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Drosophila melanogaster Prefers Compounds Perceived Sweet by Humans

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Abstract

To understand the functional similarities of fly and mammalian taste receptors, we used a top–down approach that first established the fly sweetener–response profile. We employed the fruit fly *Drosophila melanogaster*, an omnivorous human commensal, and determined its sensitivity to an extended set of stimuli that humans find sweet. Flies were tested with all sweeteners in 2 assays that measured their taste reactivity (proboscis extension assay) and their ingestive preferences (free roaming ingestion choice test). A total of 21 sweeteners, comprised of 11 high-potency sweeteners, 2 amino acids, 5 sugars, 2 sugar alcohols, and a sweet salt ($PbCl_2$), were tested in both assays. We found that wild-type *Drosophila* responded appetitively to most high-potency sweeteners preferred by humans, even those not considered sweet by rodents or new world monkeys. The similarities in taste preferences for sweeteners suggest that frugivorous/omnivorous apes and flies have evolved promiscuous carbohydrate taste detectors with similar affinities for myriad high-potency sweeteners. Whether these perceptual parallels are the result of convergent evolution of saccharide receptor–binding mechanisms remains to be determined.

Key words: comparative taste, convergent evolution, detection, high-potency sweeteners, ingestion, taste

Introduction

The ability to discriminate chemicals by taste is critical to the survival of any animal that selects its diet. In addition to nutritive sweet compounds, including carbohydrate sugars and their natural analogs, most mammals are also sensitive to a variety of high-potency sweeteners that stimulate sweetness at concentrations far below those of saccharides (Schiffman and Gatlin 1993). Although the reason for sensitivity to these nonnutritive compounds remains largely unknown, variation in mammalian responses to sweeteners are well documented and has been used to generate molecular structural explanations to sweet taste perception (Reed et al. 2004; Jiang et al. 2005; Nie et al. 2005; Li et al. 2006). Rats and mice respond appetitively to acesulfame-K, dulcin, Na saccharin, SC45647, sucralose, and sorbitol but do not detect aspartame, alitame, Na cyclamate, glycyrrhizin, neohesperidin dihydrochalcone (NHDC), or thaumatin (Sclafani and Abrams 1986; Danilova et al. 1998; Bachmanov, Tordoff, et al. 2001; Sclafani and Clare 2004). Lemurs also respond appetitively to the sweeteners dulcin, SC45647, and stevioside but do not appear to detect acesulfame-K, alitame, aspartame, Na cyclamate, monellin, NHDC, Na saccharin, sucralose, and thaumatin (Schilling et al. 2004). New world primates, such as marmosets, detect and respond to a wider range of sweeteners than do lemurs, including acesulfame-K, alitame, glycine, and sucralose (Danilova et al. 2002; Danilova and Hellekant 2004). Although they have a wider repertoire for sweeteners, new world primates do not respond appetitively to many other compounds including aspartame, Na cyclamate, D-phenylalanine, monellin, NHDC, Na saccharin, and stevioside. The great apes (including gorillas, chimpanzees, and humans) respond appetitively to all the sweeteners mentioned here (Glaser et al. 1995; Nofre et al. 1996; Hellekant, Danilova, et al. 1997; Hellekant, Ninomiya, et al. 1997).

Although their general acceptance and rejection of sapid chemicals suggest that invertebrates such as *Drosophila melanogaster* live in a gustatory world that is similar to that of humans and many other omnivorous mammals (Kennedy et al. 1997; Meunier et al. 2003; Amrein and Thorne 2005; Keller 2007), much less is known of invertebrate sweetener repertoires. The ant *Lasius niger* failed to respond appetitively to any of 16 noncarbohydrate high-potency sweeteners, despite having strong preferences for sugars (Tinti and Nofre 2001). Besides testing with sugars, however, flies have only been tested with a few high-potency sweeteners. The blowfly (*Protophormia terraenovae*) did not respond appetitively to10–100 mM Na saccharin in behavioral tests (Liscia et al. 2004). Electrophysiologically, 10 and 20 mM Na saccharin elicited spikes from water-responsive cells within labellar chemosensilla and 50–100 mM Na saccharin elicited spikes from both water and ''deterrent'' cells. Sugar cells did

not respond to Na saccharin at any of the listed concentrations. At these high concentrations, however, both the strong bitterness and the sweetener-inhibiting properties of Na saccharin are known to suppress sweet taste in most humans (Horne et al. 2002; Galindo-Cuspinera et al. 2006). Glycyrrhizin, glycerol, and Na cyclamate are the only other nonsaccharide sweeteners that have been tested in flies. Glycyrrhizin elicits a sugar-like response from the blowfly *Phormia regina* (Ahamed et al. 2001), glycerol stimulates sugar receptor cells in *Drosophila* (Koseki et al. 2004), and Na cyclamate stimulates sugar receptor cells in the blowfly *Protophormia terraenovae* (Liscia et al. 2005). The abilities of *Drosophila* to detect many sugars and bitter compounds have been shown to be qualitatively and quantitatively similar to humans' abilities (Meunier et al. 2003; Amrein and Thorne 2005); however, relatively little is known of their ability to detect nonnutritive sweeteners. Recently, a family of 7 gustatory receptors, including the trehalose receptor *Gr5a*, has been identified in *Drosophila* as responding to a wide variety of sugars and being coexpressed (Jiao et al. 2007; Slone et al. 2007).

Characterizing the receptive field of *Drosophila* to these myriad sweeteners is a first step in a top–down approach to understanding the molecular basis of fruit fly and, by comparison, human sweet taste perception. In the present study, we use 2 behavioral assays to investigate the ability of *Drosophila* to perceive 21 structurally diverse sweeteners.

Materials and methods

Flies

Canton-S (CS stock no. 1) strains of *D. melanogaster* were obtained from the Bloomington Drosophila Stock Center (Bloomington, IN). Larvae were raised on standard cornmeal/agar medium supplemented with dry yeast at 25 °C with a 12-h light/dark cycle. Flies were used for ingestion tests 1–5 days following eclosion and for measuring proboscis extension reflex (PER) 1–2 days following eclosion as older flies are more easily damaged during the handling process and produce lower responses. Prior to testing, flies were starved in vials for approximately 21 h on filter paper saturated in distilled water to prevent dehydration. All experiments were performed at 25 °C and at a relative humidity of approximately 40%.

Chemical compounds used for stimulation of flies

Twenty-one sweeteners, sweet proteins, sugars, sugar alcohols, sweet amino acids, and sweet salts (Table 1) were obtained commercially except dulcin and SC45647, which were a gift of Grant Dubois, (Coca-Cola Company, Atlanta, GA), and R43N brazzein, a gift of Goran Hellekant (University of Minnesota-Duluth). SC45647 is a zwitterionic, biphenyl, guanadinium derivative, high-potency sweetener. Solutions of each compound were made in deionized water and stored at room temperature (PERs) or made fresh for preference plates. Initial stimulus concentrations of each compound for both behavioral assays were chosen on the basis of human sensitivities to those stimuli (Table 1). Where human sensitivities were unavailable, model organism sensitivities were used. When the initial concentration failed to elicit a positive response from the flies, additional concentrations up to 10-fold higher and lower were tested (Figures 2 and 3). As per one objective of this study, to determine if flies perceived these compounds as appetitive, testing ceased for most compounds when a clearly positive response was elicited or after testing 4–5 additional concentrations without eliciting a positive response.

Food preference ingestion test

Ingestion of stimuli by animals is based upon taste stimulation at multiple possible gustatory receptor sites including all 6 tarsi, labella, and even wing edges. Preferential ingestion of chemical compounds was tested against paired control solutions using a 2-dye marker method in 60-well microplates (10 µl each well; Nunc, Fisher Scientific [Newark, DE]; Tanimura et al. 1982). Briefly, test chemicals were presented simultaneously in paired microplates in solutions containing 0.8% agar (Fisher Scientific) and either red or blue food dye (Figure 1) (Calico Food Ingredients, Kingston, ON). Paired control plates were prepared similarly, using agar containing the alternate food dye. For most compounds, the control agar solutions were made using water. For Na saccharin, paired control plates contained an equimolar concentration of NaCl to control for the modest attraction to low concentrations of sodium (Hiroi et al. 2004). Thaumatin was paired with a control solution of agar containing the partially unfolded form of the protein, denatured by boiling the test solution. This altered form of the protein was not aversive (Breslin PAS, Gordesky-Gold B, personal observation). The food dyes used were 0.187 mg/ml F,D&C Red no. 3, and 0.031 mg/ml Blue no. 1 (Calico Food Ingredients), and experiments for each test compound were replicated using each dye with the stimulus to control for dye–taste interactions. In each experiment, flies (50 males or 50 females, tested in single sex groups) were anesthetized using CO_2 and introduced simultaneously to 2 inverted plates containing solutions of the test compound in one color dye and 2 plates containing the test compound in the other color dye. After being allowed to feed at 25 °C in the dark for 75 ± 15 min, flies were anesthetized with CO_2 and their translucent abdomens scored for color of ingested foods as red, blue, or mixed. Only tests in which a minimum of 20% of animals fed were included. These scores were used to generate a preference index, according to the formula

$$PI_T = (N_T + N_M/2)/(N_T + N_C + N_M) \times 100,$$

where N_T = the number of flies scored for test compound food dye color, N_C = the number of flies scored for control food dye color, N_M = the number of flies scored for a mixed food dye color.

Table 1 Test compounds

Compounds	Fly test concentration range (mM)	Human suprathreshold sensitivity range (mM) (noted when alternate organism range used)	References
High-potency sweeteners			
Aspartame (Fluka, St Louis, MO)	1–2	0.2–10	Schiffman and Gatlin (1993)
Dulcin	0.1–5	0.18–3.1	Faurion et al. (1980)
Glycyrrhizin ammonium (Sigma)	0.1–5	0.2–4	Schiffman and Gatlin (1993)
NHDC (Sigma)	0.01–2	0.06–1.4	Schiffman and Gatlin (1993)
Na saccharin (Sigma)	1–4	0.1–6	Schiffman and Gatlin (1993)
SC45647	0.01–2	0.0003–0.9[a]	Bachmanov, Li, et al. (2001)
Stevioside (Morita Kagaku Kogyo Co., Osaka, Japan)	0.1–10	0.09–1.9	Schiffman and Gatlin (1993)
Sucralose (McNeil, McIntosh, AL)	0.01–5	0.06–3	Schiffman and Gatlin (1993)
Sweet proteins			
Brazzein	0.005–0.05	0.015	Jin, Danilova, Assadi-Porter, Markley, et al. (2003)
Thaumatin (Bioresources, Merseyside, UK)	0.005	0.0001–0.003	Schiffman and Gatlin (1993)
Arg43Asn (R43N) Brazzein	0.005–0.05	Not sweet	Jin, Danilova, Assadi-Porter, Markley, et al. (2003)
Sugars			
Fructose (Sigma)	2–100	37–555	Schiffman and Gatlin (1993)
Glucose (Sigma)	2–100	69–1160	Faurion et al. (1980)
Maltose (Sigma)	2	0.08–833[a]	Bachmanov, Li, et al. (2001)
Sucrose (Sigma)	2–100	50–400	Schiffman and Gatlin (1993)
Trehalose (Fisher Scientific)	20, 100	5–150[b]	Tanimura et al. (1982)
Sugar alcohols			
Glycerol (Sigma)	50–500	10–1000[b]	Koseki et al. (2004)
Sorbitol (Sigma)	100–400	400–2000	Schiffman and Gatlin (1993)
Sweet amino acids			
D-Aspartic acid (Fluka)	2	0.74–40.4	Schiffman et al. (1981), Budauari (1989)
Glycine (Sigma)	0.1–4	31–3300	Schiffman et al. (1981), Budauari (1989)
Sweet salts			
$PbCl_2$ (Sigma)	0.01–4	67	Kurihara (1969)

[a]Concentration used in mice.
[b]Concentration used in *Drosophila*.

Preference indices were calculated for individual plates then averaged to produce a total average PI for each solution tested. Note that PI scores at 50% indicate indifference to the stimulus versus the control and scores above 50% indicate positive preference for the stimulus.

Tarsal stimulation proboscis extension response test

Proboscis extension in response to tarsal stimulation is an appetitive reflex reaction that is normally followed by labellar stimulation and ingestion; in our use of the PER assay, both labellar stimulation and ingestion were prevented. For each experiment, flies (6–7 males and 6–7 females) were anesthetized using CO_2 and affixed by the middle of their dorsal thorax to the tip of a wooden swab stick using 2 µl of Tissue Tack Adhesive (Electron Microscopy Sciences, Fort Washington, PA). Immobilized flies were placed horizontally in a humidified chamber and allowed to recover for 3 h at 25 °C. Before initial testing, flies were permitted to drink water to satiation from a pipette tip. Similarly, flies

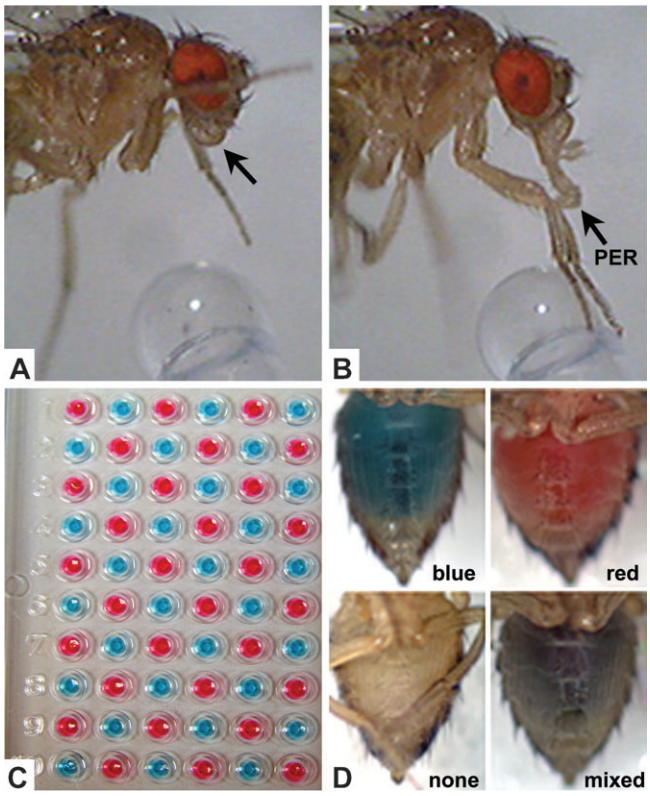

Figure 1 Behavioral tests for taste perception in *Drosophila melanogaster*. Proboscis extension response: foretarsus of fly is stimulated with a 2 µl droplet of test compound applied with a pipette for up to 5 s. Fly is observed for extension of proboscis (mouthparts). Full extension in response to stimulus is counted as a positive response. **(A)** Before tarsal stimulation with 100 mM sucrose, the proboscis is retracted. **(B)** During tarsal stimulation with 100 mM sucrose, the proboscis is extended. Feeding preference test: **(C)** 60-well test plate. Food-deprived flies are presented with a choice of 2 chemicals in an agar medium colored red or blue with commercial food dyes (Calico Food Ingredients). **(D)** Color feeding indicators can be seen through the translucent abdomen of the flies, clockwise from upper left: blue, red, mixed (ingestion of both), clear (no ingestion).

were also offered water to satiation between stimulations. Tarsal PER assays were conducted according to standard procedures (Deak 1976) using a micropipette (Eppendorf, Westbury, NY; Biohit, Neptune, NJ) to stimulate anterior tarsi bilaterally (Figure 1). Flies were retested with water applied to the tarsi at the end of each sweetener test. Trials in which flies responded to water were not included in the analysis as the responses to the sweeteners were considered nonspecific. Flies were typically tested with 1–6 stimuli during a single assay. Testing of one stimulus was completed before beginning the next stimulus with an interstimulus interval of 3–5 min.

Analyses

PER results were analyzed by a 2×2 chi-square analysis with alpha of $P < 0.05$ compared with the response to water (Zar 1984). Ingestion preference results were analyzed by a paired comparison test with an alpha of $P < 0.05$: $X = (z\sqrt{n} + n + 1)/2$, where X = significant proportion, z = transformed alpha level (0.05), n = sample size. Significance of preference indices were determined from $\% = (X/n) \times 100$ (O'Mahony 1986). Preference scores of 50% indicated indifference, 0% indicated total aversion, and 100% indicated absolute preference. In this assay, each fly (in groups of 50) was presented with a sweetener versus a nonsweet control and we asked if the fly could recognize the wells with the sweetener. Thus, the analysis was 1 tailed and based on a binomial distribution. Each fly was considered a separate choice test; flies were not retested.

Results

Behavioral responses are the primary functional basis by which the ability of flies to taste a substance can be determined. Two standard tests were used to determine the sensitivity of *Drosophila* to a range of 21 nutritive and nonnutritive sweeteners. A 60-well ingestion assay measured whole-animal preference for agar containing each test compound, whereas proboscis extension by immobilized flies was used as a measure of reflexive appetitive behavior in response to specific stimulation of tarsal taste cells by each compound (Figure 1). Several sugars, known to stimulate feeding behaviors in *Drosophila*, were used as positive controls. In any one ingestion preference test, 30–50% of the flies typically fed. As expected, flies preferentially ingested dyed agar when it contained any of the sugars. Flies also preferred the sugar alcohols (i.e., sorbitol) and sweet amino acids (i.e., L-glycine), which reflects preferences typical of primates. Furthermore, these substances all stimulated proboscis extension when applied in solution to an anterior tarsus, confirming the ability of these behavioral tests to reflect stimulation of taste receptor cells (see Table 2).

Flies also preferentially ingested dyed agar when it contained any of these sweeteners including the small high-potency sweeteners or either of the 2 sweet proteins, compared with dyed agar alone ($P < 0.05$). The lowest PI was 63% for the sweet protein thaumatin (0.005 mM), whereas sucralose (2 mM), with a mean PI of 90%, and NHDC, with a mean PI of 87%, elicited the highest responses (Table 2). The ingestion responses (high preference index, PI) to these sweeteners were quantitatively comparable to the strong preferences obtained with natural sugars. Flies appeared mildly repulsed by the sweet salt $PbCl_2$ (0.1 mM), which gave a mean PI of 47%; the nonsweet mutant (R43N) brazzein (0.005 mM), which was included as a negative control, was not preferred over dyed agar alone (PI = 52%; Table 2). Flies exhibited neither a preference nor an aversion to either of the marker dyes used in the test when both sides contained 4 mM sucrose (PI = 53%), and females did not exhibit any differences from males when tested independently for their taste response to 4 mM sucrose in both dyed agars (PI = 52%).

Table 2 Appetitive behaviors in response to test compounds

	Concentration (mM)[a]	*n*	Test	Result
High-potency sweeteners				
Aspartame	2	56	PER	+
	2	281	Ingestion	++
Dulcin	1	54	PER	+
	1	182	Ingestion	++
Glycyrrhizin	1	55	PER	0
	3	321	Ingestion	+
NHDC	2	50	PER	++
	1	357	Ingestion	+++
Na Saccharin	2	98	PER	+
	2	483	Ingestion	++
SC45647	0.01	63	PER	+++
	0.01	208	Ingestion	++
Stevioside	2	56	PER	0
	2	198	Ingestion	+++
Sucralose	2	57	PER	+
	2	247	Ingestion	++++
Sweet proteins				
Brazzein	0.05	53	PER	+
	0.005	259	Ingestion	+
Thaumatin	0.005	35	PER	+++
	0.005	131	Ingestion	+
Arg43Asn (R43N) Brazzein (negative control)	0.05	52	PER	0
	0.005	240	Ingestion	0
Sugars				
Fructose	100	96	PER	++++
	2	272	Ingestion	++
Glucose	100	64	PER	+++
	100	668	Ingestion	++++
Maltose	2	54	PER	++
	2	425	Ingestion	++++
Sucrose	100	608	PER	++++
	2	610	Ingestion	+++
Trehalose	100	52	PER	+++
	20	308	Ingestion	++
Sugar alcohols				
Glycerol	100	52	PER	+++
	2	342	Ingestion	++
Sorbitol	100	84	PER	+
	200	399	Ingestion	+++
Sweet amino acids				
D-Aspartic acid	2	50	PER	+
	2	260	Ingestion	++
Glycine	0.1	54	PER	+
	2	314	Ingestion	++
Sweet salt				
$PbCl_2$	0.1	48	PER	+
	0.1	80	Ingestion	0

Key[b]

% Flies displaying a PER	PI %
1–10 = 0 (not significant chi-square)	1–58 = 0 (not significant paired comparison test)
11–16 = +	59–68 = +
17–24 = ++	69–78 = ++
25–43 = +++	79–88 = +++
44–100 = ++++	89–100 = ++++

[a]Concentrations presented here are those that elicited the strongest response for each test solution in each assay. The same concentration did not always elicit the strongest response in each assay.
[b]Response categories for the PER assay are based on natural breaks in the distribution of fly responses.

To determine whether the ingestion responses to these sweeteners could be related to an isolated feeding reflex, flies were also examined for proboscis extension responses following direct stimulation of the tarsus with solutions of each compound. All the sweeteners were able to elicit an appetitive response, as indicated by PER, at concentrations used in the ingestion assay ($P < 0.05$), except glycyrrhizin and stevioside, which were not able to stimulate a response at any one of a range of concentrations tested (Figure 2B). Generally, proboscis extension responses were not as vigorous as responses obtained in the ingestion assay. We observed a concentration dependence in the PER responses to tarsal stimulation. For saccharides, PERs were exhibited more frequently as concentration increased (Figure 2A). However, this was not always the case with the high-potency sweeteners, which were often more effective at lower concentrations than at higher concentrations (Figure 2B).

Discussion

In this study, we used taste reactivity (PER) and ingestive (preference) behavioral assays to demonstrate that

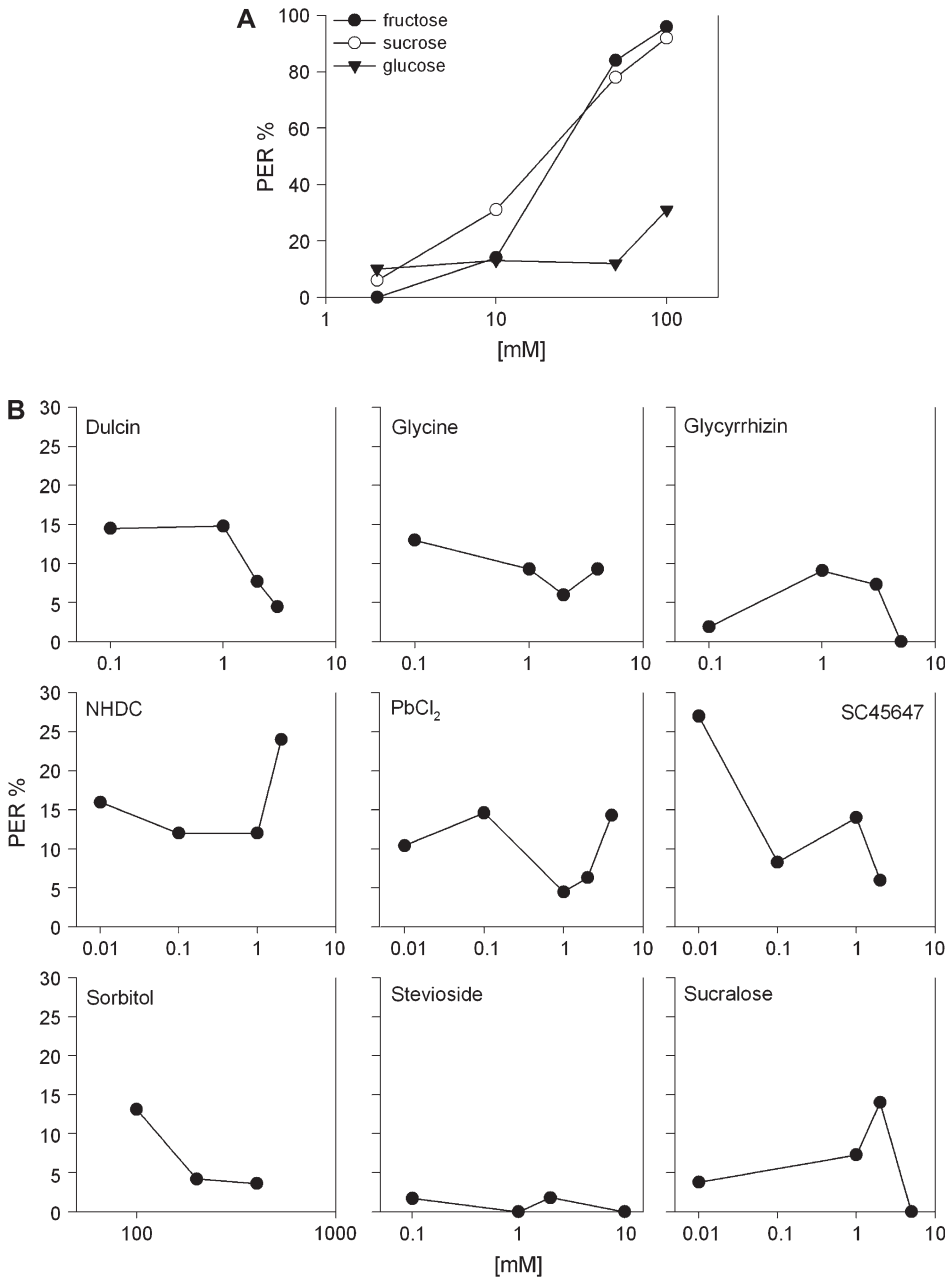

Figure 2 Concentration dependence of PER to natural sugars **(A)** and high-potency sweeteners **(B)**. Multiple high-potency sweetener concentrations were tested in the PER assay because initial concentrations tested elicited few responses. Wild-type CS flies were tested for their PER responses to a range of concentrations of 3 sugars, sucrose, fructose, and glucose (A) or 9 sweeteners, dulcin, glycine, glycyrrhizin, NHDC, $PbCl_2$, SC45647, sorbitol, stevioside, and sucralose. The *y* axis represents the percentage of flies with Proboscis Extension Responses, and the *x* axis represents concentration in mM.

Drosophila are responsive to a similarly broad repertoire of noncarbohydrate, sweet stimuli as are humans. CS flies responded appetitively to and ingested brazzein, thaumatin, NHDC, and aspartame which only humans and old world primates have, until this study, been known to prefer and ingest (Glaser et al. 1995; Nofre et al. 1996; Danilova et al. 1998; Glaser 1999; Jin, Danilova, Assadi-Porter, Aceti, et al. 2003; Danilova and Hellekant 2004). Moreover, flies failed to respond to an R43N-substituted brazzein that is not sweet to humans or rhesus monkey (*Macaca mulatta*) (Jin, Danilova, Assadi-Porter, Markley, et al. 2003). In this regard, *Drosophila* responses are more human-like than are those of many mammals, including new world monkeys. Among mammals that differ in their sweetener preferences, such as cats and mice, the sweet taste receptors are presumed homologous and any differences in sensitivities to the

sweeteners have been attributed to acquired polymorphisms (single-nucleotide polymorphism) or other alterations, such as insertion/deletion events, in the receptor gene (Bachmanov, Li, et al. 2001; Li et al. 2006). But the TAS1R2–TAS1R3 receptor surely did not evolve to detect saccharin, glycyrrhizin, and chalcones; rather, the need to detect a variety of nutritive polyols from fruits, vegetables, and perhaps some animal tissues was likely the pressure that shaped the binding pockets and receptive fields of the sweetener receptors.

Reassuringly, the 2 behavioral assays in this study, tarsal taste reactivity (PER) and ingestion (whole-animal preference testing), yielded almost completely overlapping results for each stimulus, with a couple exceptions. Two compounds, stevioside and glycyrrhizin, failed to elicit PERs upon tarsal stimulation, yet, they were preferentially ingested in the feeding test. We believe this may reflect differences in the stimulation of sweet-sensitive anatomical sites, as PER was based only on anterior tarsal stimulation (mostly a single leg and only outer segments 4 and 5). In addition to the anterior tarsus, taste receptor cells are also present in the middle and posterior tarsi and in the labella and to a lesser extent on the wings and ovipositor of *Drosophila* (Clyne et al. 2000; Scott et al. 2001; Thorne et al. 2004; Wang et al. 2004). Thus, the ingestion preference test differs significantly from our PER assay in that many more taste stimulation sites were likely involved. The observation that % PERs decreased with increasing concentration (Figure 2) for some sweeteners, such as dulcin (see also Figure 3), may be due to increasing bitterness of the stimuli (Horne et al. 2002) as occurs for humans with many sweeteners, for example, saccharin. Finally, one compound, $PbCl_2$, was observed to elicit a PER upon tarsal stimulation but was not preferred in the ingestion assay (Figure 3). This nicely demonstrates that factors other than taste stimulation of carbohydrate receptor cells govern whole-animal feeding choices in *Drosophila*, as the "sweet-tasting" toxin $PbCl_2$ would ultimately be avoided due to toxicity or perhaps malaise.

In *Drosophila*, 2 sweet receptors have been identified thus far, the trehalose taste receptor *Gr5a* and *Gr64a*, a taste receptor necessary for the detection of sucrose, glucose, and maltose (Ueno et al. 2001; Jiao et al. 2007; Slone et al. 2007). These receptors can have a high degree of specificity for saccharides as is clear in the ΔEP19 line which lacks a functional *Gr5a* receptor gene (Ueno et al. 2001) and the deleted *Gr64ab* line that exhibits drastically reduced responses to these sugars (Jiao et al. 2007). There are, however, 6 other putative taste receptor genes in *Drosophila* with strong sequence homology to *Gr5a* that are coexpressed with *Gr5a* in receptor cells (Robertson et al. 2003; Jiao et al. 2007). Because *Gr5a* has little sequence similarity with either *TAS1R2* or *TAS1R3* (Matsunami and Amrein 2003), which code for the 2 proteins comprising a mammalian sweet receptor heteromer, the similarity between human and *Drosophila* sweet taste repertoires cannot be simply explained by shared receptor ancestry. Curiously, however, *Gr5a* may require

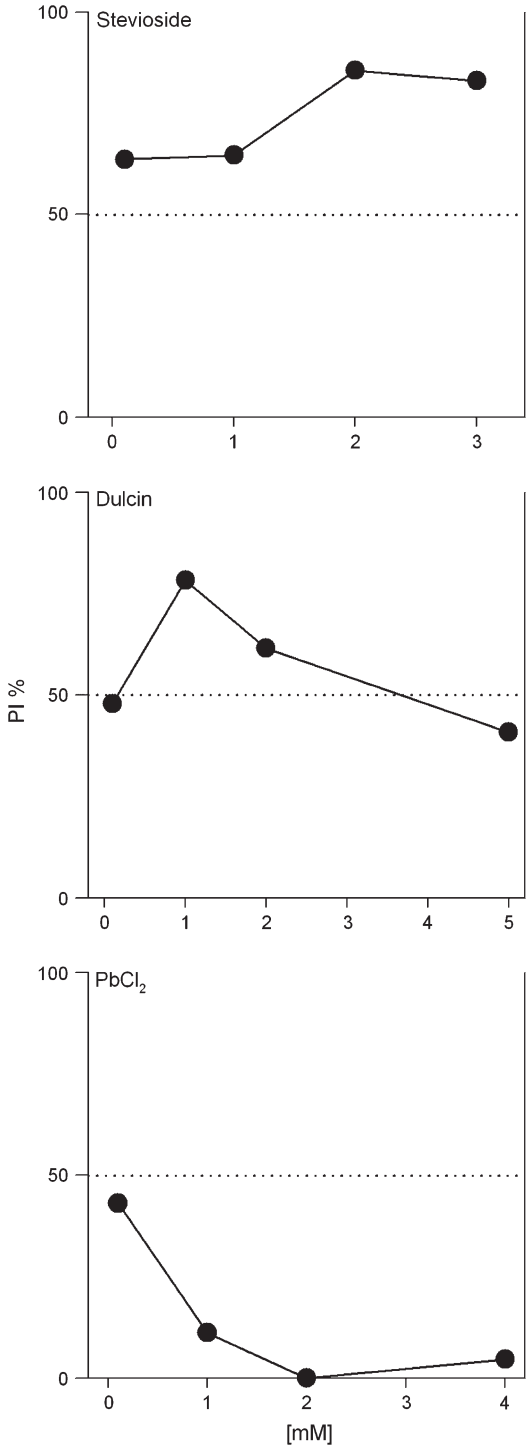

Figure 3 Concentrations tested in the preference assay for select chemicals. Multiple concentrations were tested for these 3 chemicals because the low concentration tested (0.1 mM) elicited no preference from flies in the preference assay. Wild-type CS flies were tested for their ingestion responses to a range of concentrations for stevioside dulcin and $PbCl_2$. The *y* axis represents the preference index as a percentage of animals preferring the stimulus, and the *x* axis represents concentration in mM. The horizontal dotted line at 50% represents indifference to the stimulus over plain agar. Responses above 50% indicate positive preference. The data points near 0 mM represent the response to 0.1 mM, not to water.

another receptor protein to bind saccharides, like the mammalian TAS1R heteromer receptor because deletion of *Gr64ab* removes the flies' response to trehalose (Jiao et al. 2007). Thus, fly sweet receptors may form functional heteromers paralleling the TAS1R2–TAS1R3 sweetener receptor.

If the human and fly sweetener receptors share little in sequence similarity, then what could account for their highly overlapping receptive fields? Like humans, *Drosophila* are omnivorous. They prefer plant saps and yeasts and possess a strong affinity for ripe fruits. And as a human commensal, they clearly express interest in many of the foods that appear in a typical human diet including cultivated fruits and vegetables and fermented products such as breads, beers, wines, etc (Keller 2007). The strong similarities between humans and fruit flies both in their dietary ecology (omnivory), evolutionary dietary preferences (frugivory), and overlapping evolutionary environments (African tropical and temperate zones) may have exposed them to similar evolutionary pressures that would "shape" their polyol–carbohydrate taste receptors to detect a variety of natural stimuli. Another ape, the chimpanzee (*Pan troglodyte*), one of our closest genetic relatives, bases 60–70% of its diet on fruits (Gombe, Tanzania) (Goodall 1986). We hypothesize that independently evolving sensitivity to all these naturally occurring polyol–carbohydrate sweeteners from fruits and other organisms may inadvertently require receptors that have coincident sensitivities to many high-potency noncarbohydrate sweeteners. The binding pockets of human and fly sweet receptors may be comprised of only a relatively few critically placed residues. We note that current theories of TAS1R sweet–ligand binding suggest that certain nonnutritive high-potency sweeteners such as brazzein and cyclamate require sites that differ from saccharide-binding sites. We suggest that these alternate binding regions/residues may be important to the structure of receptors that can bind the multitude of nutritive saccharide sweeteners that humans taste. Thus, the structure of fly and human saccharide-binding pockets and the placement of key residue types may be more similar than the primary protein sequences would otherwise suggest. The exact receptor mechanisms that enable these remarkable parallels in sweet ligand repertoires between humans and flies remain to be elucidated but have most likely been influenced by our common ecologies, omnivorous diets, and avidities for African fruits over evolutionary time.

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