ORIGINAL ARTICLE

Lineage-specific evolution of bitter taste receptor genes in the giant and red pandas implies dietary adaptation

Lei SHAN,1 Qi WU,1 Le WANG,1 Lei ZHANG1 and Fuwen WEI1,2,3
1Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China
2University of Chinese Academy of Science, Beijing, China and 3Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, China

Abstract

Taste 2 receptors (TAS2R) mediate bitterness perception in mammals, thus are called bitter taste receptors. It is believed that these genes evolved in response to species-specific diets. The giant panda (Ailuropoda melanoleuca) and red panda (Ailurus fulgens styani) in the order Carnivora are specialized herbivores with an almost exclusive bamboo diet (>90% bamboo). Because bamboo is full of bitter tasting compounds, we hypothesized that adaptive evolution has occurred at TAS2R genes in giant and red pandas throughout the course of their dietary shift. Here, we characterized 195 TAS2R genes in 9 Carnivora species and examined selective pressures on these genes. We found that both pandas harbor more putative functional TAS2R genes than other carnivores, and pseudogenized TAS2R genes in the giant panda are different from the red panda. The purifying selection on TAS2R1, TAS2R9 and TAS2R38 in the giant panda, and TAS2R62 in the red panda, has been strengthened throughout the course of adaptation to bamboo diet, while selective constraint on TAS2R4 and TAS2R38 in the red panda is relaxed. Remarkably, a few positively selected sites on TAS2R42 have been specifically detected in the giant panda. These results suggest an adaptive response in both pandas to a dietary shift from carnivory to herbivory, and TAS2R genes evolved independently in the 2 pandas. Our findings provide new insight into the molecular basis of mammalian sensory evolution and the process of adaptation to new ecological niches.

Key words: bitter taste receptor gene, dietary adaptation, giant panda, red panda

INTRODUCTION

The mammalian sensory system of taste, including bitter, umami, sweet, sour and salty, dedicated to the evaluation of the physical and chemical features of their diets, is vital for their survival and reproduction. Among these, bitter taste perception is particularly vital to mammalian survival, as it is a critical defense mechanism enabling animals to avoid ingesting toxic substances, because poisons usually taste bitter and cause aversion (Garcia & Hankins 1975; Li & Zhang 2014). It has been shown that the perception of bitterness is mediated by a large group of seven-transmembrane G protein-coupled receptors (GPCRs), which are encoded by the taste 2 receptor gene family in the membrane of taste receptor cells (Bachmanov & Beauchamp 2007). Studies on
taste sensory evolution have revealed that loss of taste receptor function in mammals is widespread and directly related to feeding specialization, such as pseudoge­nized sweet and/or umami taste receptor genes in cats (feliformia, obligate carnivores), giant and red pandas (caniformia, obligate bamboo feeders), vampire bats (desmodontines, obligate blood feeders), dolphins and pinnipeds (cetaceans, swallowing food whole without chewing) (Li et al. 2005; Zhao et al. 2010; Jiang et al. 2012; Sato & Wolsan 2012; Zhu et al. 2014; Hu et al. 2017). Moreover, the TAS2R repertoire vary greatly in size among species from a few to approximately 60, and losses and gains in the repertoire are frequent in mam­malian evolution (Li & Zhang 2014; Hayakawa et al. 2014; Liu et al. 2016). Furthermore, Li and Zhang (2014) found that more functional TAS2R genes in herbivores than in carnivores as plant tissues contain more toxic compounds than animal tissues do, and, thus, concluded that the number of TAS2R genes in a species correlates with the fraction of plants in its diet. Given that, dietary toxins are a major selective force driving the evolution of the TAS2R genes in mammals (Davis et al. 2010; Li & Zhang 2014), and, therefore, the degeneration and adap­tation of TAS2R genes is an evolutionary response to dietary changes.

The giant panda (Ailuropoda melanoleuca) and the red panda (Ailurus fulgens) are 2 sympatric species distributed at the edge of the Qinghai-Tibetan Plateau that have captured much interest from the communi­ty of wildlife conservationists because of their conservation status, phylogenetic distinctiveness and specialized feeding ecology (Glatston 1994; Wei et al. 1999, 2012; Yu et al. 2011). They evolved divergently from a meat-eating ancestor approximately 43 Ma (Eizirik et al. 2010), with distinct phylogenetic positions in the or­der Carnivora: the giant panda belongs to the family Ursidae and the red panda belongs to the family Ailuridae. Although they belong to the order Carnivora, both pandas are well known for their dietary shifts from carnivory to herbivory, whereby >90% of their modern diet comprises bamboo (Jin et al. 2007; Zhao et al. 2010; Hu et al. 2017). Bamboo, like most vascular plants, contains abundant bitter secondary compounds such as pheno­olics, terpenoids, alkaloids, flavonoids and cyanogenic glycosides (Keski-Saari et al. 2008; Huang et al. 2016), which appear to protect them against mammalian herbi­vores. Therefore, we hypothesized that adaptive evolution should have occurred at TAS2R genes in the giant and red pandas during their transition to a bamboo diet. To test this hypothesis, we characterized TAS2R genes from 9 carnivoran species (Fig. 1) for which genome as­semblies were available, and conducted evolutionary analyses on TAS2R genes to identify molecular mecha­nisms in response to dietary shifts in pandas.

**MATERIAL AND METHODS**

**Data mining**

---

**Figure 1** A species tree of 9 carnivores studied in this work, assessed using TimeTree v3.0 (http://www.timetree.org/, last accessed 30 June 2016) (Hedges et al. 2006)
The 9 carnivore genome assemblies were obtained from Ensembl (http://www.ensembl.org/index.html) and NCBI (https://www.ncbi.nlm.nih.gov/), which are summarized in the supplementary materials (Suppl. Table S1). We characterized TAS2R genes following a previous study (Shi & Zhang 2006) with a little modification. Initially, we retrieved previously annotated TAS2R gene sequences of these species from the NCBI, and then used these sequences as queries for TBLASTN searches in each whole-genome assembly. Because TAS2R genes are single-exon genes, we looked for intact TAS2R genes with continuous sequences from the start to stop codons, and pseudogenized TAS2R genes with ≥750 bp sequences disrupted by start loss, stop gain and/or indel, on the basis of high BLAST E-values of 1e-5. All the overlapping sequences of hits with the same orientations at the contig level were merged. The candidate TAS2R genes were verified by the TransMembrane helices prediction using hidden Markov models (TMHMM) method (online version 2.0) for the presence of 7 transmembrane domains (Krogh et al. 2001), and the intact ones were examined by BLASTP searches against the entire GenBank to ensure that the best hit with an annotation is a known TAS2R gene. All characterized TAS2R gene sequences and genomic locations are listed in the supplementary material. Here, we follow the nomenclature of the TAS2R genes established in Laurasiatheria by Hayakawa et al. (2014).

Phylogenetic and selection analyses

To determine the orthologous and paralogous relationships between the TAS2R genes, a neighbor-joining (NJ) tree was constructed based all alignable TAS2R genes used MEGA7 (Kumar et al. 2016). Then, selection analyses were conducted on all intact TAS2R genes with indels and stop codons removed, and a series of evolutionary models implemented in PAML (Yang 2007) were conducted and compared in a likelihood framework, using the species tree shown in Fig. 1. Positive selection analyses were restricted to the giant panda and red panda branch, respectively. Pseudogenization of genes is a sign of relaxed selective constraints (Conte et al. 2003), thus pseudogenized TAS2R genes were excluded from the further natural selection analyses.

A maximum-likelihood approach was used to calculate the rate ratio ($\omega$) of nonsynonymous ($d_\beta$) to synonymous ($d_\alpha$) substitutions ($\omega = d_\beta/d_\alpha$), where $d_\beta/d_\alpha > 1$ indicates positive selection, $d_\beta/d_\alpha < 1$ negative selection and $d_\beta/d_\alpha = 1$ neutrality. First, we tested one-ratio model, a more general model assuming a single $\omega$ ratio for all branches. Subsequently, we used two-ratio model that allow 2 $\omega$ ratios that differ between the background branches and the branch of interest (the giant or red panda branch). For null hypotheses, we used the one-ratio model with a fixed $\omega = 1$ for all branches and a two-ratio model with a fixed $\omega = 1$ on the branch of interest. Then, the likelihood ratio tests (LRTs) were used to evaluate the level of significance of differences between the 2 nested models, where twice the difference of log-likelihood between the models ($2\Delta lnL$) would follow a $\chi^2$-distribution, and the degree of freedom was the difference in number of parameters between the nested models. Furthermore, the recommended Test 2 was used to detect positively selected sites on the branch of interest, which compares the modified branch-site model A (model = 2 NSsites = 2) with the corresponding null model with a fixed $\omega = 1$ (fix_omega = 1 and omega =1) on the branch of interest (Yang et al. 2005; Zhang et al. 2005). The Test has been shown to have power to differentiate positive selection from relaxation of selection (Zhang et al. 2005). Finally, the posterior probabilities of positively selected sites were calculated using the Bayes empirical Bayes (BEB) method as implemented for the modified model A in the CODEML program of PAML 4 package (Yang 2007). We considered those sites with a posterior probability >0.8 as candidates undergoing positive selection.

RESULTS

Taste 2 receptor gene characterization and phylogenetic analysis

A total of 195 TAS2R genes (121 complete and 74 disrupted) were characterized from the 9 carnivore species (Fig. 2), including 26 that were characterized for the first time in this study (16 in the red panda, the TAS2R62 in the leopard, TAS2R408B in the cheetah, and TAS2R18 in all species except the polar bear). However, 3 putative TAS2R genes (TAS2R5, TAS2R16 and TAS2R408B) were not found in the cheetah, dog and red panda genome assembly, respectively. Collectively, only 10–16 intact TAS2R genes were identified for individual species. The intact TAS2R genes are those annotated without premature stop codons or frame shift mutations, thus suggesting the presence of a functional TAS2R protein. The giant and red pandas have the same number of intact TAS2R genes (both with 16), which is the largest number in comparison to the number of intact TAS2R genes from the other 7 carnivore species. Generally,
the 3 species from the Caniformia have a relatively larger number of intact TAS2R genes than the number in the 4 species from the Feliformia. The cheetah has the smallest intact TAS2R repertoire (only 10 intact TAS2R genes). In addition, the pseudogenized TAS2R genes are different between the 2 pandas. The NJ tree suggest-
ed 20 one-to-one orthologs with the exception of TAS2R408A and TAS2R408B (Fig. 2), including 3 pseudogenized across all species, and the other 17 comprising 107 intact and 45 pseudogenized TAS2R genes among the 9 species.

**Signatures of adaptive evolution**

To look for evidence of adaptive evolution of TAS2R genes in the giant and red panda branches, the 17 orthologs (with pseudogenized TAS2R genes excluded) were examined in a series of selection analyses. The one-ratio model revealed that the average ω for 12 TAS2R genes (TAS2R1, TAS2R2, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R9, TAS2R10, TAS2R38, TAS2R40, TAS2R41 and TAS2R42) are significantly less than 1 (Suppl. Table S2), indicative of a generally strong purifying selection acting on these TAS2R genes across the branches. In contrast, the average ω for the other 5 TAS2R genes (TAS2R8, TAS2R12, TAS2R39, TAS2R62, TAS2R67) are not significantly different from 1 (Suppl. Table S2), suggesting that purifying selection pressure on these receptor genes is generally relaxed. Further, a two-ratio model showed that ω for TAS2R1, TAS2R9 and TAS2R38 on the giant panda branch (ωg), and TAS2R62 on the red panda branch (ωr) are significantly less than the ratio for all other branches (ω0), indicating a divergence in the selective pressure between the giant or red panda branch and the other branches, and, thus, suggesting that the purifying selection pressure on these genes is lineage-specifically strengthened. Although the two-ratio model showed that ωg for TAS2R42, and ωr for TAS2R4 and 38 are also significantly different from the average ω, they are not significantly different from a two-ratio model where ωg or ωr = 1, respectively (Suppl. Table S2), suggesting the purifying selection pressure on these genes is lineage-specifically relaxed. The ωr for TAS2R4 is less than 1, indicative of a relaxation of selective constraint on this gene in the red panda. The ωg for TAS2R42 and ωr for TAS2R38 are greater than 1, which indicates that the TAS2R42 on the giant panda branch and TAS2R38 on the red panda branch have accumulated more nonsynonymous than synonymous mutations. Despite this, the two-ratio tests did not evidently support positive selection on the TAS2R42 and TAS2R38, as we cannot exclude the possibility of relaxation of selection. Naturally, positive selection will act on only a few sites and for a short period of evolutionary time (Shen et al. 2010); thus, the signal for positive selection usually is swamped by the continuous negative selection that occurs on most sites in a gene se-

| Site class | Background ωg | Background ωr | Foreground ωg | Foreground ωr | χ2 | df | ln(L) | ln(L) | P-value |
|------------|---------------|---------------|---------------|---------------|-----|-----|------|------|---------|
| Model A    |               |               |               |               |     |     |      |      |         |
| Proportion |               |               |               |               |     |     |      |      |         |
| Background | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |
| Foreground | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |
| Model A0   |               |               |               |               |     |     |      |      |         |
| Proportion |               |               |               |               |     |     |      |      |         |
| Background | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |
| Foreground | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |

**Table 1 Candidate amino acid sites under positive selection identified on TAS2R42 in the giant panda using the PAAML method**

In contrast, the average ω for the other 5 TAS2R genes (TAS2R8, TAS2R12, TAS2R39, TAS2R62, TAS2R67) are not significantly different from 1 (Suppl. Table S2), suggesting that purifying selection pressure on these receptor genes is generally relaxed. Further, a two-ratio model showed that ω for TAS2R1, TAS2R9 and TAS2R38 on the giant panda branch (ωg), and TAS2R62 on the red panda branch (ωr) are significantly less than the ratio for all other branches (ω0), indicating a divergence in the selective pressure between the giant or red panda branch and the other branches, and, thus, suggesting that the purifying selection pressure on these genes is lineage-specifically strengthened. Although the two-ratio model showed that ωg for TAS2R42, and ωr for TAS2R4 and 38 are also significantly different from the average ω, they are not significantly different from a two-ratio model where ωg or ωr = 1, respectively (Suppl. Table S2), suggesting the purifying selection pressure on these genes is lineage-specifically relaxed. The ωr for TAS2R4 is less than 1, indicative of a relaxation of selective constraint on this gene in the red panda. The ωg for TAS2R42 and ωr for TAS2R38 are greater than 1, which indicates that the TAS2R42 on the giant panda branch and TAS2R38 on the red panda branch have accumulated more nonsynonymous than synonymous mutations. Despite this, the two-ratio tests did not evidently support positive selection on the TAS2R42 and TAS2R38, as we cannot exclude the possibility of relaxation of selection. Naturally, positive selection will act on only a few sites and for a short period of evolutionary time (Shen et al. 2010); thus, the signal for positive selection usually is swamped by the continuous negative selection that occurs on most sites in a gene se-

| Site class | Background ωg | Background ωr | Foreground ωg | Foreground ωr | χ2 | df | ln(L) | ln(L) | P-value |
|------------|---------------|---------------|---------------|---------------|-----|-----|------|------|---------|
| Model A    |               |               |               |               |     |     |      |      |         |
| Proportion |               |               |               |               |     |     |      |      |         |
| Background | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |
| Foreground | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |
| Model A0   |               |               |               |               |     |     |      |      |         |
| Proportion |               |               |               |               |     |     |      |      |         |
| Background | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |
| Foreground | 0.00864      | 1             | 0              | 0              | 0   | 1   | -0.0051 | 0.0051 | 1       |

**Table 1 Candidate amino acid sites under positive selection identified on TAS2R42 in the giant panda using the PAAML method**
Bitter taste receptor genes in the pandas

quence (Zhang et al. 2005). Even after a short period of positive selection, this is commonly followed by a long period of purifying selection, which would obscure the selective processes (Shen et al. 2010). Therefore, positive selection on genes is difficult to detect. Branch-site models, in contrast to branch models in which \( \omega \) varies only among branches, allows variation in the selective pressure to occur at both amino acid sites and on lineages; thus, these models are considered to be powerful in distinguishing positive selection from the relaxation of purifying selection (Zhang et al. 2005). We thus used branch-site models to further examine for possible positive selective pressure on the branch leading to giant panda and red panda. Remarkably, a significant positive selection on \( \text{TAS2R42} \) was detected in the giant panda (\( P = 0.0088; \) Table 1). Nine amino acid sites were identified as candidate sites that had undergone positive selection with posterior probabilities of \( \geq 90\% \). However, no evidence for significant positive selection on \( \text{TAS2R38} \) was detected in the red panda, suggesting a relaxation of selection on this gene.

**DISCUSSION**

We included 195 \( \text{TAS2R} \) genes for 9 carnivoran mammals according to the latest assemblies. We speculate that the most recent common ancestor (MRCA) of modern carnivoran mammals had at least 19 intact \( \text{TAS2R} \) genes, but only 10–16 intact \( \text{TAS2R} \) genes were retained in modern Carnivora, which is fewer than that in herbivores (such as cows and horses) and omnivores (such as humans and mice) (Hu & Shi 2013; Li & Zhang 2014; Hayakawa et al. 2014). This result supports earlier speculation that the number of \( \text{TAS2R} \) genes in a species correlates with the fraction of plants in its diet (Li & Zhang 2014). Accordingly, the largest intact \( \text{TAS2R} \) repertoire size as found in the giant and red pandas is likely the result of adaptation to dietary shift, as more functional \( \text{TAS2R} \) genes are required to detect bitter substances in bamboo. In contrast, more pseudogenized \( \text{TAS2R} \) genes were identified in other meat-eating carnivores, which may be due to a lack of specific bitterness in their diets and greater dispensability of \( \text{TAS2R} \) genes (Conte et al. 2003; Shi et al. 2003).

Generally, purifying selection was detected for the 12 \( \text{TAS2R} \) genes, indicating the functional importance of these receptor genes in the carnivoran evolution. However, the \( \omega \) for the 12 genes excepting \( \text{TAS2R41} \) are still relatively high in comparison to an average \( \omega \) of 0.23 for mammalian genes (Zhang 2000), which shows that \( \text{TAS2R} \) genes in carnivoran mammals are generally less conserved, probably because of their diets containing less toxins. In contrast, the other 5 \( \text{TAS2R} \) genes were tested under the relaxation of selection, suggesting that these genes became functionally less important for detecting bitter tastants, where we speculate that the volume of bitter compounds in responding to these genes decreased in the mammalian evolution. However, bamboo contains a large volume of bitter compounds (Keskis-Saari et al. 2008; Huang et al. 2016); thus, pandas should experience stronger selective pressure on \( \text{TAS2R} \) genes than that in other meat-eating mammals for detecting toxins. Indeed, our study showed that purifying selection pressure is lineage-specifically strengthened on \( \text{TAS2R1}, \text{TAS2R9} \) and \( \text{TAS2R38} \) in the giant panda, and \( \text{TAS2R62} \) in the red panda, which suggests that these 4 genes are responsive to the bitter compounds from bamboo. Remarkably, the positive selection, powerful evidence suggesting an adaptive evolution, on \( \text{TAS2T42} \) in the giant panda indicates that new sites were evolved for detecting the newly encountered bitter compounds in bamboo diet, suggesting an evolutionary response to the dietary shift. However, selective constraint is markedly relaxed on \( \text{TAS2R4} \) and \( \text{TAS2R38} \) in the red panda, which suggests that some functional sites on the 2 genes have become less important, probably due to the specific bitter compounds changed during the course of the red panda’s diet shifting from carnivory to herbivory. No evidence for significant positive selection was detected on any of the \( \text{TAS2R} \) genes in the red panda; in combination with the different \( \text{TAS2R} \) genes evolved in response to the dietary shift between the 2 pandas, our results suggests that the \( \text{TAS2R} \) genes evolved independently in the 2 pandas. This may seem counterintuitive, because convergent evolution should be expected on at least some of \( \text{TAS2R} \) genes between the 2 pandas. To us, when the 2 pandas’ diet shifted to herbivory (Hu et al. 2017), bitter compounds from bamboos became the major force driving the \( \text{TAS2R} \) evolution in the 2 species. During the evolutionary process, new variations should have arisen for detecting newly encountered tastants in bamboos. Because each \( \text{TAS2R} \) receptor is commonly responsive to several bitter substances, and the same bitter tastants can activate different bitter taste receptors (Meyerhof et al. 2010), the new variations for detecting the same bitter bamboo compounds could arise independently in different \( \text{TAS2R} \) genes in the giant and red pandas. New \( \text{TAS2R} \) genes arise from the duplication of old \( \text{TAS2R} \) genes (Shi et al. 2003), which provided different possible evolutionary routes.
for achieving the same functional solution. Consequently, although the giant and red pandas face the same bitter bamboo compounds, the same compounds can be detected by different bitter taste receptors. Similar results have been found in chimpanzees and humans, where although the 2 species shared variable taste sensitivity to bitter compounds mediated by phenylthiocarbamide receptor variants, the molecular basis of the variation has arisen twice, independently, in the 2 species (Wooding et al. 2006). This phenomenon has been suggested as convergence occurred at a functional level of genes (Nery et al. 2016). The findings presented in this study provide us with new insight into the molecular basis of evolutionary adaptation in giant and red pandas to dietary shift, and support previous discoveries that bitter taste receptor genes have evolved independently in mammals in response to dietary specialization (Wooding et al. 2006; Jiang et al. 2012; Li & Zhang 2014; Liu et al. 2016; Risso et al. 2017).

ACKNOWLEDGMENTS

Financial support was provided by the National Natural Science Foundation of China (NSFC, 31670386 and 31300303) and the Chengdu Giant Panda Breeding Research Foundation (CPF2012-11).

REFERENCES

Bachmanov AA, Beauchamp GK (2007). Taste receptor genes. Annual Review of Nutrition 27, 389–414.

Conte C, Ebeling M, Marcuz A, Nef P, Andres-Barquin PJ (2003). Evolutionary relationships of the Tas2r receptor gene families in mouse and human. Physiological Genomics 14, 73–82.

Davis JK, Lowman JJ, Thomas PJ et al. (2010). Evolution of a bitter taste receptor gene cluster in a new world sparrow. Genome Biology and Evolution 3, 358–70.

Eizirik E, Murphy WJ, Koepfl R-P et al. (2010). Pattern and timing of diversification of the mammalian order Carnivora inferred from multiple nuclear gene sequences. Molecular Phylogenetics and Evolution 56, 49–63.

Garcia J, Hankins W (1975). The evolution of bitter and the acquisition of toxiphobia. In: Denton DA, Coglan JP, eds. Olfaction and Taste: Fifth International Symposium. Academic Press, New York, pp. 39–45.

Glatston AR (1994). Status Survey and Conservation Action Plan for Procyonids and Ailurids: The Red Panda, Olingos, Coatis, Raccoons, and their Relatives. IUCN, Gland, Switzerland.

Hayakawa T, Suzuki-Hashido N, Matsui A, Go Y (2014). Frequent expansions of the bitter taste receptor gene repertoire during evolution of mammals in the Euarothropes clade. Molecular Biology and Evolution 31, 2018–31.

Hedges SB, Dudley J, Kumar S (2006). TimeTree: A public knowledge-base of divergence times among organisms. Bioinformatics 22, 2971–2.

Hu LL, Shi P (2013). Smallest bitter taste receptor (T2Rs) gene repertoire in carnivores. Zoological Research 34, E75–81.

Hu Y, Wu Q, Ma S et al. (2017). Comparative genomics reveals convergent evolution between the bamboo-eating giant and red pandas. PNAS 114, 1081–6.

Huang H, Yie S, Liu Y et al. (2016). Dietary resources shape the adaptive changes of cyanide detoxification function in giant panda (Ailuropoda melanoleuca). Scientific Reports 6, 34700.

Jiang P, Josue J, Li X et al. (2012). Major taste loss in carnivorous mammals. PNAS 109, 4956–61.

Jin C, Ciochon RL, Dong W et al. (2007). The first skull of the earliest giant panda. PNAS 104, 10932–37.

Keski-Saari S, Ossipov V, Julkunen-Tiitto R et al. (2008). Phenolics from the culms of five bamboo species in the Tangjiahe and Wolong Giant Panda Reserves, Sichuan, China. Biochemical Systematics and Ecology 36, 758–65.

Krogh A, Larsson B, von Heijne G et al. (2001). Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. Journal of Molecular Biology 305, 567–80.

Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33, 1870–4.

Li D, Zhang J (2014). Diet shapes the evolution of the vertebrate bitter taste receptor gene repertoire. Molecular Biology and Evolution 31, 303–9.

Li X, Li W, Wang H et al. (2005). Pseudogenization of a sweet-receptor gene accounts for cats’ indifference toward sugar. PLoS Genetics 1, 27–35.

Liu Z, Liu G, Hailer F et al. (2016). Dietary specialization drives multiple independent losses and gains in the bitter taste gene repertoire of Laurasiatherian Mammals. Frontiers in Zoology 13, 28.
Meyerhof W, Batram C, Kuhn C et al. (2010). The molecular receptive ranges of human TAS2R bitter taste receptors. Chemical Senses 35, 157–70.

Nery MF, Borges B, Dragalzew AC, Kohlsdorf T (2016). Selection on different genes with equivalent functions: The convergence story told by Hox genes along the evolution of aquatic mammalian lineages. BMC Evolutionary Biology 16, 113.

Risso D, Behrens M, Sainz E, Meyerhof W, Drayna D (2017). Probing the evolutionary history of human bitter taste receptor pseudogenes by restoring their function. Molecular Biology and Evolution 34, 1587–95.

Sato JJ, Wolsan M (2012). Loss or major reduction of umami taste sensation in pinnipeds. Naturwissenschaften 99, 655–59.

Shen YY, Liang L, Zhu ZH, Zhou WP, Irwin DM, Zhang YP (2010). Adaptive evolution of energy metabolism genes and the origin of flight in bats. Proceedings of the National Academy of Sciences of the United States of America 107, 8666–71.

Shi P, Zhang J (2006). Contrasting modes of evolution between vertebrate sweet/umami receptor genes and bitter receptor genes. Molecular Biology and Evolution 23, 292–300.

Shi P, Zhang J, Yang H, Zhang YP (2003). Adaptive diversification of bitter taste receptor genes in mammalian evolution. Molecular Biology and Evolution 20, 805–14.

Wei F, Feng Z, Wang Z, Li M (1999). Feeding strategy and resource partitioning between giant and red pandas. Mammalia 63, 417–30.

Wei F, Hu Y, Zhu L, Bruford MW, Zhan X, Zhang L (2012). Black and white and read all over: the past, present and future of giant panda genetics. Molecular Ecology 21, 5660–74.

Wooding S, Bufe B, Grassi C et al. (2006). Independent evolution of bitter-taste sensitivity in humans and chimpanzees. Nature 440, 930–34.

Yang Z (2007). PAML 4: Phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution 24, 1586–91.

Yang Z, Wong WS, Nielsen R (2005). Bayes empirical Bayes inference of amino acid sites under positive selection. Molecular Biology and Evolution 22, 1107–18.

Yu L, Luan TP, Jin W et al. (2011). Phylogenetic utility of nuclear introns in interfamilial relationships of Caniformia (Order Carnivora). Systematic Biology 60, 175–87.

Zhao H, Yang JR, Xu H, Zhang J (2010). Pseudogenization of the umami taste receptor gene tas1r1 in the giant panda coincided with its dietary switch to bamboo. Molecular Biology and Evolution 27, 2669–73.

Zhang J (2000). Rates of conservative and radical non-synonymous nucleotide substitutions in mammalian nuclear genes. Journal of Molecular Evolution 50, 56–68.

Zhang J, Nielsen R, Yang Z (2005). Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. Molecular Biology and Evolution 22, 2472–9.

Zhu K, Zhou X, Xu S et al. (2014). The loss of taste genes in cetaceans. BMC Evolutionary Biology 14, 218.

SUPPLEMENTARY MATERIALS

Table S1 The whole-genome assemblies used in this study

Table S2 Analyses of selective pressure on the branch of the giant panda and/or red panda for 17 TAS2R genes

Cite this article as:

Shan L, Wu Q, Wang L, Zhang L, Wei FW (2018). Lineage-specific evolution of bitter taste receptor genes in the giant and red pandas implies dietary adaptation. Integrative Zoology 13, 152–9.

© 2017 The Authors. Integrative Zoology published by International Society of Zoological Sciences, Institute of Zoology/Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd