PROCEEDINGS OF THE ROYAL SOCIETY B

rspb.royalsocietypublishing.org

Research

Cite this article: Hong W, Zhao H. 2014 Vampire bats exhibit evolutionary reduction of bitter taste receptor genes common to other bats. *Proc. R. Soc. B* **281**: 20141079.
http://dx.doi.org/10.1098/rspb.2014.1079

Received: 3 May 2014
Accepted: 4 June 2014

Subject Areas:
evolution, ecology, genetics

Keywords:
vampire bats, bitter, taste receptor, pseudogenization, diet

Author for correspondence:
Huabin Zhao
e-mail: huabinzhao@whu.edu.cn

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2014.1079 or via http://rspb.royalsocietypublishing.org.

Royal Society Publishing

Vampire bats exhibit evolutionary reduction of bitter taste receptor genes common to other bats

Wei Hong and Huabin Zhao

Department of Zoology, College of Life Sciences, Wuhan University, Wuhan, People's Republic of China

The bitter taste serves as an important natural defence against the ingestion of poisonous foods and is thus believed to be indispensable in animals. However, vampire bats are obligate blood feeders that show a reduced behavioural response towards bitter-tasting compounds. To test whether bitter taste receptor genes (*T2R*s) have been relaxed from selective constraint in vampire bats, we sampled all three vampire bat species and 11 non-vampire bats, and sequenced nine one-to-one orthologous *T2R*s that are assumed to be functionally conserved in all bats. We generated 85 *T2R* sequences and found that vampire bats have a significantly greater percentage of pseudogenes than other bats. These results strongly suggest a relaxation of selective constraint and a reduction of bitter taste function in vampire bats. We also found that vampire bats retain many intact *T2R*s, and that the taste signalling pathway gene *Calhm1* remains complete and intact with strong functional constraint. These results suggest the presence of some bitter taste function in vampire bats, although it is not likely to play a major role in food selection. Together, our study suggests that the evolutionary reduction of bitter taste function in animals is more pervasive than previously believed, and highlights the importance of extra-oral functions of taste receptor genes.

1. Introduction

Mammals typically have five primary taste modalities dedicated to the evaluation of diets, of which the bitter taste serves as an important natural defence against the ingestion of poisonous foods and is thus believed to be indispensable in animals [1]. Although vertebrate bitter taste receptor genes (*T2R*s or *Tas2r*s) diverge tremendously in number from 0 in the bottlenose dolphin to 51 in the African clawed frog [2], multiple intact *T2R*s are maintained to ensure the functionality of detecting toxins in food sources for these animals, with the exception of the bottlenose dolphin [2,3]. The dolphin represents the first mammal to lack functional bitter taste receptors, probably because they swallow food whole, rendering the taste dispensable [3]. The great reduction of bitter taste function in the dolphin is surprising because natural toxins typically taste bitter, so the bitter taste represents an important natural defence against the ingestion of poisonous chemicals such as plant alkaloids and insect toxins [4–6].

Vampire bats are the only mammals that feed exclusively on blood [7] and the extreme narrowness of their diets may have rendered these bats poor tasters [8,9]. Indeed, all extant vampire bats (three species: common vampire bat, *Desmodus rotundus*; white-winged vampire bat, *Diaemus youngi* and hairy-legged vampire bat, *Diphylla ecaudata*) have lost sweet and umami tastes [9–11]; the common vampire bat behaviourally showed a reduction towards bitter-tasting compounds [11]. Furthermore, vampire bats use odour cues for prey detection [12] and use infrared sensors to locate capillary-rich areas of skin [7,13]. These capabilities may have further reduced their taste sensitivity [9]. To test whether bitter taste receptor genes (*T2R*s) have been relaxed from selective constraint in vampire bats, we sampled all three vampire bats and 11 non-vampire bats across the phylogeny and examined nine one-to-one orthologous *T2R*s that are shared in four bats representing two major groups of bats (Yangochiroptera and

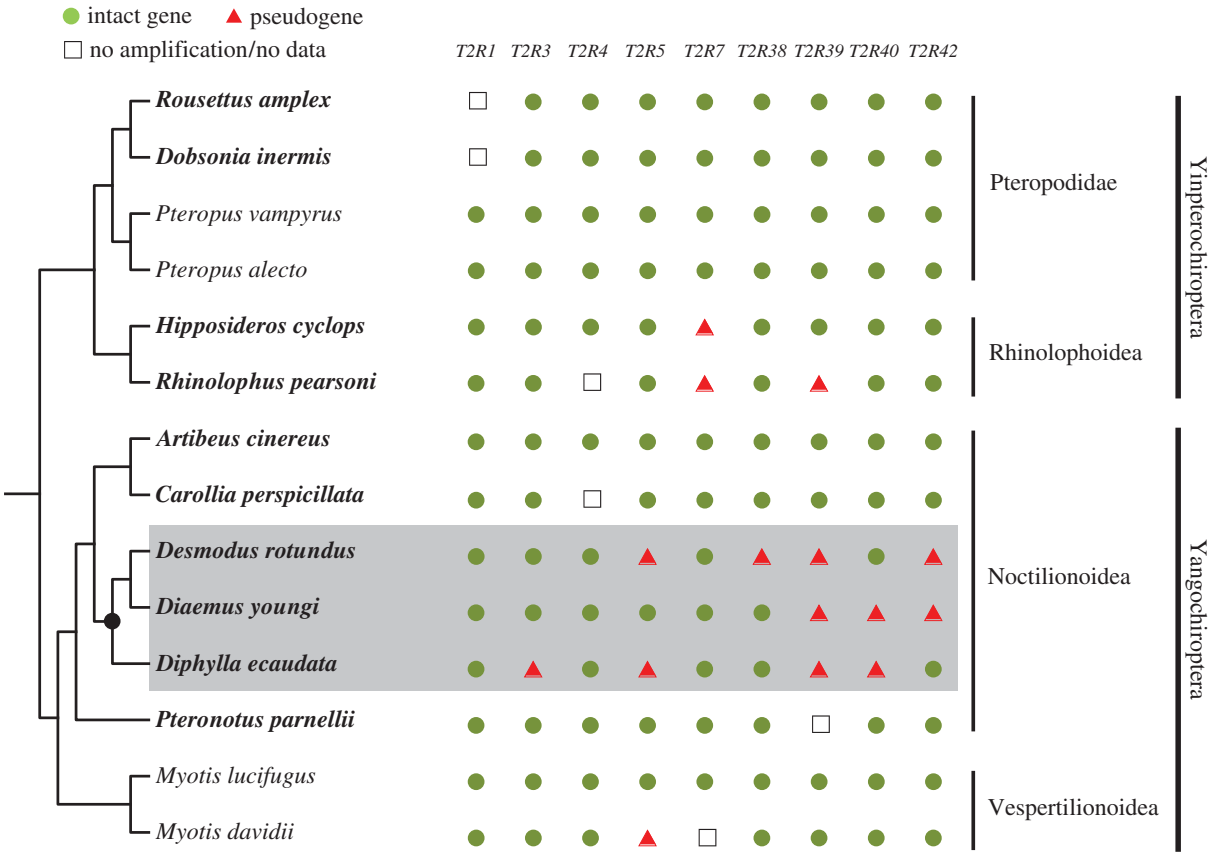

Figure 1. The species tree of the 14 bats studied, with intact and pseudogenized *T2R*s being indicated. Intact genes are characterized by an intact open reading frame (ORF), while pseudogenes are characterized by a disrupted ORF resulting from nonsense and/or frame-shifting mutations. Tree topology follows a previous study [17]. The ten species in bold are those sequenced in this study, whereas the four remaining species are those with available genome sequences. All three vampire bats are shaded in grey. The common ancestor of vampire bats discussed in the text is indicated as a black circle. (Online version in colour.)

Yinpterochiroptera) and thus are assumed to be functionally conserved in all bats. We found that, of these functionally conserved *T2R*s common to other bats, vampire bats have a significantly greater percentage of pseudogenized *T2R*s than other bats. We also found that vampire bats retain many intact and putatively functional *T2R*s.

2. Material and methods

(a) Gene identification and taxon coverage

We identified *T2R*s from the draft genome sequences of the four bats in the Ensembl genome database (*Pteropus vampyrus* and *Myotis lucifigus*) and an earlier study (*Pteropus alecto* and *Myotis davidii*) [14]. Because vertebrate *T2R*s are intronless and approximately 300 codons in length, the gene identification approach was straightforward. We used all *T2R*s from human, rat, dog and chicken as queries to TblastN against the four bat genomes following a previous study [15], and confirmed the presence of seven transmembrane domains using the TMHMM method [16]. All candidate *T2R*s were verified by the best hits with known *T2R*s using BlastN searches against the entire GenBank [15].

Our dataset of bats contained all three species of vampire bats and 11 species of non-vampire bats (figure 1). We attempted to include bat species that are both closely and distantly related to vampire bats. Specifically, two bats are affiliated with the same family Phyllostomidae as the three vampire bats; one belongs to Mormoopidae, a bat family that is most closely related to Phyllostomidae; two are from the other family in the same suborder Yangochirotera; the remaining six bats are from more distantly related families in the other suborder Yinpterochiroptera (figure 1; electronic supplementary material, table S1). The bat order Chiroptera is divided into two suborders: Yinpterochiroptera and Yangochiroptera, which comprise two and three superfamilies, respectively [17]. We sequenced *T2R*s from 10 bats and identified *T2R*s from the draft genome sequences of four additional bats. These species represent four of the five superfamilies of bats (figure 1).

(b) Polymerase chain reaction amplification and DNA sequencing

Based on the sequence alignments of *T2R*s from the four bats with available genome sequences, we designed a suite of primers (electronic supplementary material, table S2) to amplify the nine one-to-one orthologous *T2R*s in 10 bats (figure 1). All bat tissues were loaned from the American Museum of Natural History, and the identity of each bat was confirmed by sequencing the complete coding sequences of the mitochondrial cytochrome *b* (*Cytb*) gene (electronic supplementary material, figure S1). Genomic DNAs were isolated using Qiagen DNeasy kits. Polymerase chain reactions (PCRs) were performed following our previously described methods [9,10]. PCR products were sequenced directly with the same primer sets as for PCR amplifications. When the direct sequencing did not work, PCR products were cloned into the pMD19-T vector (Takara) and sequenced from both strands. We additionally amplified *T1R3* and *Calhm1* using the primer sequences listed in the electronic supplementary material, table S2. All sequences newly generated by PCRs were deposited in GenBank under accession numbers KJ55725–KJ557347.

(c) Sequence alignment and phylogenetic reconstruction

The resulting sequences were aligned with MEGA v. 5.2 [18], and checked by eye. Nucleotide sequence alignments were generated

according to protein sequence alignments and were subsequently used to reconstruct phylogenetic trees. Phylogenetic reconstruction for each dataset was conducted using a Bayesian approach, implemented in MrBayes v. 3.2 [19]. Six Markov chains were run simultaneously with as many generations as needed to ensure that the standard deviation of split frequencies was less than 0.01. We discarded the first 400 000 generations as burn-in and sampled the chains every 1000 generations. The best-fitting model of sequence evolution for each dataset was estimated by Modeltest v. 3.7 [20].

(d) Construction of ancestral sequences and tests for selection

Ancestral sequences of vampire bats were reconstructed using the Bayesian method [21] implemented in the baseml program in PAML [22] and the parsimony method [23]. To determine whether vampire bats have undergone differential selective pressures as compared to other bats, we estimated the ratio of non-synonymous to synonymous substitution rates (termed ω), which is an indicator of natural selection, with ω being less than 1, equal to 1 and more than 1 indicating purifying selection, neutral evolution and positive selection, respectively. We next undertook likelihood ratio tests of selection using branch models in the codeml program in PAML. For each gene, we conducted three tests (table 1). First, we tested whether the overall ω is significantly smaller than 1 in non-vampire bats. Second, we tested whether there is a significant difference in ω between the common ancestor of vampire bats and all other bats. Third, we tested whether there is a variation in ω before and after the divergence of vampire bats.

3. Results

(a) Survey of bitter taste receptor genes in four bat genomes

A total of 39, 34, 26 and 24 *T2R*s were identified from *M. davidii*, *M. lucifigus*, *P. alecto* and *P. vampyrus*, respectively (electronic supplementary material, table S3). Among them, 79 *T2R*s are intact with at least 270 codons, start codon, stop codon and seven transmembrane domains (electronic supplementary material, table S3); the nomenclature of bat *T2R*s followed that for human *T2R*s [24]. The deduced protein sequences of these intact genes were aligned and translated back to nucleotides and the resulting alignment was used to reconstruct a phylogenetic tree using the Bayesian approach. We found three *Myotis* specific clusters in the tree (electronic supplementary material, figure S2), suggestive of functional innovation of bitter taste in these insect-eating bats [25] because many insects rely on chemical defence against predators [6,26,27]. Notably, we identified seven clades containing four genes from each of the four bats (electronic supplementary material, figure S2), showing a one-to-one orthologous relationship. We also included two additional genes as one-to-one orthologues despite their absence in one of the four bats due to pseudogenization (*T2R5*) or incomplete sequencing (*T2R7*) (figure 1; electronic supplementary material, figure S2). The four bats analysed above belong to the two major groups of bats (Yinpterochiroptera and Yangochiroptera; electronic supplementary material, table S1) and these one-to-one orthologous *T2R*s are assumed to be functionally conserved in all bats because same bitter taste receptors tend to recognize certain bitter-tasting compounds better than other bitter-tasting chemicals, and thus tend to have the same functions [28]. We did not examine other *T2R*s because those genes are specific to certain species (electronic supplementary material, figure S2) that do not represent the conserved taste function in bats.

(b) Pseudogenization of bitter taste receptor genes in vampire bats

To test whether bitter taste function is reduced in vampire bats, we examined the nine *T2R*s in all three vampire bats, seven non-vampire bats and the four additional non-vampire bats with available genome sequences (figure 1; electronic supplementary material, table S1). We sequenced 85 *T2R* gene segments from the 10 bats, including the three vampire bats, which ranged from 528 to 872 bp in length. Phylogenetic trees reconstructed using each *T2R* gene generally agree with the established species tree [17] (electronic supplementary material, figure S3), suggesting that each *T2R* gene newly generated in various bats is orthologous. After aligning with 35 *T2R*s of the four bats with genome sequences, our analysis of 120 genes discovered 105 *T2R*s that retain intact open reading frames (ORFs), of which 89 intact ones were identified from a total of 93 genes in 11 non-vampire bats. These results strongly support the assumption that these *T2R*s are of functional importance across all non-vampire bats. By contrast, the remaining 15 *T2R*s contain ORF-disrupting mutations such as nonsense mutations and frame-shifting deletions (figure 2). In 12 of the 15 *T2R*s, the first nonsense mutations are located near the 5′ end, resulting in the loss of multiple transmembrane domains of the proteins (figure 2). The remaining three genes (*T2R5* of *Diphylla ecaudata*, *T2R7* of *R. pearsonii* and *T2R38* of *Desmodus rotundus*) contain the first nonsense mutations near the 3′ end (figure 2), which would lead to the loss of at least one transmembrane domain of the receptors because the final transmembrane domains of the bitter taste receptors are located at the very end of the coding region [29]. Therefore, none of the 15 truncated receptors is functional. Of the 15 pseudogenized *T2R*s, 11 were amplified from vampire bats, while four were identified from non-vampire bats (figure 1). For these functionally conserved *T2R*s common to non-vampire bats, the percentage of pseudogenes is significantly greater for vampire bats ($11/27 = 40.7\%$) than for non-vampire bats ($4/93 = 4.3\%$) ($p < 0.001$, Fisher's exact test), indicative of substantial reduction of bitter taste function in vampire bats.

Among vampire bats, *T2R39* is pseudogenized in all three vampire bats, two of which (*Diaemus youngi* and *Diphylla ecaudata*) share multiple frame-shifting deletions and nonsense mutations that are unshared in the third vampire bat (*Desmodus rotundus*) (figure 2). Because *Diaemus youngi* and *Diphylla ecaudata* diverged at the origin of vampire bats, the common ORF-disrupting mutations between them suggest that the pseudogenization of *T2R39* occurred in the common ancestor of vampire bats following additional mutations in *Desmodus rotundus* (figure 1). However, we cannot rule out the possibility that *T2R39* was pseudogenized independently in the three bats. Moreover, we observed *T2R42* to have one 2-bp deletion and one premature stop codon shared between *Desmodus rotundus* and *Diaemus youngi*, although this gene remains intact in *Diphylla ecaudata*. This result suggests that the pseudogenization of *T2R42* arose in the common ancestor of *Desmodus rotundus* and *Diaemus youngi* after its separation from *Diphylla ecaudata*. Additionally, *T2R5* and *T2R40* are pseudogenized in two vampire bats with no shared ORF-disrupting

Table 1. Likelihood ratio tests of selective pressures on bat *T2Rs* and *Calhm1*. *p*-values for each likelihood ratio test are given, and significant *p*-values (<0.05) are indicated in bold. See the electronic supplementary material, table S4 for details of parameter estimates.

models	model compared	genes									
		T2R1	*T2R3*	*T2R4*	*T2R5*	*T2R7*	*T2R38*	*T2R39*	*T2R42*	*T2R40*	*Calhm1*
Test 1: all non-vampire bats											
A. All branches have the same ω B. All branches have the same $\omega = 1$	B versus A	0.0651	**1.9×10^{-6}**	**3.0×10^{-8}**	**9.5×10^{-8}**	**0.002**	**1.1×10^{-24}**	**5.7×10^{-6}**	0.75	**1.1×10^{-22}**	**1.9×10^{-154}**
Test 2: all non-vampire bats plus ancestral sequence of vampire bats											
C. All branches have the same ω D. Ancestral branch of vampire bats has ω_2 and other branches have ω_1	D versus C	0.189	0.202	0.295	0.502	0.997	0.927	0.463	0.742	0.873	0.985
Test 3: all bats after removing nonsense mutations in pseudogenes											
E. All vampire bats branch have ω_2, the other branches have ω_1 F. Ancestral branch to vampire bats has ω_3, branches connecting three vampire bats have ω_2, other branches have ω_1	F versus E	0.262	0.105	0.42	1	0.362	0.327	0.869	0.633	0.117	0.119

Figure 2. Alignments of *T2R*s with the first ORF-disrupting mutations boxed. Dashes indicate alignment gaps and numbers in parentheses indicate nucleotide positions following the reference sequences. Reference sequences were from *Myotis lucifugus* or *Pteropus vampyrus* depending on the phylogenetic positions.

mutations, and *T2R3* and *T2R38* contain disruptive mutations in one of the three vampire bats, suggesting that the four genes were pseudogenized independently. Therefore, we found extensive losses of *T2R*s in vampire bats, but the common disruptive mutations that cause pseudogenization among all three vampire bats are absent, despite them sharing a common ancestry of blood-feeding [9,30,31].

(c) Likelihood ratio tests of selective pressures on bat bitter taste receptors and taste signalling pathway

To examine the functional implications of *T2R*s in non-vampire bats and to explore when the functional constraint on *T2R*s became relaxed in vampire bats, we estimated the ω ratio for each of the nine *T2R*s using a likelihood approach [22]. We undertook three tests for each gene, respectively. First, we analysed all non-vampire bats in this study, and estimated the same ω (model A in table 1) for all branches of the species tree (figure 1). The ω ratio is significantly smaller than 1 in each of the seven *T2R*s (see the comparison with model B in table 1), suggesting that these genes are under purifying selection and thus functionally important. By contrast, the remaining two genes (*T2R1* and *T2R42*) have an elevated ω ratio close to 1 (table 1), indicative of a relaxation of functional constraint on the two genes. Second, we inferred the sequence of the common ancestor of vampire bats (black circle in figure 1) for each of the nine *T2R*s using both Bayesian and parsimony approaches [21,23], and estimated ω ratios of *T2R*s for the common ancestor and other bats. We found that a model (model D in table 1) that allows a variation in ω between the common ancestor of vampire bats and all other bats is not significantly better than a simpler model (model C in table 1) that assumes the same ω across the tree for any gene (see table 1 for *p*-values). Third, we removed the nonsense mutations of pseudogenized *T2R*s in the three vampire bats and compared them with sequences from other bats. For each gene, we examined a model (model F in table 1) allowing a variation in ω between the ancestral branch of vampire bats and four branches connecting the three vampire bats. We found that the ω ratio of each gene for the ancestral branch is not significantly different from that of the four branches (see the *p*-values in table 1), after comparing with a simpler model (model E in table 1) assuming that the same ω ratio for the five branches. For details of parameter estimates for selection tests on bat *T2R*s, see the electronic supplementary material, table S4. Collectively, these results suggest that seven of nine *T2R*s are under strong functional constraint and evolutionarily conserved, and that relaxation of functional constraint resulting in pseudogenized *T2R*s may have arisen recently.

In addition to taste receptors, taste signalling pathways downstream of taste receptors are also essential for taste function. For example, CALHM1 (calcium homeostasis modulator 1) contributes to neurotransmission of taste stimuli; the loss of CALHM1 has rendered severely impaired responses to sweet, umami and bitter tastants [32]. We sequenced the complete coding sequences of *Calhm1* from all three vampire bats and seven other bats in this study (figure 1) and found these genes to be complete and intact in all bats. Likelihood ratio tests of selective pressures suggest

that *Calhm1* is under strong purifying selection in bats (table 1). For details of these selection tests, see the electronic supplementary material, text S1 and table S4. Coupled with the observations of many intact and evolutionarily conserved *T2R*s in vampire bats (figure 1 and table 1), our genetic data unambigously suggest that vampire bats still retain some bitter taste function, despite the losses of sweet and umami tastes [9,10].

4. Discussion

Behavioural tests have demonstrated that vampire bats possess poorly developed taste ability because they showed indifference to sweet and detected bitter, sour and salty tastants in high concentrations [11], and they even lost taste-aversion learning for poison avoidance [33]. Our genetic data are fully consistent with the behavioural tests. First, the sweet taste receptor gene (*T1R2*) was pseudogenized in each of the three vampire bats [9], which appeared consistent with the behavioural study [11]. In addition, we found the *T1R3* to have a common 26 bp deletion in *Desmodus rotundus* [10] and *Diphylla ecaudata*, which would shift the ORF and result in loss of most transmembrane domains of the receptor in their common ancestor (electronic supplementary material, figure S4). Because *T1R3* encodes the shared subunit of sweet and umami taste receptors [34], this finding strongly suggests that both sweet and umami tastes were lost in the common ancestor of vampire bats approximately 26 Ma [9], although the umami taste sensitivity has not been examined behaviourally [11]. Second, many pseudogenized *T2R*s in vampire bats suggest that their bitter taste is greatly reduced and the reduction of bitter taste was also observed behaviourally in *Desmodus rotundus* [11]. Third, the evolutionary conservation of several *T2R*s and taste signaling pathway strongly support the behavioural finding in which vampire bats still retain some bitter taste ability, evidenced by the detection of bitter tastants in relatively higher concentrations [11]. Consistent with the genetic data supporting the view of retaining some bitter taste in vampire bats, anatomical studies discovered normal taste buds in the canonical taste structures [35] and electrophysiological recordings identified functional taste receptors in these bats [36].

In addition to the bottlenose dolphin [3] and other whales [37], vampire bats also showed the evolutionary reduction of bitter taste function, suggesting that the reduction or major loss of bitter taste in animals is more pervasive than previously believed. All three vampire bats are obligate feeders on mammalian or bird blood [30], a food type that is unlikely ever to be bitter or toxic to these animals. This highly specialized diet with extremely narrow components would result in extensive reduction of bitter taste function in vampire bats, which would never encounter toxic foods in nature, despite many natural toxins tasting bitter [4,5]. Furthermore, instead of just taste, vampire bats use a combination of smell, echolocation and heat to find their prey and locate the skin with rich capillaries [7]. The utilization of various sensory systems may have further rendered the sense of taste less important [9]. Nonetheless, in view of the residual bitter taste conferring avoidance to higher concentrations of bitter tastants in vampire bats [11], it is not unexpected to observe many putatively functional *T2R*s in these animals. Although the functional *T2R*s are unlikely to play a major role in food selection for vampire bats, they could function in several extragustatory tissues [38]. For example, *T2R*s are expressed in the gastrointestinal and tracheal tracts [38–40]; *T2R*s are also involved in additional functions apart from bitter taste, such as regulation of glucose homeostasis [41] and delay of gastric emptying [42]. Analogous to these findings, the intact *T2R*s in vampire bats may function in extra-oral tissues. An alternative hypothesis to explain our finding of many intact *T2R*s in vampire bats is that the ancestors of vampire bats did not originally feed on blood and the specific dietary changes may have arisen recently, although these animals share a common ancestry of blood-feeding [9,30,31]. Regardless, future scrutiny of expression profiling and functional characterization of *T2R*s in vampire bats will provide a better understanding of the evolution of bitter taste in animals.

Acknowledgements. We thank Julie Feistein and Neil Duncan from the American Museum of Natural History for access to bat tissues. We are grateful to Zubing Zhan and Liang Ao for technical assistance in the laboratory.

Data accessibility. DNA sequences: Genbank accessions KJ557255–KJ557347.

Funding statement. This work was supported by National Natural Science Foundation of China (31300313) and a start-up fund from Wuhan University awarded to H.Z.

References

1. Yarmolinsky DA, Zuker CS, Ryba NJP. 2009 Common sense about taste: from mammals to insects. *Cell* **139**, 234–244. (doi:10.1016/j.cell.2009.10.001)
2. Li D, Zhang J. 2014 Diet shapes the evolution of the vertebrate bitter taste receptor gene repertoire. *Mol. Biol. Evol.* **31**, 303–309. (doi:10.1093/molbev/mst219)
3. Jiang P, Josue J, Li X, Glaserb D, Li W, Branda JG, Margolskeea RF, Reeda DR, Beauchamp GK. 2012 Major taste loss in carnivorous mammals. *Proc. Natl Acad. Sci. USA* **109**, 4956–4961. (doi:10.1073/pnas.1118360109)
4. Glendinning JI. 1994 Is the bitter rejection response always adaptive? *Physiol. Behav.* **56**, 1217–1227. (doi:10.1016/0031-9384(94)90369-7)
5. Garica J, Hankins WG. 1975 The evolution of bitter and the acquisition of toxiphobia. In *Olfaction and Taste V, Proc. of the 5th Int. Symp. in Melbourne, Australia* (eds. DA Denton, JP Coghlan), pp. 39–45. New York, NY: Academic Press.
6. de Jong P, Holloway G, Brakefield PM, de Vos H. 1991 Chemical defence in ladybird beetles (Coccinellidae) II. Amount of reflex fluid, the alkaloid adaline and individual variation in defence in 2-spot ladybirds (*Adalia bipunctata*). *Chemoecology* **2**, 15–19. (doi:10.1093/beheco/art013)
7. Altringham JD. 1996 *Bats: biology and behaviour*. Oxford, UK: Oxford University Press.
8. Zhao H, Zhang J. 2012 Mismatches between feeding ecology and taste receptor evolution: an inconvenient truth. *Proc. Natl Acad. Sci. USA* **109**, E1464. (doi:10.1073/pnas.1205205109)
9. Zhao H, Zhou Y, Pinto CM, Charles-Dominique P, Galindo-González J, Zhang S, Zhang J. 2010 Evolution of the sweet taste receptor gene *Tas1r2* in bats. *Mol. Biol. Evol.* **27**, 2642–2650. (doi:10.1093/molbev/msq152)
10. Zhao H, Xu D, Zhang S, Zhang J. 2012 Genomic and genetic evidence for the loss of umami taste in bats. *Genome Biol. Evol.* **4**, 73–79. (doi:10.1093/gbe/evr126)
11. Thompson RD, Elias DJ, Shumake SA, Gaddis SE. 1982 Taste preferences of the common vampire bat (*Desmodus rotundus*). *J. Chem. Ecol.* **8**, 715–721. (doi:10.1007/BF00988313)

12. Bahlman JW, Kelt DA. 2007 Use of olfaction during prey location by the common vampire bat (*Desmodus rotundus*). *Biotropica* **39**, 147–149. (doi:10.1111/j.1744-7429.2006.00218.x)
13. Kurten L, Schmidt U. 1982 Thermoperception in the common vampire bat (*Desmodus rotundus*). *J. Comp. Physiol. A* **146**, 223–228. (doi:10.1007/BF00610241)
14. Zhang G *et al*. 2013 Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456–460. (doi:10.1126/science.1230835)
15. Shi P, Zhang J. 2006 Contrasting modes of evolution between vertebrate sweet/umami receptor genes and bitter receptor genes. *Mol. Biol. Evol.* **23**, 292–300. (doi:10.1093/molbev/msj028)
16. Sonnhammer EL, von Heijne G, Krogh A. 1998 A hidden Markov model for predicting transmembrane helices in protein sequences. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **6**, 175–182. (doi:10.1007/978-1-59745-574-9_7)
17. Teeling EC, Springer MS, Madsen O, Bates P, O'Brien SJ, Murphy WJ. 2005 A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* **307**, 580–584. (doi:10.1126/science.1105113)
18. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739. (doi:10.1093/molbev/msr121)
19. Huelsenbeck JP, Ronquist F. 2001 MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755. (doi:10.1093/bioinformatics/17.8.754)
20. Posada D, Crandall KA. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. (doi:10.1093/bioinformatics/14.9.817)
21. Yang Z, Kumar S, Nei M. 1995 A new method of inference of ancestral nucleotide and amino acid sequences. *Genetics* **141**, 1641–1650.
22. Yang ZH. 2007 PAML 4: Phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* **24**, 1586–1591. (doi:10.1093/molbev/msm088)
23. Zhang J, Nei M. 1997 Accuracies of ancestral amino acid sequences inferred by the parsimony, likelihood, and distance methods. *J. Mol. Evol.* **44**(Suppl. 1), S139–S146. (doi:10.1007/PL00000067)
24. Shi P, Zhang J, Yang H, Zhang YP. 2003 Adaptive diversification of bitter taste receptor genes in mammalian evolution. *Mol. Biol. Evol.* **20**, 805–814. (doi:10.1093/molbev/msg083)
25. Zhou Y, Dong D, Zhang S, Zhao H. 2009 Positive selection drives the evolution of bat bitter taste receptor genes. *Biochem. Genet.* **47**, 207–215. (doi:10.1007/s10528-008-9218-y)
26. Blum M. 1981 *Chemical defense of arthropods*. New York, NY: Academic Press.
27. Weatherston J, Percy JE. 1970 *Arthropod defensive secretions*. New York, NY: Academic Press.
28. Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ. 2000 T2Rs function as bitter taste receptors. *Cell* **100**, 703–711. (doi:10.1016/S0092-8674(00)80706-0)
29. Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. 2000 A novel family of mammalian taste receptors. *Cell* **100**, 693–702. (doi:10.1016/S0092-8674(00)80705-9)
30. Fenton MB. 1992 Wounds and the origin of blood-feeding in bats. *Biol. J. Linnean Soc.* **47**, 161–171. (doi:10.1111/j.1095-8312.1992.tb00662.x)
31. Wetterer AL, Rockman MV, Simmons NB. 2000 Phylogeny of phyllostomid bats (Mammalia : Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. *Bull. Am. Mus. Nat. Hist.* **248**, 1–200. (doi:10.1206/0003-0090(2000)248<0001:POPBMC>2.0.CO;2)
32. Taruno A *et al*. 2013 CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. *Nature* **495**, 223–226. (doi:10.1038/nature11906)
33. Ratcliffe JM, Fenton MB, Galef Jr BG. 2003 An exception to the rule: common vampire bats do not learn taste aversions. *Anim. Behav.* **65**, 385–389. (doi:10.1006/anbe.2003.2059)
34. Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X. 2004 Different functional roles of T1R subunits in the heteromeric taste receptors. *Proc. Natl Acad. Sci. USA* **101**, 14 258–14 263. (doi:10.1073/pnas.0404384101)
35. Park H, Hall ER. 1951 The gross anatomy of the tongues and stomachs of eight New World bats. *Trans. Kans. Acad. Sci.* **54**, 64–72.
36. Suthers RA. 1970 Vision, olfaction, taste. In *Biology of bats* (ed. WA Wimsatt). New York, NY: Academic Press.
37. Feng P, Zheng JS, Rossiter SJ, Wang D, Zhao H. 2014 Massive losses of taste receptor genes in toothed and baleen whales. *Genome Biol. Evol.* **6**, 1254–1265. (doi:10.1093/gbe/evu095)
38. Behrens M, Meyerhof W. 2011 Gustatory and extragustatory functions of mammalian taste receptors. *Physiol. Behav.* **105**, 4–13. (doi:10.1016/j.physbeh.2011.02.010)
39. Rozengurt E, Sternini C. 2007 Taste receptor signaling in the mammalian gut. *Curr. Opin. Pharmacol.* **7**, 557–562. (doi:10.1016/j.coph.2007.10.002)
40. Wu SV, Rozengurt N, Yang M, Young SH, Sinnett-Smith J, Rozengurt E. 2002 Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. *Proc. Natl Acad. Sci. USA* **99**, 2392–2397. (doi:10.1073/pnas.042617699)
41. Dotson CD *et al*. 2008 Bitter taste receptors influence glucose homeostasis. *PLoS ONE* **3**, e3974. (doi:10.1371/journal.pone.0003974)
42. Glendinning JI, Yiin YM, Ackroff K, Sclafani A. 2008 Intragastric infusion of denatonium conditions flavor aversions and delays gastric emptying in rodents. *Physiol. Behav.* **93**, 757–765. (doi:10.1016/j.physbeh.2007.11.029)