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INVITED REVIEW

Taste and pheromone perception in the fruit fly *Drosophila melanogaster*

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Abstract Taste is an essential sense for detection of nutrient-rich food and avoidance of toxic substances. The *Drosophila melanogaster* gustatory system provides an excellent model to study taste perception and taste-elicited behaviors. "The fly" is unique in the animal kingdom with regard to available experimental tools, which include a wide repertoire of molecular-genetic analyses (i.e., efficient production of transgenics and gene knockouts), elegant behavioral assays, and the possibility to conduct electrophysiological investigations. In addition, fruit flies, like humans, recognize sugars as a food source, but avoid bitter tasting substances that are often toxic to insects and mammals alike. This paper will present recent research progress in the field of taste and contact pheromone perception in the fruit fly. First, we shall describe the anatomical properties of the *Drosophila* gustatory system and survey the family of taste receptors to provide an appropriate background. We shall then review taste and pheromone perception mainly from a molecular genetic perspective that includes behavioral, electrophysiological and imaging analyses of wild type flies and flies with genetically manipulated taste cells. Finally, we shall provide an outlook of taste research in this elegant model system for the next few years.

Keywords *Drosophila* · Gustatory receptor neurons · GTP-binding protein-coupled receptors

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Like most animals, *Drosophila* monitor their chemical world with two distinct senses: the olfactory system, which is used for the detection of volatile chemicals, and the gustatory (taste) system, which enables the fly to detect soluble compounds. The olfactory sensory system (or the fly nose) is comprised of two pairwise head appendages, the third segments of the antennae, and the maxillary palps (Fig. 1). These appendages are covered with hundreds of sensory hairs, each of which contains two to four olfactory sensory neurons (OSNs) [1]. In contrast, the gustatory system is widely distributed over the animal's body. The main taste organ, the proboscis or labial palps (i.e., the fly tongue), is located on the distal end of the labellum (also a head appendage), but flies, like many other insects, have taste sensilla on legs and wings and, in females, on the genitalia [1, 2].

The roles of the olfactory and taste systems are clearly separated with regard to the physical state of chemicals they detect (volatile vs solubilized). However, the two systems often cooperate to fulfill specific functions in related processes and behaviors, the most obvious of them being the location and identification of food. For example, chemical cues such as the specific odor of a flower and the sugar compounds present in its nectar are by nature associated with one another. Thus, the odor cue provides a primary sensory input for an insect such as a honeybee to locate a food source (nectar), whose particular chemical composition is subsequently verified by the taste system. Similarly, the typical odor of yeast, which produces high amounts of the sugar trehalose, the fly's main dietary compound, is a well-known attractant for *Drosophila* and indeed serves as an olfactory cue for the fly to find its food.

A connection between olfactory and gustatory perception is also observed in pheromone-guided behavior in *Drosophila*. The fly's many social interactions, especially

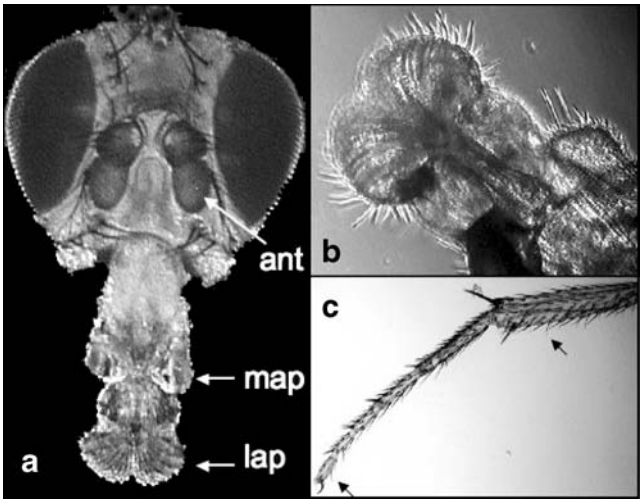

Fig. 1 Taste chemosensory structures of adult *Drosophila*. **a** Olfactory sensilla are located on two pairs of appendages of the head, the third antennal segment, and the maxillary palps. The main gustatory organ, the two labial palps, are located at the tip of the proboscis. *Ant* Third antennal segment; *map* maxillary palps; *lap* labial palps. **b** Front view of a labial palp. Most of the 31gustatory sensilla can be seen. **c** Gustatory sensilla on the forelegs. Each leg contains between 30 and 50 taste hairs, next to hundreds of mechansensory bristles, on each leg. Note that mechanosensory bristles have a straight morphology, whereas chemosensory bristles are curved (two bristles in the focal plane are indicated by *arrows*)

the well-studied courtship behavior, are to a large extent dependent on pheromone cues that can be of a volatile as well as a nonvolatile nature [3–5]. For example, aggregation behavior and some aspects of courtship (learning) behavior are mediated by the volatile compound *cis*-vaccenyl acetate [6–9], whereas courtship and courtship suppression are mediated to a large extent by contact pheromones, most likely long-chain hydrocarbons (HCs) [10–13]. However, unlike most mammals, which perceive most pheromones through a dedicated sensory structure, the vomeronasal organ [14], *Drosophila* and other insects lack a specifically dedicated organ for the sensing of pheromones. Instead, they perceive pheromones through a subset of both olfactory hairs and taste bristles located throughout both sensory systems [1]. How are food odors and tastants functionally separated from pheromone signals? Because all these neurons project mainly to the olfactory or gustatory processing centers in the brain—the antennal lobes and the subesophageal ganglion (SOG)—segregation of pheromone and other chemical input must therefore occur at this or a later level of neural processing.

The *Drosophila* taste system

Although *Drosophila* and mammals share many of the same taste preferences, their gustatory systems are organized rather differently; one hallmark of insect taste systems is the wide distribution of taste cells over much of the animal's body (Fig. 1). This feature enables the fly to gather contact chemosensory information about its environment from many reference points that may make contact with any body part (head, legs, and wings), thereby facilitating detection of potential foods or toxic compounds while walking and navigating in confined spaces. The presence of taste-sensing cells not associated with the feeding apparatus has also safety benefits, as it allows evaluation of chemicals without the potential hazard of inadvertent ingestion. The main sensory unit of all taste organs is the taste bristle (gustatory sensillum), which house two to four primary gustatory receptor neurons (GRNs) as well as a single mechanosensory neuron (MSN) [1]. The pair of labial palps, located at the end of the proboscis, form the main taste structure and contains 31 bristles each, which are classified into three types (L, S, and I type) based on their shape and location (Fig. 1) [1, 5, 15]. L- and S-type sensillum contain four GRNs, and the I type contains two GRNs. Based on electrophysiological investigations, each GRN is thought to respond exclusively to either sugar, water, low salt concentration, or high salt concentration and bitter compounds [16–20]. Taste bristle have a terminal pore at the tip, allowing taste stimuli access to the dendrite of the GRN, which extends into the bristle shaft [21]. Unlike the mammalian tongue, which contains epithelial-derived taste cells, *Drosophila* taste sensilla possess primary sensory neurons that project their axons into the SOG, where taste information is initially processed. In addition to the outer taste sensillum on the palps, legs, and wings, there are several clusters of taste neurons semi-internally or internally located. The first group is a row of taste pegs that line the inside of the labial palps and are exposed to foods when the fly 'opens' its palps and readies itself for 'sucking up' foods. The later groups consist of three sensillum clusters that line the pharynx and allow re-evaluation of the food as it passes and enters the esophagus and the digestive system. GRNs in the labial palps, the internal sensillum, and some GRNs from the legs primarily project their axons to the SOG, whereas the wing and a minority of leg GRNs project to the thoracic ganglion [22, 23].

In addition to GRNs, taste sensilla contain several other cell types, including a single MSN and several support cells [24]. Although the GRNs have received most attention, the function of the MSN in the gustatory sensillum is unknown. The vast majority of MSNs are associated with thousands of nonchemosensory bristles and hairs distributed over the entire fly body. These MSNs are known to translate mechanical forces into electrical signals that mediate hearing, positional awareness, and the coordination of movements [25, 26]. The role of the MSNs in taste sensillum may be entirely unrelated to the perception of

chemosensensation, and these neurons may just provide extra sites for tactile sensation. Alternatively, they may enable the fly to identify the texture of a surface (liquid vs gel-like vs solid) and associate it with food that is contained within, similar to the ability of the human tongue to determine the consistency and texture of foods. Experiments that alter the properties of MSNs within taste bristles will be required to determine whether these neurons are essential for appropriate gustatory behavioral response.

The role of the support cells of the gustatory bristles has not been studied in much detail either. However, the identification of several genes expressed in these cells, as well as their apparent similarity to the better-characterized support cells in olfactory sensilla, suggests that they have important roles in chemosensory perception. First, support cells in both olfactory and taste sensilla express and secrete numerous odorant-binding proteins into the olfactory and taste lymph, respectively, solutions comparable to the mucosa in the nasal epithelium of the mammalian nose. Odorant-binding proteins are thought to bind receptor ligands in the lymph and present them to receptor proteins localized on the cell membrane of sensory neuron dendrites, as well as to mask ligands or remove them from the vicinity of the receptor site. Interestingly, many of these odorant-binding proteins show spatially restricted expression, at least within the antenna, suggesting that they might exert their functions on selected sets of neurons. Some odorant-binding proteins are also expressed in taste organs [8, 27–29]. In the olfactory system, a mutation in one of these proteins, LUSH, has been shown to affect behavioral responses to *cis*-vaccenyl acetate, a volatile pheromone involved in aggregation behavior [8, 30]. Finally, a member of a new class of membrane proteins, *CheB42a*, is male-biased expressed in support cells of taste bristles also associated with the only known pheromone receptor, *Gr68a*, and plays a role in male courtship [31, 32]. Thus, several lines of evidence indicate that accessory cells of taste bristles and olfactory hairs produce and secrete proteins that may significantly modulate responses of associated neurons.

Gustatory receptor family

Like mammals, *Drosophila* utilize ion channels to detect salts and acid (sour) tastants, and gustatory receptors (GRs) to detect sugars and compounds perceived as bitter, which are the focus of this review. Putative taste receptors were discovered almost simultaneously in *Drosophila* and mammals. Although all these receptors belong to the large super family of GTP-binding (G) protein-coupled receptors (GPCRs), the fly GRs share no significant sequence similarity with their mammalian counterparts [33–42]. The fly *Gr* gene family was discovered by analyzing the *Drosophila* genome database using algorithms that identify multitransmembrane proteins or by performing reiterated Basic Local Alignment Search Tool searches with *Drosophila* olfactory receptor proteins as query sequences [35, 36, 42], and once the entire *Drosophila* genome sequence was determined, a total of 68 *Gr* genes were found (Fig. 2) [43]. The diversity of the *Gr* genes is remarkable, as similarity between most receptor pairs is only 20% or less (at the amino acid sequence level). However, there are several gene clusters containing up to six genes, presumably arisen through recent gene duplication events; these genes exhibit significantly higher similarity to each other (up to 70%). *Gr* genes that share greater than 30% sequence similarity have been grouped into several subfamilies (Fig. 2) [36]. The domain that is most conserved among all *Gr* genes is located in the region encoding the putative seventh transmembrane domain at the carboxy terminus, a domain that is also shared with the *Or* genes (this domain was used as a signature motif in one study that lead to the discovery of the fly taste receptors [36]). Interestingly, *Drosophila* odorant receptors (ORs) were recently shown to have an inverse membrane topology compared to typical GPCRs, with an intracellular amino terminus and an extracellular carboxy terminus, and it has been suggested that they may use an alternative, non-G protein-based signaling pathway [44]. Whether GRs adopt a similar membrane topology and, if so, how this topology might affect downstream signaling remain to be determined. Even so, genetic evidence supports the role of G-protein signaling molecules in *Drosophila* taste perception and is discussed further below.

There is evidence for mammalian chemoreceptors and *Drosophila* olfactory receptors with high sequence similarity to recognize structurally related ligands [45–48]. Thus, GRs within a subfamily are thought to detect structurally similar taste compounds. For example, the *Gr5a* subfamily, which consist of *Gr5a* (encoding a trehalose receptor) [49–51], *Gr61a*, and *Gr64a–f* share sequence similarity in the range of 60% and are thought to encode receptors that detect diverse sugars [15]. Likewise, a subfamily comprising six genes, *Gr39a.a*, *Gr39a.b*, *Gr39a.c*, *Gr39a.d*, and *Gr68a*, has been proposed to encode receptors for various pheromones, most likely long-chain HCs, because of the critical involvement of *Gr68a* in female pheromone sensing during male courtship [5, 52]. Because bitter compounds, in contrast to sugars and hyrdorcarbons, cover a vaste and chemically much more diverse structural space (see below), it is reasonable to assume that the majority of remaining GRs are devoted to the detection of bitter-tasting and toxic compounds. However, we should emphasize that these assumptions are largely speculative and based on analogies from mammalian chemoreceptors and *Drosophila* ORs.

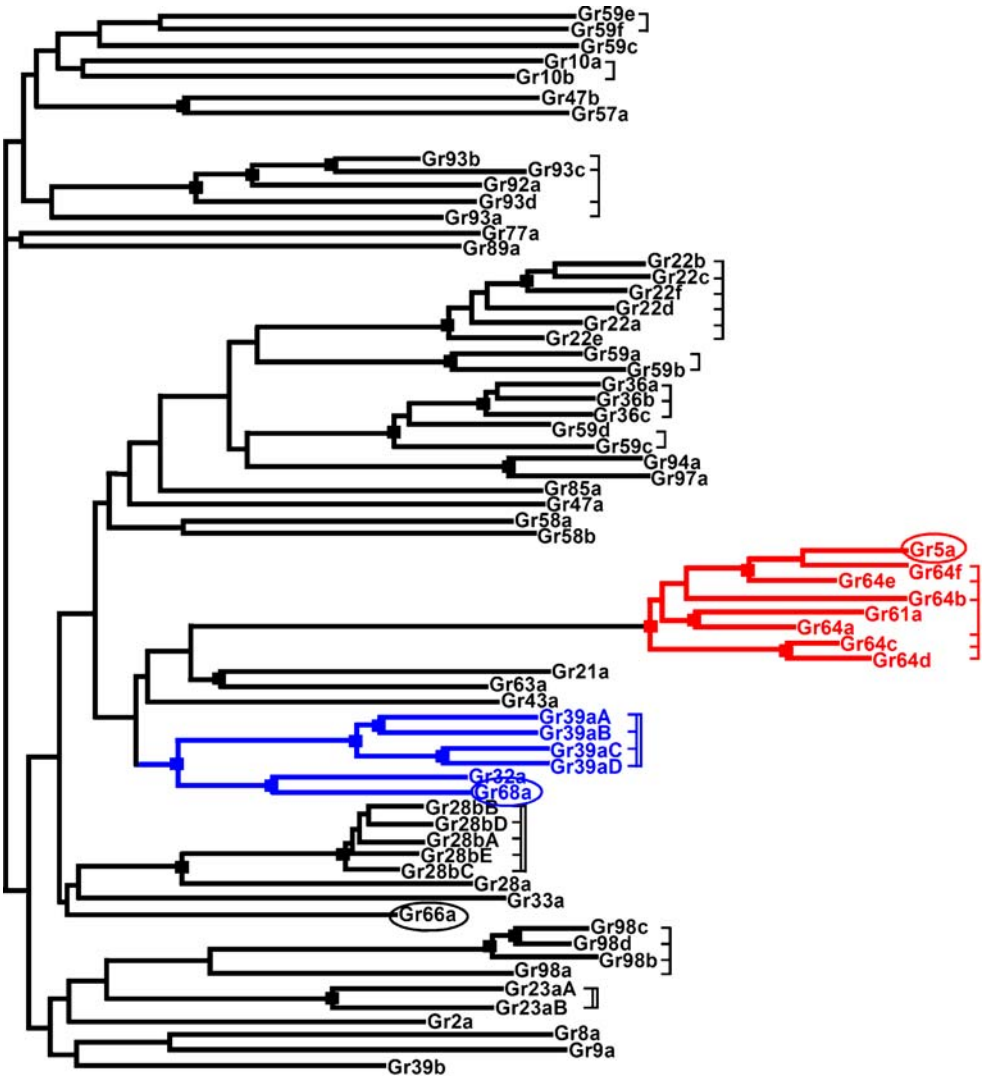

Fig. 2 Tree diagram of the *Gr* gene family. The 68 *Gr* genes share anywhere from 8 to 70% sequence similarity. Genes that are clustered are indicated by a *vertical bar*, and clustered genes that are partially identical (because of alternative promoter use and/or splicing) are indicated by a *double bar*. Genes encoding putative receptors for sugars are shown in *red* and for putative pheromone in *blue*. The remaining genes are thought to encode to a large extent for putative bitter compounds (shown in *black*). Receptors for which ligands are known or suggested by experimental data are framed with an *oval*. *Boxes* indicated branches with 75–100% bootstrap support. Modified after [43] and [15]

Gr mRNA levels are low in gustatory neurons and generally, RNA in situ hybridization experiments are not sensitive enough for determining tissue- and cell-type-specific expression [35, 36, 42]. Therefore, indirect transgenic expression methods were employed to visualize *Gr* gene expression, such as the well-established GAL4/UAS system [53]. In this method, *Gr* gene promoters are used to drive expression of the yeast transcription factor GAL4. GAL4 in turn activates transcription with high specificity via the cis-regulatory element UAS (upstream activating sequence), cloned upstream of green fluorescence protein (GFP), or β-galactosidase reporter genes [53]. Expression analyses of about a dozen *Gr* genes have clearly established that they are expressed in distinct subpopulations of GRNs, supporting their role as chemosensory receptors and providing first insights into their complex cellular expression (Table 1) [36, 42, 54, 55]. A large population of gustatory neurons expresses *Gr5a*, which has been shown to recognize the sugar trehalose [49, 51]. *Gr5a* is expressed in approximately half of all GRNs in the labial palps, including all bristle types as well as taste pegs [1, 54]. Significantly, *Gr5a* is not coexpressed with any other *Gr* analyzed so far (Table 1) [36, 42, 54, 55]. Surprisingly, as many as three GRNs associated with a single taste bristle were shown to express *Gr5a*, suggesting that multiple neurons within a sensillum may detect trehalose [54]. Finally, *Gr5a* was also found to be expressed in GRNs of all legs.

The second, widely expressed *Gr* gene is *Gr66a* [54, 55]. This *Gr* gene is expressed in one neuron of each I- and S-type sensillum, altogether in 22 GRNs per labial palp (Table 1; Fig. 2) [54, 55]. Incidentally, *Gr66a* is the only other GR for which a ligand has been identified [56]. Unlike *Gr5a*, *Gr66a* was shown to be partially coexpressed with almost all *Gr* genes that have been investigated with the GAL4 system, including *Gr22b*, *Gr22f*, *Gr22e*, *Gr28be*, *Gr32a*, *Gr47a*, and *Gr59b* [54, 55]. However, none of these are as widely expressed as *Gr66a*, and in fact, some are expressed in as few as two neurons per labial palp. Therefore, *Gr66a*-positive neurons appear to coexpress

Table 1 Summary of *Gr* gene expression

GR	Neurons/palp	Coexpressed	Neurons/legs	Internal taste sensilla	Ligand
Gr5a	~70	None	18	ND	Trehalose
G22b	10	Gr66a, Gr22e	2	VCSO	Bitter#
Gr22e	14	Gr66a	20	LSO/VCSO	Bitter#
Gr22f	4–8	Gr66a, Gr22e	0	ND	Bitter#
Gr28be	9–13	Gr66a, Gr22e	2	VCSO	Bitter#
Gr32a	6–10	Gr66a, Gr22e	11	VSCO	Bitter#
Gr59b	2–4	Gr66a, Gr22e	0	ND	Pheromone/Bitter#
Gr66a	22	Gr66a, Gr22e	21	LSO/VCSO	Caffeine
Gr68a	0	None	10[a]	ND	F pheromone
Gr39a.a–a.d	+	ND	−		Pheromone#
Gr64a–f		ND			Sugar#
Gr61		ND			Sugar#

Expression analysis of each *Gr* gene is summarized [19, 36, 42, 52, 54]. No expression analysis has been published for *Gr64a–f* or *Gr61a*. The number of Neurons in the palps and legs is given per set of legs (one first, second, and third leg).

LSO indicates labral sense organ, and *VCSO* indicates ventral cibarial sense organ, both structures in the pharynx [1]. *ND* indicates not detected. + indicates expression detected by RT-PCR, and − indicates no expression detected by RT-PCR [35]. # indicates putative ligand class based on sequence similarity of specific *Gr* gene to *Gr* genes with known functions.

[a] Expression is only detected in the male forelegs [52].

variable combinations of other *Gr* genes, which implies that possibly every *Gr66a*-expressing neuron may respond to overlapping, albeit distinct sets of chemical ligands. Although coexpression of these genes has not been investigated in detail outside the labial palps, there is at least one gene, *Gr22e*, that is expressed in GRNs of the wing that *do not* express *Gr66a* [36].

The most revealing expression profile of any *Gr* gene has been observed for *Gr68a*. This gene is expressed only in GRNs of about ten sensilla of the forelegs [52]. Intriguingly, these bristles and hence the expression of *Gr68a*, are male-specific, and they are thought to be involved in male courtship, specifically when the male taps his forelegs onto the female's abdomen and genitalia [4]; this observation suggested that GR68a is a pheromone receptor. Indeed, recent molecular genetic and behavioral studies have shown that not only the *Gr68a*-expressing neurons but the receptor itself is required for proper female courtship, supporting the notion that *Gr68a* does not encode a typical taste receptor but a receptor for a female pheromone [52].

GRNs mediate (at least) two different taste qualities

The various *Gr-Gal4* drivers have revealed not only detailed expression profiles for many *Gr* genes, but they were also crucial for the initial functional characterization of discrete groups of GRNs (i.e., neurons that express a given receptor) in chemosensory behaviors. How are such behaviors evaluated and quantified? Several different assays have been developed to either measure feeding itself or to determine a behavioral feeding reflex upon exposure to chemicals; the two most commonly used methods are briefly summarized in this paper. The two choice preference feeding assay evaluates the fly's actual food intake by providing a starved animal two artificially colored food sources, thereby allowing identification and (semi-)quantification of the ingested food [57]. A more direct behavioral measure of GRN stimulation is the proboscis extension reflex (PER). If taste bristles in the foreleg are brought into contact with an attractive tastant, such as a sugar solution, the fly responds with the extension of its proboscis [2, 58, 59]. Conversely, if a sugar solution is contaminated with a toxic or bitter compound, the PER is severely reduced. Two laboratories investigated such behavioral responses in flies carrying various *Gr-Gal4* drivers in combination with reporters that either induce cell death or block neural transmission (*UAS-dipteria toxin* or *UAS-tetanus toxin*, respectively) [54, 55]. These investigations revealed that GRNs fall into at least two functional groups dedicated to distinct taste modalities: *Gr5a*-expressing neurons were found to be essential for the detection of trehalose and, in the PER assay, for other sugars; *Gr66a* neurons, on the other hand, were necessary for the detection of bitter compounds, especially caffeine. These analyses indicate that two distinct groups of GRNs mediate the response to attractive and repulsive chemical compounds. This arrangement is somewhat reminiscent of the mammalian taste system, where distinct cells in the taste buds of the tongue express either the T1Rs or T2Rs, receptors that detect sweet/umami or bitter-tasting substances, respectively [33, 34, 48, 60]. However, several questions about *Drosophila* bitter and sweet sensation remain. Thus far, expression of

only one putative receptor for a sugar, *Gr5a*, has been analyzed, therefore whether other suspected sweet receptors, such as *Gr61a* or the *Gr64a* genes, are coexpressed with *Gr5a* and if so, to what extent remains unclear. Moreover, the complex, partial coexpression of the suspected bitter-taste receptors suggests that various bitter-taste neurons are not identical with regard to their ligand recognition profile and therefore will be activated differentially. Thus, whether *Drosophila* possesses such a simple, mammalian-like sweet–bitter taste system or whether its taste system is set up in a more complex fashion that allows discrimination within these two broad taste qualities remains to be investigated.

Pheromones control social behavior in *Drosophila* via the gustatory system

A third group of GRNs not involved in taste but in mediating pheromone signals has also been functionally characterized. Inactivation of the male-specific neurons expressing *Gr68a* significantly impairs female-directed male courtship behavior but does not affect taste perception, suggesting that these neurons detect an attractive female pheromone compound [52].

Pheromones represent a profoundly different class of chemicals than tastants because they mediate complex social behaviors. In *Drosophila*, pheromones can be both volatile and nonvolatile in nature, and hence their detection occurs through both the olfactory and gustatory systems. The role of nonvolatile pheromones is evident in courtship [3–5]. *Drosophila* courtship is mainly driven by the male and is composed of a series of distinct innate behavioral steps that are carried out in a loose sequence and ultimately leads to copulation. The male first orients himself toward the female, a manifestation that relies mostly on olfactory and visual cues. He then taps the female abdomen with his forelegs, whereby he samples the cuticular pheromones of his potential mating target, a mixture consisting mostly of long-chain HCs. He then extends and vibrates his wings to generate a highly species-specific courtship song, giving the female an important auditory cue as to his identity. The male continues his ritual by next licking and tapping her genitalia to gain additional pheromone information about his target and then attempts and finally succeeds in copulation with the female [4]. Although these behavioral steps follow one another in a more or less sequential manner, a male might return to a previous step occasionally, and he usually repeats most steps multiple times before moving forward to the next.

Extensive use of gustatory organs during courtship, such as the labial palps and forelegs, obvious sexual dimorphism of taste bristles [1, 21, 61] and the nonvolatile nature of known pheromones [10, 11] led to the suggestion that a group of GRs may serve as pheromone receptors [5]. Currently, there is only one clear GR candidate for a pheromone receptor: GR68a was shown to be required during the second (tapping) step of courtship, as males in which expression of this receptor was down regulated via RNA interference (RNAi) failed to proceed efficiently into the later stages of courtship [52]. Potential ligands for GR68a include 7,11-heptacosadiene and/or 7,11-nonacosadiene, two female-specific cuticular HCs that act as male stimulatory pheromones [12]. Interestingly, GR68a is a member of a small subfamily of receptors, which include GR32a and four partially identical proteins derived from alternative promoter usage in the *Gr39a* gene (Fig. 2), leading to the hypothesis that these six receptors might be dedicated not to the perception of taste but to the detection of pheromones. For example, additional receptors expressed in labial GRNs, such as *Gr32a*, are likely to provide pheromone information during the licking step of courtship.

In addition to stimulatory pheromones important for efficient female courtship, inhibitory pheromones are likely to play a role in repressing male–male courtship. Male-directed courtship occurs when pheromone signals are either not properly received by the chemosensory system or integrated into the neural network that controls courtship, such as might be the case in males that carry specific mutation in the *fru* gene [62–64], or it can be induced by altering the pheromone signature of a target male [65]. Candidate inhibitory pheromones include the non sex-specific long chain HCs 7-tricosene and 7-pentacosene (see below; Fig. 4), with candidate receptors possibly represented by additional members of the GR68a protein family. However, genetic analyses of these *Gr* genes will be necessary to investigate these predictions.

Male courtship is essentially hardwired, and first insights into neural circuits that control courtship may be revealed by analyzing the axonal projections of sensory neurons that mediate pheromone cues. However, such analyses have not been performed yet, and hence, no specific structures in the central nervous system (CNS) involved in *Gr68a*-initiated male courtship behavior are currently known. However, the mushroom bodies (MB), a pair of structures localized to the dorso-posterior region of the brain, have been implicated in volatile pheromone-stimulated courtship behavior [66]. Expression of the temperature-sensitive Dynamin mutation shi^{ts1} in the MB inhibits neurotransmission without disrupting brain development. Blocking MB synaptic transmission in males resulted in a delay in courtship initiation and courtship activity toward virgin females, but had no effect on courtship behavior toward young males that display a different pheromone profile. However, MBs are mainly known to receive input from the olfactory, but not the gustatory system [1]. In addition to the MBs, other

structures in the CNS, including region in the thoracic ganglion and the protocerebrum, have been implicated in receiving and integrating sensory input during courtship from genetic mosaic analyses [67–70]. In this context, it is worth noting that some GRNs in the legs project their axons to the thoracic ganglia, rather than the SOG [54, 55].

GR ligands

Despite the broad functional characterization of the fly's taste system, very little is known about the particular role of individual GR proteins. What has emerged from the sparse number of functional studies is that the GR proteins are likely to recognize a vast array of chemicals including sugars, many diverse bitter and toxic compounds, and probably long-chain HCs (Fig. 4). Given the complex expression profiles, it has become increasingly clear that a combination of genetic and heterolgous expression analyses will be necessary to elucidate their diverse roles in taste and pheromone perception. Successful implementation of heterologous expression systems has led to recent progress in deorphanization of mammalian olfactory receptors [71] but has yet to be broadly applied to *Drosophila* chemoreceptors [50, 72]. Thus, genetic studies currently provide the major resource of knowledge about *Drosophila* taste receptors and their ligands. The first receptor for which a specific function was identified is GR5a. An allelic variant of a gene involved in the detection of the sugar trehalose was initially identified in a laboratory strain [57]; flies in this strain showed a reduced sensitivity for the sugar trehalose but not other sugars such as sucrose, and this allele was later mapped to the *Gr5a* gene [49, 51]. Subsequently, null alleles of *Gr5a* were generated, as well as transgene rescue constructs, and flies lacking *GR5a* altogether were shown to display diminished behavioral as well as electrophysiological response to trehalose but not other sugars [51]. Importantly, the lack of GR5a does not lead to complete loss of trehalose detection but rather to a reduced sensitivity for this sugar. Although these results suggest that GR5a plays a significant role in trehalose detection, other receptors can apparently also function as low-affinity trehalose receptors. The existence of distinct high affinity receptors dedicated to the detection of specific sugars has also been proposed based on electrophysiological–biochemical studies. For example, proteolytic treatment of gustatory sensilla with papain eliminates the neuronal response to fructose but not sucrose or glucose [73]. Furthermore, the sweet compound glycerol has been suggested to bind to an unknown GR in *Drosophila* [74]. The distinct sugar specificities are likely to be mediated at least in part by members of the *Gr5a* gene family (Table 1; Fig. 2); however, flies defective in *Gr5a*-related genes must be generated and analyzed to identify specific high-affinity ligands of other sugar receptors.

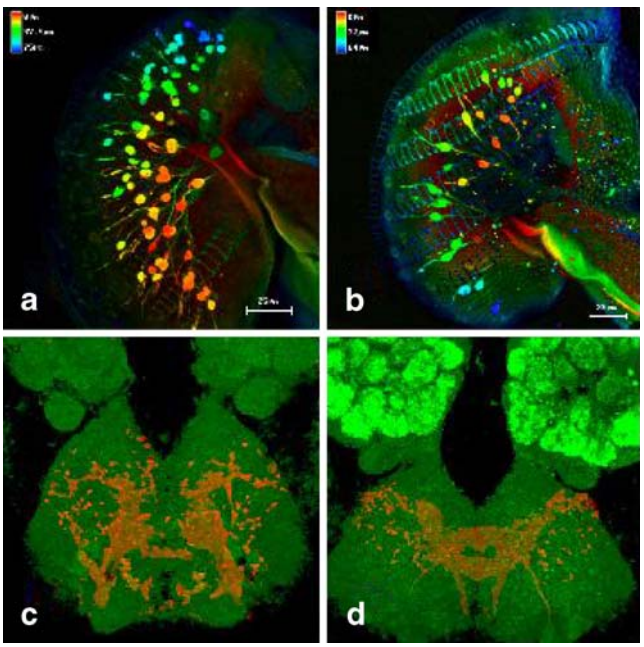

Fig. 3 Expression of two *Gr* genes and axonal projections of their neurons. All photomicrographs were taken from labial and brain preparations, which were stained with rabbit anti-gfp primary antibody and goat anti-rabbit Cy3 secondary antibody. Expression of *Gr5a*, revealed in *Gr5a:Gal4; UAS-GFP* flies (**a**/**c**) and *Gr66a*, revealed in *Gr66a:Gal4; UAS-GFP* flies (**b**/**d**) in the labial palps (**a**/**b**) and the brain (**c**/**d**). For detail see text

The second receptor for which a ligand has been identified is *Gr66a* [56]. *Gr66a*-deficient flies are defective in avoiding caffeine-containing sucrose but not sucrose containing other bitter compounds, such as quinine. Tip recordings of action potentials from gustatory sensilla showed that *Gr66a*-deficient GRNs did not respond to caffeine but did respond to quinine, berberine, or denatonium in a similar manner as wild type GRNs (Fig. 4).

Finally, a small but highly relevant group of chemicals, long-chain HCs secreted from the oenocytes in the abdomen of adult males and females, play critical roles in social behaviors of *Drosophila*, especially courtship (see above). These pheromones have been proposed to be detected by members of the GR68a subfamily, which include GR68a, GR32a, and four related proteins encoded by the *Gr39a* locus [5]. Although none of these receptors have been shown to recognize HCs, there is indirect evidence that GR68a is a receptor for a stimulatory HC, such as 7,11-nonacosadiene or 7,11-heptacosadiene [52]. In any case, these long-chain HCs are structurally very similar to each other (Fig. 4), and several of them have been strongly implicated to have roles in male courtship of females, as well as suppression of male courtship towards other males [10–12, 65, 75–77]. Because long-chain HCs are not water soluble, identifying cognate GRs for these

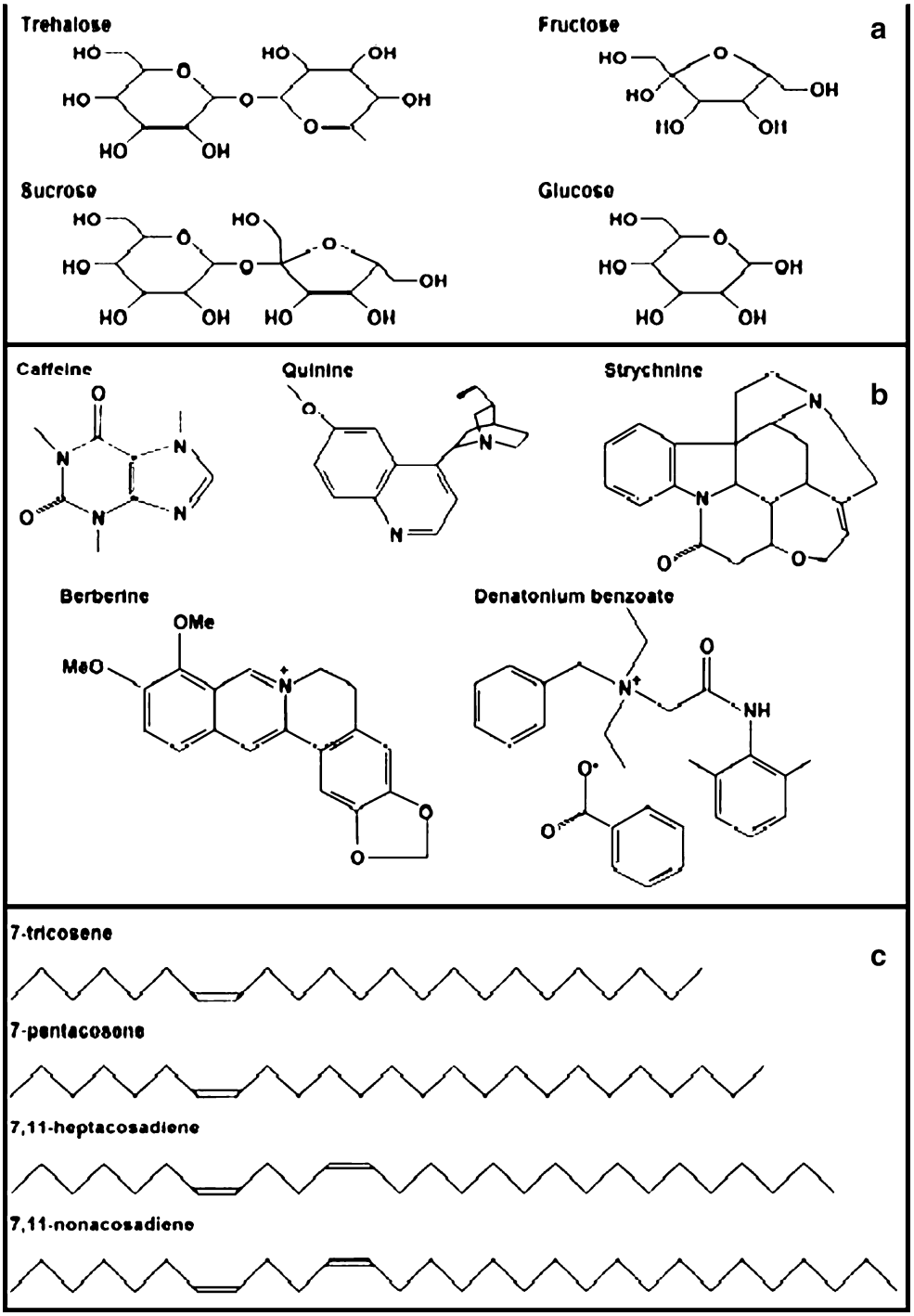

Fig. 4 Ligands of GR proteins. **a** Sugar ligands recognized by the GR proteins, probably by the eight genes of the *Gr5a* subfamily, consisting of *Gr5a*, *Gr61a*, and the six genes *Gr64a–f*. The lonely ligand to which a receptor has been matched is trehalose (GR5a). **b** Bitter-tasting and toxic chemical to which flies are known to respond via the gustatory system. Note that the listed chemicals are only a tiny selection of the hundreds of compounds flies are thought to recognize. Caffeine is a known ligand for the GR66a receptor. **c** Four of the major long chain hydrocarbons that are found on the abdominal cuticle of adult *Drosophila*. Note that two of these chemicals, 7,11-heptacosadiene and 7, 11-nonacosadiene, are essentially female specific. GR68a is possibly the receptor for one of these ligands. Other long chain hydrocarbons are thought to be detected by other members of the GR68a family, which include GR32a and GR39a.a–a.d

compounds will be especially challenging. We cannot exclude the possibility that receptors unrelated to the GR family may play a primary role in the detection of (some) nonvolatile pheromones. Regardless, with the emergence of homologous recombination to create gene knockouts and the large resource of P-element strains that can serve as mutagens to generate targeted deletions of nearby *Gr* genes, an infusion of new *Gr* mutations can be expected and should help elucidate their specific role in taste and pheromone perception.

Signal transduction

The least understood aspect of *Drosophila* taste perception is the signaling mechanism, which ultimately leads to the generation of an action potential of the GRN. GRs are putative GPCRs, suggesting that chemosensory detection utilizes a typical GPCR signaling pathway. Upon binding of an extracellular ligand, a GPCR activates the intracellular G-protein consisting of an $\alpha\beta\gamma$ heterotrimer bound to GDP [78, 79]. The α subunit exchanges GDP for GTP and

dissociates from the βγ complex, enabling both the α and βγ subunits to activate downstream signaling molecules. Despite expressing thousands of GPCRs, mammals have only four subfamilies of α subunits that are grouped according to the signaling pathway an α subunit activates [78].

Coupling between the GPCR and the α subunit of the G-protein is the key event in triggering an intracellular response. However, how GPCRs recognize their cognate G-protein partner is unknown. No predictive consensus motif has been identified among GPCRs that recognize the same G-protein. Structural studies of GPCRs and G-proteins suggest that the globular G-protein interacts with multiple intracellular regions of the GPCR [80–83].

G-protein subunits that mediate mammalian taste perception have been identified. A member of the mammalian Gα subfamily, Gs, stimulates adenylyl cyclase, thus elevating cAMP concentrations [78, 79]. This cAMP signal transduction pathway has been shown to be involved in sugar perception [84–86]. Additionally, the Gα subunit of the taste receptor-specific G-protein gustducin is required for mammalian behavioral response to bitter and sweet compounds [87]. Another G-protein subunit, Gγ13, is involved in bitter signal transduction [88]. Thus, G-proteins are important for signal transduction in the mammalian gustatory system.

The *Drosophila* genome encodes hundreds of GPCRs, including the 68 GRs and 60 ORs [43]. However, only 16 genes predicted to encode G-protein subunits have been identified: 11 α subunits, three β subunits, and two γ subunits [89]. The relatively small number of G-proteins compared to the number of GPCRs indicates that many GPCRs activate the same signaling pathway. For example, it seems quite reasonable to assume that all GRs act though the same set of signaling molecules, albeit separate pathways for different taste modalities cannot be ruled out. To date, only two G-protein subunits, Gsα and Gγ, have been implicated in the GR pathway, and both subunits are required for sugar detection [90, 91]. The *Drosophila* Gs homolog, DGsα was recently shown to be expressed in approximately half of the GRNs in the labellum using specific antibodies, suggesting a role in gustatory signal transduction [91]. Heterozygous dGsα null mutant flies show reduced sugar intake compared to wild-type controls but no change in preference for bitter substances. Likewise flies expressing UAS-DGsα double-stranded RNA under the control the *Gr5a*-Gal4 transgene system show reduced trehalose intake; however, the effect on the intake of other sugars was not examined. Moreover, DGsα RNAi was not used to examine the possible role in other GRNs, particularly bitter-sensing neurons. Additional studies will be required to determine whether other Gα subunits are involved in gustatory signaling. For example, the Gα subunit DGqα-3 is also expressed in a subset of GRNs, suggesting a role in taste transduction [92].

The G-protein subunit, Gγ1, is expressed in the GRNs of the labellum [90]. Flies defective for Gγ1 show reduced behavioral response to sucrose, glucose, fructose, and trehalose, indicating that Gγ1 is involved in the signal transduction of sugar taste perception. This result also supports the idea that multiple sugar GRs activate molecules in the same signaling pathway. The Gβ subunit associated with Gγ1 remains to be identified. Both *Gβ13F* and *Gβ5* are expressed in the labial palps, but functional studies for these subunits are lacking and their roles in sugar (or bitter) perception are currently not known. Thus, like the GRs, targeted mutagenesis of specific G-protein subunits in the GRNs, coupled with behavioral and electrophysiological analyses, will further our understanding of G-protein coupled signaling in taste transduction.

The G-protein subunits activate a signaling cascade that results the generation of an action potential in the GRN. In mammalian taste cells, phospholipase2β (PLCβ2) is activated, which ultimately leads to activation of TRPM5, a transient receptor potential (TRP) channel, and subsequent cell depolarization [47, 93–96]. Animals that lack either PLCβ2 or TRPM5 fail to detect sugars, bitter compounds, or amino acids [47]. Therefore, the same downstream signaling molecules are implemented in mammalian taste cells, regardless of the nature of the taste receptor (T1Rs or T2Rs) that is activated.

Drosophila may use similar downstream signaling molecules as mammals, as putative gustatory PLC and TRP proteins are encoded in the *Drosophila* genome. The *Drosophila* PLC homolog *norpA* is required for photo and olfactory transduction, but also has been shown to be expressed in the fly gustatory organs [97]. Flies carrying a mutation in *norpA* do show reduced intake of both trehalose and sucrose as compared to wild-type flies, suggesting that *norpA* mutants are defective in sensing sugars [98]. In addition, the *Drosophila* genome contains 13 *trp*-related genes [99]. One *trp* homolog, *painless* is expressed in the GRNs of the labial palps, legs, and wings [100]. Flies defective in *painless* show normal responses to sugars and bitter compounds, suggesting that *painless* is not required for detection of these tastants. However, *painless* is required for avoidance of isothiocyanate, the pungent ingredient of wasabi, and is thought to be required for nociception signal transduction. The role of other *trp* homologs in chemosensory detection remains to be determined.

Taste signal processing and taste sensory maps

Drosophila must evaluate chemosensory input and translate this information into an appropriate behavioral response,

which may include feeding, cessation of feeding, search for alternative food source, courtship, or egg-laying. Any behavioral response is likely to be dependant on several parameters that include the nature of the taste ligand and its concentration, as well as the location of the taste neurons that are in contact with that ligand. For example, detection of sugar by GRs of the labial palps induces a sucking response, whereas sugar detection by the legs induces proboscis extension [2, 101]. In addition, there are likely to be other parameters, such as satiation level and 'competing' olfactory and visual sensory input, that affect behavioral output. To understand the information flow from activation of specific GRNs to behavioral output, it will be important to gain insight into the neuronal wiring of the gustatory system at each level of information processing.

Unlike mammalian taste cells, fruit fly GRNs are primary sensory neurons that project axons directly to the brain and central nervous system. Axons of GRNs in the ovipositor, wings, and some leg sensilla project to the thoracic ganglia, whereas all other taste neurons are thought to project to the SOG [22, 23]. Although labial palp and some leg GRNs project to the SOG, visualization of organ-specific GRN projections revealed that GRNs in different taste organs project to different regions in the SOG [22, 23]. Signaling from GRNs of the legs activates a different region of the SOG than GRNs of the labial palps, providing a neuroanatomical rationale for different behavioral outputs of neurons responding to the same ligand but located in different taste tissues. Significant advances in defining neural taste coding were made possible by visualizing axonal projections of GRNs that express the same receptors. Specifically, *Gr-Gal4* drivers were combined with GFP reporters that localize to axons and synapses, such as *syn*-GFP or CD8-GFP, followed by analysis of whole brain preparations using confocal microscopy. These studies have revealed that, unlike in the fly olfactory system, where precise axonal projections of OSNs to specific antennal lobe glomeruli are the rule [102, 103], taste neurons send their axons to loosely defined, widely circumscribed zones in the SOG or thoracic ganglia [36, 42]. For example, labial palp GRNs that express *Gr5a* project to large areas in the lateral and anterior region of the SOG, whereas the *Gr66a*-expressing GRNs project to the medial part of the SOG (Fig. 3) [54, 55]. Furthermore, GRNs that express other *Gr* in a subpopulation of *Gr66a*-expressing neurons send their axons to the subregion of *Gr66a* projection sites in the SOG.

Most recently, taste responses have been monitored in live flies expressing the calcium-sensitive indicator G-CaMP in an effort to map "functional" domains in the brain [104]. Stimulation of the fly proboscis with either sucrose or trehalose resulted in activation of the Gr5a neuronal projections; stimulation of the fly proboscis with either caffeine or denatonium resulted in activation of the Gr66a neuronal projections. These results demonstrate directly that different taste compounds activate distinct neural ensembles in the SOG. Interestingly, activation of specific neurons is sufficient to elicit a behavioral response, regardless of nature of the ligand/receptor. Flies that express the mammalian VR1 [105] in *Gr5a*-expressing neurons prefer capsaicin-containing agar, but flies that express VR1 in *Gr66a*-expressing neurons avoid capsaicin-containing agar [104]. Thus, activation of the GRN determines the fly's behavior, rather than the *Gr* expressed in a particular neuron, supporting the idea that taste behavioral responses are hardwired. However, one potential caveat in this study is that fruit flies have indeed been shown to respond to capsaicin-containing sucrose based on a two-dye food preference tests, suggesting that wild-type flies can detect capsaicin [100].

Conclusions

The identification of key molecules involved in gustatory perception has provided new insights into taste perception of *Drosophila*. Similar to mammals, specific taste modalities can be associated with a specific set of taste cells/neurons, which express distinct members of the GR gene family (T1Rs and T2Rs in mammals). Furthermore, different sets of neurons can target distinct regions in the SOG, providing at least a rough spatial map of taste preferences in the primary taste center of the brain. Finally, the GRs appear to comprise the only group of receptors responsible for the detection of very structurally diverse chemicals, including sugars, toxic/bitter-tasting compounds (alkaloids, etc.) and long-chain carbons (Fig. 4). Yet, many questions remain. We have little knowledge about which specific substrates the vast majority of GRs recognize or how GR-ligand interactions are mediated within the cell to elicit an electrical current sufficient for the neuron to fire an action potential. Beyond the primary projection targets of taste neurons, we know nothing of how the taste map in the SOG is connected to the next order processing centers (protocerebrum), a crucial factor in generating meaningful behavioral output (proboscic extension reflex, sucking reflex, feeding avoidance, courtship, etc.). Elucidating the neural circuits for taste perception will have a major impact in some fascinating areas of associative learning and behavior. For example, association of odors with certain nutrients, a phenomenon that many animals use to find food sources, will likely be reflected by the way various neural circuits are interconnected with each other.

The in vivo characterization of the *Gr* genes will play a crucial role in addressing some of these issues. Mutations will certainly be of great help to identify their cognate ligands, and newly created *Gr-Gal4* drivers will be essential

to fine map the SOG and other target regions in the CNS (thoracic ganglia, etc.). In combination with novel trans-synaptic markers, *Gr-Gal4* might even become useful for mapping second-order neurons functionally connected to specific GRs (i.e., taste modalities). However, heterologous expression systems, in which chemorecptors can be efficiently expressed at the cell surface and their interaction with ligands measured using Ca^{++} indicators, will be necessary to make broad progress in deorphanizing the large family of GRs.

Finally, increased attention has certainly been paid to the identification of signaling molecules that mediate taste responses. Despite the few reports on some suspected candidates, none of the suggested molecules have passed rigorous criteria as "the critical component" in *Drosophila* taste transduction. One possible caveat that may slow down progress in this area is the possibility that taste signaling components are also involved in other processes that are essential for viability. Hence, mutations in such genes may cause lethality and investigations into their possible role in taste transduction of adult flies may therefore not be straight forward. However, new technologies, such as conditional knockout strategies, can be implemented to circumvent such scenarios.

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