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Analyses of Sweet Receptor Gene (*Tas1r2*) and Preference for Sweet Stimuli in Species of Carnivora

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Abstract

The extent to which taste receptor specificity correlates with, or even predicts, diet choice is not known. We recently reported that the insensitivity to sweeteners shown by species of Felidae can be explained by their lacking of a functional *Tas1r2* gene. To broaden our understanding of the relationship between the structure of the sweet receptors and preference for sugars and artificial sweeteners, we measured responses to 12 sweeteners in 6 species of Carnivora and sequenced the coding regions of *Tas1r2* in these same or closely related species. The lion showed no preference for any of the 12 sweet compounds tested, and it possesses the pseudogenized *Tas1r2*. All other species preferred some of the natural sugars, and their *Tas1r2* sequences, having complete open reading frames, predict functional sweet receptors. In addition to preferring natural sugars, the lesser panda also preferred 3 (neotame, sucralose, and aspartame) of the 6 artificial sweeteners. Heretofore, it had been reported that among vertebrates, only Old World simians could taste aspartame. The observation that the lesser panda highly preferred aspartame could be an example of evolutionary convergence in the identification of sweet stimuli.

Key words: *carnivore, diet, sweeteners, sweet receptor, taste testing*

The sense of taste plays a major role in one of the most important daily decisions an animal makes: whether to ingest a substance or to reject it. This complex and exacting behavior has been subject to natural selection, consisting, at least partly, of a dynamic interplay among food selection, nutrient quality, and the specificity of the taste receptors that ultimately evaluate the food consumption.

The interplay among receptor specificity, diet selection, and food intake can be seen in the confluence of receptor specificity for sweet compounds and the preference shown for sweet stimuli. Several comparative studies on taste receptor specificity and receptor structure have shed light on the sometimes very subtle changes in amino acid sequence that can dramatically affect stimulus intake. For example, analysis of the genotype–phenotype associations in 30 inbred mouse strains showed that I60T of T1R3, one component of the sweet taste receptor, had the strongest association with saccharin preference (Bachmanov et al. 2001; Reed et al. 2004).

Yet, as much as can be learned from the consideration of strain differences in single species, considerable insight can be gained in understanding receptor evolution from studies of closely related species that differ in food habits. Although the preference for sweet substances is common to many animals, there are also some telling between-species differences, as with the perception of the nonnutritive sweetener aspartame. Most rodents and even some primate species are indifferent to the taste of aspartame (Naim et al. 1982; Sclafani and Abrams 1986; Thomsen et al. 1988). Yet, other primates, including humans, are sensitive to it. Glaser et al. tested 42 species of primates; all 18 Old World primates were aspartame tasters, whereas all 24 New World primates were nontasters. The authors concluded that the ability to recognize aspartame as sweet is a recent evolutionary

development, occurring in a common ancestor of Old World simians (Glaser et al. 1992, 1995, 1996). The data we present here from Carnivora question this restriction.

The Order, Carnivora, is composed of 2 suborders, Feliformia (with 4 families) and Caniformia (with 8 families) (Figure 1). This order houses species whose diet ranges from obligate carnivores to strict herbivores (Arnason et al. 2002; Flynn et al. 2005; Nowak 2005). All species in the Felidae family (suborder, Feliformia) are obligate carnivores. Species in Odobenidae, Otariidae, and Phocidae are piscivorous (fish eaters). Species in Procyonidae, *Ailurus*, and Ursidae are either omnivorous or almost exclusively herbivores, such as the giant and red pandas (folivores). The remaining species of the other 5 families are either opportunistic carnivores or omnivores. These large differences in diet (food choice) across species of Carnivora, in the face of a likely close phylogenetic relationship, make this an attractive group in which to study the comparative behavior of taste and the corresponding molecular biology and molecular genetics of taste.

To date, most studies of food selection in carnivores have been limited to food intake and nutrition/diet interplay, having generally ignored taste testing. An exception to this has been taste testing of cats and dogs. Cats, both wild and domestic, appear to be indifferent to substances we call sweet yet can detect chemicals of the other 4 basic qualities (bitter, sour, salty, umami [a Japanese word meaning savory or delicious, imparted in human primarily by the amino acid glutamate]) (Carpenter 1956; Beauchamp et al. 1977; Bradshaw et al. 1996; Glaser 2002; Li et al. 2005). Most domestic dogs apparently have the ability to perceive all 5 taste qualities (Carpenter 1956; Boudreau and White 1978; Kumazawa et al. 1991), and reportedly, they show preferences to most carbohydrate sugars including sucrose, glucose, fructose, and lactose but not maltose (Grace and Russek 1968; Houpt et al. 1979; Ferrell 1984). However, Glaser (2002) reported that dogs do not show a preference for sucrose, fructose, and several artificial sweeteners. These disparate results could be due to different testing procedures but are, more likely, a result of breed difference.

The taste receptors for sweet, umami, and bitterness are G-protein–coupled receptors. Those for bitterness comprise a family of structurally related receptors, but curiously, the number of bitter receptor genes is variable across species (Go 2006). In contrast, there is only one major receptor for umami and one (or perhaps 2) for sweetness. These sweet and umami receptors belong to the class C type and function as heterodimers. There are 3 such taste type 1 receptors, labeled as T1R1, T1R2, and T1R3. The umami receptor is the T1R1/T1R3 heterodimer. The sweet receptor is the heterodimer, T1R2/T1R3. There is also some evidence for a second sweet receptor, the homodimer, T1R3/T1R3. This

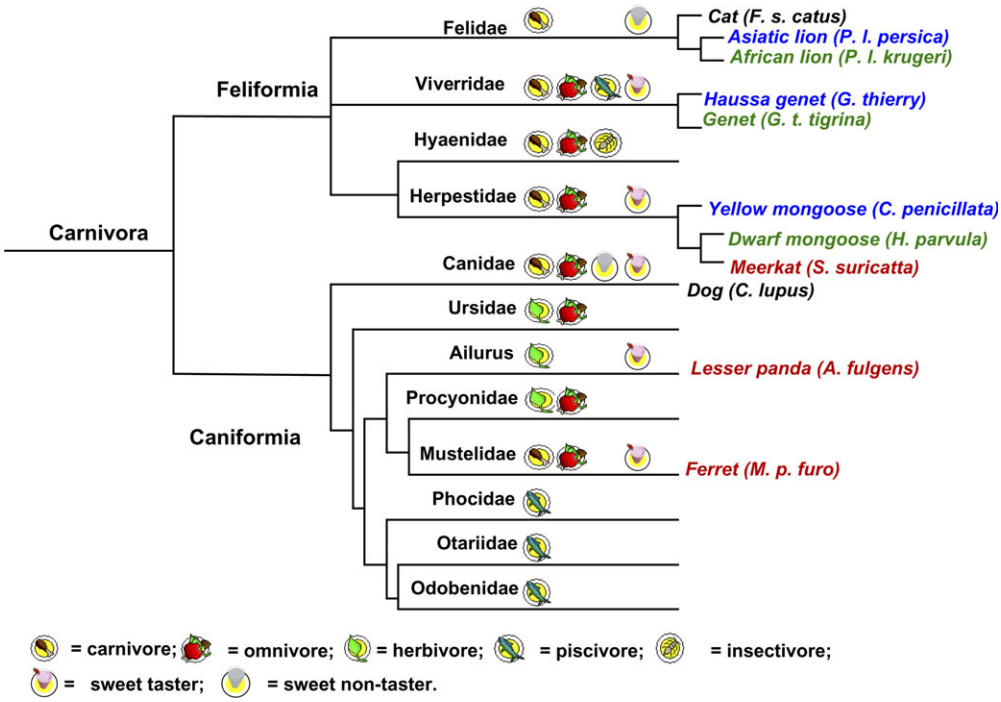


Figure 1. A simplified evolutionary tree of the Order Carnivora (Flynn 2005; Nowak 2005). The 2 major branches of the Order Carnivora, Feliformia, and Caniformia, diverged approximately 45 Ma. The dish symbols represent the different diets preferred within each family. A cup of strawberry smoothie indicates the animals tested within the family prefer sweet stimuli over plain water, and a glass of water indicates the animals tested within the family show no preference to sweet stimuli over plain water. At the end of each branch, species used in both sequencing and taste testing are marked in red, species only used in behavioral testing are marked in blue, and species only used in sequencing are marked in green.

homodimer responded only to very high concentrations of sugars (>300 mM) (Zhao et al. 2003). In contrast, the human heterodimer, T1R2/T1R3, recognizes both naturally occurring sweeteners and synthetic ones. The T1R2/T1R3 heterodimer of rat and mouse recognizes natural sugars but only a few artificial sweeteners (Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Bachmanov et al. 2001; Li et al. 2002; Nelson et al. 2002; Damak et al. 2003; Zhao et al. 2003).

Based on our work in cats and mice, and work of others in dogs (Grace and Russek 1968; Houpt et al. 1979; Ferrell 1984; Li et al. 2006), one may predict that the specificity of the sweet receptors will be different in selected carnivore species that vary substantially in dietary habits. In the extreme cases, such as cats versus dogs, difference in dietary habits is reflected in the different functional states of the receptors for sweet taste, that is, dogs are omnivores, whereas cats are obligate carnivores; dogs have a functional *Tas1r2*, whereas cats do not (Li et al. 2005, 2006).

We know little of the relationship between sweet taste perception and sweet receptor structure in other members of Carnivora. The main goal of this research is to examine variations of the sweet receptor gene *Tas1r2* in species of the order Carnivora and to correlate these with the animals' responses to sweet stimuli.

Materials and Methods

Selection of Species

Six species from Carnivora were selected for behavioral taste testing: lesser panda (*Ailurus fulgens*, *Ailurus*), domestic ferret (*Mustela putorious furo*, Mustelidae), Haussa genet (*Genetta thierry*, Viverridae), meerkat (*Suricata suricatta*, Herpestidae), yellow mongoose (*Cynictis penicillata*, Herpestidae), and Asiatic lion (*Panthera leo persica*, Felidae). These species were selected for the following reasons.

1. Phylogenetically, these species are representative of 5 of the 12 families within the order Carnivora and are relatively close to the domestic cat or dog (see distribution in Figure 1).
2. Even though these species are all within the order Carnivora, their diets are quite different. For example, lesser pandas are almost exclusively herbivores (Nowak 2005); ferrets, genets, and mongooses feed on small mammals, birds, and fruits (Rasa 1973a, 1973b; Virgós et al. 1999); meerkats are mostly insectivorous (Nowak 2005); and lions are obligate carnivores (Nowak 2005).
3. From a practical perspective, the chosen species were readily available in captive settings that were amenable to the behavioral tests.

For analyses of *Tas1r2* coding sequences, we used the same species as in behavioral experiments when DNA samples were available; otherwise, we used a closely related subspecies, for example, genet (*Genetta tigrina tigrina*), dwarf mongoose (*Helogale parvula*), and African lion (*Panthera leo krugeri*) (Figure 1).

Behavioral Taste Testing

Animals

Animals in this study were made available to us by 2 zoos in Switzerland. From the Rapperswil Zoo, we tested 5 meerkats (one 8-year-old male, one 7-year-old female, and three 2-year-old juveniles: 2 females and 1 male), 3 yellow mongooses (one 3-year-old female, one 6-year-old male, and one 2-year-old male), and 2 domestic ferrets (1 female and 1 male both 6 years old). From the Zoological Garden of Zurich, we tested 2 genets (2 males 5 and 6 years old), 1 lion (8-year-old male), and 2 lesser pandas (1 female and 1 male, both 11 years old). All these animals were born in captivity. They were maintained and tested according to the Monell Chemical Senses Center animal protocol (Institutional Animal Care and Use Committee No. 1112) and with the permission and oversight of the zoos involved.

Two-Bottle Tests

A traditional 2-bottle (or bowl) 24-h preference tests were employed to determine taste responses to 12 compounds perceived as sweet by humans. The test compound dissolved in tap water was in one bottle, with tap water in the other. The smaller animals were offered the choice of 2 bottles attached to the cage. The medium-sized animals were provided with 2 drinking bowls placed inside the cages. The larger animals were tested with their usual drinking bowls (Glaser et al. 2000).

Animals were divided and tested in groups according to species, with the exception of genets. To reduce a side or position bias, the positions of the drinking receptacles were reversed after 12 h. The test period began at 09:30 AM and ended 24 h later.

After 24 h, the volume of fluid consumed from each bottle was determined by difference. Preference scores were calculated as the ratio of taste solution intake to total fluid intake × 100%. A "strong preference" for the test compound is defined here as a preference score greater than 80%. In Table 3, preferences above 80% are identified by a plus sign. The strongly preferred compounds generally evoked a very robust response, with animals consuming much more sweet fluid than water, leaving no doubt that the stimulus preference was driven by its sweet taste. However, we did not define other stages of preference, such as weakly preferred or even rejected, because the total intake for some of the species is relatively low, making the difference score not as reliable (Glaser et al. 2000).

Selection of Sweeteners

To acquire general information on sweet preferences in these animals, we selected 6 natural sugars and 6 artificial sweeteners (see Table 1). The 6 sugars were chosen because they are commonly present in many fruits and are included in animals' natural diet; the 6 artificial sweeteners were chosen because they have been tested previously in cats and dogs (Beauchamp et al. 1977; Glaser 2002), effectively giving us access to preference data from 2 additional species

Table 1. Compounds and concentrations selected for 2-bowl tests

Compounds	Sweetness potency in humans (on a molar basis)	Ranges tested
Natural		
Sucrose	1	0.3–1.2 M
Maltose	0.33	0.3–1.2 M
Lactose	0.33	0.3–1.2 M
Glucose	0.25	0.3–1.2 M
Fructose	0.5	0.3–1.2 M
Galactose	0.2	0.3–1.2 M
Artificial		
Neotame	11 000	0.0008–25 mM
Sucralose	1160	0.008–25 mM
Saccharin–Na	215	0.03–20 mM
Aspartame	170	0.03–20 mM
Acesulfame–K	150	0.03–20 mM
Na–cyclamate	17.6	0.3–20 mM

of Carnivora. In addition, these 6 artificial sweeteners represent several binding sites on both T1R2 and T1R3.

Because species may differ in detection thresholds for a given sweetener, we tested 2–5 different concentrations to determine the limits of the animal's ability to detect sweeteners. Because the detection thresholds for these carnivores are unknown, tests started with a range of solution concentrations close to detection and preference thresholds of other animals including humans (Table 1) (Schiffman and Gatlin 1993).

Carryover effects can be a concern when taste testing multiple compounds. For instance, testing with a previous compound may influence the animal's behavior to a later compound. With natural sugars, in long-term 2-bowl tests, the carryover effects may be related to the potential contribution of postingestive effects of sweetener consumption (Spector 2003). To reduce the influence of possible carryover effects, tap water was given for 2 days between each series of tests.

To gain a more complete understanding of the spectrum of taste preferences in these animals, we also selected several artificial sweeteners. These sweeteners are without significant calories and have few, if any, metabolic effects. Any preference for these sweeteners may therefore be directly related to peripheral taste response. We tested substances in the order listed in Table 1 for all species.

Tas1r2 Coding Sequence Analyses

Collection of DNA Samples

DNA samples from lesser panda (*A. fulgens*), ferret (*M. putorious furo*), genet (*G. tigrina tigrina*), mongoose (*C. penicillata*), and African lion (*P. leo krugeri*) were provided by the Conservation and Research for Endangered Species program at the San Diego Zoo, and a DNA sample from meerkat (*S. suricatta*) was made available from the laboratory of Dr S.J.O'B. at the National Cancer Institute.

Primer Design

To examine the *Tas1r2* gene from selected carnivore species, we designed degenerate primers to amplify *Tas1r2* coding sequence from DNA samples. To design these primers, we aligned the *Tas1r2* from human, cat, and dog and manually picked primers that spanned the boundaries of each exon based on the conserved region of *Tas1r2* among these species (see Table 2). To determine if the previously reported 247-bp microdeletion in exon 3 of cats exists also in the carnivore species tested here, we aligned human and dog *Tas1r2* and designed degenerate primers to amplify the region spanning the microdeletion.

Sequencing of Tas1r2

Polymerase chain reaction (PCR) was conducted to amplify exons 1–6 of *Tas1r2* from the 6 species using degenerate primers designed from conserved exon–intron boundary sequences. The PCR reagents and *Taq* DNA polymerase were from Roche (Indianapolis, IN). The PCR parameters were 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 66 °C for 45 s, and 72 °C for 2 min; 72 °C for 10 min; and a 4 °C hold. PCR products were separated on 2% agarose gels (FMC Bioproducts, Rockland, ME) containing 0.5 mg/ml ethidium bromide, visualized by ultraviolet transillumination. The PCR products were purified by using Qiagen gel extraction kit (Valencia, CA) and sequenced at the Sequencing Facility of the University of Pennsylvania. The coding sequences of *Tas1r2* from all the selected carnivore species were assembled by Sequencher 4.8 (Gene Codes Corp, Ann Arbor, MI).

Results

Analyses of Preference for Sweet Taste

Because each species may have a different threshold for different sweeteners, we tested several concentrations of all 12 sweeteners in all 6 species. The range of concentration of each stimulus under test for each species is displayed in Table 1. The concentrations of each stimulus tested in all 6 species can be seen in Table 4, and the responses to these concentrations are shown in Table 3. When higher concentrations of a particular stimulus were tested, the preference pattern shown in Table 3 did not change.

Considering the sugars, 5 of the 6 species tested preferred sucrose, maltose, and glucose over water, the exception being the Asiatic lion (Table 3). These 5 species showed different responses to fructose, lactose, and galactose. Mongooses and ferrets preferred fructose, whereas genets, meerkats, and probably lesser panda did not. Only the lesser panda preferred lactose and galactose.

Considering the artificial sweeteners, we anticipated that none of the test animals of Carnivora would show a preference toward any of the lower 6 compounds of Table 1. Such was true for all animals except the lesser

Table 2. Primers used to amplify coding regions of *Tas1r2* from selected species

Name	Primer Sequence (5′–3′)	Anneal (°C)
T1R2CarDeE1F1	GCTCTCTGATGAGGCAGGGCCACCTCC	67
T1R2CarDeE1F2	CGGGGACCHCTCACTTCCCAGCCATGGGAC	66
T1R2CarDeE1F3	TTCCCAGCCATGGGACCCCGGGCCARG	67
T1R2CarDeE1R1	CTGGMGACTCACTYCTTGCACTGGGGCACCT	67
T1R2CarDeE1R2	ACTCACTYCTTGCACTGGGGCACCTGCAGG	67
T1R2CarDeE2F1	CACTCTGGACCTGCYTCYYACCCCACC	67
T1R2CarDeE2F2	CTGCYKCYYACCCCACCCHACATGGC	67
T1R2CarDeE2F3	CCCCAGGTATGAAATRAAGGTGTTGGGCTAC	63
T1R2CarDeE2R1	GGGCCTCMCCTGTGGAAGGAGGAAGAG	67
T1R2CarDeE2R2	CCTGTGGAAGGAGGAAGAGDGAGAGGAAGC	66
T1R2CarDeE3F1	GCAGATCACCTACAGCGCCATCAGTGACGAG	67
T1R2CarDeE3F2	CCTACAGCGCCATCAGTGACGAKCTRCGG	67
T1R2CarDeE3R1	GTGCAGRACCGGGTCGATGGCCCAGGA	67
T1R2CarDeE3R2	CCGGGTCGATGGCCCAGGACTCGGA	68
T1R2CarDeE3F3	CCGCGAGGTGCTCCGCCAGAACYTCA	67
T1R2CarDeE3F4	ACGGGCGYCGTGYGGATCGCCTCC	68
T1R2CarDeE3R3	CCTCACCTGCCAGGGRTAGACSACCYC	67
T1R2CarDeE3R4	GAGTACACGYTGTAGACCACGCGCTCGCC	67
T1R2CarDeE4F1	GSCCTYCYAGCTGCTTMAGGAAATCTGGAAG	67
T1R2CarDeE4F2	GGAAGGTCAACTTCACCCTYCTGGGCCAC	67
T1R2CarDeE4R1	GCTGACCGTGTTGTTGGCSGTGTGCCAG	67
T1R2CarDeE4R2	GTGTTGTTGGCSGTGTGCCAGGAGACGTC	67
T1R2CarDeE5F1	CTCAGGRKCTCTTGCCYTCCTCCCTCCAGATC	67
T1R2CarDeE5F2	ATCCCCGTGTCCATGTGTTCCAAGGACTGCC	67
T1R2CarDeE5R1	GGGGTGYGGGTCTGYRAGTCCCAYCTGCA	67
T1R2CarDeE5R2	GTCCCAYCTGCAGTTYGGTTGAGGAAGGTGCC	67
T1R2CarDeE6F1	CTGACGGGARCTGCYGTGGGCTCTTG	67
T1R2CarDeE6F2	GTGGGCTCTTGCAGACGARTTTGRCTGCC	67
T1R2CarDeE6R1	AAGACRYAGGGCCCGTGGYAGCGSACC	67
T1R2CarDeE6R2	CCCAGTAGCCGTAGGCRCGCGGGAG	68
T1R2CarDeE6F3	CCCTCTGCTTCACCRTCTGYATCTCCYG	66
T1R2CarDeE6F4	CCAGATCGTCYGCRTCTTCAASATGGCCAG	67
T1R2CalDeE6R3	GCTAGTCCTTCCSCRTGGTGTAGCCCTGAATC	67
T1R2CalDeE6R4	GGTGTAGCCCTGAATCATGCTGCTGAAGTAGAC	66

panda. Interestingly, these animals showed strong preference for 3 of the 6 artificial sweeteners, neotame (89%), sucralose (91%), and aspartame (99%). The average fluid intake of the artificial sweeteners versus tap water for neotame was 498 ml versus 59 ml of water; for sucralose, 496 ml versus 52 ml of water; and for aspartame, 486 ml versus 3 ml of water. Such robust responses to the preferred stimuli seem to be a consistent observation, with the

Table 3. Summary of sweet preferences in 8 carnivore species by 2-bowl tests

Compounds	Lesser panda (*Ailurus*)	Domestic ferret (Mustelidae)	Haussa genet (Viverridae)	Meerkat (Herpestidae)	Yellow mongoose (Herpestidae)	Asiatic lion (Felidae)	Domestic cat[a] (Felidae)	Domestic dog[a] (Canidae)
Sucrose	+	+	+	+	+	−	−	+/−
Maltose	+	+	+	+	+	−	−	−
Glucose	+	+	+	+	+	−	−	+/−
Fructose	−	+	−	−	+	−	−	+/−
Lactose	+	−	−	−	−	−	−	+/−
Galactose	+	−	−	−	−	−	−	−
Neotame	+	−	−	−	−	−	−	−
Sucralose	+	−	−	−	−	−	−	−
Saccharin	−	−	−	−	−	−	−	−
Aspartame	+	−	−	−	−	−	−	−
Acesulfame–K	−	−	−	−	−	−	−	−
Na–cyclamate	−	−	−	−	−	−	−	−

+: Preference score above 80%; −: preference score below 80%. Note that the responses to sweeteners were based on the concentrations described in Table 4.

[a] Cat data are from Glaser 2002, and dog data are from Glaser 2002, Grace and Russek 1968, and Houpt et al. 1979.

exception of fructose. The pandas showed a 64% preference for fructose, yet they consumed 298 ml of fructose water along with 169 ml of water. This intake of total fluid, 467 ml, is quite high when compared with the total fluid intake of test days when offered the other 3 sweeteners: 21 ml of total fluid intake with saccharin, 19 ml with acesulfame–K, and 109 ml with cyclamate. And while total fluid intake is low for these latter 3 conditions, there is no evidence from these data that the pandas respond negatively to the bitterness of saccharin and of acesulfame–K.

The apparent excessive intake of the sweet test solutions observed in lesser panda is seen only in genet when being tested with sucrose (347 ml of sucrose solution vs. 5 ml of water). The other preferences for sugars are apparent but occur without the very high total intake. Too few animal subjects consuming too small amounts of fluid indicate that we cannot, with confidence, suggest that some of the species are showing rejection of the artificial sweeteners because of off tastes.

Analyses of Coding Sequences of *Tas1r2*

To examine variations in the *Tas1r2* receptor gene, we designed degenerate primers as described in the Materials and Methods and, using PCR amplification of the corresponding genomic DNA, obtained sequences of the 6 exons of *Tas1r2*. We then aligned the deduced T1R2 amino acid sequences from the 6 species of carnivores used in this study, along with a reference sequence of human T1R2 and the sequence of cat T1R2 up to the point where a premature stop codon is encountered after the 247-bp deletion. This results in a deduced amino acid sequence of 391 amino acids (Figure 2).

The sequence similarity of the *Tas1r2* gene between each pair of the 5 non-Felidae carnivores ranges from 87% to 95%. Such a high degree of similarity indicates that *Tas1r2* is highly conserved among these species, reflecting the phylogenetic relatedness of species of Carnivora; for example, meerkat and mongoose (both are Herpestidae) are closer to each other than to other carnivores. At both nucleotide and amino acid levels, the sequence similarity of this gene between each pair of animals including human is more than 76%, with the exception of lion and cat. The lion and cat show similarity values above 90%, and the similarities with other carnivores are low. The sequence analysis shows that, as in domestic cats, the *Tas1r2* in lions is a pseudogene, possessing the microdeletion in exon 3 and several stop codons in exons 4 and 6. In a previous study (Li et al. 2005), we reported that cheetah and tiger also have the same microdeletion in exon 3, suggesting that the purifying selection may facilitate the retaining of the truncated open reading frame of the *Tas1r2* gene within family of Felidae. For the other 5 carnivore species, no deletion or stop codons were detected across this region.

Because of the preference shown by lesser panda for 3 artificial sweeteners, we examined more closely the sequence alignment of T1R2 from human, lesser panda, several other carnivores from the current study, as well as other mammalian species available from public domain (Figure 2). Six Old World simians including human along with the lesser panda are aspartame tasters, whereas 3 New World simians, 7 carnivores, cow, and rodents are aspartame nontasters. Interestingly, we did not see any amino acids that are clearly different between the 2 groups (i.e., taster vs. nontaster). However, we did find 16 unique amino acid sites in lesser panda that are different from any of the other species (highlighted in Figure 2 using asterisk symbols). These sites span both extracelluar and transmembrane domains and are potential sites that may help define the unique specificity of the lesser panda.

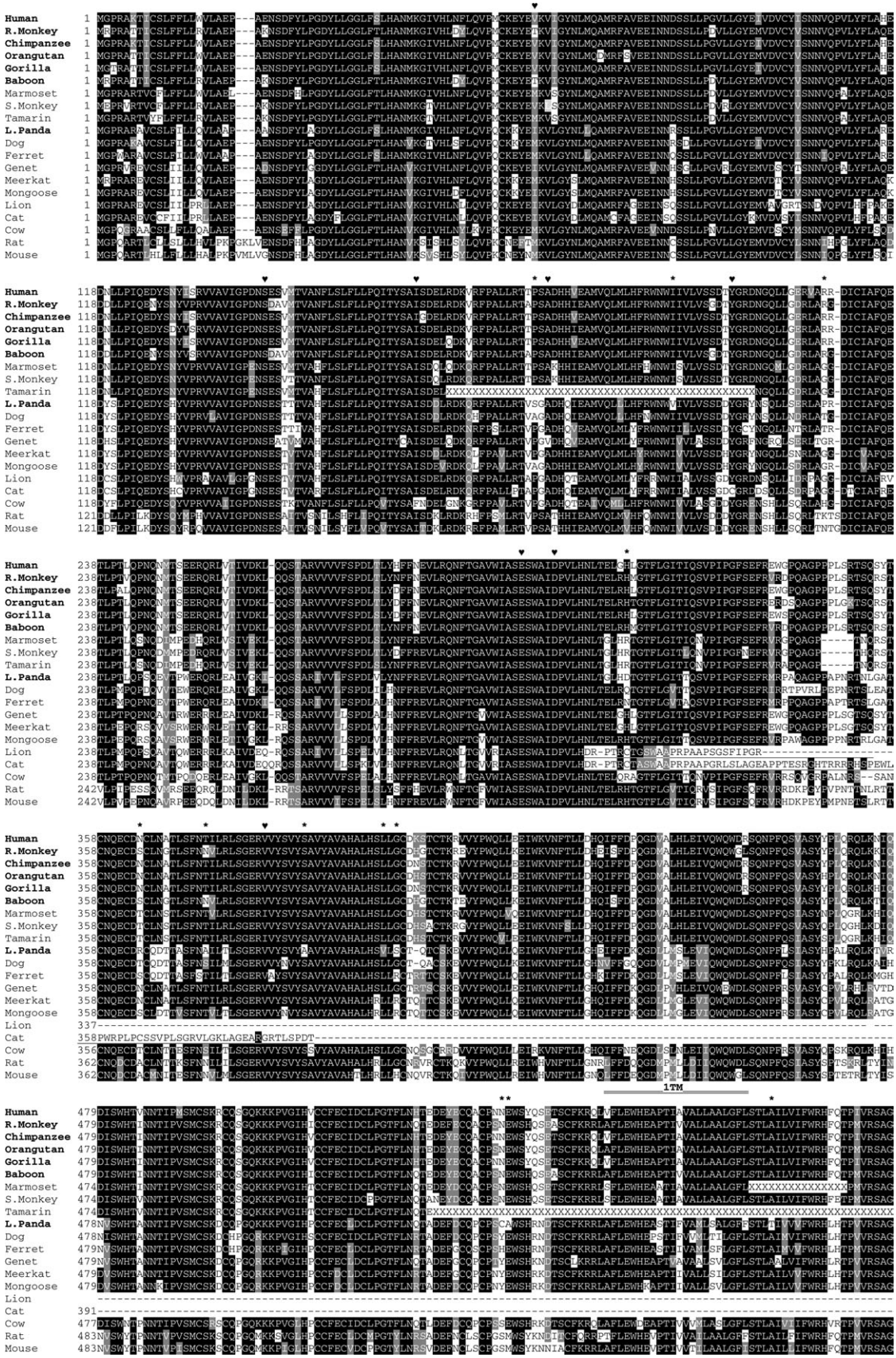
Discussion

Variations of preference for sweeteners and correlative knowledge of sweet taste receptor structure should allow for predictive structure/activity studies. In addition, insight into the manner in which taste receptor structure influences diet selection can only be gained by studying groups of animals closely related phylogenetically but showing high variability in food preference. Our initial assessment of these parameters in species of Carnivora is the subject of this work.

Our current study was constrained by the following circumstances. 1) Because most of the carnivores are wild animals, we have limited access to the animals in zoos, and at times we were not able to obtain the DNA samples from exact matching species. Thus, the sample size for the species included in the study is small, and some of the species examined in sequencing and behavioral testing are not perfectly matched. 2) The current work involved complete sequencing of *Tas1r2*. This gene only codes for one subunit of the T1R2/T1R3 dimer. It is possible that the species differences in sweet taste preference may also involve variation in *Tas1r3*. To evaluate this possibility, future studies will fully sequence this gene as well.

The results of taste testing of 12 stimuli in 6 species show that the lion, a Felidae, is indifferent to all 12 stimuli, whereas the other 5 show varying apparent preferences for sugars. In addition, the lesser panda, unlike any of the other animals tested here, also displays a robust preference for 3 of the 6 artificial sweeteners: neotame, sucralose, and aspartame (Tables 3 and 4). Our results show that each species of Carnivora tested displayed a unique pattern of preference for the 12 stimuli. These varying responses to natural sugars likely reflect differences in T1R2 and/or T1R3 sweet receptors.

Recent studies have shown that the likely binding sites for both aspartame and sucralose are on the extracellular domain of human T1R2 (Li et al. 2002; Jiang et al. 2004; Nie et al. 2006). The structural similarity of neotame and aspartame argues that these sweeteners bind to the same or similar sites. Because no preference is seen for cyclamate (the binding site for which reportedly lies on human T1R3) (Jiang et al. 2005), the ability of the lesser panda to recognize these artificial sweeteners is very likely the result of sequence variation in T1R2.


Human
R.Monkey
Chimpanzee
Orangutan
Gorilla
Baboon
Marmoset
S.Monkey
Tamarin
L.Panda
Dog
Ferret
Genet
Meerkat
Mongoose
Lion
Cat
Cow
Rat
Mouse
1TM

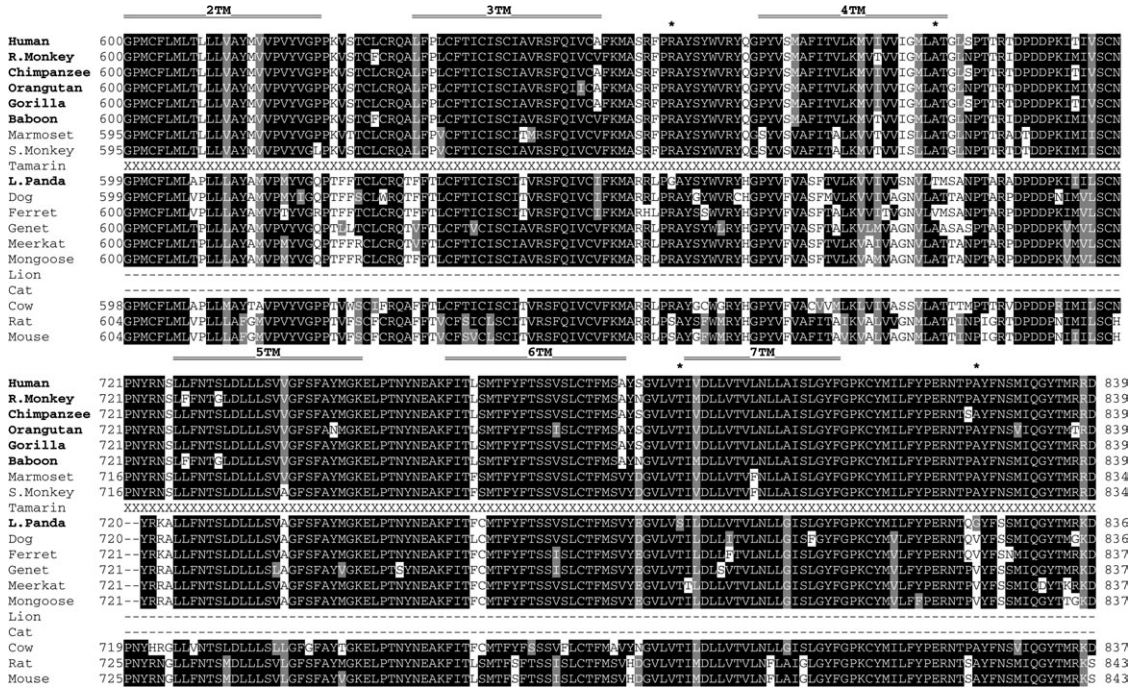


Figure 2. (Continued).

Because of apparent importance of T1R2 subunit for binding of the artificial sweeteners (e.g., aspartame, neotame, and sucralose), and the preference of both humans and lesser pandas for these sweeteners, we examined the T1R2 sequence alignment of human, 8 primates, 8 carnivores, cow, and 2 rodents. For the 8 binding sites in T1R2 that were predicted based on ligand binding sites of metabotropic glutamate receptor-subtype 1 from rat (Figure 2, marked in heart symbols), we cannot find any sites that can distinguish aspartame tasters from nontaster species. Among these 8 sites, it was reported that mutations of hT1R2 at S144A and E302A abolished the sensitivity to aspartame and neotame (Xu et al. 2004). We also examined all other sites that may be responsible for the behavioral differences of these species in the sequence alignment. We could not find sites that distinguished these 2 groups. We did find 16 sites unique to the lesser panda. Some or all of these could be important for generating a favorable binding surface for these 3 artificial sweeteners. Further studies are necessary to gain a better understanding of the importance of these variations of T1R2.

In vitro studies have shown that both cat and dog T1R3 receptors are functional proteins (Li X, Li W, Xu J, Beauchamp G, Brand J, in preparation), and both in situ

Figure 2. Alignment of deduced amino acid sequence of T1R2 from 20 mammalian species. Among the 20 species listed, human, chimpanzee, orangutan, gorilla, rhesus monkey, baboon, and lesser panda are aspartame tasters (marked in bold); marmoset, squirrel monkey, tamarin, dog, ferret, genet, meerkat, mongoose, lion, cat, cow, rat, and mouse are aspartame non tasters. The sequences of human, cow, rat and mouse are from GenBank, and sequences from the rest of the species are generated and deposited in GenBank by our group. Amino acids that are identical among species are shaded in black, conservative amino acid substitutions are shaded in gray, and non conservative amino acid substitutions are not shaded. The cat T1R2 sequence shows high similarity with that of lion: They both have premature stop codon and predict a truncated protein. The underlined amino acids in cat beginning at 313 and lion at 312 show the frameshift caused by the microdeletion in exon 3. Note that the deduced T1R2 amino acid sequences of lesser panda, ferret, genet, meerkat, and mongoose predict apparently normal protein showing high similarity with those reference sequences from other mammals. The transmembrane (TM) prediction was based on human T1R2 using computer program (http://www.ebi.ac.uk/~moeller/transmembrane.html#TMHMMs). The TMs of each species were similar among those species having normal T1R2 proteins. The asterisks show the amino acid sites that differentiate lesser panda from other species. For example, at position 185, lesser panda is S (Ser), the rest of species either have P (Pro) or A (Ala). These sites are potentially important for interaction with artificial sweeteners in lesser panda. The heart symbols indicate the 8 ligand-binding sites in mGluR1, presumably also the ligand-binding sites in T1R2 (Li et al. 2002; Xu et al. 2004). "X" indicates the missing sequences.

Table 4. Consumption of taste solution and water from 6 carnivore species

	Lesser panda (*Ailurus fulgens*) ($n = 2$)			Ferret (*Mustela putorious furo*) ($n = 2$)			Genet (*Genetta thierryi*) ($n = 1,1$)		
Sweeteners	*T* (ml)	*W* (ml)	Preference (%)	*T* (ml)	*W* (ml)	Preference (%)	*T* (ml)	*W* (ml)	Preference (%)
Sucrose (0.5 M)	491	17	97	79	2	98	347	5	99
Maltose (0.7 M)	319	72	82	89	22	80	84	8	91
Glucose (0.8 M)	477	33	94	91	6	94	59	9	87
Fructose (0.8 M)	298	169	64	92	16	85	15	7	68
Lactose (0.5 M)	498	74	87	78	65	55	16	4	80
Galactose (0.8 M)	261	9	97	35	38	48	11	6	65
Neotame (10.5 mM)	498	59	89	13	76	15	21	13	62
Sucralose (5.03 mM)	496	52	91	9	17	35	21	16	57
Saccharin (6.2 mM)	16	5	76	2	75	3	12	8	60
Aspartame (10 mM)	486	3	99	2	11	15	14	12	54
Acesulfame–K (6.0 mM)	13	6	68	1	12	8	5	4	56
Na–cyclamate (6.2 mM)	71	38	65	14	23	38	9	6	60
	Meerkat (*Suricata suricatta*) ($n = 5$)			Yellow mongoose (*Cynictis penicillata*) ($n = 3$)			Lion (*Panthera leo persica*) ($n = 1$)		
Sweeteners	*T* (ml)	*W* (ml)	Preference (%)	*T* (ml)	*W* (ml)	Preference (%)	*T* (ml)	*W* (ml)	Preference (%)
Sucrose (0.5 M)	57	14	80	18	3	86	212	269	44
Maltose (0.7 M)	26	5	84	28	4	88	76	399	16
Glucose (0.8 M)	48	8	86	29	6	83	48	57	46
Fructose (0.8 M)	63	66	49	62	10	86	82	71	54
Lactose (0.5 M)	58	26	69	54	30	64	54	156	26
Galactose (0.8 M)	21	20	51	4	11	27	177	560	24
Neotame (10.5 mM)	2	17	11	3	12	20	53	121	30
Sucralose (5.03 mM)	18	9	67	8	5	62	85	61	58
Saccharin (6.2 mM)	13	31	30	6	47	11	75	71	51
Aspartame (10 mM)	18	23	44	2	7	22	51	323	14
Acesulfame–K (6.0 mM)	7	21	25	20	13	61	105	214	33
Na–cyclamate (6.2 mM)	25	27	48	6	5	55	374	372	50

T: intake of taste solution (mean); *W*: intake of water (mean); preference (%) = $[T/(T + W)] \times 100\%$.

hybridization and immunohistochemistry show that cat T1R3 is expressed in taste buds (Li X, Li W, Xu J 2005). Therefore, we expect the *Tas1r3* gene for T1R3 to be intact in other selected carnivores. Indeed, without a functional T1R3, neither the sweet heterodimer nor the umami heterodimer would be functional, making it problematic that such individuals could thrive.

It is known that there are large species differences in preference for nonnutritive sweeteners. Heretofore, the preference shown to aspartame was reported to be confined to Old World simians (Glaser et al. 1992, 1995, 1996). Glaser recently reported that Old World simians can taste neotame, whereas the New World simians cannot (Glaser 2007). On the other hand, sucralose is preferred by both Old World and New World simians (Hellekant et al. 1996; Danilova and Hellekant 2004). Among the 3 artificial sweeteners preferred by the lesser panda, aspartame has been the most extensively studied across many different species.

This is the first case we know of where a non-Primate recognizes and avidly consumes aspartame. The preference for 3 artificial sweeteners here in the lesser panda, and the known preference shown by Old World primates, may represent a case of convergent evolution. Such a process could reflect dietary similarities and associated similarities in selection pressures. One might assume that more precise genetic comparisons of the sweet taste receptor genes of Old World primates and the lesser panda may point to specific structural features of sweet receptors that allow them to interact with these artificial sweeteners. However, this is not the case; when comparing carnivore sequences with primate sequences (Old World and New World simians), there are no common variations that differentiate the aspartame taster species from the nontaster species. Instead, we found 16 sites that differentiate lesser panda from any of the other species listed, and these sites may be important for interaction with artificial sweeteners. It is likely that there are some other underlying mechanisms that affect the tertiary structure of the heterodimer, T1R2/T1R3. One possibility is that independent domains can be either individually activated or can respond in a cooperative manner, much as one might envision an allosteric response.

In the present study, we found the lion to have the same microdeletion as the domestic cat. We detected no deletion or stop codon across this region in the other 5 carnivore species. This observation is consistent with the suggestion that the pseudogenization of *Tas1r2* occurred within the lineage of

Felidae, after it split with Hyaenidae from common ancestor of Feliformia around 30–35 million years ago (Bininda-Emonds et al. 1999; Nowak 2005; Koepfli et al. 2006) (Figure 1).

It is likely that differences seen here in taste preference for sweet compounds are primarily dependent on quaternary structural differences in the sweet receptor. However, other mechanisms could also be involved, for example, copy number variations in taste receptor genes, changes in expression levels of taste receptors, or differences in sweet-induced signal transduction anywhere from taste cell level to the central nervous system. We are currently expanding our studies to test a wider range of species from each family of order Carnivora in order to obtain sufficient data for detailed structure–activity relationships. To evaluate other possible cellular mechanisms that may account for the differences in taste preference, we plan to conduct studies such as detecting copy number variations of the taste receptor genes, determining taste receptor expression. Ultimately, we will gain a more complete understanding of the correlation between sweet receptor gene function and sweet taste preference and how these interactions impact dietary choice.

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