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Differentiated adaptive evolution, episodic relaxation of selective constraints, and pseudogenization of umami and sweet taste genes *TAS1Rs* in catarrhine primates

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Abstract

Background: Umami and sweet tastes are two important basic taste perceptions that allow animals to recognize diets with nutritious carbohydrates and proteins, respectively. Until recently, analyses of umami and sweet taste were performed on various domestic and wild animals. While most of these studies focused on the pseudogenization of taste genes, which occur mostly in carnivores and species with absolute feeding specialization, omnivores and herbivores were more or less neglected. Catarrhine primates are a group of herbivorous animals (feeding mostly on plants) with significant divergence in dietary preference, especially the specialized folivorous Colobinae. Here, we conducted the most comprehensive investigation to date of selection pressure on sweet and umami taste genes (*TAS1Rs*) in catarrhine primates to test whether specific adaptive evolution occurred during their diversification, in association with particular plant diets.

Results: We documented significant relaxation of selective constraints on sweet taste gene *TAS1R2* in the ancestral branch of Colobinae, which might correlate with their unique ingestion and digestion of leaves. Additionally, we identified positive selection acting on Cercopithecidae lineages for the umami taste gene *TAS1R1*, on the Cercopithecinae and extant Colobinae and Hylobatidae lineages for *TAS1R2*, and on *Macaca* lineages for *TAS1R3*. Our research further identified several site mutations in Cercopithecidae, Colobinae and *Pygathrix*, which were detected by previous studies altering the sensitivity of receptors. The positively selected sites were located mostly on the extra-cellular region of *TAS1Rs*. Among these positively selected sites, two vital sites for *TAS1R1* and four vital sites for *TAS1R2* in extra-cellular region were identified as being responsible for the binding of certain sweet and umami taste molecules through molecular modelling and docking.

Conclusions: Our results suggest that episodic and differentiated adaptive evolution of *TAS1Rs* pervasively occurred in catarrhine primates, most concentrated upon the extra-cellular region of *TAS1Rs*.

Keywords: Catarrhine primates, *TAS1Rs*, Adaptive evolution, Positive selection, Episodic relaxation of selective constraints, Pseudogenization

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Background

Umami and sweet tastes are important sensations allowing animals to recognize diets with nutritious proteins and carbohydrates, respectively. In mammals, sweet and umami taste perceptions are conferred by taste receptor cells through the use of G protein-coupled receptors (GPCRs) TAS1R, which are encoded by the *TAS1R* gene family [1,2]. Of these, TAS1R1 and TAS1R2 are expressed in separate taste receptor cells, although both are co-expressed with TAS1R3. The TAS1R1 protein forms a heterodimer with TAS1R3 to form a two-part umami taste receptor, and the TAS1R2 and TAS1R3 heterodimer functions as the sweetness receptor [3,4].

The gene structure of *TAS1R* family is conserved among species [5]. Human *TAS1R* genes span from 3 kb-20 kb with 6 exons and 5 introns, and the cDNA of *TAS1R* genes consist of ~2500 bp. TAS1R proteins are characteristic of seven domains spanning the plasma membrane, which have a large N-terminal extracellular domain composed of the Venus flytrap module (VFTM) and the small cysteine-rich domain (CRD), followed by the transmembrane domain (TMD) and the C-terminal intracellular domain (CID) [6]. These domains are required for recognizing sweeteners and umami compounds, such as aspartame, neotame, monellin, cyclamate, neohesperidin dihydrochalcone, brazzein and L-amino acids [7-12]. Furthermore, studies using mutagenesis, molecular modeling and functional expression have demonstrated multiple potential binding sites in the heterodimeric receptors [8-11,13-19]. For example, it is reported that two amino acid substitutions (A110V and R507Q) in the VFTM domain of TAS1R1 and two substitutions (F749S and R757C) in the TMD domain of TAS1R3, severely impair the response to monosodium glutamate (MSG) in humans [17]. Above all, genetic factors have been shown to play a crucial role in the variability of sensitivity to tastants.

Until recently, analyses of umami and sweet taste receptors have been performed on various domestic and wild animals. For example, *TAS1R1* is a pseudogene in the herbivorous giant panda (family Ursidae) [20,21], and the *TAS1R2* gene is inactivated in cats (family Felidae), vampire bats, chickens, zebra finches, the western clawed frog, and some carnivorous mammals [5,22,23]. However, research on the evolution of sweet and umami taste genes revealed that taste perception of sweet and umami is not be as conserved as previously thought, and the structural integrity of *TAS1R* is sometimes inconsistent with the known functions of these genes and the tastes involved [24]. For example, although *TAS1R2* is a pseudogene in some species of carnivores, some other obligate carnivores (such as ferrets and Canadian otters) still possess an intact *TAS1R2* gene [25]. Moreover, *TAS1R2* is absent in all bird genomes sequenced thus far, irrespective of their diet [21]. These puzzling cases indicate that our understanding of

the physiological functions of sweet and umami tastes and/or their receptor genes is far from complete [24].

While most previous studies in mammals focused on the pseudogenization of taste genes which occur mostly in carnivores and species with absolute feeding specialization, omnivores and herbivores were more or less neglected, except for the systematic study of bats and the giant panda [26]. Additionally, it is known that plant foods usually contain much more complex and variable taste-inducing compounds than animal food [27,28]. The taste system of omnivores and herbivores may be more complex than in carnivores, and pseudogenization of taste genes is less possible to occur massively in omnivores and herbivores (except for the giant panda). To thoroughly understand the physiological functions and adaptive evolution of sweet and umami tastes and/or their receptor genes, more comprehensive studies should be performed in omnivorous and herbivorous animals with close phylogenetic relationships.

Catarrhine primates are a group of herbivorous animals (feeding mostly on plants) with a significant divergence in dietary preference. The Colobinae, also called leaf-monkeys, feed predominantly on relatively low-energy leaves and other plant parts [29], and are unique among primates in that they have a complex stomach to permit efficient digestion of leaves [30]. By contrast, the Cercopithecinae feed predominantly on relatively high-energy foods such as fruits, seeds, insects, and vertebrates. Gibbons are fruit-pulp specialists, and the foods eaten by the great apes and modern humans generally include a wide variety of items such as fruits, assorted types of vegetation, bark, seeds, insects and meat, although the great apes are predominantly frugivorous. Consequently, as a group of animals including omnivores and herbivores, catarrhine primates exhibit a certain degree of diet specialization and differentiation, which makes them ideal objects for studying the evolution of sweet and umami taste genes.

As diets have evolved and differentiated during the radiation of catarrhine primates, presumably tastes have responded adaptively in order to maximize energy intake. Species with the highest taste sensitivity for sugars and other soluble nutrients tend to improve foraging efficiency, which could be the target of natural selection. Thus, we predicted that the umami and sweet taste genes of species in catarrhine primates with different diets have experienced variation in selection pressure through evolutionary history. To test this hypothesis, we performed a comprehensive investigation of evolution in *TAS1R* genes for representative species of herbivorous catarrhine primates. Our research aimed to explore possible specific adaptive evolution of *TAS1R* in catarrhine primates with known dietary specializations, and to enrich our understanding of the physiological functions of sweet and umami tastes receptor genes.

Results

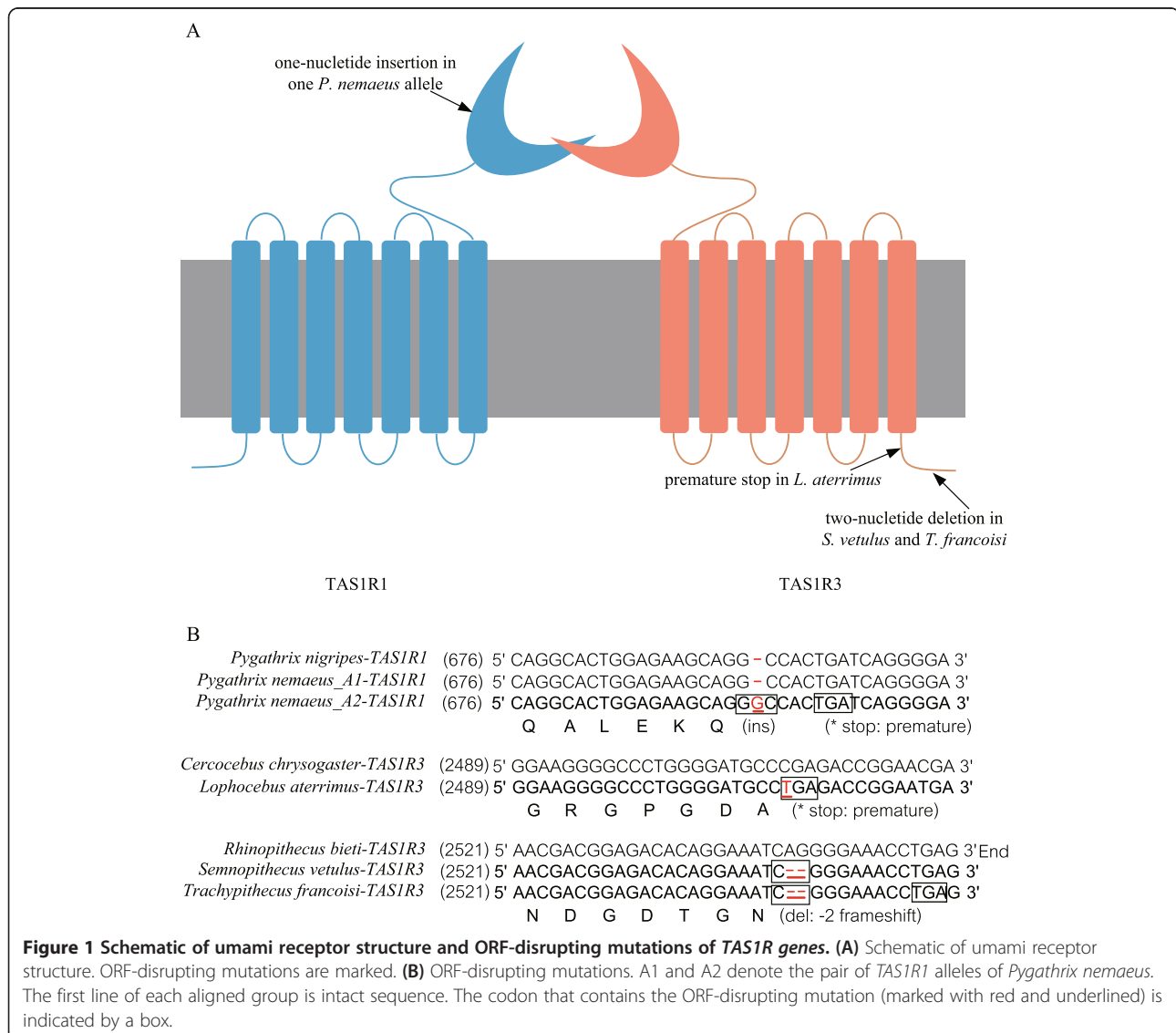
Characterization of the *TAS1R* genes in catarrhine primates

Complete coding regions of *TAS1R* were obtained from 30 catarrhine primate species. Most of them were highly conservative without premature stop codons or frame shift mutations. In total, 2523 bp, 2517 bp and 2556 bp DNA sequences were generated from six exons of *TAS1R1*, *TAS1R2* and *TAS1R3*, respectively. Additionally, we also downloaded available *TAS1R* sequences of Hominidae from GenBank: all *TAS1R* of *Pan troglodytes*, *Homo sapiens* and *Gorilla gorilla gorilla*; *TAS1R1* and *Tas1r2* of *Pan paniscus*; *TAS1R1* and *TAS1R3* of *Pongo abelii*; *Tas1r2* and *TAS1R3* of *Pongo pygmaeus* (see Additional file 1). Alignments of *TAS1R* revealed a total of 334 (13.24%), 398 (15.81%) and 469 (18.35%) variable sites for *TAS1R1*, *TAS1R2* and *TAS1R3*, respectively. Among these sites, 243, 318 and 414 synonymous mutations were found in *TAS1R1*, *TAS1R2*

and *TAS1R3*, respectively. Correspondingly, 185, 263 and 269 non-synonymous mutations were detected in *TAS1R1*, *TAS1R2* and *TAS1R3*, which revealed the most variable sites but the lowest non-synonymous ratio of *TAS1R3* (see Additional file 2). The alignments of amino acid sequences are provided as Additional files 3, 4 and 5.

ORF-disrupting mutations in *TAS1R* genes

The open reading frame (ORF) of *TAS1R* was disrupted in some species of Cercopithecoidea, including insertions, deletions and transitions (Figure 1). Both of two individuals of *Pygathrix nemaues* had an allele of *TAS1R1* with an insertion of a G in exon 3 (nucleotide position 695, codon number 232), leading to a frame shift mutation and a premature stop codon. Thus, *TAS1R1* is likely a pseudogene in *P. nemaues*, resulting in the truncation of the protein in the extracellular N-terminus (VFTM).



However, this gene is intact in all other *Pygathrix* species *P. nigripes* and *P. cinerea*, indicating a recent origin of this pseudogene. *Trachypithecus francoisi* and *Semnopithecus vetulus* share a 2-nucleotide deletion at the very end of exon 6 (nucleotide position 2,543-2,544, codon number 848) of *TAS1R3*, leading to three amino acid substitutions and a two amino acid residue shorter C-terminal intracellular domain (CID) in comparison with other species. Thus, this mutation of *TAS1R3* was suggested to occur in the common ancestor of *Semnopithecus* and *Trachypithecus*, which are the closest genera in Colobinae. Interestingly, a premature stop codon was also found in *TAS1R3* of *Lophocebus*

aterrimus because of a transition from C to T in exon 6 (nucleotide position 2,512, codon number 838). All these mutations were confirmed by multiple PCR experiments and colonies of different cloning procedures.

Relaxation of selective constraint on *TAS1R* genes within catarrhine primates

In the branch model, the free-ratio model was detected to be significantly different from the one-ratio model only for *TAS1R2*, indicating that d_N/d_S ratios among lineages of *TAS1R2* were different (Table 1). These ratios varied from 0 ($d_N = 0$) to infinity ($d_S = 0$). Then, the two-ratio model was

Table 1 CODEML analyses of selective pattern on the *TAS1R2* in catarrhine primates

Models	lnL	Compared	2ΔlnL	P-value	Parameter estimates
Branch model					
M0:one-ratio	-7258.8072				$\omega = 0.1608$
M1:free-ratio	-7211.2267	M0 vs. M1	95.1610	$p < 0.05$	
Foreground branch: ancestral Colobinae					
H1: ω_0, ω_1	-7256.0276	M0 vs. H1	5.5592	$p < 0.05$	$\omega_0 = 0.1548, \omega_1 = 0.5583$
H0: $\omega_0, \omega_1 = 1$	-7256.4033	H0 vs. H1	0.7514	$p > 0.05$	$\omega_0 = 0.1543, \omega_1 = 1.0000$
Foreground branch: ancestral <i>Hylobates</i>					
H1: ω_0, ω_1	-7256.8606	M0 vs. H1	3.8932	$p < 0.05$	$\omega_0 = 0.1570, \omega_1 = 0.5554$
H0: $\omega_0, \omega_1 = 1$	-7257.1816	H0 vs. H1	0.6420	$p > 0.05$	$\omega_0 = 0.1570, \omega_1 = 1.0000$
Site model					
M1a	-7149.5623				$p_0 = 0.8609, p_1 = 0.1391, \omega_0 = 0.0451, \omega_1 = 1.0000$
M2a	-7145.1097	M1a vs. M2a	8.9052	$p < 0.05$	$p_0 = 0.8669, p_1 = 0.1198, p_2 = 0.0133, \omega_0 = 0.0499, \omega_1 = 1.0000, \omega_2 = 3.6223$
M8a	-7149.3577				$p_0 = 0.8725 (p_1 = 0.1275), p = 0.4874, q = 7.5964, \omega = 1.0000$
M8	-7143.4448	M8a vs. M8	11.8258	$p < 0.01$	$p_0 = 0.9744 (p_1 = 0.0257), p = 0.1750, q = 1.1154, \omega = 2.8718$
Branch-site model					
Lineages of Cercopithecidae					
Null	-7146.1093				$p_0 = 0.8463, p_1 = 0.0953, p_{2a} = 0.0525, p_{2b} = 0.0059, \omega_0 = 0.0407, \omega_1 = 1.0000, \omega_2 = 1.0000$
Alternative	-7144.2553		3.7080	$p < 0.05$	$p_0 = 0.8683, p_1 = 0.0924, p_{2a} = 0.0354, p_{2b} = 0.0038, \omega_0 = 0.0481, \omega_1 = 1.0000, \omega_2 = 2.0345$
Lineages of Colobinae					
Null	-7145.5063				$p_0 = 0.8299, p_1 = 0.1176, p_{2a} = 0.0460, p_{2b} = 0.0065, \omega_0 = 0.0396, \omega_1 = 1.0000, \omega_2 = 1.0000$
Alternative	-7143.7154		3.5818	$p < 0.05$	$p_0 = 0.8542, p_1 = 0.1162, p_{2a} = 0.0261, p_{2b} = 0.0036, \omega_0 = 0.0428, \omega_1 = 1.0000, \omega_2 = 2.4997,$
Lineages of Cercopithecinae					
Null	-7148.7133				$p_0 = 0.8512, p_1 = 0.1292, p_{2a} = 0.0170, p_{2b} = 0.0026, \omega_0 = 0.0440, \omega_1 = 1.0000, \omega_2 = 1.0000$
Alternative	-7143.4285		10.5696	$p < 0.01$	$p_0 = 0.8639, p_1 = 0.1228, p_{2a} = 0.0116, p_{2b} = 0.0017, \omega_0 = 0.0466, \omega_1 = 1.0000, \omega_2 = 5.8766$
Lineages of Hylobatidae					
Null	-7149.1766				$p_0 = 0.8489, p_1 = 0.1326, p_{2a} = 0.0161, p_{2b} = 0.0025, \omega_0 = 0.0442, \omega_1 = 1.0000, \omega_2 = 1.0000$
Alternative	-7143.6646		11.0240	$p < 0.01$	$p_0 = 0.8612, p_1 = 0.1297, p_{2a} = 0.0079, p_{2b} = 0.0012, \omega_0 = 0.0462, \omega_1 = 1.0000, \omega_2 = 11.8832$

used to identify whether lineages with $0.50 < \omega < 1$ (detected by free-ratio) had undergone different selection pressure from other lineages, which was addressed as hypothesis 1 (H1) in Table 1. After comparing with the one-ratio model, we found that selection pressure acting on the ancestral lineage of Colobinae for *TASIR2* (branch ACo in Figure 2) was significantly different from other lineages ($\omega_1 = 0.5583$, $p < 0.05$, Table 1). We also hypothesized a fixing $\omega_1 = 1$ for the ancestral lineage of *TASIR2* in Colobinae (H0, Table 1), and H1 was not significantly supported compared with H0. The analysis revealed that selection constraints were relaxed on *TASIR2* of ancestral Colobinae, but it was not totally removed. The relaxation of selective constraints was also found in *TASIR1* of the common ancestor of human, chimpanzee and gorilla ($\omega_1 = 0.7772$, $p < 0.05$, Table 2, Figure 3), and in *TASIR2* of the common ancestor of the genus *Hylobates* ($\omega_1 = 0.5554$, $p < 0.05$, Table 1, Figure 2).

Positively selected sites on *TASIR* genes within catarrhine primates

In site models, LRTs showed that the incorporate selections (i.e. M2a and M8) fitted significantly better than

neutral models (i.e. M1a and M8a) for *TASIR1* and *TASIR2*, whereas no significant evidence of positive selection was found for *TASIR3* (Tables 1, 2 and 3). In the M2a model, six and one sites for *TASIR1* and *TASIR2*, respectively, were under positive selection. Model M8 revealed six and 10 positively selected sites for *TASIR1* and *TASIR2*, respectively, identified by the BEB approach with posterior probabilities larger than 0.85 (see Additional file 6).

The branch-site model was then used to test for positive selection in potential codons in lineages of separate groups of catarrhine primates, i.e., in Cercopithecoidea, Cercopithecoidea, Colobinae, Hylobatidae, and Hominoidea (Tables 1, 2 and 3). The LRT results showed that Hylobatidae-specific lineages for *TASIR2*, and Cercopithecoidea-specific lineages for both *TASIR1* and *TASIR2* were subjected to strong positive selection. Further analysis indicated that lineages of Cercopithecoidea and Colobinae for *TASIR2* (Figure 2) and *Macaca*-specific lineages for *TASIR3* were also under strong positive selection (Figure 4). In addition, 11 codons (*TASIR1*: 391; *TASIR2*: 8, 21, 175, 404, 411, 413, 510 and 733; *TASIR3*: 195 and 225) were also

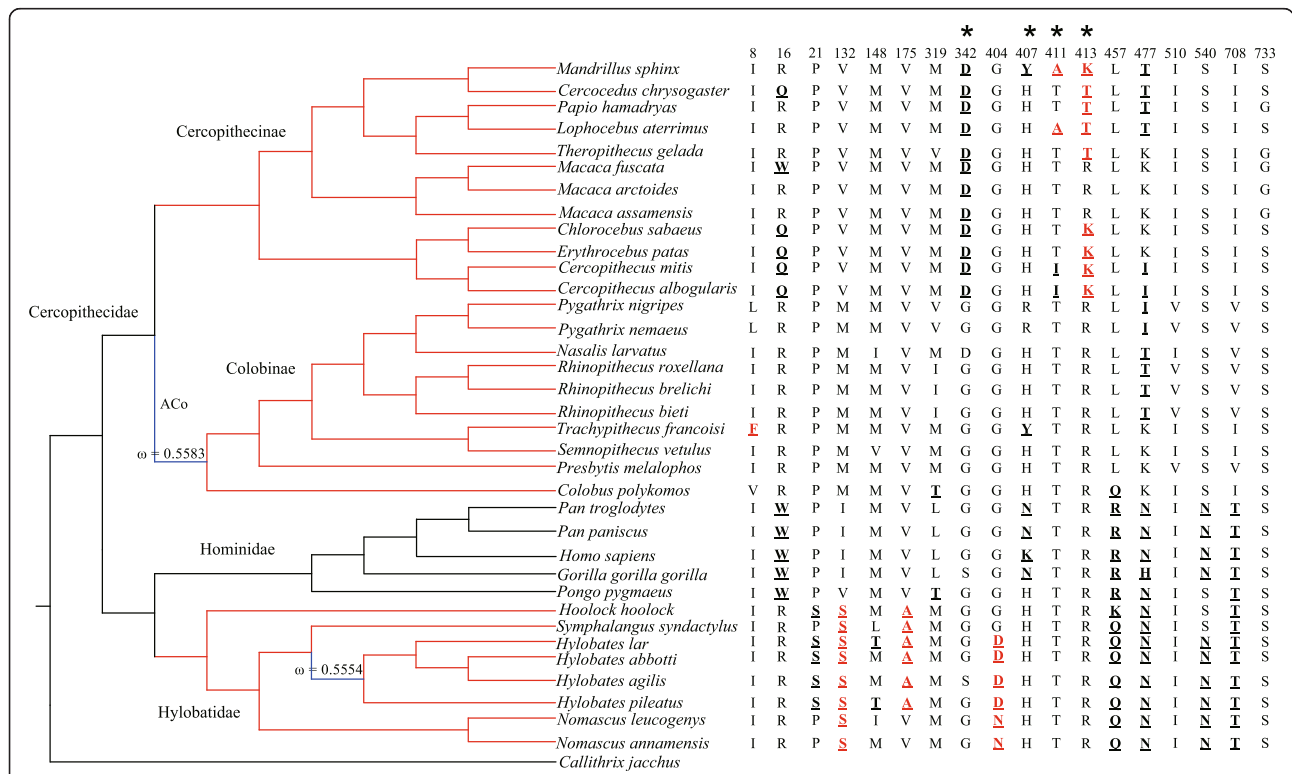


Figure 2 Radical amino acid changes in selected sites across the catarrhine primates phylogeny of *TASIR2*. The input tree used for pressure analysis of *TASIR2* is shown on the left. Positively selected sites detected by site models and branch-site models are shown on the right. Radical changes are shown in bold and underlined, and changes detected by branch-site models are marked in red additionally. *Callithrix jacchus* was used as outgroup, but not included in the CODEML analysis. Lineages under positive selection are marked in red, while lineages experienced relaxation of selective constraints are marked in blue.

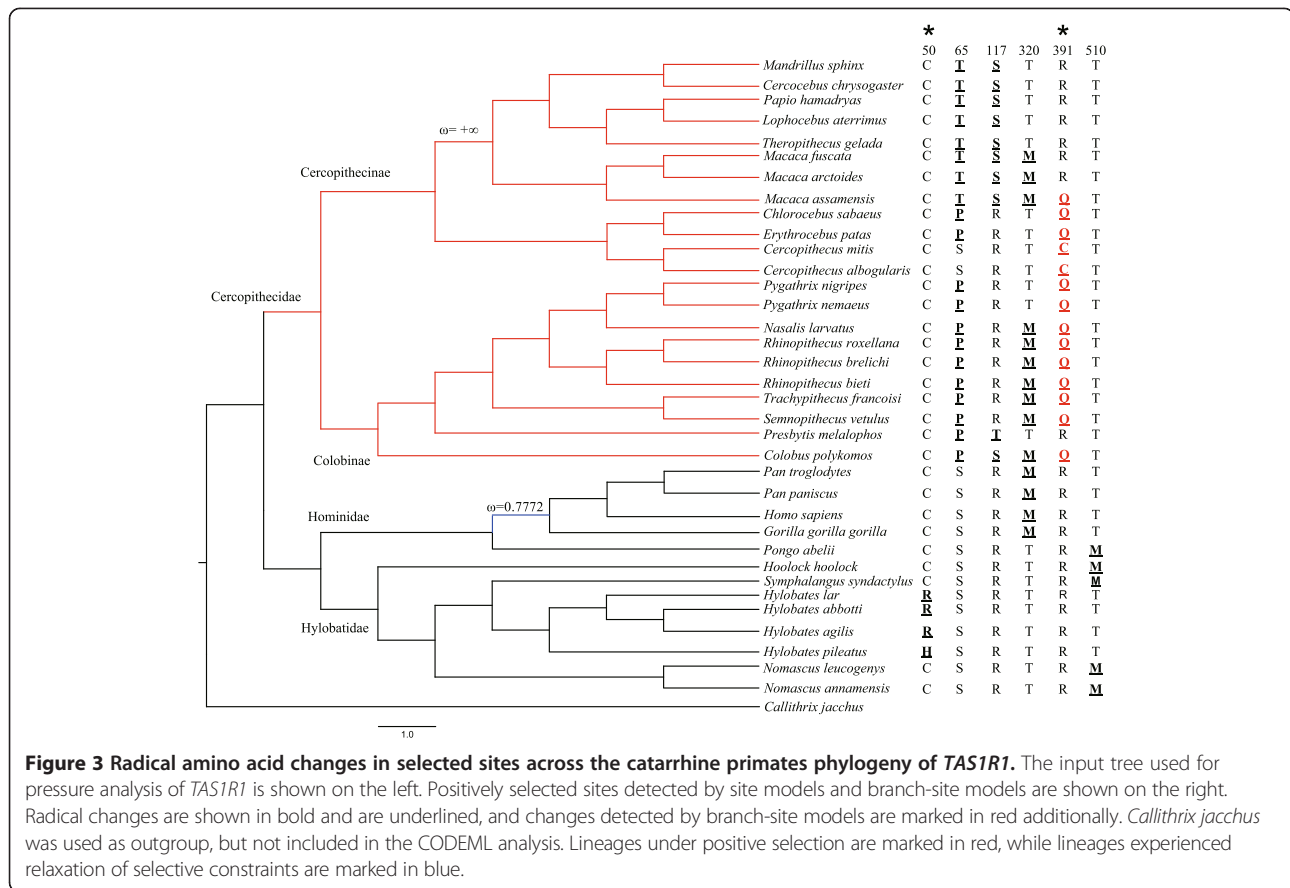
Table 2 CODEML analyses of selective pattern on the *TAS1R1* in catarrhine primates

Models	lnL	Compared	2ΔlnL	P-value	Parameter estimates
Branch model					
M0:one-ratio	-6347.5127				$\omega = 0.2486$
M1:free-ratio	-6316.3339	M0 vs. M1	62.3576	$p > 0.05$	
Foreground branch: all lineages of Cercopithecidae except for the ancestral branch					
H1: ω_0, ω_1	-6344.8700	M0 vs. H1	5.2854	$p < 0.05$	$\omega_0 = 0.3112, \omega_1 = 0.1981$
H0: $\omega_0, \omega_1 = 1$	-6412.3663	H0 vs. H1	134.9926	$p < 0.01$	$\omega_0 = 0.3149, \omega_1 = 1.0000$
Foreground branch: ancestral of <i>Mandrillus</i> , <i>Cercocebus</i> , <i>Papio</i> , <i>Lophocebus</i> , <i>Theropithecus</i> and <i>Macaca</i>					
H1: ω_0, ω_1	-6343.4866	M0 vs. H1	8.0522	$p < 0.01$	$\omega_0 = 0.2419, \omega_1 = +\infty$
Foreground branch: ancestral of <i>Pan</i> , <i>Homo</i> and <i>Gorilla</i>					
H1: ω_0, ω_1	-6345.0477	M0 vs. H1	4.9300	$p < 0.05$	$\omega_0 = 0.2379, \omega_1 = 0.7772$
H0: $\omega_0, \omega_1 = 1$	-6345.1667	H0 vs. H1	0.2380	$p > 0.05$	$\omega_0 = 0.2379, \omega_1 = 1.0000$
Site model					
M1a	-6316.1817				$p_0 = 0.8312, p_1 = 0.1688, \omega_0 = 0.0859, \omega_1 = 1.0000$
M2a	-6310.5399	M1a vs. M2a	11.2836	$p < 0.01$	$p_0 = 0.8708, p_1 = 0.1159, p_2 = 0.0133, \omega_0 = 0.1137, \omega_1 = 1.0000, \omega_2 = 4.7335$
M8a	-6316.2094				$p_0 = 0.8318(p_1 = 0.1682), p = 9.4660, q = 99.0000, \omega = 1.0000$
M8	-6310.7199	M8a vs. M8	10.9790	$p < 0.01$	$p_0 = 0.9794(p_1 = 0.0206), p = 0.4478, q = 1.7622, \omega = 4.0147$
Branch-site model					
Lineages of Cercopithecidae					
Null	-6316.1817				$p_0 = 0.8312, p_1 = 1688, p_{2a} = 0.0000, p_{2b} = 0.0000, \omega_0 = 0.0859, \omega_1 = 1.0000, \omega_2 = 1.0000$
Alternative	-6308.6453		15.0728	$p < 0.01$	$p_0 = 0.8384, p_1 = 1583, p_{2a} = 0.0027, p_{2b} = 0.0005, \omega_0 = 0.0936, \omega_1 = 1.0000, \omega_2 = 13.4610$

$+\infty$ means infinite, and in this case $d_s=0$ thus $\omega(d_N/d_S)=+\infty$.

Table 3 CODEML analyses of selective pattern on the *TAS1R3* in catarrhine primates

Models	lnL	Compared	2ΔlnL	P-value	Parameter estimates
Branch model					
M0:one-ratio	-7676.8782				$\omega = 0.1342$
M1:free-ratio	-7638.3982	M0 vs. M1	76.9600	$p > 0.05$	
Site model					
M1a	-7623.3051				$p_0 = 0.8913, p_1 = 0.1087, \omega_0 = 0.0632, \omega_1 = 1.0000$
M2a	-7623.3051	M1a vs. M2a	0.0000	$p > 0.05$	$p_0 = 0.8913, p_1 = 0.0609, p_2 = 0.0478, \omega_0 = 0.0632, \omega_1 = 1.000, \omega_2 = 1.0000$
M8a	-7619.6648				$p_0 = 0.9461(p_1 = 0.0539), p = 0.2391, q = 1.8178, \omega = 1.0000$
M8	-7619.7175	M8a vs. M8	0.1054	$p > 0.05$	$p_0 = 0.9678(p_1 = 0.0322), p = 0.2634, q = 1.9053, \omega = 1.1121$
Branch-site model					
Lineages of <i>Macaca</i>					
Null	-7622.2362				$p_0 = 0.8389, p_1 = 0.1004, p_{2a} = 0.0543, p_{2b} = 0.0065, \omega_0 = 0.0619, \omega_1 = 1.000, \omega_2 = 1.0000$
Alternative	-7620.4248		3.6228	$p < 0.05$	$p_0 = 0.8877, p_1 = 0.1058, p_{2a} = 0.0059, p_{2b} = 0.0007, \omega_0 = 0.0621, \omega_1 = 1.000, \omega_2 = 21.4208$



identified by the BEB approach with posterior probabilities larger than 0.85 in branch-site analysis (see Additional file 6). Finally, most of these changes detected by both site models and branch-site models were identified to be critical (see Additional file 6).

Prediction of amino acid sites of TAS1R1 and TAS1R2 responsible for umami and sweet taste molecules binding

In order to examine whether positively selected sites detected in this study were responsible for binding of some umami and sweet taste molecules, we built homology models of TAS1R1 and TAS1R2 VFTM domain and docked with some sweet and umami taste molecules (Figure 5). Then, we got a series of docking results and found that most of these molecules bound with taste receptors at specific regions of TAS1R1 and TAS1R2. Finally, we mapped positive selection sites detected in our study onto models of TAS1R1 and TAS1R2 and found that sites 50 and 391 of TAS1R1 may interact with inosine monophosphate (IMP) and L-glutamate (see Additional file 7). Similarly, sites 342, 407, 411 and 413 of TAS1R2 were also found to be within binding domains of D-tryptophan, D-galactose, D-glucose, fructose, galactose and sucrose (see Additional file 8).

Critical sites influencing the response of TAS1R genes to tastants

Previous functional expression data showed that mutations in two amino acid residues (S40T and I67S) of TAS1R2 were predicted to affect receptor response to aspartame and neotame, i.e., S40T abolished aspartame sensitivity and I67S reduced response to neotame slightly [31] (Table 4). Investigation of the amino acid residue 40 of TAS1R2 revealed a serine residue (S) in Hylobatidae and Hominiidae, but a threonine residue (T) in Cercopithecoidea. Furthermore, residue 67 is isoleucine (I) in most species, but methionine (M) in Colobinae (Table 4). Substitution at residue 733 (733A in Hylobatidae and Hominiidae, 733 V in Cercopithecoidea) of TAS1R3 was previously suggested to reduce its sensitivity to lactisole [32]. Furthermore, substitution R757C in human TAS1R3 seems to reduce responses to monosodium glutamate (MSG) and to increase binding with monopotassium glutamate (MPG) [13,16] (Table 4). This site shows histidine (H) in the genus *Pygathrix* and arginine (R) in other species (Table 4).

Discussion

The present study conducted the most comprehensive investigation to date of selection pressure on sweet and

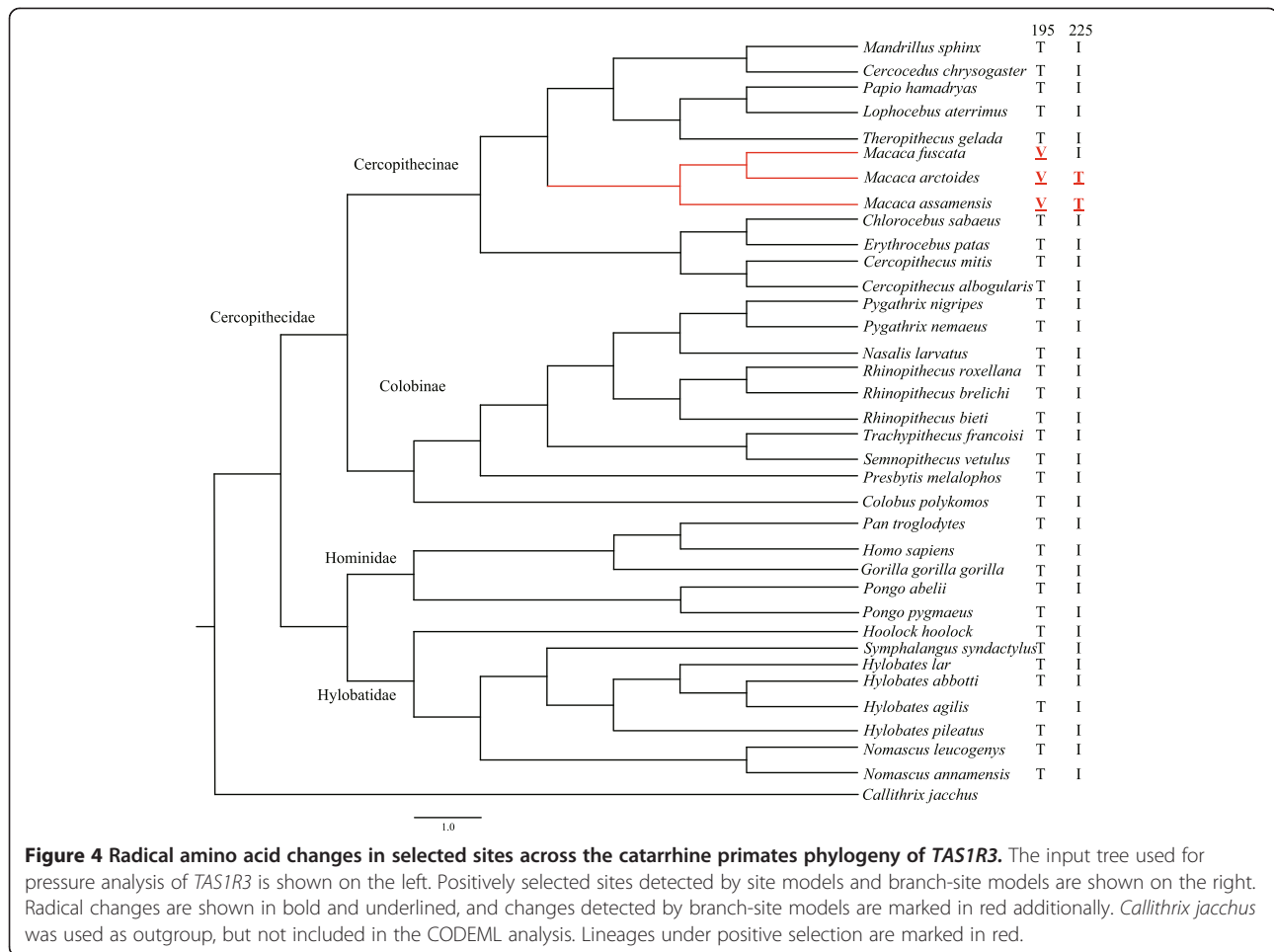


Figure 4 Radical amino acid changes in selected sites across the catarrhine primates phylogeny of *TAS1R3*. The input tree used for pressure analysis of *TAS1R3* is shown on the left. Positively selected sites detected by site models and branch-site models are shown on the right. Radical changes are shown in bold and underlined, and changes detected by branch-site models are marked in red additionally. *Callithrix jacchus* was used as outgroup, but not included in the CODEML analysis. Lines under positive selection are marked in red.

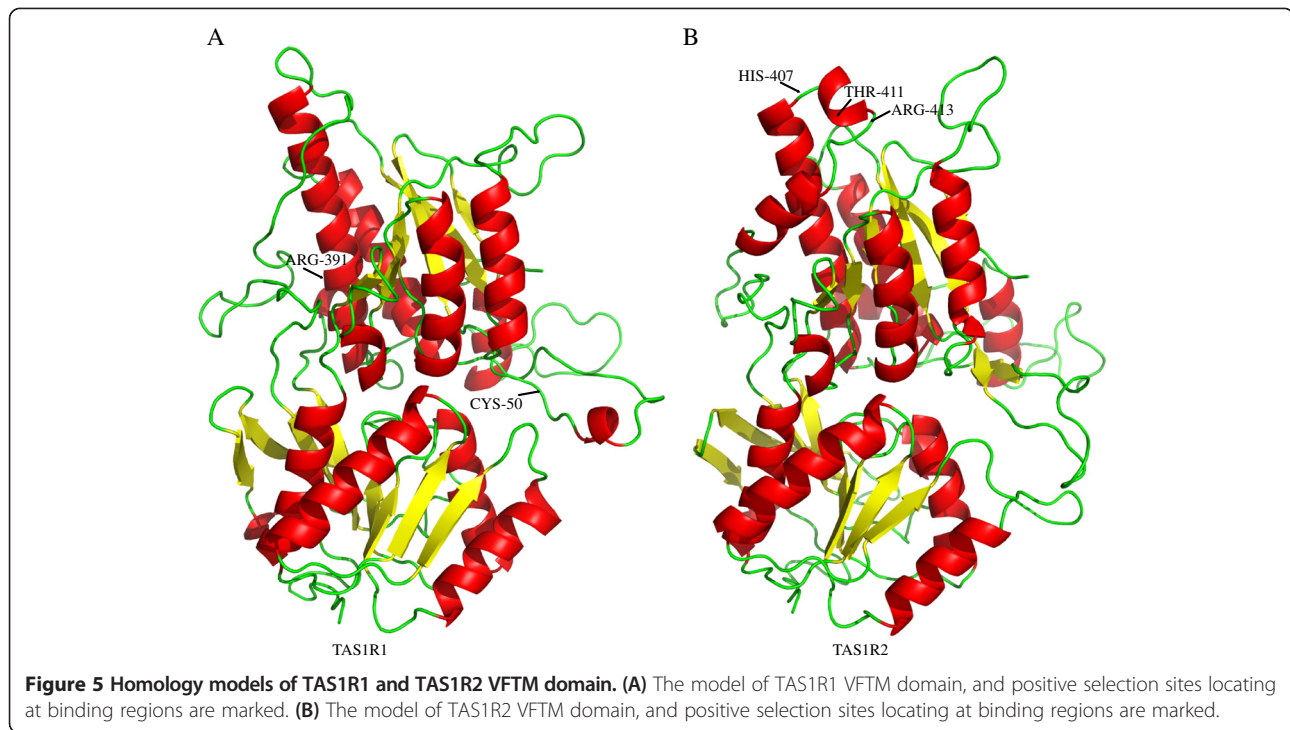
umami taste genes (*TAS1Rs*) in catarrhine primates to test whether specific adaptive evolution occurred during their diversification in association with particular plant diets. Our results support differentiated evolution and episodic relaxation of selective constraints on *TAS1R* in herbivorous/omnivorous catarrhine primates.

Non-massive pseudogenization of *TAS1Rs* in catarrhine primates

As predicted above, no massive pseudogenization of *TAS1Rs* in catarrhine primates were founded in our data. However, disrupting mutations of *TAS1Rs* were revealed in *P. nemaues*, *S. vetulus*, *T. francoisi* and *L. aterrimus*, which were supposed to influence species-specific functional differences in sweet and/or umami tastes (Figure 1). In the two species of *Pygathrix* (*P. nigripes* and *P. nemaues*) tested in this study, one pseudogenized allele resulting in the truncation of the protein in VFTM was found in *TAS1R1* of *P. nemaues*, which was otherwise intact in *P. nigripes*. Subsequent analysis of the *TAS1R1* gene in *P. cinerea* (data not shown) confirmed that this pseudogenized allele occurred only in *P. nemaues*. Thus,

considering their close phylogenetic relationships, the cause and biological influence of this one-allele pseudogenization of *TAS1R1* seems to be informative for a comparative analysis of *Pygathrix*. This pseudogenization of one *TAS1R1* allele in *P. nemaues* likely weakens the biological function of this gene and decreases categories of discernible umami compounds. Suggestively, *P. nemaues* shows the lowest diversity of recorded consumed plants compared to *P. cinerea* and *P. nigripes* [33].

Unlike the pseudogenized allele of *TAS1R1* in *P. nemaues* resulting in truncation of the protein in VFTM, the two-nucleotide deletion of *TAS1R3* in *S. vetulus* and *T. francoisi* and the transition from C to T of *TAS1R3* in *L. aterrimus* occurred in the C-terminus of *TAS1R3* and led to a premature stop codon in C-terminal intracellular domain (CID). Until now, mutations in VRTM, CRD, TMD of *TAS1Rs* have been reported to influence the sensitivity of *TAS1Rs* to a variety of substances [13,15,17,31]. However, the d_N/d_S ratio is significantly lower than 1 for CID in rodent *TAS2R* genes, demonstrating the operation of purifying selection and a vital role of CID [34]. The experimental verification of the function of CID for *TAS1Rs* is lacking,



and the influences of these mutations on the CID of TAS1R3 await further research.

Pseudogenization of *TAS1R* genes has been reported in various species with dietary specialization, such as the giant panda (*Ailuropoda melanoleuca*), vampire bats (*Desmodus* spp.) and whales (Cetacea) [21,23,24]. Although the Colobinae display a certain degree of dietary specialization and feeds mainly on leaves, no massive loss of *TAS1R* genes was found. Furthermore, the pseudogene of *TAS1R2* found in *P. nemaus* was heterozygous. For Cercopithecidae, the premature stop codons occurring in CID of *TAS1R3* in *S. vetulus*, *T. francoisi* and *L. aterrimus* seemed not to disrupt the main structure of *TAS1R3*, as they only shortened the C-terminus. While, as endangered species, it is difficult to get living organisms for experimental analysis to examine whether these stop codons would terminate *TAS1R3* function in these species. But as the only species of catarrhine primates with amino acids length mutations in such sequence conservative *TAS1Rs*, formation mechanisms and biology function of these mutations are interesting and need further research. Moreover, neither pseudogene nor premature stop codon was found in Hylobatidae, which are typical frugivores. These results imply a functional role of *TAS1R* genes in most (or even all) species of catarrhine primates.

Episodic relaxation of selective constraints and subsequent positive selection on *TAS1R2* in leaf-eating Colobinae

As specific leaf-eating primates, Colobinae attracted our primary attention in this study. The lineage-specific

analysis showed that the ancestral *TAS1R2* of Colobinae (branch ACo in Figure 2) has undergone significantly different selection pressure and relaxation of selective constraints ($\omega_1 = 0.5583$, $p < 0.05$) compared with other lineages (Table 1). The folivorous colobines mostly prefer leaves, and some unripe fruits with low simple and water-soluble sugars; microbes in the foregut of colobines can also degrade crude fibers of leaves and produce sugar, proteins and vitamins [35,36]. Additionally, colobines can recover nutrients by breaking down and digesting bacteria in a true stomach using various enzymes [37,38]. Lysozyme, one of those bacteriolytic enzymes, was documented to be under positive natural selection in a common ancestor of the foregut-fermenting colobine monkeys, which coincided with the establishment of leaf-eating and foregut-fermentation [39]. Therefore, sugar content in food was apparently not very important for ancestral colobines. Moreover, colobines are vulnerable to dietary change because the foregut microbes are not buffered by acidic defenses of the stomach [40]; the high simple sugar content of ripe and pulpy fruits will lower fore-stomach pH value, which can disrupt fermentation and induce bloating or acidosis, resulting in death [41-44]. As a consequence, the function or sensitivity of sweet taste may have been less necessary for the common ancestor of Colobinae, and selection pressure acting on functional sweet taste gene underwent a certain degree of relaxation. Consistent with this assumption, we indeed detected such a relaxation in the ancestral Colobinae *TAS1R2* gene. In addition, we also

Table 4 Functional TAS1R binding sites for sweet and umami tastants from previous studies and the mutation in catarrhine primates

Compounds	Interact receptors	Amino acid mutations	Results	Mutations in catarrhine monkeys	References
Aspartame	hTAS1R2 ¹	S40T	abolish aspartame sensitivity	S40T in Cercopithecidae	Liu et al. [31]
Neotame	hTAS1R2	I67S	reduce response to neotame slightly	I67M only in Colobinae	Liu et al. [31]
Lactisole	hTAS1R3	A733V	reduce sensitivity to lactisole	A733V in Cercopithecidae	Jiang et al. [32]
Monosodium glutamate (MSG)	hTAS1R3	R757C	reduce sensitivity to MSG	R757H in <i>Pygathrix</i>	Raliou et al. [16]
Monopotassium glutamate (MPG)	hTAS1R3	R757C	increase sensitivity to MPG	R757H in <i>Pygathrix</i>	Chen et al. [13]

¹h denotes human.

found fourteen specific amino acid substitutions in the ancestral *TAS1R2* gene of Colobinae (I67M, E118D, D119N, D225N, P348S, E423K, I436L, S547R, M616V, A635V, A649T, T686M, M697I and V741L) through alignment with the ancestral *TAS1R2* of Cercopithecidae and Cercopithecinae. Interestingly, except for D225N and A649T, all other amino acid substitutions were unique and fixed in colobine species, suggesting that these amino acid changes reflect a necessary adaption to their specific diet.

Interestingly, branch-site analysis indicated that *TAS1R2* was subjected to strong positive natural selection ($\omega_2 = 3.5818$, $p < 0.05$) in lineages of extant colobines, and also suggested substitutions I8F and I510V (posterior probability ≥ 0.850) as candidate sites for selection (Table 1). It seems to be a paradox that *TAS1R2* would have undergone positive selection in extant leaf-eating colobines, which prefer low simple and water-soluble sugars. However, they actually prefer young leaves and only few fruits (especially ripe fruits), which contain certain proportion of proteins and sugars and are also preferred by other catarrhine primates [43-46]. It has been reported that *P. cinerea* eat 49.5% young leaves, 21.9% ripe fruits, 19.1% unripe fruits and only 9.3% mature leaves; the nutritional component in selected food of *P. cinerea* consists of protein at 11.4%, dry matter, lipids at 2.6%, minerals at 5.0%, sugars at 4.9%, starch at 12.8%, and neutral detergent fibers at 40.8% [33]. The sense of sweetness is associated with sugars, some amino acids, a few proteins, and glucosides [47-49]. Perhaps the sensitivity of *TAS1R2* for sweeteners in foods is essential for extant colobines. After the establishment of leaf-eating and foregut-fermenting, extant colobines would have undergone further selection to detect new nutrient substance through *TAS1R2*.

Positive selection of *TAS1R1* in Cercopithecidae

The branch-site model revealed that the *TAS1R1* lineage of all Cercopithecidae underwent positive selection, and that substitutions R391C and R391Q are candidate sites for selection. Primates can sense the presence of protein through the taste of umami, which is elicited by glutamate and glutamic acid [50,51]. Commonly incorporated in leaf protein, glutamic acid is also reported from nectar

and figs [52,53]. Besides dietary differences between Cercopithecinae and Colobinae, both groups also require proteins in plant foods (and animal foods). According to Yeager & Kool [54], colobines select foods of high nutritional value, and young leaves are preferred over mature leaves. Young leaves contain more protein and less fiber than mature leaves [44]. Furthermore, it was reported that *Rhinopithecus bieti* tended to choose leaves with high content of crude protein, as well as insects and even birds [55]. Thus, it is assumed that the taste of umami is important not only for Cercopithecidae but also for Colobinae.

Positive selection of *TAS1R2* in Cercopithecinae

Branch-site analysis showed that *TAS1R2* in Cercopithecinae is subject to strong positive natural selection ($\omega_2 = 5.8766$, $p < 0.01$), and the substitutions T411A, R413I and R413K (posterior probability ≥ 0.850) were indicated as candidate sites for selection (Table 1). However, compared to colobines, for which *TAS1R2* is also under positive natural selection, the candidate sites of positive natural selection in Cercopithecinae are different. The functions of the different positively selected sites of *TAS1R2* in Cercopithecinae and Colobinae await further research, and perhaps these mutations have changed sensitivity for certain substances. It might reflect the common importance of sweetness for Cercopithecinae and extant colobines, along with variation in types and proportions of different kind of sweet substances.

Positive selection of *TAS1R3* in Macaques

TAS1R3 showed positive natural selection in the genus *Macaca*. As described above, *TAS1R3* is the most conserved member among the *TAS1R* gene family due to its basic function in both sweet and umami taste receptors. Therefore, as the only group of lineages in which *TAS1R3* was detected to be under strong positive natural selection, the genus *Macaca* was suggested to possess special sweet and umami tastes and food perceptions different to that of other taxa. The genus *Macaca* represents one of the most successful primate radiations. While most cercopithecines are confined in Africa,

except for the Barbary macaque of northern Africa [56], macaques are widely distributed in Asia, north to Japan and west to Afghanistan, which represented the only Asian lineage in Cercopithecinae [57-59]. According to fossil data, the earliest macaque originated in northeast Africa around 7 million years ago [60,61], and spread through most of Eurasia [62]. Now, they are found in a wide range of habitats, from evergreen forests to grassland and even areas modified by humans, from tropical forests to temperate ecosystems, and from continents to deep-water islands [57]. This range is likely reflected in dietary diversity as well.

Positive selection of *TAS1R2* in Hylobatidae

Branch-site analysis showed that lineages of Hylobatidae were under significant positive natural selection for *TAS1R2* ($\omega_2 = 11.8832$, $p < 0.01$), indicating adaptive evolution of sweet taste in gibbons. As well-documented frugivores, fruits (especially ripe fruits), account for more than 50% of gibbons' diet, except for the more folivorous siamang (*Symphalangus syndactylus*) [63-66]. It is commonly agreed that food choices of primates are correlated with the nutritional and toxic contents [65,67,68]. Compared with other parts of plant, fruits typically have the highest content of soluble carbohydrates, i.e., sweet tasting compounds [69,70]. As typical frugivorous primates, gibbons seem to intake food with more soluble sweet taste contents than other primates. For example, white-handed gibbons were reported to include a high proportion of carbohydrate-rich fruits in their natural diet [71] and showed clear preferences toward ripe fruits, which typically have the highest content of soluble carbohydrates [69,70]. Furthermore, a food preference test of captive white-handed gibbons with ten types of fruits revealed a highly significant positive correlation between the food preference ranking and total carbohydrates [67]. Accordingly, total carbohydrate content in foods might be an important determinant of food choice for gibbons [72-77], which may contribute to specific adaptive evolution of *TAS1R2*. Four amino acid substitutions (M132S, V175A, G404D and G404N) were detected as candidates for natural selection, which may have influenced the sensitivity of *TAS1R2* in gibbons and adapted to their special frugivorous diet.

Why is there no positive selection of *TAS1R* genes in Hominidae?

Interestingly, no signs of positive selection were detected in Hominidae. This could be explained by external and internal reasons. Hominid species are able to acquire, process and consume a wide variety of foods such as fruits, assorted types of vegetation, bark, seeds, insects and meat. They are able to change the composition of diet due to local ecological conditions as they have the ability of tool usage to acquire and consume foods [78].

Thus, sensitivity to sweet and umami taste might not play an overwhelming role in their feeding ecology. From another aspect, sensitivity of taste is also correlated with the body size of primates [47]. A large area of the lingual mucosa in large animals may increase taste performance. This means that the larger the species, the better their taste acuity [47]. As the largest species among the primates, the sensitivity of umami and sweet taste in Hominidae might be sufficiently sensitive for foraging.

Additionally, an episodic relaxation of selective constraints ($\omega_1 = 0.7772$, $p < 0.05$) was also found in *TAS1R1* for the common ancestor of human, chimpanzee, bonobo and gorilla (Table 2, Figure 3). In contrast, diet and food availability for orang-utans exert a much more restrictive influence. These large, arboreal great apes rely predominantly on fruit, such as figs (*Ficus* spp.) and durian (*Durio* spp.) This difference in diet might explain the variation in the evolutionary history of *TAS1R1* between orang-utans and other great apes (humans included).

Functional sites concentrated on extra-cellular region of taste receptors and phylogenetically scattered

Nearly all of the positive selected sites (92.3%, 24 out of 26 positive selected sites, see Additional file 2) were localized in the VFTM and CRD domains (extra-cellular region) of *TAS1R*, which are responsible for binding of small molecules [79]. In order to examine potential influence of these positively selected mutations, we conducted the commonly used molecular modeling and docking methods to detect binding of some sweet and umami taste molecular with *TAS1R*s receptors. Interestingly, we located some positive selection sites at binding regions of several sweet and umami taste molecules based on the molecular modelling and docking results. Sites 50 and 391 of *TAS1R1*, and sites 342, 407, 411 and 413 of *TAS1R2* were identified to interact with several taste molecules, such as D-tryptophan, IMP, L-glutamate, fructose and sucrose (see Additional files 7, 8). It is remarkable that these amino acid sites were coincidentally revealed to be positively selected sites. All of these results offer us potential active regions and important amino acid sites which are likely to influence the binding of several tastants, which also implies that the extra-cellular regions play a vital role in adaptive evolution. Certainly, comparing functional expression experiments, molecular modeling and docking have certain limitations. However, as the widely used molecular structure analysis methods, molecular modeling and docking can predicate some potential binding domains and binding sites between receptors and ligands, theoretically. In our study, as the complementary analysis to predicate potential influence on binding with ligands of positively selected sites detected in our studies, the modeling and docking studies were suggested to be necessary and critical.

Remarkably, most of these radical changes in amino acids occurred in a few sub-terminal and terminal lineages across the phylogeny of catarrhine primates, without obvious phylogeny-related occurrence (Figures 2, 3 and 4). In other words, relatives belonging to the same phylogenetic group (family or genus) usually did not exhibit uniform amino acids in a single radical change site, and even several of these radical changes occurred irregularly in a single or a few distantly related species. This suggests specific adaptive evolution of *TAS1R* genes in catarrhine primates, resulting from habitat change and complexity of plant-based diets.

Potential variation in tasting of aspartame, neotame, lactisole, monosodium glutamate and monopotassium glutamate among catarrhine primates

Although a potential functional change induced by these positively selected sites was not substantiated in this study, we referred to multiple mutation/substitution sites of *TAS1R* identified by previous functional expression studies in other species, and some of them also occurred in the sequences described here (Table 4). For example, the site substitution S40T of *TAS1R2* was shown to abolish the receptor response to aspartame [31], suggesting that Cercopithecidae species showing this substitution might have lost the taste of aspartame (Table 4). However, other studies suggested that aspartame is perceived as sweet by humans, apes and some species of Cercopithecidae [20,80]. Variable perception strength to aspartame between Cercopithecidae and other species of catarrhine primates was suggested to be the best explanation for this 'contradictory' situation. We hypothesized that this amino acid substitution did not really abolish response of Cercopithecidae to aspartame, but rather led to differences in sensitivity of aspartame perception between Cercopithecidae and other species. Further functional expression studies about the influence of S40T on sensitivity of *TAS1R2* in catarrhine primates should be launched in future. Additionally, the A733V substitution in *TAS1R3* was one of the important changes detected for diminished receptor sensitivity to lactisole, according to research conducted in humans and rodents [32]. The same substitution (A733V) occurred in Cercopithecidae, where A was found in Hylobatidae and Hominidae. It is reasonable to hypothesize that the A733V substitution in *TAS1R3* of Cercopithecidae potentially led to more diminished sensitivity to lactisole than in Hylobatidae and Hominidae.

The substitution I67S of *TAS1R2* in humans was confirmed to slightly reduce the response to neotame. Furthermore, the substitution R757C of *TAS1R3* in humans reduces sensitivity to monosodium glutamate (MSG) and increases sensitivity to monopotassium glutamate. Substitutions at the same sites of *TAS1R2* and *TAS1R3* but not the same amino acid changes were found in Colobinae (Table 4) and their potential influence on *TAS1R2* and

TAS1R3 in Colobinae is expected to be evaluated during future studies.

Though some of these tastants were artificial compounds and have less of relationship with adaption of the sweet and umami tastes, these work supplied examples which reflect the mutation-induced sensitivity variation for certain compounds no matter natural or artificial. It highlights the importance of a number of sites and domains for ligands binding. Similar work can be implemented in research on catarrhine primates incorporating more natural compounds to examine the potential influence of positive selection sites detected in our study.

Conclusions

Unlike animal food, the number and diversity of nutritional compounds of leaves and fruit varies with plant species, location, position on the tree, stage of development, and even time of day [27,81-84]. Therefore, it is reasonable to assume that catarrhine primates require more sensitive and specific sweet and umami tastes when compared to carnivorous animals. Our results demonstrate family-, subfamily-, genus- and even species-specific adaptive evolution of *TAS1R* genes, and suggest pervasively differentiated evolution of sweet and umami tastes during the divergence of catarrhine primates. Episodic relaxation of selective constraints and pseudogenization were also found, both of which might be accompanied by and even contribute to dietary transition. Remarkably, positive selected sites were concentrated on extra-cellular region of taste receptors, which indicated that extra-cellular region of *TAS1R*s play a vital role in adaption for variations in sweet and umami taste molecules in different diets.

The relationships among gustatory sense, food intake and foraging efficiency are complicated [85]. Both physical and chemical properties of food play important roles in food choice in primates [86]. Thus, the influence of both external and internal factors on taste receptor gene evolution is complex. Given such multiple factors, the impact of any particular factor is likely to be quantitative rather than qualitative, and a small number of counterexamples do not automatically refute any potential. Given these complications, to avoid spurious results, it is imperative to examine a large and diverse group of species when testing the potential impact of any given ecological factor on taste receptor gene evolution [87]. Our study provides new insights into the evolutionary history of taste genes in primates, as well as a database of mutations/substitutions in taste genes which will facilitate future work assessing the ecological correlates between taste sensitivity and food choice in primates.

Methods

Ethical approval

Blood samples were taken during routine health checks by experienced veterinarians and not specifically for this

study. Fresh tissue samples were taken from deceased animals. All research complied with protocols approved by the Animal Welfare Body of the DPZ in Germany and the Wildlife Conservation Association in China, and adhered to the legal requirements of the countries, in which research was conducted. The study was carried out in compliance with the principles of the American Society of Primatologists for the ethical treatment of non-human primates (<https://www.asp.org/society/resolutions/EthicalTreatmentOfNonHumanPrimates.cfm>). No animals were sacrificed for this study.

Polymerase chain reaction and DNA sequencing

Based on an alignment of currently available *TASIR* sequences of human, chimpanzee, and macaque, we designed a set of primer pair to amplify *TASIR* sequences from DNA samples of 30 species in catarrhine monkeys (see Additional file 1). The polymerase chain reaction (PCR) mixtures (50 μ l) contained 5 μ l (50 ng/ μ l) genomic DNA, 25 μ l of 2 \times buffer, 7.5 μ l (50 mM) MgCl₂, 5 μ l (10 mM) of each primer, and 1 U Taq DNA polymerase (Takara). PCRs were performed in a DNA Engine Dyad Cycler (BioRad) under the following condition: 5 min of initial denaturation, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 60 s; and a final extension at 72°C for 5 min. PCR products were examined on agarose gels and subsequently cloned in the PMD18-T (Promega) cloning vector. Positive clones were sequenced on an ABI 3130 xl DNA Sequencer. Three to five clones of each PCR product were sequenced from both directions to validate the results. All intact *TASIR* sequences of Hominidae were downloaded from GeneBank (see Additional file 1). Additionally, we also aligned our sequences with available primate genomes from GeneBank and the genome assembly of snub-nosed monkeys (unpublished), confirming that *TASIR* are single-copy genes in genomes of catarrhine primates. The nucleotide and deduced amino acid sequences of each gene were aligned with CLUSTALX 1.81 [88] and modified with Bio-Edit 7.0.4 [89].

Selective pressure detection

A powerful approach to detecting molecular evolution by positive selection derives from comparison of the relative rates of synonymous (d_s) and non-synonymous substitutions (d_N) [90-92]. The rate ratio ω (d_N/d_s) is a measure of selective pressure, where $\omega = 1$, $\omega < 1$ and $\omega > 1$ correspond to neutral evolution, purifying and positive selection [93]. The ω ratio was estimated using a codon-based maximum-likelihood method implemented in CODEML program of the PAML v. 4.4 package [94]. A well-accepted phylogeny of primates was used as the input tree in all analyses [95]. Because only one or two intact *TASIR* were available in some hominoids, input trees used in

the analysis of *TASIR1*, *TASIR2*, and *TASIR3* were slightly different within lineages of Hominidae (Figures 2, 3 and 4).

A combination of branch, site and branch-site models was used to analyze the selection of *TASIR* in catarrhine primates. Firstly, to test whether the ω ratio of each gene was different among lineages, the 'free-ratios' model (M1), which assumes an independent ω ratio for each branch, was compared with the 'one-ratio' model (M0), which assumes the same ω ratio for all branches [93]. Subsequently, site models in which ω can vary among sites were implemented to identify candidates of positively selected sites in the *TASIR* genes [96,97]. Therefore, two pairs of site models were tested: M1a (nearly neutral) versus M2a (positive selection), and M8a (nearly neutral; beta distribution) versus M8 (positive selection; beta distribution).

Additionally, positive selection often operates episodically on a few amino acid sites in a small number of lineages in a phylogenetic tree [98]. Therefore, in the case of branch-site model, modified branch-site model A was performed for each gene in the lineages of Cercopithecoidea, Cercopithecoidea, Colobinae, Hylobatidae and Hominidae, separately. Finally, two-ratio model assuming the branches of interest having a d_N/d_s (ω_1) different from the background ratio (ω_0) was used for lineages with $0.50 < \omega < 1$ (detecting by free ratio model) to identify potential functional relaxation [99,100]. Ancestral *TASIR* sequences were inferred based on empirical Bayesian methods implemented in the CODEML program of the PAML package.

The likelihood ratio test (LRT), which calculates twice the log-likelihood ($2\Delta\ln L$) of the difference following a chi-square distribution, was used to evaluate the significance of differences between each pair of models. All of the pseudogenes were removed in the PAML-based analysis. To evaluate the probabilities of positively selected sites on *TASIR* sequenced in this research, the Bayes empirical Bayes (BEB) analysis was used to calculate posterior probabilities of positively selected sites implemented in the CODEML program of PAML. Based on BEB analysis, sites with a posterior probability > 0.85 were considered as candidates for selection. Finally, according to charge, polarity and volume of amino acids, positive selection sites detected by site models and branch-site models were used to estimate amino acids change patterns (conservative or radical substitutions) along the evolution lineages of primates [98].

Molecular modelling and docking

Homology models of *TASIR1* and *TASIR2* VFTM (amino acids 29-496 and 24-494, respectively) were built with the Modeller9.11 [101,102] and EasyModeller4.0 [103] using the mGluR1-VFTM crystal structure (PDB entry: 2U4E) [104] as the template. Then, automatic molecular docking

programs, Autodock Vina1.1.2 [105] and MGLTools1.5.6 [106,107] were used for the docking studies with some sweet and umami taste molecules (umami molecules: glycine, IMP, L-alanine, L-glutamate; sweet molecules: D-tryptophan, fructose, D-fructose, galactose, D-galactose, D-glucose and, sucrose, neotame and aspartame), and these results were viewed modified by PyMol (The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC).

Additional files

Additional file 1: Information about the species and genes present in this study.

Additional file 2: Summary of mutation sites and positive selected sites in TAS1Rs proteins.

Additional file 3: Alignment of TAS1R1 amino acid sequences of 35 catarrhine primates. Variant residues are marked with red.

Additional file 4: Alignment of TAS1R2 amino acid sequences of 35 catarrhine primates. Variant residues are marked with red.

Additional file 5: Alignment of TAS1R3 amino acid sequences of 35 catarrhine primates. Variant residues are marked with red.

Additional file 6: Candidate amino acid sites under positive selection identified in Tas1rs using the site models and branch-site models.

Additional file 7: Molecular docking results of TAS1R1. Positive selection sites detected in our research are marked with red.

Additional file 8: Molecular docking results of TAS1R2. Positive selection sites detected in our research are marked with red.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ML, ZL, CR, LW and GL participated in the study design. GL and ZL conducted the experiments, analysed the data and wrote the manuscript. ML, LW and CR offered samples, helped to draft the manuscript and revised it critically. ST, XT and FS helped to analyse data and provided valuable suggestions. All authors read and approved the final manuscript.

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