Current Biology, Vol. 15, R673–R684, September 6, 2005, ©2005 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2005.08.021

Gustatory Perception and Behavior in *Drosophila melanogaster*

Review

Hubert Amrein and Natasha Thorne

The sense of taste is essential for the survival of virtually all animals. Considered a 'primitive sense' and present in the form of chemotaxis in many bacteria, taste is also a sense of sophistication in humans. Regardless, taste behavior is a crucial activity for the world's most abundant (insects) and most successful (mammals) inhabitants, providing a means of discrimination between nutrient-rich substrates, such as sugars and amino acids, from harmful, mostly bitter-tasting chemicals present in many plants. In this review, we present an update on progress in understanding taste perception in the model fruit fly *Drosophila melanogaster*. An introduction to the fly's taste system will be presented first, followed by a description of relevant behavioral assays developed to quantify taste perception at the organismal level and a short overview of electrophysiological studies performed on taste cells. The focal point will be the recent molecular-genetic investigations of the gustatory receptor (*Gr*) genes, which is complemented by a comparison between *Drosophila* and mammalian taste perception and transduction. Finally, we provide a perspective on the future of *Drosophila* taste research, including three specific proposals that seem uniquely applicable to this exquisite model system and cannot, at least currently, be pursued elsewhere.

Introduction

Drosophila, with its rich history of genetic research, is considered the classic insect model for studying many sensory systems, including taste and olfaction. Coincidentally, the fruit fly has food preferences and dislikes similar to those of humans. For example, carbohydrates are a major food source for both humans and adult *Drosophila*. Likewise, many compounds that taste bitter and are harmful to humans are also toxic to flies and are therefore avoided. Furthermore, both salts and acids are integral parts of *Drosophila* foods, and just as in mammals, the detection and proper amount of uptake of these chemicals are crucial for electrolyte homeostasis. The fly's sensitivity for detecting these chemicals is quite exquisite and within the detection range of mammals. For example, sucrose is easily detected at levels of 1 mM, and many bitter tasting and/or toxic compounds are detected in the micromolar range. Thus, the fly's gustatory system is in many aspects an ideal model system for studying the perception of taste.

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, USA. E-mail: hoa1@duke.edu

Organization of the *Drosophila* Taste Sensory System

The gustatory (or taste) system of insects is complex and, unlike that of mammals, not restricted to a single taste organ [1,2]. The main taste tissue in *Drosophila* is composed of the two labial palps located at the distal end of the proboscis — the fly equivalent of the human tongue (Figure 1). Each palp is covered w ith 31 stereotypically arranged taste bristles (sensilla). Based on morphological criteria, these bristles fall into three classes: small (s), intermediate (i) and long (l), containing two (i) or four (s and l) taste sensory neurons [2]. The two palps close off the entrance to the pharynx. During active feeding, the labial palps open and expose additional, poorly characterized sensilla, the taste pegs, which make contact with the food as it enters the pharynx. Three separate taste cell clusters, the labral sense organ and the ventral and the dorsal cibarial sense organs, line the interior wall of the pharynx and 'monitor' the food as it is ingested (Figure 1A). The specific roles of these internal taste organs are not known, but they might serve either as sensors for harmful substances that, if activated, elicit a 'regurgitate' response, or alternatively, to verify desirable substances and promote sucking reflexes.

Taste bristles and pegs have a terminal pore at the tip to allow direct access of the food substances to the dendritic processes of the gustatory receptor neurons (GRNs), which extend into the bristle shaft (Figure 1B) [3]. As with olfactory sensory neurons, the space between the dendrite and the inner surface of the bristle is filled with lymph, a secretion from support cells that are associated with each taste sensillum [4]. Little is known about the composition of the taste lymph, but it is likely to have similar functions as the lymph of olfactory sensilla, which is thought to modulate accessibility of odorants to their cognate receptors [5,6]. Indeed, several 'odorant binding proteins' are also expressed in taste sensilla [7,8] and might play a general role in shuttling both volatile and soluble chemicals from the environment to the dendrite of chemosensory neurons which express specific taste (or gustatory) receptors.

Additional taste bristles, interspersed between the more abundant mechanosensory bristles, are located on the legs and the anterior wing margins [2,3,9] (Figure 1B; see also Figure 5). Each leg contains at least 30 taste bristles and the anterior wing margin of each wing has an additional 40 such bristles [10,11], bringing the total number of taste sensilla on the body to about 260 (in comparison, the labial palps contain 62 taste bristles). The wide distribution of taste cells throughout the fly's body underscores the critical role that chemosensory stimuli represent in the fly's world.

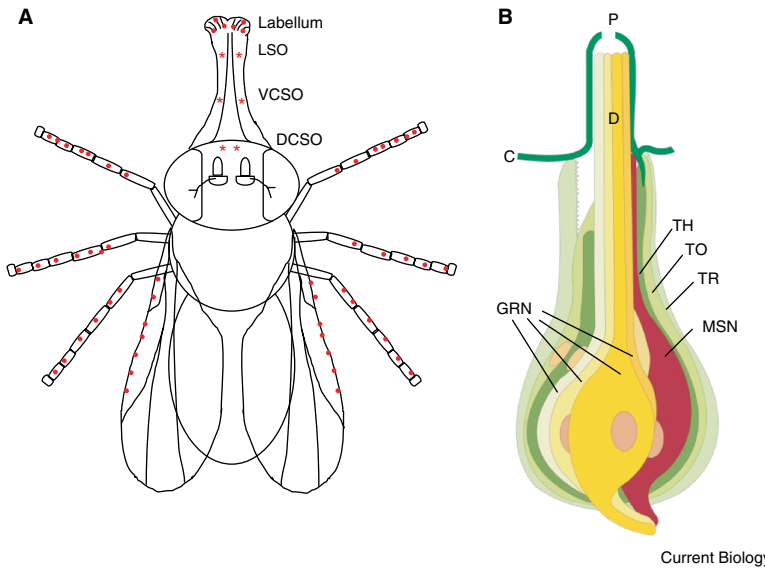

Figure 1. Organization of the *Drosophila* chemosensory system.

(A) Location of taste sensilla. Gustatory receptor neurons (GRNs) are distributed throughout the fly's body and are located at the base of taste sensilla (red dots/asterisks). Most of these sensilla are bristles with the sensory cells located at the base. The main taste organs are the labial palps, which harbor 62 sensilla. Some GRNs appear to detect chemicals as the fly feeds, namely those of taste pegs (at the inner border of the palps) and three clusters of neurons (red asterisks), the labral sense organ (LSO) and the dorsal and ventral cibarial sense organs (DSO and VSO) located in the pharynx. In addition, all legs contain GRNs, as does the anterior wing margin (see text). Activation of GRNs is directly relayed to the CNS via their axonal extensions, which target different regions in either the subesophageal ganglion (GRNs in the labial palps and pharynx and some GRNs in the legs) or the thoracic ganglion (GRNs in the legs and presumably the wings). (B) Structure of a taste bristle. The taste bristles contain two to four GRNs, each of which extends a dendrite into the bristle. Soluble chemicals can enter the bristle shaft through the pore (P) at the tip and get in contact with the dendrite (D) and the receptors on their cell surface. The space between the dendrite and bristle is filled with a secretion from some of the surrounding support cells, which include the thecogen (TH), the tormogen (TO) and the trichogen (TR) cell, and is thought to facilitate interaction between chemicals and receptor proteins. Many, but not all, taste sensilla also contain a mechanosensory neuron (MSN). C, cuticle.

A sexual dimorphism has been noted in the number of taste bristles on the forelegs; males have on average 50 such bristles, whereas females have about 37 [3,12]. Interestingly, the male taps the female's abdomen extensively during courtship, presumably to sample pheromone chemicals secreted from specialized cells in this body part [13,14]. A crucial role for these specialized, male-specific taste bristles in pheromone detection during courtship has recently been reported [15].

Measuring Taste Behavior

Several behavioral assays, crucial for a quantitative assessment of taste at the organismal level, have been developed (Figure 2). Attraction or aversion to soluble chemicals can be evaluated based on the adult fly's feeding behavior (two-choice preference test) or by a robust reflex behavior that involves a poorly characterized neural circuit (proboscis extension reflex). The two-choice preference test, first developed by Tanimura and co-workers [16], is a simple but powerful assay for measuring feeding behavior (Figure 2A). To promote feeding, flies are first starved for 24–32 hours, after which they are given the choice of two food substrates for 60–90 minutes. The two substrates are colored with 'tasteless' red or blue chemical compounds which allow for rapid examination of the ingested food by scoring the color of the gut through the semitransparent abdomen of the fly. A feeding preference index (PI) is calculated, indicating the preference for either one of the two chemicals (Figure 2A).

The proboscis extension reflex is a more direct measure of taste response of specific GRNs, as opposed to the overall perception at an organismal level [1,17,18]. Flies are immobilized and observed under a binocular lens as specific taste hairs, usually located on the legs, are brought into contact with a test solution (Figure 2B). Attractive substances, such as sucrose or trehalose, elicit a large increase in the frequency of proboscis extension, a behavior that occurs spontaneously, but increases with feeding activity. Proboscis extension can also measure the effects of repulsive chemicals when they are mixed with attractive compounds, such as sucrose, as this leads to a significant reduction of proboscis extension reflexes [1].

Electrophysiological Studies: *Drosophila* GRNs Detect Salts, Sugar, Bitter Compounds and Water

Initial studies of taste perception focused on electrophysiological investigations using single sensilla recordings of bristles on the labial palps or the legs [1,19–22]. An electrode is inserted into the base of a taste bristle, which is bathed in a solution that can be rapidly exchanged. Electrical activity of the neurons within the sensillum is recorded before and during application of various chemical compounds in the form of voltage spikes (action potentials) of cells within the sensillum [22–24]. It is important to note that this technique records the activity of all (two or four) neurons within the sensillum simultaneously. Thus, based on distinct characteristics of spiking patterns — reflecting the cells' different physiological and molecular nature — it is inferred which cell, if any, within a sensillum is stimulated upon exposure to specific ligands. The main observations from these early electrophysiological investigations can be summarized as follows (Figure 3A): L-type sensilla are composed of distinct sensory neurons, one of which

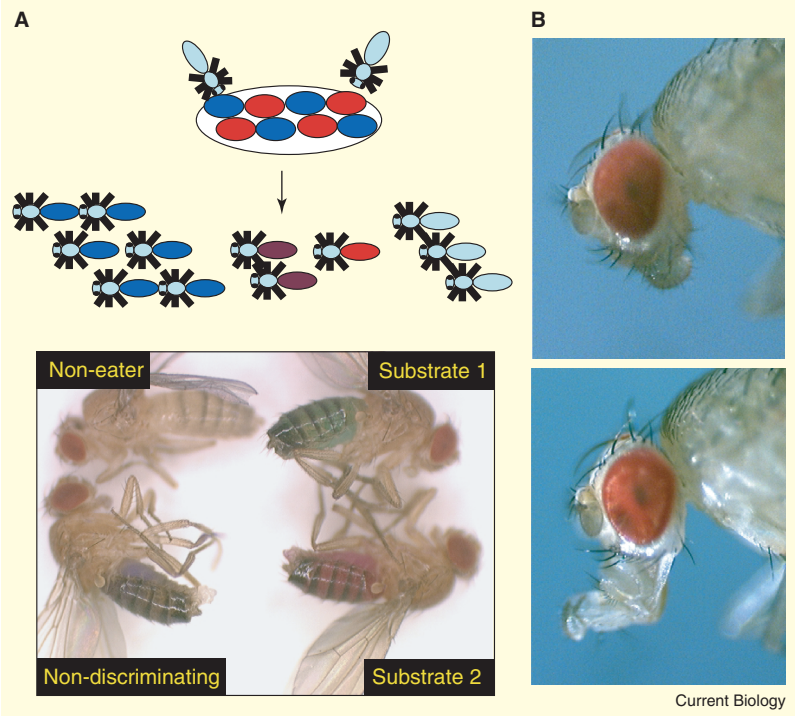

Figure 2. Assays of feeding behavior.

(A) Two-choice preference assay. Flies are first starved for a day and then provided with the choice of two chemicals, presented on a multi-well plate at specific concentrations in an agar medium. The two food substrates also contain different tasteless compounds that have intrinsic colors. For example, sucrose might be added in one half of the wells on a titer plate, along with the sulforhodamine B dye (red) and trehalose, along with an erioglaucine dye (blue) on the other half of the wells. The feeding is carried out in darkness (to exclude any influence of color preference) and the abdomen of the flies is inspected visually. The number of flies feeding on either substrate (red or blue abdomen) and both substrates (purple abdomen) are used to determine the feeding preference index: PI (sucrose) = N(red) + 0.5N(purple) / N(red)+N(blue)+N(purple). (B) Proboscis extension reflex. This does not measure feeding behavior, but rather a reflex behavior associated with feeding. Starved flies are narcotized and immobilized and then let to awaken and recover. Upon satiation with water, the forelegs of the fly — the GRNs — are stimulated with a chemical and the number of proboscis extensions are counted over a short period directly following the stimulation. The proboscis is usually withdrawn (top), but upon stimulation of the foreleg with a feeding stimulus (for example a sugar solution), is frequently extended (bottom). The number of extensions is directly correlated with the attractiveness of the stimulus.

responds (best) to water (W cell), one to sugars (S cell), one to low (L1 cell) and one to high salt concentrations (L2 cell) [24]. Even though each sensilla appears to contain only one S cell, electrophysiological data suggest that this cell has distinct receptor sites (receptor proteins, see below) which recognize different sugars (one specific for fructose and one for most other sugars) [20]. I-type sensilla, with only two chemosensory neurons, are composed of a sugar and high-salt sensitive cell [25]. S-type sensilla are more difficult to access and recordings from only a couple have been performed, revealing a similar neuronal composition as I-type sensilla [25] (Figure 3A).

More recent electrophysiological investigations by the Tanimura laboratory [25–27] have revisited the specificity of GRNs in various types of taste bristles and, not surprisingly, a more complex picture has begun to emerge that is better aligned with the complex expression profile of the putative gustatory receptor genes (see below). First, S-cells from the three sensilla types show significant differences in spike frequency when stimulated with various sugars [25]: for example, the S-cell in I-type sensilla is two to three fold more sensitive to sugars when compared to the S-cell of s-type sensilla. Second, the S-cell of s-type sensilla appears to detect only a subset of sugars, responding only to sucrose and fructose, but not glucose and trehalose.

Another recent study from the Tanimura lab [27] was the first in-depth investigation of GRNs' responsiveness to various bitter compounds. It is well established that herbivorous insects, such as Lepidoptera, have gustatory neurons that are activated by harmful compounds, eliciting feeding inhibition [28,29]. However, surprisingly little was known about 'bitter taste' perception in *Drosophila* until recently, when Meunier *et al.* [27] recorded from taste sensilla located on the prothoracic leg of females (18 sensilla) and males (28) and identified six sensilla that house a neuron activated by bitter compounds. These six sensilla fall into two groups: four were activated when stimulated with quinine but not berberine, and two were activated by berberine but not quinine. All six sensilla showed similar responses to denatonium and strychnine, two other bitter tasting compounds.

Most interestingly, the bitter-sensing cell within these six sensilla was found to correspond to the L2 cell, known to be activated by high concentrations of NaCl (a repulsive stimulus). Thus, the L2 cell is a widely tuned neuron that responds to chemically diverse repulsive compounds (see below). Many other prothoracic taste bristles, however, did not appear to house an L2 cell activated by bitter tasting compounds used in this study; interestingly, however, the firing pattern of the W and S cells in these sensilla — stimulated by water or sugar — was significantly inhibited in the presence of quinine. These findings indicate that the detection of chemical compounds avoided by the fly are mediated through (at least) two different mechanisms, one that leads to the activation of an avoidance neuron (L2 cell) and one that leads to

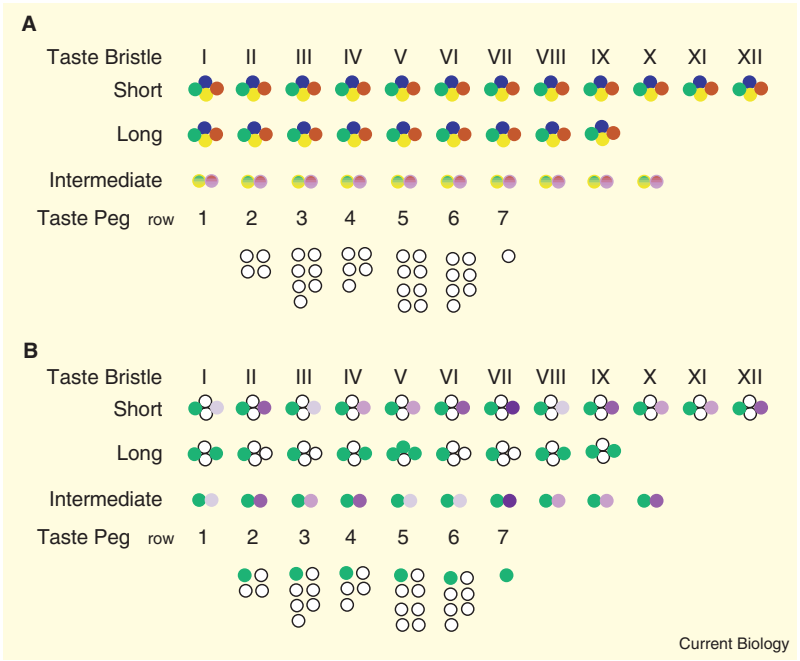

Figure 3. Functional organization of taste sensilla in the labial palps.

Each of the two labial palps contains 12 short (s) bristles (four chemosensory neurons), 9 long (l) bristles (four) and 10 intermediate (i) bristles (two). (A) Functional organization according to electrophysiological studies: Colors of neurons correspond to substrates to which they respond. Sugar, green; water, blue; low salt, yellow; high salt, red; bitter, purple; not characterized, white. Two neuron types are associated with i-type bristles: one that responds to both sugar and low salt (a combination of S and L1 cells), green and yellow; and one that responds to both high salt and bitter compounds, red and purple (bitter). Only the L2 cell of labellar i-type bristles and L2 cells on the tarsi have been shown to be activated by bitter substrates. It is not known whether the L2 cells of s-type and l-type bristles of the labellum also respond to bitter compounds. GR expression profiles would suggest so for s-type bristles. There are seven rows of taste pegs in females (six in males; shown) located between the pseudotracheae. Each taste peg is associated with one or two chemosensory sensilla. None have been electrophysiologically characterized. (B) Organization of *Gr* gene expression in the labial palps. Expression of most *Gr* genes (except *Gr5a*) in gustatory neurons are mainly associated with s-type bristles [25,51,52]. Some genes were expressed in a single neuron associated with every s-type bristle (*Gr66a, Gr22e,* and *Gr28be*), while others were only expressed in a few neurons, indicating a complex pattern of partial co-expression in labellar neurons, with some neurons expressing as many as six of the receptors examined. Note that *Gr66a* is also expressed in one of the neurons of i-type sensilla. It was found that these neurons are involved in mediating sensitivity to bitter tastants. Neurons that are sensitive to bitter substances and express at least *Gr22e* and *Gr66a* are shown in distinct shades of purple. The *Gr5a* gene, encoding a receptor for the sugar trehalose, is expressed in a distinct group of neurons, associated with each bristle type, as well as with taste pegs [51] (shown in green).

the inhibition of neurons (S cell) involved in the detection of attractive substrates, such as sugars.

Finally, Hiroi *et al.* [26] also investigated the firing pattern of labellar neurons stimulated by bitter-tasting compounds, focusing on i-type sensilla, which have only two neurons, facilitating experimental interpretation of spike patterns. Previous investigations indicated that i-type sensilla contain an S cell and an L2 cell, responding to sugars and high salt concentrations, respectively. However, Hiroi *et al.* [26] showed that the L2 cell in these sensilla is also activated by very low concentrations of various bitter compounds, including strychnine, berberine, quinine and caffeine [26]. In conclusion, the L2 cells of many, but not all taste bristles located on the labial palps and the legs appear to be broadly tuned and respond to various repulsive stimuli including chemically diverse bitter-tasting compounds as well as high concentrations of salts (Figure 3A).

A Large Family of G-Protein Coupled, Putative Taste Receptors

Significant breakthroughs in our understanding of olfactory perception and coding in both mammals and invertebrates have come from the identification of the genes encoding the olfactory receptors [30–37]. Similarly, the cloning of two gene families encoding G-protein coupled taste receptors, the T1Rs and T2Rs, has provided a molecular basis of bitter and sweet taste perception in mammals, respectively [38–45]. Hence, major efforts were directed toward the identification of the *Drosophila* taste receptor genes.

The putative taste receptor gene family was first described by Clyne *et al.* [46], who developed an algorithm for identifying DNA sequences encoding transmembrane proteins,'trained' to distinguish G-protein coupled seven-transmembrane receptors from other multi-transmembrane proteins. Subsequent analysis revealed the presence of 43 related, putative candidate *gustatory receptor* (*Gr*) genes, 19 of which were shown by RT-PCR to be expressed in at least one of several tissues containing GRNs. Later, Scott *et al.* [47] extended the family to 56 genes through reiterative database searches using the candidate genes identified by Clyne *et al.* [46]. Expression analysis of several of these genes confirmed their specific expression in GRNs of the labial palps and legs, as well as the internal taste organs and the terminal taste organ of larvae. A different strategy was employed by Dunipace *et al.* [48], who observed that BLAST searches with the *Drosophila* odorant receptor sequences identified numerous, previously unknown seven transmembrane receptor proteins. Using these new sequences for reiterative BLAST searches, they identified a novel gene family that turned out to correspond to the genes described by Clyne *et al.* [46]; moreover, Gal4 analysis of some of these candidate taste receptor genes revealed expression in taste

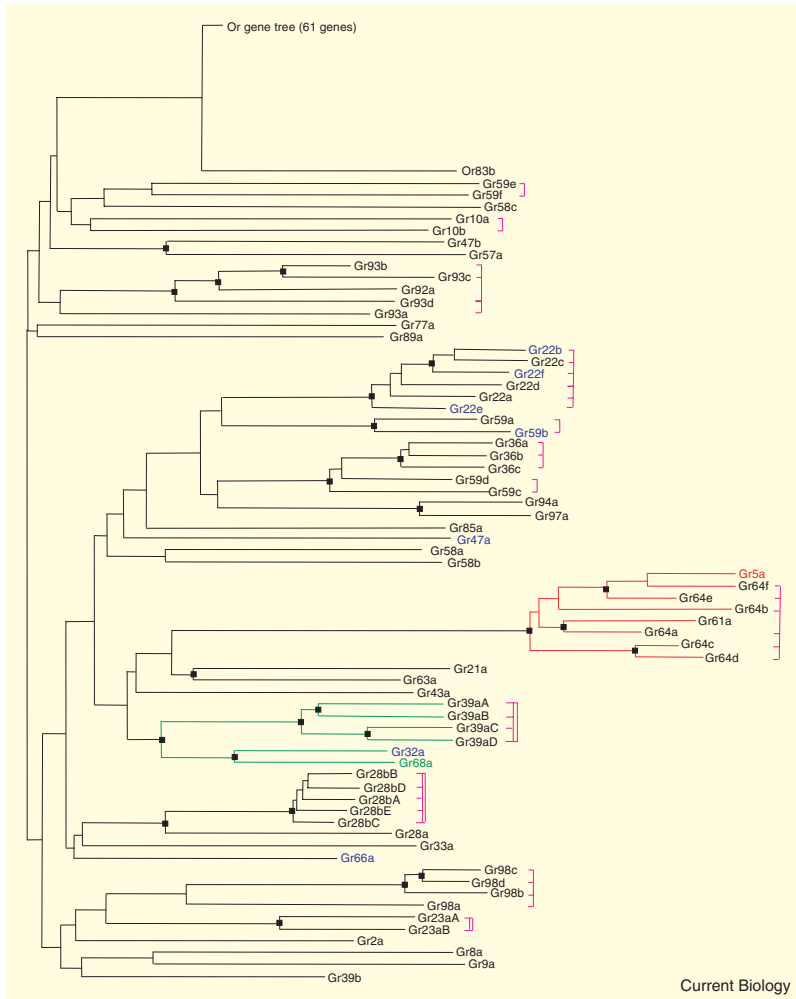

Figure 4. Organization of the gustatory receptor gene family.

The *Gr* genes are structurally related to the *Or* genes, and the direct link between the two families is *Or83b*. The *Gr* genes appear to have evolved rather rapidly through gene duplication and transposition events. Genes that are clustered share significantly more similarity than genes in distinct genomic regions. Clustered genes are indicated by brackets. Clustered and alternatively spliced genes, controlled by distinct promoters, are indicated by double-brackets. The putative sweet receptor gene family (shown in red) consists of six clustered genes on the left arm of chromosome 3 (*Gr64a–f*), as well as *Gr61a* (also on 3L) and the trehalose receptor *Gr5a* (on X). Bitter taste receptor genes might comprise genes from many subfamilies (see text). A putative pheromone receptor family, in addition to *Gr68a* (green), might also contain *Gr39a-d* and *Gr32a.* Black boxes indicate branches with 75–100% bootstrap support. Other *Gr* genes with known expression — possibly involved in bitter taste perception — are shown in blue. (Figure adapted from [49].)

neurons throughout the fly's body, a result also observed by Scott *et al.* [47].

Once the entire genome sequence was released, the chemosensory gene repertoire of the fruit fly was systematically classified [49]. It contains almost 130 genes which fall into two large subfamilies, the *Or* genes (61) and the *Gr* (68) genes (Figure 4). Overall, the sequence similarity between different gustatory receptors is fairly low (8–12% amino acid identity), even lower than that of the diverse olfactory receptors. The *Or83b* gene, which appears to encode a co-receptor for other (ligand-binding) olfactory receptors [50], represents the evolutionary link between the two sub-families. About two-thirds of *Gr* genes appear as clusters harboring up to six genes; often, individual genes are separated by only a few hundred nucleotides. Clustered genes share much higher sequence similarity to each other than to the remaining *Gr* genes (up to 70%). In three of these loci, the genes are alternatively spliced: promoters control transcription of unique 5′ exons that are spliced to common 3′ exons, generating transcripts that encode receptors with identical carboxyl termini, but different amino termini. Unlike mammals, *Drosophila* does not appear to have a distinct family of pheromone receptors, but instead appears to have recruited specific members of the *Or* and *Gr* gene family for these social behaviors [15].

Complex Expression Profile of *Gr* Genes

The rapid progress in elucidating *Drosophila* olfactory coding was possible, not only because of the many powerful molecular genetic tools that are routine in this system, but also because of the relative simplicity of the olfactory system, the manageable number of *Or* genes to be analyzed and the structural features of olfactory sensory neurons (their direct physical connection with the primary processing centers in the CNS through their axons). Yet, despite the structural similarity of olfactory and gustatory neurons and the close relationship and similar sizes of *Gr* and *Or* gene families, establishment of gene expression profiles for *Gr* genes and determination of axonal projections of GRNs to primary processing centers in the brain turned out to be rather challenging. Unlike *Or* mRNAs, *Gr* mRNAs are found at exceedingly low levels in taste neurons, making RNA *in situ* hybridization an unreliable method and requiring more laborious, indirect detection strategies [46–48]. Moreover, taste neurons are widely distributed on many appendages throughout the body of the fly, many of which (wings and legs, for example) are not amenable to *in situ* hybridization

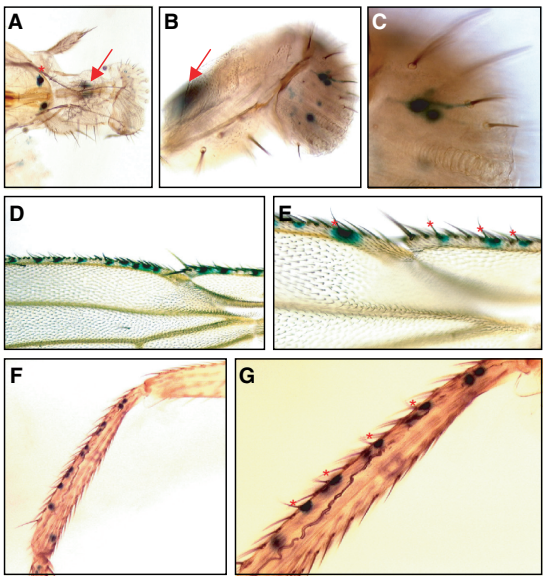

Figure 5. Expression of a *Gr* gene in GRNs of various taste organs.

(A) β-gal staining of *p[Gr22e]-Gal4; UAS*-β*Gal* flies reveal expression of this gene in the ventral cibarial organ (asterisks) and labral sense organ of the pharynx (arrow), as well as taste neurons of the labellum (B). These taste neurons clearly project their dendrites into the shaft of the bristle with which they are associated (C). *Drosophila* chemosensory bristles are characteristically curved, whereas mechanosensory bristles are virtually straight. *Gr22e* is expressed in taste neurons associated with these bristles on both wings and all legs (D plus E, F plus G, respectively). Curved chemosensory bristles expressing *Gr22e* are indicted by asterisks.

methods. In addition, GRNs from these different appendages project to distinct CNS structures, the subesophageal ganglion (SOG) in the brain as well as the thoracic ganglions. These projection centers are far less structured than the antennal lobes [2], complicating the interpretation of axon tracing experiments from GRNs.

Despite these challenges, the limited analysis of the *Gr* genes has yielded significant new insights and revealed some intriguing complexities of the sense of taste in the fly [51–53] (see below). This progress was possible by employing the bimodal Gal4/*UAS* expression system: a putative *Gr* gene promoter is used to drive expression of the yeast transcriptional activator Gal4, which tightly regulates the expression of a *Gal4* dependent *UAS*-reporter gene [54]. This system has the distinct advantage over RNA *in situ* hybridization experiments in that it allows visualization of different structures of the *Gr*-expressing neuron via GFP targeting to the nucleus (occupying the main cell body), the dendrites and axons, or the axon terminals, depending on the molecular nature of the specific *UAS* reporter used. Even though the Gal4 system usually reproduces endogenous gene expression accurately, it should be kept in mind that it is an indirect method, and hence, it is possible that in some rare cases, a *Gr* expression profile obtained with *Gr–Gal4* drivers might not precisely represent that gene's endogenous expression.

Initial expression studies revealed a rather complex expression profile for the *Gr* gene family as a whole [47,48]. For example, at least one gene, *Gr22e*, was found to be expressed in some but not all GRNs of each organ containing taste sensilla, whereas expression of most other *Gr* genes were found to be restricted to a few neurons in one or two taste organs (Figure 5) [47,48]. All genes tested were found to be expressed in a fraction of GRNs (between 1 and 5%), but no detailed comparison between different *Gr* genes were performed and it remained unclear whether and to what extent co-expression of different *Gr* genes occurred.

The true complexity of *Gr* gene expression was revealed in more recent studies [51,52] (Figure 3B). Nine *Gr* genes were analyzed in great detail and their expression was directly compared in a pairwise combination analysis. These studies revealed several major new findings. First, *Gr5a*, a gene recently shown to encode a receptor for the sugar trehalose [55,56], has a unique expression profile and is not co-expressed with any of the other receptor genes that have been analyzed. *Gr5a*-expressing neurons are also significantly more abundant than neurons expressing any other *Gr* gene previously analyzed. For example, this gene was expressed in about 120 of the approximately 240 GRNs in each labial palp [51]. Most surprisingly, GRNs expressing this receptor are sometimes clustered in groups of up to three neurons and hence are housed within the same sensillum, indicating the presence of more than one *Gr5a* expressing cell (and hence more than one S cell) in at least a fraction of bristles. Finally, each row of taste pegs contained such a cell (Figure 3B).

The remaining eight genes analyzed in these studies — *Gr22b*, *Gr22f*, *Gr22e*, *Gr28be*, *Gr32a*, *Gr47a*, *Gr59b* and *Gr66a* — were expressed in complex patterns of partially overlapping set of cells, at least in the main taste organ, the labial palps [51,52]. The gene with the broadest expression is *Gr66a*, which was found in a single GRN of each s-type and i-type sensillum and hence was expressed in about 22 neurons per labial palp. Fourteen of these cells, mostly associated with s-type sensilla, also express *Gr22e* (Figure 3B). The remaining genes are expressed in a smaller number of these fourteen cells, in partially overlapping sets of neurons. Thus, GRNs appear to fall in at least two distinct groups based on *Gr* expression profiles. A large set of GRNs expresses *Gr5a* and none of the other genes analyzed, whereas a relatively small set of neurons show partially overlapping expression of members of the remaining *Gr* genes (Figure 3B). The neurons of this latter set are therefore defined by specific receptor gene codes, and hence are likely to have unique, albeit partially overlapping, ligand recognition repertoires.

The distinct role of *Gr5a*-expressing neurons and GRNs expressing (some of) the other *Gr* genes is also revealed by their projection patterns to the CNS. Using reporter genes that encode synaptobrevin-GFP or CD8-GFP fusion proteins, which preferentially

localize to synapses or membranes, both the Scott lab [51] and ours [52] have shown that these neurons target distinct regions within the SOG. For example, *Gr66a*- and *Gr22e*-expressing neurons converge mostly to a fairly small area in the dorsolateral region of the SOG, whereas *Gr5a*-expressing neurons do not project to any specific area, but instead terminate throughout the SOG. In addition, there are extensive contralateral projections of axons from *Gr22e*- and *Gr66a*-expressing neurons, whereas axons of *Gr5a*-positive neurons appear to be confined mostly to the same side of the brain as their cell bodies in the labial palps. Therefore, the expression profile in the peripheral taste organs is translated into a distinct projection map in the first processing center in the brain, the SOG, which provides a neuroanatomical basis for the distinct behaviors mediated by these neurons (see below).

It should also be noted that a few *Gr-Gal4* drivers fail to reveal expression in GRNs or any other cells in the adult, and a few appear to be expressed in other neurons of both the CNS and the PNS [47,48]. Either the *Gal4* driver in these cases lacked specific elements for appropriate expression or these *Gr* genes might have acquired other functions altogether. Moreover, some *Gr* genes appear to have chemosensory functions unrelated to taste. *Gr68a,* and presumably other *Gr* genes, encode pheromone receptors and are involved in male courtship and possibly other social interactions between individuals [15], whereas three other *Gr* genes are expressed in olfactory neurons and might recognize odorants, rather than tastants [15,47,48]. For example, *Gr21a* is expressed in narrowly tuned olfactory neurons that detect carbon dioxide (CO_2), a stress chemical released under crowding conditions [57].

Distinct Sets of GRNs Mediate Bitter and Sweet Taste Modalities

To date, the function of *Gr* genes has mainly been addressed indirectly by 'inactivating' specific sets of GRNs (that is, sets that express a specific *Gr* gene). Both the Scott lab [51] and ours [52] have used this approach to provide a conceptual framework of how the fly can discriminate between different taste qualities. Using the available Gal4 drivers, flies were created in which defined sets of GRNs were killed or rendered non-functional by expressing diphtheria or tetanus toxin reporter genes. The behavior of such flies was then assayed by either the two-choice preference test or the proboscis extension reflex assay. Lack of functional *Gr66a*-expressing neurons was found to cause reduced sensitivity for bitter compounds, but not to affect trehalose or sucrose sensitivity. Conversely, when *Gr5a*-expressing neurons were killed or rendered functionally inactive, detection of bitter compounds was not altered, whereas that of trehalose was significantly reduced. The main conclusion from these studies was that the population of *Gr*-expressing taste cells can be divided into two functional groups, one required for the perception of sugars and possibly other nutrient-rich compounds (amino acids) and a second group for the detection of undesirable substances — chemicals perceived as bitter (by humans).

One difference between the two studies [51,52] should be noted: the loss of sensitivity in the two choice preference tests was rather specific to caffeine and trehalose, respectively, because the flies did not reveal significant changes in their avoidance behavior to other bitter compounds (quinine, berberine and denatonium) when *Gr66a*-expressing neurons were inactivated or the sugar sucrose when *Gr5a*-expressing neurons were inactivated [51]. However, the proboscis extension reflex was affected, albeit to a lesser extent, when flies with diptheria-toxin ablated *Gr66a* or *Gr5a* neurons were challenged with these compounds [52]. One obvious explanation for this difference might be related to the fact that the proboscis extension reflex monitors the activity of only a small fraction of neurons expressing a given (set of) receptors (namely those in the forelegs), whereas in two choice feeding preference tests, all neurons expressing a given driver can have an impact on the feeding behavior. In addition, even though the same neurons in each report were targeted, the method of incapacitation was different and might not be equally effective (inhibition of synaptic transmission versus cell ablation).

Do Specific Receptor Sub-Families Recognize Chemically Related Compounds?

Even though the distinct expression profiles of the *Gr* genes and the behavioral analysis of flies lacking specific sets of GRNs (see above) are consistent with the notion that *Grs* code for gustatory receptors, direct evidence has been reported only in a single case. More than 20 years ago, Tanimura and co-workers [16] identified a fly strain (*tre*) with reduced sensitivity to the sugar trehalose, an abundant metabolic product of yeast and an important food source of *Drosophila melanogaster*. Recently, two groups [55,56] showed that lower sensitivity to trehalose of the *tre* strain and certain other fly strains was associated with specific amino acid substitutions within GR5a, and that absence of the *Gr5a* gene altogether resulted in a similar phenotype. Moreover, a genomic transgene construct containing the *Gr5a* gene from a strain with high sensitivity to trehalose can rescue the phenotype of a low trehalose sensitivity strain [56]. Finally, Carlson and co-workers [58] showed that *GR5a* expressed in *Drosophila* tissue culture cells elicited Ca^{2+} influx in the presence of trehalose-containing medium, but not of sucrose- or maltose-containing medium.

Compared to mammals, the high specificity by which *Gr5a* affects taste perception to trehalose, but not other sugars, is striking. In mice, for example, heterodimeric T2R1–T2R3 receptors are essential for detection of all sugars and artificial sweeteners, which explains the apparent inability of mice (and humans) to discriminate between different sugars [44,59]. In contrast, it seems certain that, in *Drosophila*, *Gr* genes other than *Gr5a* encode receptors for other sugars such as glucose, sucrose and maltose, which are abundantly present in other fly food sources (fruit); it has been suggested that the *Gr64* subfamily encode

such candidate receptors [51,52,58,60]. This subfamily, which is characterized by unusually high sequence similarity, includes a cluster of six genes in the cytological region 64A (*Gr64a-f*), as well as *Gr61a* and *Gr5a*, and probably arose through recent gene duplication and transposition events [49]. Mutations in these genes along with detailed expression profiles should provide a better understanding of sugar perception in the fly. Of particular significance will be to determine the extent of overlap in expression between *Gr5a* and other members of this subfamily.

The number as well as the chemical complexity of bitter-tasting compounds is much larger than that of biologically relevant sugars. Hence, it seems unlikely that receptors for these compounds are restricted to one (or even a few) sub-family. Rather, it appears that receptors for these compounds are found among most other sub-families, possibly with the exception of the *Gr64* and the *Gr39* subfamilies, which might represent sugar (see above) and pheromone receptor subfamilies [15,53 60], respectively.

Comparison of Sweet and Bitter Taste between Insects and Mammals

The behavioral studies of flies lacking specific sets of taste neurons have provided clear evidence that the GRNs can be functionally characterized by their critical role in detecting two major substrate categories: sugars, including a major metabolite of yeast (trehalose) and preferred fly food; and bitter-tasting compounds that are often harmful and toxic [51,52]. The fly taste system is thus not unlike that of the mammalian tongue, where two distinct and mutually exclusive sets of taste cells express either the bitter or sweet/umami receptors [60]. Bitter tasting compounds, which are avoided by most mammals, are recognized by a single class of about 30 taste receptor genes (*T2R*s) [40,61,62], all of which appear to be co-expressed in a single set of taste neurons in the taste buds of the tongue [39]. Sweet and umami (the taste of glutamate) tasting chemicals, on the other hand, are detected by distinct taste cells that express members of the *T1R* family [43,59,63,64].

A notable difference in the organization of putative bitter taste receptor expression between *Drosophila* and mammals remains and demands an interpretation. Whereas bitter taste cells in the mouse appear to express all, or virtually all, *T2R* genes [39], avoidance neurons in the fly labellum express different combinations of *Gr* genes, which may provide the fly with neurons that exhibit distinct ligand recognition properties (see above) [51–53]. Assuming that these gustatory receptors are indeed involved in the detection of toxic and noxious chemicals, the *Drosophila* taste system might then be capable of discriminating among various harmful compounds, enabling the animal to respond in a differentiated and 'measured' fashion to harmful stimuli.

How can such a hypothesis be tested? Ability for odor discrimination in flies has been established using conditioned odor learning paradigms [65,66]. However, such assays have yet to be developed for fly taste perception. Whether *Drosophila*, which feed almost exclusively on yeast and fruit, have a requirement for bitter taste discrimination is unclear at best, and questionable at worst, but it is almost certain that other insects, especially herbivores, often encounter bitter and potentially toxic compounds present in plants. Even though it is well establish that many of these insects employ specific detoxification strategies to neutralize ingested, toxic chemicals [67–69], another strategy would be the prevention of intake of the most harmful chemicals altogether. In fact, many herbivorous insects are feeding specialists and feed on very few plants, which they must discriminate from similar, but (more) toxin-containing relatives. Such discrimination might very well involve the bitter taste system [28,29], although other modalities such as attractive soluble compounds or even olfactory cues might be employed in this process. Thus, it is also conceivable that the distinct expression profile of putative bitter taste receptors in *Drosophila* might represent an evolutionary remnant of a relevant feature of herbivorous insects, but bears no functional significance in the fly.

Sweet and Bitter Taste Transduction

Relatively little is known of how the interaction of taste ligand and receptor translates into neural activity. In mammals, sweet and bitter taste receptors use the same signaling cascade, in which the receptor-ligand interaction leads to the activation of a G-protein, which in turn activates phospholipase C (PLC) and ultimately a TRP channel, leading to the generation of an action potential [70]. As *Drosophila* GRs are thought to belong to the family of G-protein coupled receptors, it is likely that heterotrimeric G-proteins link the receptors to downstream signaling molecules.

Expression of genes encoding G-alpha subunits in the chemosensory organs has been reported [71]; however, flies mutant for these genes are either not available or have not been studied with regard to taste behavior or the electrophysiological properties of their gustatory neurons. Similarly, the PLCβ encoded by the *norpA* gene, which is known to be essential for *Drosophila* photo and olfactory transduction, is also expressed in neurons of taste organs [72], suggesting that this enzyme might also be involved in taste transduction. In contrast, It will be interesting to see whether any of the 14 *Drosophila trp* channels are expressed in GRNs. Ultimately, however, only functional genetic analysis will reveal whether any of these components have similar roles to their mammalian counterparts and are indeed involved in sweet and bitter taste signaling of *Drosophila* (Figure 6A).

Genetic Analyses of Taste

Numerous genetic approaches have been tried for identifying genes involved in taste perception [16,24,73–77]. Many mutations cause fairly broad and pleiotropic taste phenotypes and likely affect other sensory modalities as well. For example, many of these genes could encode proteins involved in a variety of processes, including signaling of neuronal activity within the cell, development and differentiation

of specific types of GRNs, or proteins directly involved in the detection of chemicals, including receptors or peri-receptor proteins, such as odorant binding proteins. Curiously, the one mutation/variation that has been directly linked to a gene was *tre*, which encodes the trehalose receptor GR5a (see above). The absence of linkage between other identified phenotypes and specific genes might reflect a lack in effort by the researchers or inherent difficulty and complexity of the mapping process *per se*, as the phenotypes are usually fairly subtle and often influenced by the genetic background.

Many gustatory mutations, such as *gustC* and *gustM*, affect the behavioral perception of different types of chemicals including sugars, salts and quinine (a bitter tasting compound). Some, however, have a relatively specific phenotype and affect only the perception of pyranose (*gustA*), salt (*gustB* and *gustE*) or bitter compounds (*gustD*). Interestingly, some mutations with relatively restricted phenotypes map in regions that harbor one or several *Gr* genes; for example, *gustC* and *gustE* (10E), *gustM* (93C/D) and *gustR* (64B/C) are in relatively close proximity to *Gr* gene clusters (*Gr10a/b*, *Gr64a-f* and *Gr93a-d*) at these cytological positions. Thus, it remains to be seen whether any of these mutations map to a *Gr* gene.

Detection of Salts

Salty and sour tastes are not just human perceptions, but also cause behavioral responses in insects and are especially relevant for fluid and electrolyte homeostasis. Whereas the perception of sour taste has not been explored in much detail in *Drosophila*, detection of salts, which regulates the uptake of Na^+ and other cations, has been studied both at the electrophysiological and more recently at the molecular-genetic level. For example, the fly exhibits differentiated responses to salts and feeds on it at low to moderate concentrations (up to 100 mM), but avoids it at concentrations of more than 200 mM. Based on electrophysiological studies, these two opposing behaviors appear to be mediated by distinct neurons in the taste sensilla, the L1 and L2 cells, respectively, (see above).

In mammals, salts (NaCl or KCl) are thought to be detected by epithelial-type sodium channels (ENaCs), several of which are expressed in taste cells and are thought to be involved in the detection of extracellular Na^+ ions that directly activate taste cells [78]. Liu and collaborators [79] specifically addressed the potential role of these channels, also known as the *pickpocket* (*ppk*) gene family, in *Drosophila* taste. They found that at least six *ppk* genes (of a total of about 25) are expressed in the larval and/or adult taste system, among other tissues. A role in salt perception for two of these, *ppk11* and *ppk19*, was addressed by expressing dominant negative forms of these channels, which are thought to inhibit channel function through the creation of non-functional multimers, as well as by RNA interference experiments to prevent expression of the specific channels altogether. These experiments revealed that both *ppk* genes are required for the detection of low concentration of salt

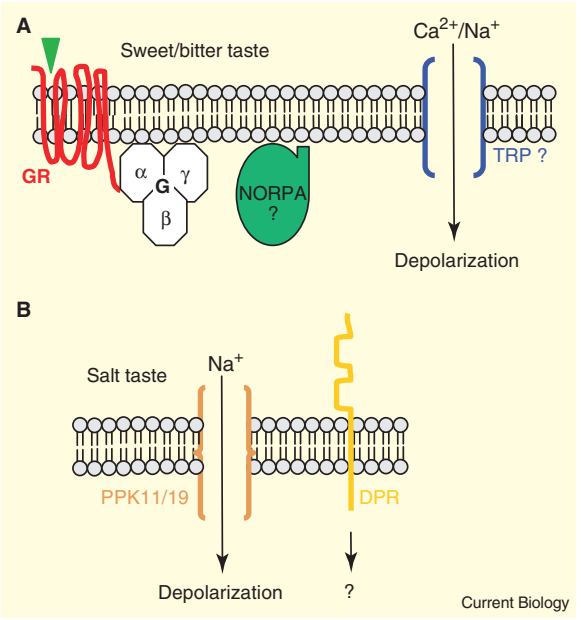

Figure 6. Taste signal transduction for sweet, bitter and salt compounds.

(A) Sweet and bitter tastants are initially detected by GRs, such as GR5A and possibly GR66s. As the *Gr*s are thought to encode G-protein coupled receptors, their interaction with ligand probably activates a trimeric G-protein. The proposed signaling mechanisms are hypothetical and solely based on the observations that two components, phospholipase C (NORPA) and G_{α}, are expressed in taste neurons, and that mammalian sweet/bitter GPCRs are known to signal through a phospholipase C and a TRP channel [70]. (B) Salt taste is thought to be mediated by direct influx of sodium (or potassium) ions into the cell via DEG/ENaC channels such as PPK11 and PPK19 [79]. Additionally, members of the *DPR-Ig* family of genes, such as *dpr1*, are also involved in salt signaling [80]. However, their specific role in the signaling cascade remains to be elucidated.

in the larva. Moreover, aversion to high salt concentration was markedly reduced, especially in adult flies (Figure 6B). These data indicate that epithelia-type Na^+ channels of *Drosophila* are required for the detection of salts and that this taste modality appears to be mediated by the same molecular machinery in mammals and insects.

Another type of membrane-associated protein was recently shown to be involved in the detection of salts. Nakamura and co-workers [80] mapped a mutation in the *defective proboscis extension responsive 1* (*dpr1*) mutant strain to a member of the *DPR-Ig* family of genes, which are characterized by two Ig repeats and a single transmembrane segment. None of the other twenty or so *dpr*-like genes have been characterized and it is not known whether any of these are involved in the detection of salts, nor is it known whether the mammalian counterparts have a role in taste sensation.

Perspective

The remarkable increase in number and sophistication of studies on various *Drosophila* sensory systems over the last few years has led to a more comprehensive and detailed understanding of how the fruit fly

perceives its world and processes sensory stimuli. This has been particularly true for olfaction and, most recently, taste, as we have begun to uncover the molecular basis for discriminating olfactory and feeding behaviors. The first functional studies using mutant flies or flies lacking specific sets of taste receptor neurons have provided a framework for how insects detect the large number of chemicals they encounter in their environment and how they discriminate between the basic taste modalities. Just as in the vertebrate chemosensory systems and the *Drosophila* olfactory system, this progress has been possible through the identification of the *Drosophila* taste receptor genes, and further progress will certainly be facilitated with these genes now at our disposal.

What are the next steps in *Drosophila* taste research, and what impact might they have for the broader field of chemosensory perception? Some of the obvious questions are similar to those currently being tackled in olfaction: What are the natural ligands for specific GRs? How broadly tuned are the GRs? What is the nature of other sugar receptors and how are they expressed with respect to each other and to *Gr5a*? What is the consequence of the complex expression patterns of the many *Gr* genes, presumably involved in the detection of bitter tasting compounds? Are there different 'avoidance' or 'bitter taste' qualities that the fly can discriminate? And likewise, can it discriminate between different sugars? How is the activation of GRNs translated into taste perception in the brain — how are second and higher order neurons that link functionally related GRNs, organized in the brain to elicit a specific behavioral output? Are tastes memorized, just like odors, and put into a specific context? In other words, how can the fly associate taste with other sensory experiences and retrieve such information when similar combinations occur at a later point in its life history?

To address the many questions that will capture our attention in the near future, we would like to propose three lines of investigation that we believe deserve special consideration, as they appear currently only possible in this unique model system.

Can Flies Discriminate between Compounds within a Taste Modality?

In contrast to bitter taste in mammals, all expression data available to date strongly argues that, in *Drosophila*, different avoidance neurons expressing distinct combinations of putative bitter taste receptors exhibit unique ligand recognition properties. Exposure to different bitter tasting chemicals thus leads to activation of different, albeit overlapping, sets of GRNs and hence to the activation of distinct neural ensembles in taste processing centers, such as the SOG and the thoracic ganglion. Thus, in principle, such distinct activation patterns might provide a means for bitter taste discrimination. Whereas *Drosophila melanogaster* feeding on fruit or yeast might not have a need for discrimination of different bitter tasting compounds, other *Drosophila* species have completely different food resources and some feed in fact on plants directly, rather than on the fruit they bear [81–83]. Thus, it is of interest to investigate whether taste discrimination between different bitter compounds indeed exists. To do so, it will be necessary to develop new behavioral assays, similar to those that were employed for investigating olfactory discrimination [66]. These assays are based on the ability of flies to associate the perception of a specific odor with other sensory input that is perceived simultaneously. Thus, analogous experiments in which bitter tasting stimuli are associated with a conditioning stimulus should eventually reveal whether flies — and by inference other insects — have the ability to discriminate different chemicals within this taste modality. Naturally, similar investigations for determining the potential of discrimination between different sugar compounds could be carried out as well.

Generating a Taste-Receptor-Less Fly

As taste neurons express multiple *Gr* genes, functional analysis of specific GRN subsets will not allow determination of individual gene function. Heterologeous expression of G-protein coupled receptors has often been successful for identifying ligands for specific receptors. Such strategies have been difficult for chemosensory receptors, however, presumably because of the need for specific chaperons for cell surface expression [84]. How can the natural ligands for insect chemosensory receptors, specifically the GRs, be identified? We believe that a genetic characterization of possibly the entire *Gr* gene repertoire is feasible in *Drosophila*, mainly through two recent and remarkable innovations: homologous recombination and PiggyBac element-generated deletions [85–89]. These two methods allow the targeted deletion of a gene of interest in a precise (or fairly precise) and efficient manner, and it is therefore possible to generate a large collection of fly strains that lack specific *Gr* genes and test their behavior.

To knock-out 68 genes may seem like a labor-intensive endeavor, but the extensive gene clustering would allow knocking out more than two thirds of all *Gr* genes with only 12 knockout events (Figure 4). As *Gr* genes within a cluster are more similar to each other, and are therefore likely to detect similar ligands, such an approach may lead to the identification of natural ligands for many receptors (or subfamilies of receptors). Moreover, effects on specific taste modalities (in the absence of the entire repertoire of GRs of another taste modality) could be investigated.

Evolution of GRNs within *Drosophila* and Insects

The genomes of several *Drosophila* species and other insect species, including *Musca domestica* (housefly), *Apis mellifera* (honeybee) and *Anopheles gambiae* (mosquito), have recently been sequenced. This provides a unique opportunity to investigate the evolution of this large receptor gene family. For example, the high sequence conservation of the odorant receptor *Or83b* across species was an early indication that this gene might serve an important (and possibly unique) function [49,90]. Indeed OR83b was shown to be required for cell surface expression of other ORs and is thought to function as a co-receptor

[50]. Similarly, the relatively high conservation of *Gr5a* is consistent with an important role for this receptor in sugar detection in many insect species (our lab's unpublished results; Hugh Robertson, personal communication). Thus, *Gr* genes that show comparatively high sequence conservation across species might indicate receptors that detect ubiquitously relevant substrates. On the other hand, *Gr* genes poorly conserved or conserved only among a small group of different insect species might reveal genes with highly specialized functions. In any case, comparative genomics might provide an important criterion for deciding whether to pursue detailed analyses of selected members of this diverse gene family.

Acknowledgments

We thank Akemi Toyama and Steve Bray for assistance with the figures. This work was supported by grants from the NIH (Nr. GMDC05606-01 and NSF (Nr. 0349671) to H.A.

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