ELSEVIER

PII: S0308-8146(96)00337-8

Food Chemistry, Vol. 60, No. 3, pp. 311–321, 1997

0308-8146/97 $17.00+0.00

Genetic analyses of sweet taste transduction

Linda M. Kennedy,* Shachar Eylam, Jason E. Poskanzer & Anna-Riika Saikku

Department of Biology, Neuroscience Program, Clark University, Worcester, MA 01610-1477, USA

(Received 29 August 1995; revised version received 11 October 1996; accepted 11 October 1996)

A genetic analysis of sweet taste transduction was initiated using fruitflies and humans. In flies, behavioral and receptor cell electrophysiology data indicate different mechanisms for fructose and glucose in or before the receptor cells. Fructose nontasters (FN) and glucose nontasters (GN) were selected from natural populations of adults and larvae. The FN are the first fructose taste variants isolated in any species. There were no significant differences between male and female frequencies for either variant type, and no observable differences in banding patterns between wildtype and FN X-chromosomes. These and preliminary cross-breeding data suggested a polygenic trait, either completely autosomal, or including coding by a defective X-chromosomal gene for which an autosomal gene(s) can compensate. In humans, psychophysical functions also indicated different fructose and glucose mechanisms. Variability analysis suggested separate signals for the monosaccharides and an additive sucrose signal. Potential GN were identified by elevated glucose thresholds. © 1997 Elsevier Science Ltd

INTRODUCTION

The molecular mechanisms for sweet taste transduction in receptor cells are not well understood. Perceptual, physiological and biochemical data suggest multiple membrane receptors for sweet stimuli (Shallenberger & Acre, 1967; Shimada *et al.*, 1974; Faurion, 1987) and a role for G-protein-dependent increases in cyclic nucleotides (cAMP or cGMP) leading to a receptor potential, perhaps via activation of a protein kinase that inactivates K^+ channels (Striem *et al.*, 1989; Tonosaki & Funakoshi, 1988; Daley & Vande Berg, 1976; Avenet *et al.*, 1988; Cummings *et al.*, 1993; Schiffmann & Gatlin, 1993). A somewhat different version is suggested by data from our studies with taste-altering compounds: multiple sweet taste receptors may be coupled to a single sweet taste second messenger system (Kennedy, 1991; Kennedy *et al.*, 1993) involving stimulus-activated **decreases** in cyclic nucleotides (Foster & Kennedy, 1994*a*,*b*). Beyond the cloning from taste tissues of a G-protein, which is similar to G-proteins involved in visual transduction (McLaughlin *et al.*, 1992, 1993), the specific molecules and mechanisms remain unknown.

An understanding of such protein receptors and transduction systems can be facilitated by the application of classical and molecular genetics techniques to identify specific proteins, together with behavioral and electrophysiological studies to indicate the functions of those proteins. Such research can be conceptualized as a mutant-to-gene-to-protein process. We have adopted this approach in studies with both animal models (fruitflies) and humans. This paper summarizes key results which characterize the fruitfly responses to sugars, reports the isolation of fruitfly taste variants for fructose (FN) and glucose (GN), and describes ongoing work to characterize the FN. Our FN are the first fructose taste variants isolated in any species. We also report studies which indicate separate fructose and glucose mechanisms in humans and potential human variants for glucose. Portions of this work are published in abstract form (Her & Kennedy, 1991; Poskanzer *et al.*, 1992; Poskanzer & Kennedy, 1993; Kennedy & Poskanzer, 1994; Eylam *et al.*, 1995).

Genetic studies with fruitflies as models

Fruitflies (*Drosophila* species) are the premier animals for genetic studies, because of their small sizes, large numbers of progeny, short generation times, and ease of culture (Hartl, 1992). Their giant polytene chromosomes and small genomes (three pairs of autosomes and one pair of sex chromosomes) facilitate cytological and molecular genetic localization (Merriam *et al.*, 1991; Hartl, 1992). Overall, the relative simplicity of *Drosophila* is a major advantage for an integrated use of

*To whom correspondence should be addressed.

classical and molecular genetics. In addition, chemical structural homology is conserved well enough for application of the analyses to mammalian systems (Gilman, 1987; Barnard, 1989; Neer *et al.*, 1990), and this 'evidence of basically similar molecular mechanisms... has proven to be widely obeyed' (Barnard, 1989).

Three 'sugar cell' mutants, which show altered behavioral and receptor cell responses to some sugars, have been reported in the small fruitfly, *D. melanogaster* (Bhavsar *et al.*, 1983; Rodrigues & Siddiqi, 1981; Tanimura *et al.*, 1988; Tanimura, 1991). There is evidence for the cytological location of the defective genes for two of these: the 'Tre' gene, which controls trehalose sensitivity, is on the X-chromosome, and the gene for glucose sensitivity is on chromosome 3 (Tanimura *et al.*, 1988). However, characterizations of mutant receptor cell responses are incomplete in *D. melanogaster*, presumably because of difficulties in identifying the cells firing. These difficulties are thought to be due to the small size of receptor cell dendrites in this species (Fujishiro *et al.*, 1984).

We have been studying the genetics of responses to sugars in the Hawaiian fruitfly, *D. adiastola*. The large size of this species facilitates behavioral and receptor cell electrophysiological characterization of wildtype and variant taste responses. Such characterization is essential for a thorough understanding of the defective and normal physiological transduction mechanisms. A particular advantage is that we can reliably obtain clear, interpretable recordings of action potentials from *D. adiastola* receptor cells, without the confounding variations in amplitude and waveform reported (Fujishiro *et al.*, 1984) for *D. melanogaster*. Here we summarize data which characterize behavioral and receptor cell responses to sugars in the wildtype *D. adiastola*, report the isolation of variants for fructose and glucose, and provide statistics for the occurrence of the variant traits and the male/female distributions in a natural population.

A disadvantage of *D. adiastola* is that there exist neither a genetic linkage map nor a corresponding supply of known mutants, as there are for *D. melanogaster*. However, the banding patterns and rearrangements of the larval polytene chromosomes of this and other closely related Hawaiian species have been described (Carson & Stalker, 1968). Thus, cytological localization by physical mapping techniques, and isolation of relatively short lengths of DNA for molecular analysis, should be possible. Here we report the isolation of larval FN and preliminary results from studies of chromosomal banding patterns in wildtype and FN *D. adiastola* larvae.

A second disadvantage with *D. adiastola* is difficulty in maintaining laboratory cultures and, consequently, difficulty in conducting cross-breeding experiments. Therefore, we also have isolated nontasters in *D. melanogaster* and report here initial results from FN cross-breeding experiments with these animals.

Human responses to sucrose, fructose and glucose

Response functions obtained by constant-stimulus paired-comparisons (Pangborn, 1963) and by intensity/time judgments (Portmann *et al.*, 1992) of various concentrations of sucrose, fructose and glucose vary in ways similar to our data from *Drosophila* and also indicate different mechanisms for fructose and glucose. Functions from our study with a forced-choice paradigm analogous to that we used for the *Drosophila* are similar, as well. These similarities, together with similar response functions from rat chorda tympani recordings (Hagstrom & Pfaffmann, 1959), and with the apparent conservation of molecular mechanisms from *Drosophila* to mammals, suggest that information from studies with *Drosophila* can be relevant to human taste. The human study reported here shows the similarities between *Drosophila* and humans when similar test paradigms are used. This demonstrated similarity will enable appropriate conclusions in future genetic research with both species.

In contrast to the human studies just mentioned, Breslin *et al.* (1994) compared sensitivities for sucrose and fructose with glucose to obtain U-shaped functions from which they concluded that the three sugars are indiscriminable. However, the different functions in the Pangborn (1963) and our data presented here are for concentrations lower than those tested by Breslin *et al.* (1994), and our functions converge at our highest concentrations, in agreement with the Breslin *et al.* (1994) data. Also, the Portmann *et al.* (1992) and Breslin *et al.* (1994) data cover the same concentration range, with the Portmann *et al.* (1992) functions converging at concentrations where the Breslin *et al.* (1994) subjects were unable to distinguish the sugars. Thus, although the data are interpreted differently, the results do not contradict each other.

The evidence for different glucose and fructose mechanisms in humans suggests that we should be able to find some individuals who are defective in one or the other, as we have in *Drosophila*. Here we report data for some individuals whose glucose response functions do indeed vary markedly from those of the population, as well as population data which support different mechanisms for fructose and glucose.

MATERIALS AND SUBJECTS

Taste materials

Sugars were [alpha]-D(+)-glucose, [beta]-D(−)-fructose, and [alpha]-D-glucopyranosyl-[beta]-D-fructofuranoside (Sigma, ACE reagent) in water or agar (Bacto-agar, DIFCO certified), and the food dyes were a commercial product ('Dec*a*cake', Durkee-French Foods, Inc.).

For the fly experiments, water was distilled from Worcester city water in the Clark University Science

Center still and filtered through a charcoal filter; the purity is checked every three months by a consultant (Mitchell Associates, Milford, MA). Test media were prepared 1 h prior to experiments.

For the human experiments, Poland Spring distilled water, the purity of which is checked bimonthly (Poland Spring representative), was used. Sugar solutions were prepared at least 24 h before use, stored at 5°C, and brought to room temperature (~23°C) for tests.

Flies and larvae

The flies were *D. adiastola*, a Hawaiian painted-wing fruitfly descended from *D. grimshawi* (Carson & Stalker, 1968), or *D. melanogaster*. The *D. adiastola* were cultured at the Hawaiian Environmental Biology Program, University of Hawaii, Honolulu and shipped to our laboratory. Adults arrived at 7 days of age and larvae at 75 h after hatching. Adults were maintained in mass-feeding vials with sucrose, fructose and glucose, 2.5% w/v each (in 2% w/v agar) *ad lib*, at 18–20°C and allowed to adjust to a 12:12 h day:night cycle for at least two days before tests. Larvae were maintained in the shipping medium under the same conditions until testing. *D. melanogaster* adults and pupae were obtained from Connecticut Valley Biological Supply Co., Inc., cultured at 18°C, with 12:12 h light:dark, on Blue *Drosophila* medium (Connecticut Valley Biological Supply Co.). All tests were conducted with adult progeny from these cultures.

Human subjects

This research has been approved by the Clark University Committee on the Rights of Human Participants in Research and Teaching Programs. Ten male and 10 female subjects (mean age 41.6, range 18–72 years) were recruited from the multicultural Clark University community, and included 5 Asian or Pacific Island individuals, as well as Caucasian Americans. They completed a confidential questionnaire with information about age, sex, race/ethnic origin, smoking, medications and family history of diabetes. Any reporting known diabetes in themselves were thanked and told that we could not test them as subjects. The nondiabetic individuals who were included in the experiment were considered representative of a 'natural' human population.

METHODS

Fly behavioral tests

Behavioral data were obtained using a two-choice paradigm, as adapted from Tanimura *et al.* (1982) by Her and Kennedy (1991) and Poskanzer *et al.* (1992). Flies chose to feed on red or blue agar media, containing (a) sugars of various concentrations vs plain agar, or (b) fructose vs glucose at concentrations which had equal taste potency.

Characterization of the natural population

Groups of 43–55 flies were food-deprived, with 1% agar in water *ad lib* for 48 h. The flies then were anaesthetized by cooling in a freezer and placed on water-saturated filter paper in the opaque section of a double-sectioned test chamber, and the partition between the opaque and transparent sections removed. Upon awakening, the flies were attracted to, and moved toward, the light in the transparent section. Since the opening between the sections was low, the flies were forced to walk on the wet filter paper. Thus their water receptors were stimulated and those flies that were thirsty drank. In this way, the paradigm controlled for ingestion of a test solution by a thirsty fly for the water, rather than for the sugar. Once all the flies had moved into the transparent section, the opaque section was closed off and the sliding shelf removed. There, they chose among 12 Petri dishes, randomly arranged on the floor of the testing chamber; six contained a single concentration of a single sugar in 1% agar (red) and six contained sugar-free 1% agar (blue). The ranges of sugar concentrations in the media were sucrose 1.5–150 mM, fructose 1.5–250 mM, and glucose 20–250 mM.

The flies were allowed to feed for 4.5 h. Then the flies' abdominal coloring was checked, and the flies categorized as red only, blue only, both red and blue, or neither red nor blue. A preference index (*PI*) was computed according to the following equation: $PI = (R + 1/2M)/(R + M + B)$, where R = number of red flies (those eating only the sugar-containing solution), M = number of mixed (red and blue) flies, and B = number of blue flies (those eating only the plain agar solution). The test was repeated using different concentrations and different sugars, and the resulting *PI* values were plotted against sugar concentration (Her & Kennedy, 1991).

Selection of adult variants

Behavioral tests for variant selection in adult flies were as for the population characterization with some modifications. Test media were 20 mM fructose and 40 mM glucose in 1% agar, slightly suprathreshold concentrations for which the *PI* was 0.6. In the first of two screening tests, fructose was colored blue and glucose was colored red. After the tests, flies were placed into individual vials containing a medium of 2.5% each sugar in 2% agar. On the second post-test day, the vials were examined for colored spots, and the flies classified as above. Those that were both red and blue were designated 'wildtype' (WT), since they ate both sugars. Those that were either only red or only blue were subjected to a second screening test after 48 h food-deprivation, with the same concentrations of sugars, but with the colors reversed. Any flies which showed the same color in both tests had eaten both sugars and were thus WT. Those who excreted red in the first test and blue in

the second were designated 'fructose nontasters' (FN), as they had eaten only glucose, and thus presumably could taste only glucose. Similarly, those who excreted blue in the first test and red in the second were 'glucose nontasters' (GN) (Poskanzer *et al.*, 1992; Poskanzer & Kennedy, 1993).

Selection of larval variants

A 96-well Elisa microplate (CorningR) was fitted with dental wax (Lactona) to enclose an octagonal test area 7.5 cm in diameter, containing 48 wells. A center 'start zone' of four wells was filled with 1% agar. Around this area, blue and red media of fructose 20 mM and glucose 40 mM (each in 1% agar) were placed in alternating wells, in ABAB fashion. Thus the larvae were required to not simply feed in the most-easily reached wells, but rather to make a choice by tasting two media, as they had to pass by A to get to another B. Spaces between wells were filled with plain agar so that larvae could not fall into these areas and become trapped.

Since food-deprivation may cause chromosomal rearrangements in this species (K. Kaneshiro, 1994, personal communication), the larvae were not food-deprived before testing. They were removed from the shipping medium, allowed to crawl on water-saturated filter paper for 5–10 min, and then placed in the start zone of the test chamber. They were allowed to feed 6–8 h and then classified according to the colors seen in the intestines by light microscopy (Saikku, 1994).

Chromosomal preparations

After classification of a larva, the salivary glands were removed, stained with 1% orcein in 45% acetic acid and fixed (Sorsa, 1988). The glands then were squashed between glass slides, viewed by light microscope, photographed, and traced by camera Lucida projections. The banding patterns were compared with standard maps for *D. adiastola* (Carson & Stalker, 1968) and *D. melanogaster* (Sorsa, 1988) and also compared between the *D. adiastola* WT and FN (Saikku, 1994).

Receptor cell neurophysiology

The action potential responses of receptor cells in single labellar sensilla were tip-recorded (a form of loose patch-clamp recording) in isolated *D. adiastola* proboscis preparations as in our previous work with blowflies (Kennedy & Halpern, 1980; Kolodny & Kennedy, 1988). A concentration series of each sugar was determined from the effective concentration ranges in the previous behavioral tests: sucrose or fructose 5, 10, 20, 40, 80, 160 mM; glucose 30, 40, 60, 80, 100, 150 mM (all in choline chloride 100 mM). The various concentrations of a given sugar, and also 'water' (75 μM NaCl) and 100 mM choline chloride, were presented in random order to each of nine sensilla as 5 s stimulations, with 5-min disadaptation periods in between. Data analyses were on the first 100 ms responses, as in our previous work (Kennedy & Halpern, 1980; Kolodny & Kennedy, 1988).

Human psychophysical tests

Procedure

Subjects tasted and compared the sweetness of various concentrations of sucrose, fructose and glucose (2, 4, 8, 16, 32, 64 and 128 mM) with that of distilled water (21 pairs) in each of three sessions. The concentration range was selected to include the threshold as in the literature and according to results from preliminary tests. The series for all sugars was selected to range from a low concentration that no subject could recognize as sweet to a high concentration that all would recognize as sweet. The range also was selected to give clear resolution of a concentration recognized as sweet by > 50% of the subjects.

Cups of solutions (10 ml) were placed on a table in a labeled order for presentation (21 pairs of sugar and water). The order was random for the concentration of each sugar, and for the water/sugar order within a pair, with the three sugars presented serially. The serial order of sugars was systematically varied for each of three sessions—sucrose/glucose/fructose, glucose/fructose/sucrose, fructose/sucrose/glucose.

Subjects were asked to not eat for at least 1 h before testing. The following instructions were read by them, and then read to them:

> 'First rinse with a comfortable amount of water and then at the instruction 'start' from the experimenter, take cup 1-A from the first pair of cups, sip the solution and taste it. Hold the solution in your mouth until the instruction 'out' is given. Then spit the sample into the spittoon cup and rinse with a comfortable amount of water. At the instruction 'next', take cup 1-B from the first pair and sip, taste, spit and rinse as before. Then indicate which of the 2 solutions taste sweeter by circling A or B on the line labeled 1 on the data sheet. Then, at the instruction 'next', take cup 2-A from the second pair of cups, and sip, taste, spit and rinse as before. We will continue this procedure until you have tasted and picked the sweeter solution of all the pairs on the table.'

The temporal sequence of the experiment was controlled by the experimenter seated behind the subjects, according to a digital stop watch. The sequence was as follows: 30 s for each sample in a pair, including 10 s tasting, then spitting and rinsing, with an interpair interval for recording the data of 15 s (total of 1 min, 15 s for each cycle). This temporal sequence was selected as one which would provide enough time between tastings to avoid sensory adaptation but would not be so long between solutions as to allow the subjects to

forget the sweetness of the previous solution within the pair (Eylam *et al.*, 1995).

Response functions

Recognition indices (*RI*), indicating the proportion of the population that correctly recognized the sugar solution in each pair as sweet, were calculated. Subjects' responses were collected as a binary series of numbers: correct recognition = 1, and incorrect (indicating the water as the sweeter solution) = zero. If the subject guessed in the forced-choice procedure, he/she had a 50% chance of being correct. Each subject was tested three times with the same sugars and concentration range, but in a different random order. Responses from all subjects were summed and divided by the N to calculate *RI* values for the population data. An incorrect response in three replications of a given concentration of a particular sugar for all the subjects would give a population *RI* of 0. As the concentration range increases, there is a greater likelihood of a correct answer, until the proportion of the population that can correctly recognize the sugar reaches a maximum where it levels off ($RI = 1.0$) (Eylam *et al.*, 1995).

Variability analysis

Positive *ID* values (the lowest concentration for which the sugar was correctly distinguished from water 100% of the time) were calculated for each subject. Positive *ID* cumulative percentiles for the population were plotted and inspected for similarities and differences in the functions for the three sugars. Then positive *ID* concentrations and logs of positive *ID* concentrations (normal and lognormal distributions) were plotted vs. computed Z-scores for the percentiles (0 and 100 were not used). By this analysis the intercept gives the mean, and the slope gives the standard deviation. The fits of these plots were inspected to determine whether they indicated separate signals for fructose and glucose. For interpretation, a true lognormal distribution would be expected to result from a situation in which many factors contribute to variation in the measured parameter, and the factors all act multiplicatively. A linear normal distribution would be expected when the factors act additively. A bimodal distribution composed of two separate lognormal signals is thought to appear normal (Hattis & Silver, 1994; Eylam *et al.*, 1995).

RESULTS AND CONCLUSIONS

Responses of *D. adiastola* natural population

Behavioral and receptor cell responses for the population to the various sugar concentrations are summarized in Table 1. The behavioral thresholds (highest concentrations for which the $PI = 0.5$) were 5 mM for sucrose and fructose. For glucose, however, the threshold was higher, at 30 mM. The range from the threshold concentration to the lowest concentration at which the *PI* values began to level off (maximal response) was similar for sucrose and fructose, but narrower for glucose.

Likewise, the first 100 ms receptor cell action potential responses showed concentration-dependent firing over similar concentration ranges for sucrose and fructose. However, the concentration-dependent firing elicited by glucose increased more rapidly to higher maximal rates at the higher concentrations.

Fits of the behavioral *PI* values and receptor cell firing rates (numbers of action potentials in first 100 ms responses, normalized to *PI* scale) to the equation for a rectangular hyperbola were significant ($p \leqslant 0.001$, ANOVA). Again, the parameters (concentrations for the half-maximal and maximal response values) from

Table 1. *Drosophila adiastola* population behavioral and receptor cell responses to sugars[a]

Sugar	Threshold Concentration[b] (mM)	Response Parameters[c]	
Measure		Concentration for Half-maximal Response (±S.E.) (mM)	Maximal Response[d] (±S.E.)
Sucrose			
Behavior	5	2.5 (0.5)	0.75 (0.02)
Neurophysiology		3.7 (1.3)	0.93 (0.06)
Fructose			
Behavior	5	3.7 (0.8)	0.80 (0.02)
Neurophysiology		2.3 (0.5)	0.82 (0.02)
Glucose			
Behavior	30	18.8 (5.6)	0.93 (0.06)
Neurophysiology		16.5 (7.0)	0.95 (0.09)

[a]Summarized from Her and Kennedy (1991) and Poskanzer *et al.* (1992).
[b]Lowest concentration for which $PI = 0.5$ (see Methods). These concentrations elicited 1–3 action potentials in first 100 ms responses (mean 1.8, S.D. ±0.4).
[c]For fits of the data to the equation for a rectangular hyperbola ($p \leqslant 0.0001$, ANOVA).
[d]Behavior: *PI*. Neurophysiology: mean number of action potentials in first 100 msec responses, normalized to *PI* scale.

these fits were similar for sucrose and fructose, but higher for glucose (Table 1). These data suggest that there are different mechanisms for fructose and glucose in this species.

Table 1 also shows that for each sugar, the parameters for the behavioral and receptor cell responses correspond. To confirm this correspondence, the rising phases ($PI = 0.5-0.8$) of the response curves were fitted by linear regression. The fits and slopes were significant ($p \leqslant 0.02$, ANOVA), with good correlations ($r^2 = 0.79-0.96$). Moreover, the slopes for fructose and glucose were significantly different by Analysis of Covariance ($p < 0.001$). These data suggest that the different fructose and glucose mechanisms are located in or before the receptor cells (Her & Kennedy, 1991; Poskanzer *et al.*, 1992; Kennedy & Poskanzer, 1994).

Control tests with 50 mM sucrose (red) vs plain agar (blue) in 9 flies and 50 mM sucrose (blue) vs plain agar (red) in 10 flies yielded similar *PI* values (0.722 and 0.700, respectively). Thus the color of the solution did not affect the flies' choice (Her & Kennedy, 1991).

D. adiastola fructose and glucose taste variants

Of 1224 adult flies from the natural population, all were screened for the FN trait, and 905 were screened for the GN trait. These tests selected FN and GN at frequencies (±S.E.) of 5.7 (±0.7) and 3.6 (±0.6), respectively. The FN are the first variants for fructose taste isolated in any species.

There were no differences in frequencies between males and females for either nontaster type ($p > 0.05$, χ^2 Test) (Table 2). Since all the flies tested had the normal phenotypic appearance, they could be expected to be normal diploid specimens with no multiple chromosomes. This statistical result then suggests that the gene for the variant trait is not on the *X*-chromosome. Rather, it must be located on an autosomal chromosome (Poskanzer *et al.*, 1992; Poskanzer & Kennedy, 1993; Kennedy & Poskanzer, 1994).

D. adiastola WT and FN chromosomes

A total of 112 larvae were screened to yield 6.25% FN and 3.57% GN, frequencies which are comparable to those obtained in the adults. Microscopic inspections of 11 WT chromosomal preparations showed similar banding patterns to those in chromosomal maps of Carson and Stalker (1968) for *D. adiastola*.

Comparison of the clearest WT preparation with maps for *D. melanogaster* (Sorsa, 1988) showed both similarities and differences. However, comparison of the WT preparation with 6 different FN chromosomal preparations, with particular attention to the *X*-chromosomes, revealed no apparent differences in banding patterns. Since the patterns were not clearly resolved in the entire genome of any one FN preparation, firm conclusions require additional data. However, these preliminary results from larval chromosomes support the suggestion from the adult fly behavioral data that the FN trait is not located on the *X*-chromosome. That is, they support an autosomal location for the FN trait (Saikku, 1994).

D. melanogaster cross-breeding experiments

Fructose nontasters (3 females) also were isolated from a group of 40 laboratory population *D. melanogaster* with a frequency (7.5%) that was not markedly different from that for the natural population of *D. adiastola* (5.7%). The cross of a WT male with an FN female yielded 87 first filial (F_1) progeny, including 20 FN, which represents a much higher frequency (23%) from that in the parental (*P*) *D. melanogaster* population. There was no clear difference in numbers of male and female FNs (11 and 9, respectively) within the F_1 progeny, but the proportion of male FNs among the progeny (9 out of 87) was, again, greater than those in the *P* population (0 out of 40). There appeared to be no lethal alleles.

Cross of an F_1 WT female with an F_1 WT male yielded 16 second filial (F_{2a}) progeny with no FN. Cross of an F_1 FN female with an F_1 FN male yielded 10 F_{2b} progeny, including 3 FN at a greater frequency (30%) than that in the *P* population (7.5%). None of the F_{2b} FN were female. There were no apparent lethal alleles in either F_2 progeny. While the numbers of flies in these crosses are too low for reasonable χ^2 analysis, these preliminary data agree with the suggestion from the *D. adiastola* behavioral and chromosomal data that the trait does not involve a single gene on the *X*-chromosome. Rather, the FN trait appears to be polygenic, and

Table 2. Frequencies of nontasters isolated from a *Drosophila adiastola* natural population[a]

	All flies	females	males	Female/male differences[b]
Fructose Nontasters				
N	1,224	619	605	
Frequency (% [±S.E.])	5.7 [0.7]	5.6 [0.9]	5.8 [0.9]	$0.90 < p < 0.95$
Glucose Nontasters				
N	905	467	438	
Frequency (% [±S.E.])	3.6 [0.6]	4.3 [0.9]	3.0 [0.8]	$0.25 < p < 0.50$

[a]Summarized from Poskanzer *et al.* (1992), Poskanzer and Kennedy (1993), and Kennedy and Poskanzer (1994).
[b]χ^2 TEST.

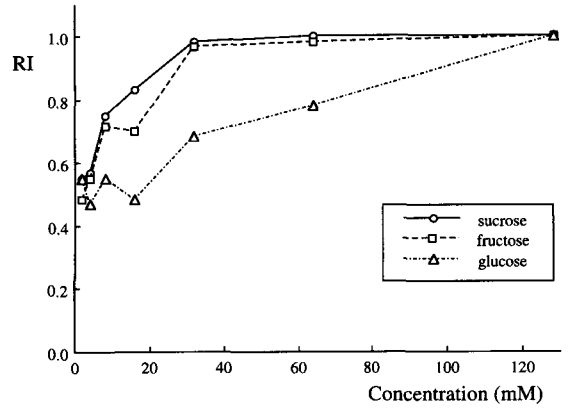

Fig. 1. Human natural population proportions of correct recognition.

either completely autosomal or partially coded by a defective *X*-chromosomal gene for which one or more normal autosomal genes can compensate.

Psychophysical responses of the natural human population

Population recognition index (*RI*) values, which indicate the proportion of the population that correctly recognized the sugar in each pair as sweet (Methods), are in Fig. 1. The response curves fluctuate at the lower sugar concentrations and then increase steadily with increasing concentrations in the middle of the range. While the curves for sucrose and fructose reach a maximum at 32 mM, the curve for glucose continues to increase throughout. The threshold concentrations ($RI = 0.5$) are similar for sucrose and fructose (0 and 2 mM, respectively), while that for glucose is higher ($[6 + 15 + 18]/3 = 13$ mM). Likewise, concentrations for correct recognition by 65% of the population ($RI = 0.65$) are identical for sucrose and fructose (6 mM), but higher for glucose (30 mM). These results support, but do not prove, separate physiological mechanisms for fructose and glucose taste in humans (Eylam *et al.*, 1995).

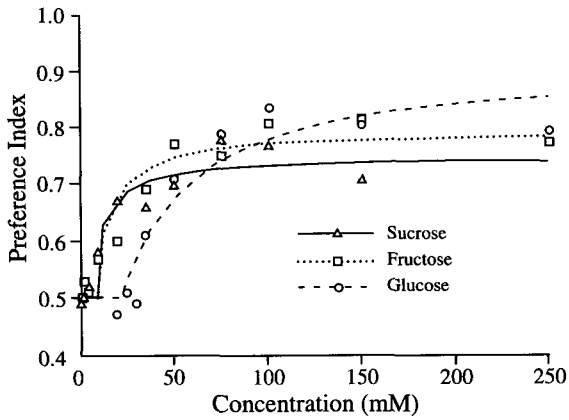

Fig. 2. *D. adiastola* adult natural population behavioral preference index functions (fitted to the equation for a rectangular hyperbola).

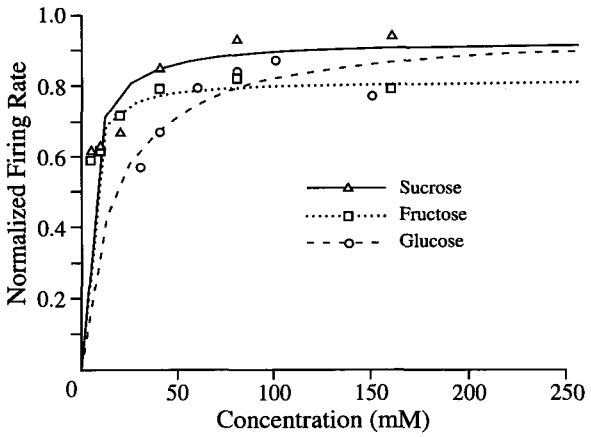

Fig. 3. *D. adiastola* adult natural population normalized receptor cell firing rates (fitted to the equation for a rectangular hyperbola).

These response curves agree with those obtained from humans by Pangborn (1963) using constant-stimulus paired-comparisons, and by Portmann *et al.* (1992) using intensity/time judgements in the similarities of sucrose and fructose functions and difference of glucose functions and in the general forms of the functions (converging for sucrose and fructose, expanding for glucose). They also agree with data of Hagstrom and Pfaffmann (1959) for rat chorda tympani responses to sucrose and glucose.

The three curves from our present study converge at the highest concentration (128 mM), 100% correct recognition ($RI = 1.0$). This result is consistent with the monogeusia reported by Breslin *et al.* (1994) for concentrations higher than those tested here.

In addition, these human data are similar to data for behavioral and receptor cell responses of *D. adiastola* in the forms of the functions (Figs 2 and 3) and also in the sucrose, fructose, and glucose concentrations for correct recognition by 65% of the human population (6, 6 and 30 mM, respectively) and 60% of the *D. adiastola* population (10, 10 and 35 mM, respectively) (data of

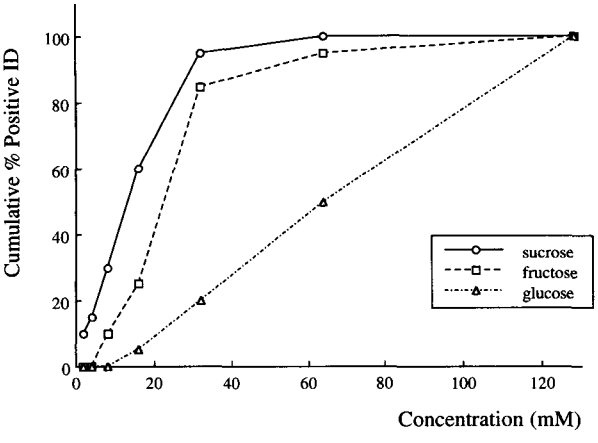

Fig. 4. Human natural population cumulative percentile positive *ID* functions.

Her & Kennedy, 1991; Poskanzer *et al.*, 1992). These similarities support the suggestion that basic mechanisms in *Drosophila* may be similar to those in humans and that *Drosophila* species may be a good model for the human mechanisms (Eylam *et al.*, 1995).

Variability analysis

Figure 4 gives the population cumulative percentiles of positive *ID* values for each subject (the lowest concentration for which the sugar was correctly distinguished from the water 100% of the time by that subject) (see Methods). This graph again supports the hypothesis of separate mechanisms for the monosaccharides, since the sucrose and fructose functions are similar, while the glucose function is different.

Figure 5 shows variability distributions calculated from the cumulative percentiles of positive *ID* values, and Table 3 shows the regression parameters for the distributions. The data for fructose and glucose are better fitted by a lognormal distribution, while that for sucrose is better fitted by a normal distribution. This difference in fits suggests that the monosaccharides, glucose and fructose elicit separate signals, while the disaccharide sucrose elicits an additive response, perhaps of the separate glucose and fructose signals (see Methods and Hattis & Silver, 1994; Eylam *et al.*, 1995).

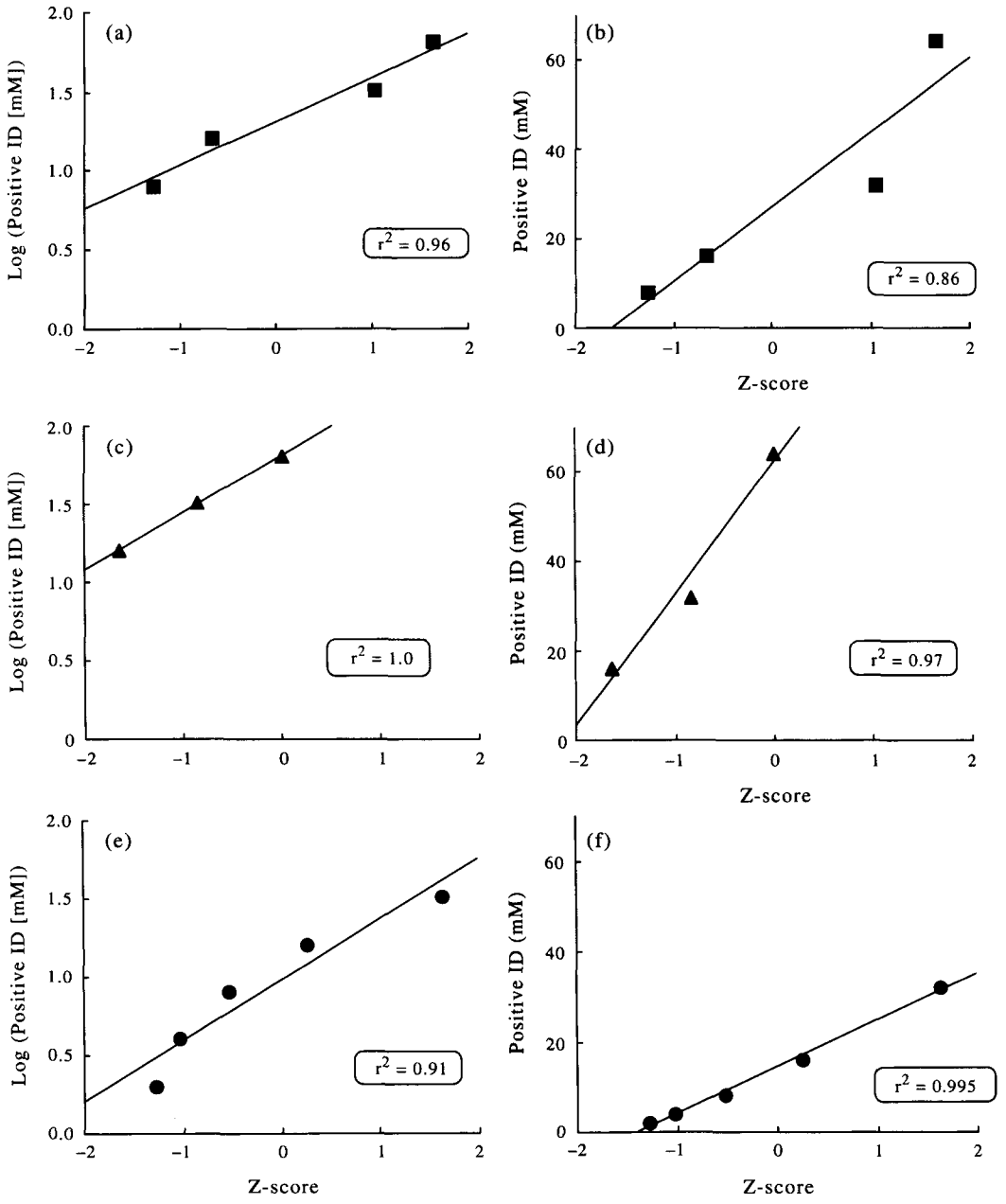

Fig. 5. Distributions of human natural population positive *ID* values: (a) fructose lognormal distribution, (b) fructose normal distribution, (c) glucose lognormal distribution, (d) glucose normal distribution, (e) sucrose lognormal distribution, (f) sucrose normal distribution.

Table 3. Regression parameters

Sugar	Variability distribution					
	Normal			Lognormal		
	Mean (intercept) (mM)	S.D. (slope) (mM)	r^2	Geometric mean (intercept) (mM)	Geometric S.D. (slope) (mM)	r^2
Sucrose	14.3	10.3	0.995	10	2.4	0.91
Fructose	27	16.6	0.86	20	1.9	0.96
Glucose	61.6	29.3	0.97	63	2.3	1

Potential glucose nontaster

Figure 6 shows the *RI* values from a subject who may be a GN. Her function for fructose is similar to that for the population, but her glucose function shows a poorer sensitivity to this sugar. The concentration for her $RI = 0.65$ ($\sim$80 mM) is considerably higher than that for the population ($\sim$13 mM). Like the population, she can recognize 128 mM glucose as sweet in comparison with water 100% of the time.

DISCUSSION

In *D. adiastola* adults from the natural population, parameters for behavioral and receptor cell responses to sucrose and fructose were similar, while those for glucose were different. These results suggest that physiological taste mechanisms for the two monosaccharides are different. That parameters for the behavioral and receptor cell responses corresponded, indicates that the different physiological mechanisms are located in or before the taste receptor cells.

The existence of different mechanisms is further supported by the isolation of variants for fructose (FN) and for glucose (GN) from the adult natural *D. adiastola* population. There were no significant differences in frequencies between male and female FN or GN frequencies. This result suggests that the variant traits are not sex-linked, i.e. that they are located on an autosomal chromosome. The FN are the first variants for fructose taste isolated in any species.

D. adiastola larval FN and GN were also isolated from a natural population at frequencies comparable to those in the adults. The existence of these larval variants supports the suggestion that the FN and GN traits are inherent, rather than acquired, characteristics of the adult populations. Inspection of larval chromosomes suggested that banding patterns in the wildtype (WT) larvae were similar to those in published maps for this species, yet different from those in maps for *D. melanogaster*, and demonstrated that differences could be discriminated in our preparations. However, comparison of *D. adiastola* FN and WT preparations, with focus on the *X*-chromosomes, revealed no apparent differences. Although some additional preparation might yet resolve a difference, these preliminary results agree with the adult behavioral data to support an autosomal location for the FN variant trait.

Both FN and GN were isolated from a laboratory population of *D. melanogaster* adults at frequencies that were not markedly different from those for the adult *D. adiastola* natural population. These results indicate that it should be possible to exploit some of the major advantages of *D. melanogaster*—ease of culture for cross-breeding experiments, the extensive genetic linkage maps and corresponding stocks of known mutants, and the increasing base of information about the molecular genetics and development of molecular techniques for this species—in future genetic work on sweet taste transduction.

The results from three crosses with *D. melanogaster* FN variants yielded no apparent lethal alleles. The FN frequencies for the progeny suggest a polygenic trait that is either completely autosomal or includes coding by a defective *X*-chromosome gene for which one or more normal autosomal genes can compensate. The numbers of flies mated and progeny obtained thus far are low, however; therefore, these cross-breeding data must be considered preliminary.

Human psychophysical functions from a forced-choice procedure similar to that used for the *Drosophila* also were similar for sucrose and fructose but different for glucose, and variability analysis indicated separate signals for fructose and glucose. Moreover, the concentrations of the three sugars for 65% *RI* (correct recognition by humans) and 60% *PI* (feeding preference

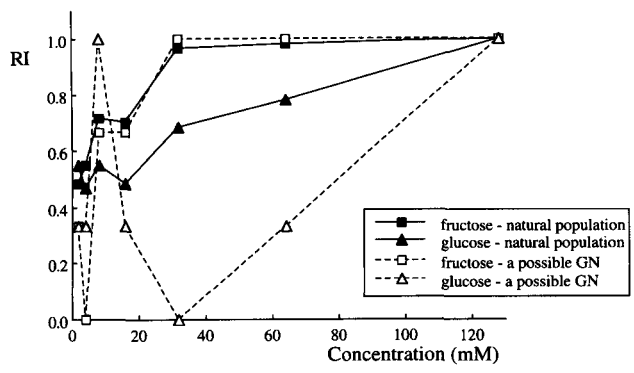

Fig. 6. Comparison of *RI* functions for a possible human glucose nontaster with *RI* functions for the natural population.

by *Drosophila*) were virtually the same. These similarities support the suggestion that basic molecular sweet taste transduction mechanisms may be similar in *Drosophila* and humans. Thus the advantages of a genetic analysis in *Drosophila* could be most useful in the attempt to understand human sweet taste mechanisms.

The evidence for separate fructose and glucose mechanisms in humans suggests that variants for one or the other mechanism could be identified in humans. The functions obtained from a potential human GN support this suggestion. The identification of such variants from a natural population and taste-testing of members of the variants' families to construct pedigrees for the variant traits could be most useful.

The studies reported here have probed the initial steps in a variant-to-gene-to-protein approach to sweet taste transduction. Overall, the results suggest that future classical and molecular genetics studies with both *Drosophila* and humans within the context of this approach should be most useful.

ACKNOWLEDGEMENTS

This research was supported by NSF BNS-9118858, NIH DC01563, NIH DC02663 and a Clark University Faculty Development Award to LMK. We thank K. Kaneshiro for flies, and F. Hanson, D. Hattis, T. Livdahl, T. Lyerla, and D. Stevens for discussion. Portions of this research were presented at meetings of the Association for Chemoreception Sciences (1992, 1993, 1994), European Symposium on Insect Taste and Olfaction (1993, 1995), International Chemoreception Workshop on Insects (1993, 1995), and International Symposium on Olfaction and Taste XI (1994).

REFERENCES

Avenet, P., Hofmann, F. & Lindemann, B. (1988). Transduction in taste receptor cells requires cAMP-dependent protein kinase. *Nature*, **333**, 351–354.

Barnard, E. A. (1989). Molecular neurobiology—an introduction. In *Molecular Neurobiology*, ed. D. M. Glover & B. D. Hames Oxford University Press, Oxford, pp. 1–7.

Bhavsar, P. N., Rodrigues, V. & Siddiqi, O. (1983). *J. Biosci.*, **5**, 279–285.

Breslin, P. A. S., Kemp, S. & Beauchamp, G. K. (1994). Single sweetness signal. *Nature*, **369**, 447–448.

Carson, H. L. & Stalker, H. D. (1968). Polytene chromosome relationships in Hawaiian species of *Drosophila*. III The *D. adiastola* and *D. punalua* subgroups. *Studies in Genet.*, **4**, 367–380.

Cummings, T. A., Powell, J. & Kinnamon, S. C. (1993). Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides. *J. Neurophysiol.*, **70**, 2326–2336.

Daley, D. L. & Vande Berg, J. S. (1976). Apparent opposing effects of cyclic AMP and dibutyryl-cyclic GMP on the neuronal firing of the blowfly chemo-receptor. *Biochem. Biophys. Acta*, **437**, 211–220.

Eylam, S., Kennedy, L. M. & Stevens, D. A. (1995). Potential human variants for glucose taste suggest separate fructose and glucose mechanisms. *Chem. Senses*, **20**, 690–691 (abstract).

Faurion, A. (1987). Physiology of the sweet taste. *Prog. Sensory Physiol.*, **8**, 130–201.

Foster, K. D. & Kennedy, L. M. (1994*a*). IBMX and hodulcin-induced suppression of receptor cell responses to sucrose in *Phormia regina*. *Chem. Senses*, **19**, 470 (abstract).

Foster, K. D. & Kennedy, L. M. (1994*b*). Sweet taste transduction mechanisms: additive effects of hodulcin and IBMX. *Chem. Senses*, **20**, 176 (abstract).

Fujishiro, N., Kijima, H. & Morita, H. (1984). Impulse frequency and action potential amplitude in labellar chemosensory neurones of *Drosophila melanogaster*. *J. Insect Physiol.*, **30**, 317–325.

Gilman, A. G. (1987). G proteins: transducers of receptor-generated signals. *Ann. Rev. Biochem.*, **56**, 615–649.

Hagstrom, E. C. & Pfaffmann, C. (1959). The relative taste effectiveness of different sugars for the rat. *J. Comp. Physiol. Psychol.*, **52**, 259–262.

Hartl, D. L. (1992). Genome map of *Drosophila melanogaster* based on yeast artificial chromosomes. In *Genome Analysis, Vol. 4: Strategies for Physical Mapping*, ed. K. E. Davies & S. M. Tilghman, Cold Spring Harbor Laboratory Press, U.S., pp. 39–69.

Hattis, D. & Silver, K. (1994). Human interindividual variability—a major source of uncertainty in assessing risks for noncancer health effects. *Risk Analysis*, **14**, 421–431.

Her, C. & Kennedy, L. M. (1991). Behavioral and neurophysiological responses to taste stimuli in Hawaiian fruitflies. *Chem. Senses*, **16**, 533(abstract).

Kennedy, L. M. & Halpern, B. P. (1980). Extraction, purification and characterization of a sweetness-modifying component from *Ziziphus jujuba*. *Chem. Senses*, **5**, 123–147.

Kennedy, L. M. (1991). Suppression of responses to sweet stimuli by the gymnemic acids, ziziphins and hodulcin. *Chem. Senses*, **16**, 388.

Kennedy, L. M., Bourassa, D. M. & Rogers, M. E. (1993). The cellular and molecular neurobiology of sweet taste: Studies with taste-altering compounds. In *Sweet Taste Reception*, eds. M. Mathlouthi, J. A. Kanters & G. G. Birch, Elsevier Applied Science, London, pp. 317–351.

Kennedy, L. M. & Poskanzer, J. E. (1994). Isolation of variants for fructose or glucose taste from a natural population of Hawaiian *Drosophila adiastola*. In *Olfaction and Taste XI*, eds. K. Kurihara, N. Suzuki & H. Ogawa, Springer-Verlag, Tokyo, p. 237 (abstract).

Kolodny, D. E. & Kennedy, L. M. (1988). A model system for receptor cell studies with the taste modifier, hodulcin. *Chem. Senses*, **13**, 545–557.

McLaughlin, S. K., McKinnon, P. J. & Margolskee, R. F. (1992). Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature*, **357**, 563–569.

McLaughlin, S. K., McKinnon, P. J., Robichon, A., Spickofsky, N. & Margolskee, R. F. (1993). Gustducin and transducin: a tale of two G proteins. In *The Molecular Basis of Smell and Taste Transduction* (Ciba Foundation Symposium 179), Wiley, Chichester, pp. 186–200.

Merriam, J., Ashburner, M., Hartl, D. L. & Kafatos, F. C. (1991). Toward cloning and mapping the genome of *Drosophila*. *Science*, **254**, 221–225.

Neer, E. J., Chow, Y. K., Garen-Fazio, S., Michel, T., Schmidt, C. J. & Silbert, S. (1990). In *Biology of Cellular Transducing Signals*, ed. J. Y. Vanderhoek, Plenum Press, New York, pp. 83–92.

Pangborn, R. M. (1963). Relative taste intensities of selected sugars and organic acids. *J. Food Sci.*, **28**, 726–733.

Portmann, M. O., Serghat, S. & Mathlouthi, M. (1992). Study of some factors affecting intensity/time characteristics of sweetness. *Food Chem.*, **44**, 83–92.

Poskanzer, J. E., Rudnick, L., Her, C. & Kennedy, L. M. (1992). Isolation of Hawaiian *Drosophila* variants which prefer glucose to fructose at equipotent suprathreshold concentrations. *Chem. Senses*, **17**, 684 (abstract).

Poskanzer, J. E. & Kennedy, L. M. (1993). Fructose and glucose non-tasters in the Hawaiian, *Drosophila adiastola*. *Chem. Senses*, **18**, 615 (abstract).

Rodrigues, V. & Siddiqi, O. (1981). A gustatory mutant of *Drosophila* defective in pyranose receptors. *Mol. Gen. Genet.*, **181**, 406–408.

Saikku, A.-R. (1994). Studies for cytological localization of a gene known only by it's variant phenotype: a step in the variant-to-gene-to-protein analysis of sweet taste. Biology Honors Thesis, Clark University, Worcester, MA.

Schiffman, S. S. & Gatlin, C. A. (1993). Sweeteners: state of knowledge review. *Neurosci. Biobehav. Rev.*, **17**, 313–345.

Shallenberger, R. S. & Acre, T. E. (1967). Molecular theory of sweet taste. *Nature*, **216**, 480–482.

Shimada, I., Shiraishi, A., Kijima, H. & Morita, H. (1974). Separation of two receptor sites in a single labellar sugar receptor of the flesh-fly by treatment with *p*-chloromercuribenzoate. *J. Insect Physiol.*, **20**, 605–621.

Sorsa, V. (1988). *Chromosome maps of Drosophila*, Vol. I and II, CRC Press, Boca Raton.

Striem, B. J., Pace, U., Zehavi, U., Naim, M. & Lancet, D. (1989). Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes. *Biochem. J.*, **260**, 121–126.

Tanimura, T., Isono, K., Takamura, T. & Shimada, I. (1982). Genetic dimorphism in the taste sensitivity to trehalose in *Drosophila melanogaster*. *J. Comp. Physiol.*, **147**, 433–437.

Tanimura, T., Isono, K. & Yamamoto, M.-T. (1988). Taste sensitivity to trehalose and its alteration by gene dosage in *Drosophila melanogaster*. *Genetics*, **119**, 399–406.

Tanimura, T. (1991). Genetic alteration of the multiple taste receptor sites for sugars in *Drosophila*. In *Chemical Senses III: Genetics of Perception and Communication*, eds. C. J. Wysocki & M. R. Kare, Marcel Dekker, Inc., New York, pp. 125–135.

Tonosaki, K. & Funakoshi, M. (1988). Cyclic nucleotides may mediate taste transduction. *Nature*, **331**, 354–356.