebel LJ, Carlson JR. 2004. Olfaction: mosquito receptor for human-sweat odorant. *Nature* 427:212–13
37. Hallem EA, Ho MG, Carlson JR. 2004. The molecular basis of odor coding in the *Drosophila* antenna. *Cell* 117:965–79
38. Heimbeck G, Bugnon V, Gendre N, Haberlin C, Stocker R. 1999. Smell and tatse perception in *Drosophila melanogaster* larva: toxin expression studies in chemosensory neurons. *J. Neurosci.* 19:6599–609
39. Heinbockel T, Kaissling KE. 1996. Variability of olfactory receptor neuron responses of female silkmoths (*Bombyx mori* L.) to benzoic acid and (±)-linalool. *J. Insect Physiol.* 42:565–78
40. Hekmat-Scafe D, Steinbrecht A, Carlson J. 1996. Co-expression of two odorant-binding protein homologs in *Drosophila:* implications for olfactory coding. *J. Neurosci.* 17:1616–24
41. 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.* 12:1357–69
42. Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu. Rev. Neurosci.* 20:595–631
43. Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, et al. 2002. G protein-coupled receptors in *Anopheles gambiae*. *Science* 298:176–78
44. Hiroi M, Marion-Poll F, Tanimura T. 2002. Differentiated response to sugars among labellar chemosensilla in *Drosophila*. *Zool. Sci.* 19:1009–18
45. Hiroi M, Meunier N, Marion-Poll F, Tanimura T. 2004. Two antagonistic gustatory receptor neurons responding to sweet-salty and bitter taste in *Drosophila*. *J. Neurobiol.* 61:333–42
46. Hodgson E, Lettvin J, Roeder K. 1955. Physiology of a primary chemoreceptor unit. *Science* 122:417–18
47. Hodgson ES, Roeder KD. 1956. Electrophysiological studies of arthropod chemoreception. I. General properties of the labellar chemoreceptors of Diptera. *J. Cell Physiol.* 48:51–75
48. Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJP, Zuker CS. 1999. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* 96:541–51
49. Imamura K, Mataga N, Mori K. 1992. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. *J. Neurophysiol.* 68:1986–2002
50. Inomata N, Goto H, Itoh M, Isono K. 2004. A single-amino-acid change of the gustatory receptor gene, *Gr5a*, has a major effect on trehalose sensitivity in a natural population of *Drosophila melanogaster*. *Genetics* 167:1449–58
50a. Johnson BA, Woo CC, Leon M. 1998. Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb. *J. Comp. Neurol.* 393:457–71
51. Jones WD, Nguyen TA, Kloss B, Lee KJ, Vosshall LB. 2005. Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Curr. Biol.* 15:R119–21
52. Kaissling K-E. 1998. A quantitative

model of odor deactivation based on the redox shift of the pheromone-binding protein in moth antennae. *Ann. NY Acad. Sci.* 855:320–22

53. Kaissling K-E, Kasang G, Bestmann HJ, Stransky W, Vostrowsky O. 1978. A new pheromone of the silkworm moth *Bombyx mori*. *Naturwissenschaften* 65:382–84
54. Keller A, Sweeney ST, Zars T, O'Kane CJ, Heisenberg M. 2002. Targeted expression of tetanus neurotoxin interferes with behavioral responses to sensory input in *Drosophila*. *J. Neurobiol.* 50:221–33
55. Kent KS, Hildebrand JG. 1987. Cephalic sensory pathways in the central nervous system of larval *Manduca sexta* (Lepidoptera: Sphingidae). *Philos. Trans. R. Soc. London B Biol. Sci.* 315:1–36
56. Kim MS, Repp A, Smith DP. 1998. LUSH odorant-binding protein mediates chemosensory responses to alcohols in *Drosophila melanogaster*. *Genetics* 150:711–21
57. Koseki T, Koganezawa M, Furuyama A, Isono K, Shimada I. 2004. A specific receptor site for glycerol, a new sweet tastant for *Drosophila*: structure-taste relationship of glycerol in the labellar sugar receptor cell. *Chem. Senses* 29:703–11
58. Kreher SA, Kwon JY, Carlson JR. 2005. The molecular basis of odor coding in the *Drosophila* larva. *Neuron* 46:445–56
59. Krieger J, Grobe-Wilde E, Gohl T, Breer H. 2005. Candidate pheromone receptors of the silkmoth *Bombyx mori*. *Eur. J. Neurosci.* 21:2167–76
60. 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* 101:11845–50
61. 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* 189:519–26

61a. Krieger J, Raming K, Dewer YME, Bette S, Conzelmann S, Breer H. 2002. A divergent gene family encoding canditate olfactory receptors of the moth *Heliothis virescens*. *Eur. J. Neurosci.* 16:619–28

62. Laissue P, Reiter C, Hiesinger P, Halter S, Fischbach K, Stocker R. 1999. Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *J. Comp. Neurol.* 405:543–52
63. 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* 43:703–14
64. Laurent S, Masson C, Jakob I. 2002. Whole-cell recording from honeybee olfactory receptor neurons: ionic currents, membrane excitability and odourant response in developing workerbee and drone. *Eur. J. Neurosci.* 15:1139–52
65. Leal WS. 2004. Pheromone reception. *Top. Curr. Chem.* 240:1–36
66. Leal WS, Chen AM, Ishida Y, Chiang VP, Erickson ML, et al. 2005. Kinetics and molecular properties of pheromone binding and release. *Proc. Natl. Acad. Sci. USA* 102:5386–91
67. Lindemann B. 1996. Taste reception. *Physiol. Rev.* 76:718–66
68. Liscia A, Masala C, Crnjar R, Sollai G, Solari P. 2004. Saccharin stimulates the "deterrent" cell in the blowfly: behavioral and electrophysiological evidence. *Physiol. Behav.* 80:637–46
69. Liscia A, Solari P. 2000. Bitter taste recognition in the blowfly: electrophysiological and behavioral evidence. *Physiol. Behav.* 70:61–65
70. Liu L, Leonard AS, Motto DG, Feller MA, Price MP, et al. 2003. Contribution of *Drosophila* DEG/ENaC genes to salt taste. *Neuron* 39:133–46
71. Malnic B, Hirono J, Sato T, Buck LB.

1999. Combinatorial receptor codes for odors. *Cell* 96:713–23

72. McKenna M, Hekmat-Scafe D, Gaines P, Carlson J. 1994. Putative *Drosophila* pheromone-binding proteins expressed in a subregion of the olfactory system. *J. Biol. Chem.* 269:16340–47
73. Meijerink J, Braks MA, Van Loon JJ. 2001. Olfactory receptors on the antennae of the malaria mosquito *Anopheles gambiae* are sensitive to ammonia and other sweat-borne components. *J. Insect Physiol.* 47:455–64
74. Meijerink J, van Loon JJ. 1999. Sensitivities of antennal olfactory neurons of the malaria mosquito, *Anopheles gambiae*, to carboxylic acids. *J. Insect Physiol.* 45:365–73
75. Melo ACA, Rutzler M, Pitts RJ, Zwiebel LJ. 2004. Identification of a chemosensory receptor from the yellow fever mosquito, *Aedes aegypti*, that is highly conserved and expressed in olfactory and gustatory organs. *Chem. Senses* 29:403–10
76. Meunier N, Ferveur JF, Marion-Poll F. 2000. Sex-specific non-pheromonal taste receptors in *Drosophila*. *Curr. Biol.* 10:1583–86
77. Meunier N, Marion-Poll F, Rospars JP, Tanimura T. 2003. Peripheral coding of bitter taste in *Drosophila*. *J. Neurobiol.* 56:139–52
78. Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, et al. 1996. Visualizing an olfactory sensory map. *Cell* 87:675–86
79. Morita H, Shiraishi A. 1968. Stimulation of the labellar sugar receptor of the fleshfly by mono- and disaccharides. *J. Gen. Physiol.* 52:559–83
80. Nakagawa T, Sakurai T, Nishioka T, Touhara K. 2005. Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. *Science* 307:1638–42
81. Nayak S, Singh R. 1983. Sensilla on the tarsal segments and mouthparts of adult *Drosophila melanogaster* meigen (Diptera: Drosophilidae). *Int. J. Insect Morphol. Embryol.* 12:273–91
82. Nayak SV, Singh RN. 1985. Primary sensory projections from the labella to the brain of *Drosophila melanogaster* meigen (Diptera: Drosophilidae). *Int. J. Insect Morphol. Embryol.* 14:115–29
83. Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJP, Zuker CS. 2001. Mammalian sweet taste receptors. *Cell* 106:381–90
84. Neuhaus EM, Gisselmann G, Zhang W, Dooley R, Stortkuhl K, Hatt H. 2005. Odorant receptor heterodimerization in the olfactory system of *Drosophila melanogaster*. *Nat. Neurosci.* 8:15–17
85. Ng M, Roorda RD, Lima SQ, Zemelman BV, Morcillo P, Miesenbock G. 2002. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. *Neuron* 36:463–74
86. Nikonov AA, Leal WS. 2002. Peripheral coding of sex pheromone and a behavioral antagonist in the Japanese beetle, *Popillia japonica*. *J. Chem. Ecol.* 28:1075–89
87. Ochieng SA, Park KC, Baker TC. 2002. Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 188:325–33
88. Oppliger FY, Guerin PM, Vlimant M. 2000. Neurophysiological and behavioural evidence for an olfactory function for the dorsal organ and a gustatory one for the terminal organ in *Drosophila melanogaster* larvae. *J. Insect Physiol.* 46:135–44
89. Ozaki M, Takahara T, Kawahara Y, Wada-Katsumata A, Seno K, et al. 2003. Perception of noxious compounds by contact chemoreceptors of the blowfly, *Phormia regina*: putative role of an odorant-binding protein. *Chem. Senses* 28:349–59

90. Park SK, Shanbhag SR, Dubin AE, de Bruyne M, Wang Q, et al. 2002. Inactivation of olfactory sensilla of a single morphological type differentially affects the response of *Drosophila* to odors. *J. Neurobiol.* 51:248–60
91. Pikielny C, Hasan G, Rouyer F, Rosbash M. 1994. Members of a family of *Drosophila* putative odorant-binding proteins are expressed in different subsets of olfactory hairs. *Neuron* 12:35–49
92. 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* 101:5058–63
93. Pollack GS, Lakes-Harlan R. 1995. Birth times of neurons in labellar taste sensilla of the blowfly *Phormia regina*. *J. Neurobiol.* 26:17–32
94. Python F, Stocker RF. 2002. Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons. *J. Comp. Neurol.* 445:374–87
95. Rajashekhar KP, Shamprasad VR. 2004. Maxillary palp glomeruli and ipsilateral projections in the antennal lobe of *Drosophila melanogaster*. *J. Biosci.* 29:423–29
95a. Ramaekers A, Magnenat E, Marin EC, Gendre N, Jefferis GS, et al. 2005. Glomerular maps without cellular redundancy at successive levels of the *Drosophila* olfactory circuit. *Curr. Biol.* 15: 982–92
96. Rawson NE, Eberwine J, Dotson R, Jackson J, Ulrich P, Restrepo D. 2000. Expression of mRNAs encoding for two different olfactory receptors in a subset of olfactory receptor neurons. *J. Neurochem.* 75:185–95
97. Rehder V. 1989. Sensory pathways and motoneurons of the proboscis reflex in the suboesophageal ganglion of the honey bee. *J. Comp. Neurol.* 279:499–513
98. Ressler KJ, Sullivan SL, Buck LB. 1994. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79:1245–55
99. Robertson HM, Warr CG, Carlson JR. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 100:14537–42
100. Rodrigues V, Siddiqi O. 1978. Genetic analysis of chemosensory pathways. *Proc. Indian Acad. Sci.* 87B:147–60
101. Rospars JP. 1988. Structure and development of the insect antennodeutocerebral system. *Int. J. Insect Morphol. Embryol.* 17:243–94
101a. Rubin BD, Katz LC. 1999. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* 23:499–511
102. Sakurai T, Nakagawa T, Mitsuno H, Mori H, Endo Y, et al. 2004. Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. *Proc. Natl. Acad. Sci. USA* 101:16653–58
103. Schneiderman AM, Matsumoto SG, Hildebrand JG. 1982. Trans-sexually grafted antennae influence development of sexually dimorphic neurones in moth brain. *Nature* 298:844–46
104. Scott K, Brady R, Cravchik A, Morozov P, Rzhetsky A, et al. 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* 104:661–73
105. Shanbhag S, Muller B, Steinbrecht A. 1999. Atlas of olfactory organs of *Drosophila melanogaster.* 1. Types, external organization, innervation and distribution of olfactory sensilla. *Int. J. Insect Morphol. Embryol.* 28:377–97
106. Shanbhag S, Singh RN. 1992. Functional implications of the projections of neurons from individual labellar sensillum of *Drosophila melanogaster* as revealed by neuronal marker horseradish

peroxidase. *Cell Tissue Res.* 267:273–82

107. Shanbhag SR, Hekmat-Scafe D, Kim MS, Park SK, Carlson JR, et al. 2001. Expression mosaic of odorant-binding proteins in *Drosophila* olfactory organs. *Microsc. Res. Tech.* 55:297–306
108. Shanbhag SR, Muller B, Steinbrecht RA. 2000. Atlas of olfactory organs of *Drosophila melanogaster*. 2. Internal organization and cellular architecture of olfactory sensilla. *Arthropod Struct. Dev.* 29:211–29

108a. Shanbhag SR, Smith DP, Steinbrecht RA. 2005. Three odorant-binding proteins are co-expressed in sensilla trichodea of *Drosophila melanogaster*. *Arthropod Struct. Dev*. 34:153–65

109. Shields VDC, Hildebrand JG. 2000. Responses of a population of antennal olfactory receptor cells in the female moth *Manduca sexta* to plant-associated volatile organic compounds. *J. Comp. Physiol. A* 186:1135–51
110. Shields VDC, Hildebrand JG. 2001. Recent advances in insect olfaction, specifically regarding the morphology and sensory physiology of antennal sensilla of the female sphinx moth *Manduca sexta*. *Microsc. Res. Tech.* 55:307–29
111. Shimada I, Horiki H, Ohrui H, Meguro H. 1985. Taste response to 2,5-anhydro-D-hexitols; rigid stereospecifity of the furanose site in the sugar receptor of the flesh fly. *J. Comp. Physiol. A* 157:477–82
112. Shimada I, Isono K. 1978. The specific receptor site for aliphatic carboxylate anion in the labellar sugar receptor of the fleshfly. *J. Insect Physiol.* 24:807–11
113. Shimada I, Shiraishi A, Kijima H, Morita H. 1974. Separation of two receptor sites in a single labellar sugar receptor of the flesh-fly by treatment with *p*-chloromercuribenzoate. *J. Insect Physiol.* 20:605–21
114. Singh RN, Singh K. 1984. Fine structure of the sensory organs of *Drosophila melanogaster* meigen larva (Diptera: Drosophilidae). *Int. J. Insect Morphol. Embryol.* 13:255–73
115. Slifer EH. 1970. The structure of arthropod chemoreceptors. *Annu. Rev. Entomol.* 15:121–42
116. Steinbrecht R. 1998. Odorant-binding proteins: expression and function. *Ann. NY Acad. Sci.* 855:323–32
117. Stensmyr MC, Dekker T, Hansson BS. 2003. Evolution of the olfactory code in the *Drosophila melanogaster* subgroup. *Proc. R. Soc. London B Biol. Sci.* 270: 2333–40
118. Stensmyr MC, Giordano E, Balloi A, Angioy AM, Hansson BS. 2003. Novel natural ligands for *Drosophila* olfactory receptor neurones. *J. Exp. Biol.* 206: 715–24
119. Stocker R. 1994. The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.* 275:3–26
120. Stocker RF, Lienhard MC, Borst A, Fischbach KF. 1990. Neuronal architecture of the antennal lobe in *Drosophila melanogaster*. *Cell Tissue Res.* 262:9–34
121. Stocker RF, Schorderet M. 1981. Cobalt filling of sensory projections from internal and external mouthparts in *Drosophila*. *Cell Tissue Res.* 216:513–23
122. Stortkuhl K, Kettler R. 2001. Functional analysis of an olfactory receptor in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 98:9381–85
123. Stortkuhl KF, Kettler R, Fischer S, Hovemann BT. 2005. An increased receptive field of olfactory receptor Or43a in the antennal lobe of *Drosophila* reduces benzaldehyde-driven avoidance behavior. *Chem. Senses* 30:81–87
124. Suh GS, Wong AM, Hergarden AC, Wang JW, Simon AF, et al. 2004. A single population of olfactory sensory neurons mediates an innate avoidance

behaviour in *Drosophila*. *Nature* 431: 854–59

125. Tanimura T, Isono K, Takamura T, Shimada I. 1982. Genetic dimorphism in the taste sensitivity to trehalose in *Drosophila melanogaster*. *J. Comp. Physiol.* 147:433–37
126. Tanimura T, Isono K, Yamamoto M. 1988. Taste sensitivity to trehalose and its alteration by gene dosage in *Drosophila melanogaster*. *Genetics* 119:399–406
127. Tanimura T, Shimada I. 1981. Multiple receptor proteins for sweet taste in *Drosophila* discriminated by papain treatment. *J. Comp. Physiol.* 141:265–69
128. Tegoni M, Campanacci V, Cambillau C. 2004. Structural aspects of sexual attraction and chemical communication in insects. *Trends Biochem. Sci.* 29:257–64
129. Thorne N, Chromey C, Bray S, Amrein H. 2004. Taste perception and coding in *Drosophila*. *Curr. Biol.* 14:1065–79

129a. TNO. 2004. *Volatile compounds in food. Qualitative and quantitative data*. http://www.voeding.tno.nl/vcf/VcfNavigate.cfm

130. Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI. 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* 83:207–18
131. Ueno K, Ohta M, Morita H, Mikuni Y, Nakajima S, et al. 2001. Trehalose sensitivity in *Drosophila* correlates with mutations in and expression of the gustatory receptor gene *Gr5a*. *Curr. Biol.* 11:1451–55
132. van den Broek IV, den Otter CJ. 1999. Olfactory sensitivities of mosquitoes with different host preferences (*Anopheles gambiae* s.s., *An. arabiensis*, *An. quadriannulatus*, *An. m. atroparvus*) to synthetic host odours. *J. Insect Physiol.* 45:1001–10
133. Vassar R, Chao SK, Sitcheran R, Nunez JM, Vosshall LB, Axel R. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* 79:981–91
134. Vermeulen A, Rospars JP. 2004. Why are insect olfactory receptor neurons grouped into sensilla? The teachings of a model investigating the effects of the electrical interaction between neurons on the transepithelial potential and the neuronal transmembrane potential. *Eur. Biophys. J.* 33:633–43
135. Vogt RG, Riddiford LM. 1981. Pheromone binding and inactivation by moth antennae. *Nature* 293:161–63
136. Vogt RG, Riddiford LM, Prestwich GD. 1985. Kinetic properties of a pheromone degrading enzyme: the sensillar esterase of *Antheraea polyphemus*. *Proc. Natl. Acad. Sci. USA* 82:8827–31
137. Vosshall L, Wong A, Axel R. 2000. An olfactory sensory map in the fly brain. *Cell* 102:147–59
138. Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* 96:725–36
139. Wang JW, Wong AM, Flores J, Vosshall LB, Axel R. 2003. Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell* 112:271–82
140. Wang Z, Singhvi A, Kong P, Scott K. 2004. Taste representations in the *Drosophila* brain. *Cell* 117:981–91
141. Wetzel C, Behrendt H, Gisselmann G, Stortkuhl K, Hovemann B, Hatt H. 2001. Functional expression and characterization of a *Drosophila* odorant receptor in a heterologous cell system. *Proc. Natl. Acad. Sci. USA* 98:9377–80
142. Wieczorek H, Wolff G. 1989. The labellar sugar receptor of *Drosophila*. *J. Comp. Physiol. A* 164:825–34
143. Xu P, Atkinson R, Jones DNM, Smith DP. 2005. *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. *Neuron* 45:193–200

144. Yetman S, Pollack GS. 1986. Central projections of labellar taste hairs in the blowfly, *Phormia regina* meigen. *Cell Tissue Res.* 245:555–61
145. Zacharuk RY. 1980. Ultrastructure and function of insect chemosensilla. *Annu. Rev. Entomol.* 25:27–47
146. Zou Z, Li F, Buck LB. 2005. Odor maps in the olfactory cortex. *Proc. Natl. Acad. Sci. USA* 102:7724–29

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