Evolution of the Sweet Taste Receptor Gene Tas1r2 in Bats

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Abstract
Taste perception is an important component of an animal’s fitness. The identification of vertebrate taste receptor genes in the last decade has enabled molecular genetic studies of the evolution of taste perception in the context of the ecology and dietary preferences of organisms. Although such analyses have been conducted in a number of species for bitter taste receptors, a similar analysis of sweet taste receptors is lacking. Here, we survey the sole sweet taste–specific receptor gene Tas1r2 in 42 bat species that represent all major lineages of the order Chiroptera, one of the most diverse groups of mammals in terms of diet. We found that Tas1r2 is under strong purifying selection in the majority of the bats studied, with no significant difference in the strength of the selection between insect eaters and fruit eaters. However, Tas1r2 is a pseudogene in all three vampire bat species and the functional relaxation likely started in their common ancestor, probably due to the exclusive feeding of vampire bats on blood and their reliance on infrared sensors rather than taste perception to locate blood sources. Our survey of available genome sequences, together with previous reports, revealed additional losses of Tas1r2 in horse, cat, chicken, zebra finch, and western clawed frog, indicating that sweet perception is not as conserved as previously thought. Nonetheless, we found no common dietary pattern among the Tas1r2-lacking vertebrates, suggesting different causes for the losses of Tas1r2 in different species. The complexity of the ecological factors that impact the evolution of Tas1r2 calls for a better understanding of the physiological roles of sweet perception in different species.

Key words: bats, vampire bats, Tas1r2, sweet, taste, pseudogene.

Introduction
The sense of taste provides important dietary information and is crucial for the survival of animals (Bachmanov and Beauchamp 2007). All tastes are combinations of five basic modalities: sweet, umami, bitter, salty, and sour (Kinnamon and Margolskee 1996; Lindemann 1996). Sweet and umami tastes allow the recognition of diets with nutritious carbohydrates and proteins, respectively, and often cause appetitive reactions; bitter and sour tastes warn against the ingestion of potentially harmful foods and often cause aversive reactions; the salty taste detects sodium and other minerals that are needed with varying concentrations in different species (Herness and Gilbertson 1999; Bachmanov and Beauchamp 2007).

Taste perception is conferred by taste receptor cells through the use of either ion channels (sodium and sulty) or G protein-coupled receptors (GPCRs) (sweet, umami, and bitter) (Roper 1989; Lindemann 1996; Bachmanov and Beauchamp 2007). Two families of GPCRs, Tas1rs and Tas2rs, function as sweet/umami and bitter taste receptors, respectively (Hoon et al. 1999; Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001; Nelson et al. 2002; Zhao et al. 2002; Fischer et al. 2005; Go et al. 2005; Shi and Zhang 2006; Meyerhof 2005). Because the bitter taste is mainly responsible for the detection of toxins in food sources, which vary greatly among different animals, Tas2r genes have been hypothesized to differ substantially among species. This hypothesis is supported by multiple evolutionary studies (Conte et al. 2003; Shi et al. 2003; Wang et al. 2004; Fischer et al. 2005; Go et al. 2005; Shi and Zhang 2006). For example, there is a great variation in Tas2r gene number among mammals, from 21 in dog to 42 in rat (Shi and Zhang 2006).

By contrast, the Tas1r gene repertoire is small with only three genes in most mammals studied: Tas1r1, Tas1r2, and Tas1r3 (Hoon et al. 1999; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001; Zhao et al. 2003; Shi and Zhang 2006). Of these, Tas1r1 and Tas1r2 are expressed in separate taste receptor cells, although they are both coexpressed with Tas1r3. The Tas1r1 + Tas1r3 heterodimer functions as the umami taste receptor, whereas the Tas1r2 + Tas1r3 heterodimer functions as the sweet taste receptor (Nelson et al. 2001; Li et al. 2002; Mombaerts 2004). Thus, Tas1r2 is likely the sole sweet-specific taste receptor. Indeed, Tas1r2-knockout...
mice exhibit severely impaired responses to sweet but not other tastants and show human-like sweet taste preference after the subsequent knockin of human *Tas1r2* (Zhao et al. 2003).

Compared with the evolution of *Tas2rs*, the evolution of *Tas1rs* is poorly characterized. Because the sweet and umami tastes are regarded as necessary for nutrient uptake, they are believed to be conserved among species (Shi and Zhang 2006) and therefore have not been well studied. Indeed, limited surveys conducted thus far found one *Tas1r1*, one *Tas1r2*, and one *Tas1r3* in every mammal (Shi and Zhang 2006), with only two exceptions: *Tas1r1* is pseudogenized in the giant panda (family Ursidae) (Li et al. 2010; Zhao et al. 2010) and *Tas1r2* is inactivated in the cat family Felidae (Li et al. 2010). However, because the number of species that have been examined is small (Shi and Zhang 2006), it is unclear whether the observed conservation of *Tas1rs* is generally true. More importantly, the ecological causes of the two exceptions are unclear. For example, if the loss of *Tas1r1* in the giant panda is due to its dietary shift from meat to bamboo, why do herbivores such as the cow and horse still have a functional *Tas1r1* gene (Zhao et al. 2010)? Similarly, the inactivation of *Tas1r2* in the cat family but not in other carnivores is puzzling.

To examine the impact of diet on *Tas1r* evolution while avoiding confounding factors, it is preferable to examine relatively closely related species that nonetheless have diverse dietary preferences.

We choose to study the evolution of the sweet taste receptor gene *Tas1r2* in 42 bat species because bats have a huge diversity in their diets, including insects, other arthropods, fish, reptiles, amphibians, mammals, birds, fruits, flowers, nectar, pollen, foliage, and blood (Altringham 1996). Around 70% of bat species are insectivorous, although some of these are also carnivorous or piscivorous. Only three species are sanguivorous, feeding exclusively on blood. Two groups of bats consume mainly plant products: Old World fruit bats (members of the suborder Yinpterochiroptera) exclusively eat fruits, flowers, pollen, and nectar, whereas New World (NW) fruit bats (members of the suborder Yangochiroptera) feed on some insects in addition to their main diets of fruits or nectar and pollen (Altringham 1996). For simplicity, we classify bats into three main dietary groups: insect eaters (species primarily feeding on insects and other arthropods), fruit eaters (species primarily feeding on plant products), and blood feeders (species feeding on blood) (fig. 1). We show that *Tas1r2* is pseudogenized in blood feeders, whereas conserved in other bats. By surveying currently available genome sequences, we further report losses of *Tas1r2* in horse and zebra finch and a possible loss in pig. We discuss possible ecological factors influencing the evolution of *Tas1r2*.

### Materials and Methods

**Polymerase Chain Reaction and DNA Sequencing**

The genomic DNAs of 40 bat species (supplementary table S1, Supplementary Material online) were isolated from wing membrane biopsies or liver tissue samples using the Qiagen DNeasy kit. Based on an alignment of currently available *Tas1r2* sequences of human, mouse, dog, cow, flying fox, and little brown bat, we designed a pair of degenerate primers (SFF: 5′-GACRCYTCGSTGCTTCAAGCCGGC-3′ and SFR: 5′-GGTTGACACRGTCACCACGAGC-3′) to amplify ~720 nucleotides of the sixth exon of *Tas1r2* from 39 of the 40 bats. For the remaining species (hairy-legged vampire bat), the above primers did not work. We thus redesigned a forward primer (SFF23: 5′-CGACTGGCCCTTCCTAAAT-3′) according to the *Tas1r2* sequence acquired from the common vampire bat; SFF23 worked when coupled with SFR. The polymerase chain reaction (PCR) mixtures (10 μl) contained 1 μl (50 ng/μl) genomic DNA, 5 μl of 2× buffer, 1.5 μl (50 mM) MgCl₂, 1 μl (10 μM) of each primer, and 1 U Taq DNA polymerase (Takara). PCRs were conducted on 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 were cloned into the pMD19-T vector (Takara). Positive clones were sequenced on an ABI DNA sequencer using the sequencing primer pair (M13–M13 primer set). Sequences were deposited into the GenBank with the accession numbers AY604444, AF382889, DQ077398, FJ155475, FJ155476, AF316447, AF316445, AF316444, AF316454, and AF316438.

**Evolutionary Analysis**

Deduced amino acid sequences were aligned with CLUSTALX 1.81 (Thompson et al. 1997) and modified with BioEdit 7.0.4 (Hall 1999). The nucleotide sequence alignment was generated according to the protein sequence alignment. Bat phylogeny and divergence dates were taken from Miller-Butterworth et al. 2007). Ancestral sequences were reconstructed using the Bayesian method (Yang et al. 1995) implemented in the BASEML program in PAML 4.1 (Yang 2007) and the parsimony method. Nonsynonymous (dN) and synonymous (dS) nucleotide substitution rates were estimated using the likelihood method implemented in the CODEML program in PAML 4.1 (Yang 2007).

The bat Cytb and RAG2 gene sequences were retrieved from the GenBank, with the accession numbers AY604444, AF382889, DQ077398, FJ155475, FJ155476, AF316447, AF316445, AF316444, AF316454, and AF316438.

### Results

**Equally Strong Purifying Selection on *Tas1r2* of Fruit-Eating Bats and Insect-Eating Bats**

*Tas1r2* has six coding exons, encoding an approximately 800 amino acid peptide, which is composed of a long...
extracellular N-terminus, seven transmembrane domains, three extracellular and three intracellular loops, and an intracellular C-terminus (Li et al. 2002). From 40 bat species spanning the entire phylogeny of bats (fig. 1), we sequenced 720 nucleotides of the sixth exon of Tas1r2. The sequenced segment corresponds to the region from the end of the N-terminus to the beginning of the seventh transmembrane domain (fig. 2). We also obtained the DNA sequences of the same region from the draft genome sequences of two additional bat species (M. lucifugus and P. vampyrus). The maximum-likelihood tree reconstructed using the 42 bat Tas1r2 sequences is not significantly different from the established bat species tree (Teeling et al. 2005) (Shimodaria-Hasegawa test, \( P = 0.079 \)), suggesting that the sequences acquired are orthologous. We thus used the established species tree in all subsequent analyses.

With the exception of vampire bats, the open reading frame (ORF) of Tas1r2 is maintained in the sixth exon in all bats surveyed. In addition, we sequenced all six exons of the Tas1r2 gene from M. lucifugus and P. vampyrus and confirmed that the entire Tas1r2 ORF is intact in these species (supplementary fig. S1, Supplementary Material online). To verify that Tas1r2 is under purifying selection and thus is putatively functional in non-vampire bats and to explore the possibility of differential selection on Tas1r2 in fruit eaters and insect eaters, we estimated the ratio \((\omega)\) of nonsynonymous to synonymous substitution rates by a likelihood method. We conducted three analyses on the dataset of 39 species, excluding the
vampire bats. First, under the assumption of a uniform $\omega$ for all branches of the tree of the 39 bats (model A in Table 1), $\omega$ was estimated to be 0.197, which is significantly smaller than 1 ($P < 10^{-100}$; see the comparison with model B in Table 1). This result suggests that $Tas1r2$ is under an overall strong purifying selection in these bats. Second, we tested whether a model that allows different $\omega$ values for fruit eaters and insect eaters (model C in Table 1) fits the data significantly better than a simpler model that does not allow the difference (model D in Table 1). In this analysis, each branch that cannot be classified into the clades of fruit eaters or insect eaters has its own $\omega$ (fig. 1). We did not find model C to fit the data significantly better than model D ($P > 0.4$), indicating similar levels of purifying selection on the $Tas1r2$ genes of fruit eaters and insect eaters. Third, we examined a model in which every branch has its own $\omega$ (model E). This model is not significantly better than the uniform $\omega$ model (model A), suggesting that the variation of $\omega$ among different lineages of nonvampire bats is minimal.

**Pseudogenization of Tas1r2 in Vampire Bats**

In striking contrast to the conservation of $Tas1r2$ in all fruit-eating and insect-eating bats, the ORF of $Tas1r2$ is disrupted in each of the three vampire bats, which feed exclusively on blood. The ORF-disrupting mutations include nonsense mutations, insertions, and deletions (fig. 2). Specifically, the hairy-legged vampire bat *Diphylla ecaudata* has a 12-nucleotide deletion and two premature stop codons that would result in the loss of all transmembrane domains in its $Tas1r2$ protein. The white-winged vampire bat *Diaemus youngi* has a 12-nucleotide deletion and a 4-nucleotide insertion, which leads to a frame shift and a premature stop codon, resulting in the truncation of the protein in the third transmembrane domain. The common vampire bat *Desmodus rotundus* has a five-nucleotide deletion, a six-nucleotide deletion, a nine-nucleotide deletion, and a premature stop codon, resulting in the loss of the last two transmembrane domains in its $Tas1r2$.

Despite the observation of several ORF-disrupting mutations in each vampire bat, none of these mutations are shared among any pair of the three species. However, because the region we sequenced is relatively short, it remains possible that shared ORF-disrupting mutations exist in the regions of $Tas1r2$ that are not sequenced here. Even if no shared ORF-disrupting mutations exist, it is still possible that the purifying selection on $Tas1r2$ was completely removed before the divergence of the three vampire bats because it takes a substantial amount of time for an

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**Table 1**

<table>
<thead>
<tr>
<th>Model</th>
<th>$\omega$ Estimate</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.197</td>
<td>$&lt; 10^{-100}$</td>
</tr>
<tr>
<td>B</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2**

Alignment of $Tas1r2$ nucleotide sequences from the three vampire bats and one New World fruit bat for the regions examined in this work. Dashes indicate alignment gaps and question marks represent unamplified nucleotides. Codons in the correct reading frame are indicated by shading and premature stop codons are boxed. Regions corresponding to the seven transmembrane (TM) domains are indicated.
ORF-disrupting mutation to occur and be fixed in a species. Below, we evaluate whether the relaxation of the functional constraint on \( \text{Tas1r2} \) started before or after the divergence of the three vampire bats.

We inferred the \( \text{Tas1r2} \) sequence of the common ancestor of vampire bats (i.e., the black circle in fig. 1) using a combination of Bayesian and parsimony methods. Because the Bayesian method (Yang et al. 1995) cannot deal with indels, we used this method to infer the ancestral sequence from the alignment of all 42 \( \text{Tas1r2} \) sequences for regions without indels. For indel regions, we used parsimony to infer the ancestral sequence. We then estimated \( \omega \) using the inferred ancestral vampire bat \( \text{Tas1r2} \), which has an ORF, with the \( \text{Tas1r2} \) sequences from the 39 other bats. Assuming that all branches in the tree of the 39 extant sequences and one ancestral sequence have the same \( \omega \) (model F in Table 1), we estimated \( \omega = 0.211 \). If we assume that the branch leading to the ancestral vampire bats has \( \omega_2 \), whereas all other branches have \( \omega_1 \) (model G in Table 1), we found that \( \omega_2 = 0.994 \) and model G fits the data significantly better than model F \( (P < 10^{-8}) \). We further found by comparing model G and model H (\( \omega_2 \) is fixed at 1; see Table 1) that \( \omega_2 \) is not significantly different from 1. In addition to this analysis, we removed premature stop codons in the three vampire bat sequences and analyzed them together with the 39 other bat sequences. We found that a model that allows a variation in \( \omega \) between the stem vampire bat branch and the four branches connecting the three vampire bats (model J in Table 1) is not significant better than a simpler model that assumes the same \( \omega \) for these five branches (model I in Table 1) \( (P = 0.083) \). Together, these results support the hypothesis that a relaxation of functional constraint occurred in the branch leading to the common ancestor of vampire bats and that \( \text{Tas1r2} \) may have been evolving neutrally since the divergence of vampire bats from NW fruit bats.

If the above conclusion is correct, what is the probability of the absence of any shared ORF-disrupting mutation in the \( \text{Tas1r2} \) region sequenced in the three vampire bats? To answer this question, it is necessary to date the evolutionary events under investigation. We searched GenBank for DNA sequences available in all three vampire bats and found two suitable genes for this analysis: cytochrome b \( (\text{Cytb}) \) and recombination activating gene 2 \( (\text{RAG2}) \). The DNA sequences of these two genes are also available in two NW fruit bats, which we use as outgroups (fig. 3a). We concatenated the two genes and reconstructed a neighbor-joining (NJ) tree (fig. 3a), based on Tamura-Nei distances estimated by the maximum composite likelihood method implemented in MEGA4 (Tamura et al. 2007). We could not reject the molecular clock hypothesis using Tajima’s test (Tajima 1993) and therefore converted the NJ tree to a linearized tree (Takezaki et al. 1995). Using the calibration time of 26 My since the divergence of

### Table 1. Likelihood Ratio Tests of Selective Pressures on Bat \( \text{Tas1r2} \) Gene.

<table>
<thead>
<tr>
<th>Models</th>
<th>( \omega ) ( (d_{ni}/d_{se}) )</th>
<th>( \ln L^a )</th>
<th>( np^b )</th>
<th>Models Compared</th>
<th>( 2\Delta(\ln L)^c )</th>
<th>( P ) Values(^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dataset I: 39 sequences (all except vampire bats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. All branches have one ( \omega )</td>
<td>( \omega = 0.192 )</td>
<td>-6223.77</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. All branches have the same ( \omega = 1 )</td>
<td>( \omega = 1 )</td>
<td>-6485.21</td>
<td>77</td>
<td>B vs. A</td>
<td>522.88</td>
<td>( 1.0 \times 10^{-115} )</td>
</tr>
<tr>
<td>C. Branches in yellow have ( \omega_1 ), branches in blue have ( \omega_2 ), and branches a, b, c, and d each has its own ( \omega )</td>
<td>( \omega_1 = 0.183, \omega_2 = 0.205 )</td>
<td>-6211.42</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Branches in yellow have ( \omega_1 ), branches in blue have ( \omega_2 ), and branches a, b, c, and d each has its own ( \omega )</td>
<td>( \omega_1 = \omega_2 = 0.196 )</td>
<td>-6211.42</td>
<td>83</td>
<td>D vs. C</td>
<td>0.64</td>
<td>0.422</td>
</tr>
<tr>
<td>E. Each branch has its own ( \omega )</td>
<td>Variable ( \omega ) by branch</td>
<td>-6189.76</td>
<td>153</td>
<td>A vs. E</td>
<td>68.02</td>
<td>0.703</td>
</tr>
<tr>
<td>Dataset II: 40 sequences (dataset I plus the ancestral sequence of vampire bats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. All branches have the same ( \omega )</td>
<td>( \omega = 0.211 )</td>
<td>-6575.83</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. Branch e (ancestral to vampire bats) has ( \omega_1 ) and other branches have ( \omega_2 )</td>
<td>( \omega_1 = 0.192, \omega_2 = 0.994 )</td>
<td>-6559.21</td>
<td>81</td>
<td>F vs. G</td>
<td>33.23</td>
<td>( 8.2 \times 10^{-9} )</td>
</tr>
<tr>
<td>H. Branch e has ( \omega_2 = 1 ) and other branches have ( \omega_1 )</td>
<td>( \omega_1 = 0.192, \omega_2 = 1 )</td>
<td>-6223.77</td>
<td>79</td>
<td>H vs. G</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dataset III: 42 sequences (all bats, after removing gaps in pseudogenes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Branch e and branches connecting the three vampire bats have ( \omega_2 ), whereas other branches have ( \omega_1 )</td>
<td>( \omega_1 = 0.193, \omega_2 = 0.424 )</td>
<td>-6158.29</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. Branch e has ( \omega_2 ), branches connecting the three vampire bats have ( \omega_2 ), and other branches have ( \omega_1 )</td>
<td>( \omega_1 = 0.193, \omega_2 = 0.393, \omega_3 = 911.06 )</td>
<td>-6156.79</td>
<td>86</td>
<td>I vs. J</td>
<td>3.00</td>
<td>0.083</td>
</tr>
</tbody>
</table>

\( ^a \) The natural logarithm of the likelihood value.
\( ^b \) Number of parameters.
\( ^c \) Twice the difference in \( \ln L \) between the two models compared.
\( ^d \) \( P \) values lower than 0.05 are shown in bold.
NW fruit bats and vampire bats (Teeling et al. 2005), we estimated the divergence times of the three vampire bats (fig. 3). One can see that the time between the divergence of vampire bats from NW fruit bats and the first speciation within vampire bats is relatively short (1.1 My), whereas the time between the two speciation events within vampire bats is relatively long (7.2 My). We then estimated the probability that a \textit{Tas1r2} sequence contains no fixed ORF-disrupting mutations after neutral evolution for a given amount of time, using the PSEUDOGENE program (Zhang and Webb 2003). This estimation requires the neutral rates of point mutations and indel mutations. We estimated from a genomic comparison between mouse and rat (Gibbs et al. 2004) that the neutral point mutation rate is $4.83 \times 10^{-9}$ per site per year and the indel mutation rate is $3.13 \times 10^{-10}$ per site per year. Furthermore, previous studies estimated that about 83% of indel mutations are not of multiples of three nucleotides and therefore are ORF disrupting (Podlaha and Zhang 2003; Zhang and Webb 2003). Using these parameters and a NW fruit bat \textit{Tas1r2} sequence, we estimated that the half-life of \textit{Tas1r2} is 2.02 My, meaning that the probability that the ORF is still present in the sequenced \textit{Tas1r2} region is 50% after 2.02 My of neutral evolution. Using this half-life, we estimated that the probability of no fixation of ORF-disrupting mutations is $P_1 = 0.085$ after 7.2 My of neutral evolution and is $P_2 = 0.058$ after 8.3 (7.2 + 1.1) My of neutral evolution. Thus, it is possible that no shared ORF-disrupting mutations are observed in \textit{Tas1r2} even if the purifying selection on the gene has been completely removed since the separation of vampire bats from NW fruit bats. In the above calculation, we assumed that the mutation rate per site per year is the same in bats and murids. Because the generation time of vampire bats is longer than that of murids, the mutation rate per year may be lower in bats than in murids. Thus, the actual probabilities may be somewhat higher than the above estimates of $P_1$ and $P_2$.

As a comparison, we also estimated the selective pressures on \textit{Cytb} and \textit{RAG2} in vampire bats and NW fruit bats. We designated the four branches connecting the three vampire bats as vampire bat branches and the two branches connecting the two NW fruit bats as NW fruit bat branches (fig. 3a). For \textit{RAG2}, there is no significant difference in $\omega$ between the two groups of branches ($P > 0.7$). For \textit{Cytb}, $\omega$ is significantly higher for vampire bats than for NW fruit bats ($P < 0.01$). Nonetheless, for both genes, vampire bat $\omega$ is significantly lower than 1 (0.158 for \textit{RAG2} and 0.028 for \textit{Cytb}). Thus, the pseudogenization of vampire bat \textit{Tas1r2} is not part of any genome-wide relaxation of purifying selection but rather a gene-specific event.

Our multiple analyses of the selective pressure and pseudogenization process are consistent with each other. Together, they suggest that, although no shared ORF-disrupting mutations are observed in the \textit{Tas1r2} genes of the three vampire bats, the relaxation of functional constraint already happened in their common ancestor.

**Survey of \textit{Tas1r2} in Mammalian Genome Sequences**

To gain a broader picture of \textit{Tas1r2} evolution, we used TBLASTN and query sequences from closely related species to search for \textit{Tas1r2} in 32 currently available mammalian genome sequences (fig. 4; see the list of species names and gene accession numbers in supplementary table S2, Supplementary Material online). For the majority of the species surveyed, we were able to identify \textit{Tas1r2}. Although in many low-coverage genome sequences, the identified
sequences were incomplete, there were no premature stop codons. However, we were not able to find *Tas1r2* from the horse (6.79× coverage) and pig (4×) genome sequences (fig. 4). In human and most species surveyed, *PAX7* and *ALDH4A1* are the two genes that are adjacent to *TAS1R2* on the chromosome. We applied TBlastN to screen these two genes in horse and pig. In horse, *PAX7* and *ALDH4A1* were found to be next to each other, suggesting that *Tas1r2* is truly lost. In pig, *PAX7* but not *ALDH4A1* was found, suggesting that the absence of *Tas1r2* could be due to the incompleteness of the genome sequence, which may not be surprising given the low coverage (4×) of the sequence.

Consistent with earlier behavioral (Ganchrow et al. 1990) and electrophysiological (Halpern 1962) studies, *Tas1r2* was reported to be absent in the chicken genome (Shi and Zhang 2006). Interestingly, we found that *Tas1r2* is also missing from zebra finch, the only other bird with an available genome sequence (6× coverage), despite the presence of the two adjacent genes *PAX7* and *ALDH4A1*. Because chicken and zebra finch belong to two distantly related avian orders (Hackett et al. 2008), if the absences of their *Tas1r2* genes were due to the same evolutionary loss, *Tas1r2* must be absent in a large number of birds including nectar feeders. It seems more likely that the
absence of Tas1r2 in chicken and zebra finch were due to independent gene losses, but additional sampling of birds is required to test this hypothesis.

**Discussion**

In this study, we surveyed the sweet-specific receptor gene Tas1r2 from 42 species of bats to examine how different dietary preferences influence the evolution of Tas1r2. Because fruits and flowers contain glucose, fructose, and sucrose, one may expect Tas1r2 and the sweet taste to be much more important for fruit eaters than insect eaters. However, we found Tas1r2 to be under equally strong purifying selection in fruit eaters and insect eaters. Although the Tas1r2 region we surveyed does not include the putative ligand-binding domain (Cui et al. 2006), a relaxation of selective constraint is unlikely to be limited to that region. In fact, a previous study that documented a relaxation of selective constraint in human bitter taste receptors showed that the relaxation happens in all domains including those not involved in ligand binding (Wang et al. 2004). Furthermore, using the complete Tas1r2 sequences that include ligand-binding domains from P. vampyrus (fruit eater), M. lucifugus (insect eater), and dog, we confirmed that ω is not significantly different between the two bat lineages (P > 0.4). Based on the draft genome sequences, P. vampyrus and M. lucifugus have an intact gene for Tas1r3, which binds to Tas1r2 to form a functional sweet receptor. Thus, the sweet taste is likely under equally strong constraints in fruit eaters and insect eaters. It would be interesting to confirm our results by behavioral studies.

We found that Tas1r2 is a pseudogene in all three blood-feeding vampire bats. This finding appears consistent with previous behavioral studies. Specifically, the taste ability of common vampire bats is poorly developed compared with other mammals; they are able to discriminate salty, sour, and bitter tastants in high concentration but show indifference to sweet tastants even at the highest concentration tested (Thompson et al. 1982). Interestingly, a recent behavioral test found that common vampire bats lack taste-aversion learning, which is critical to poison avoidance (Ratliffe et al. 2003). It would be interesting to study other taste receptors in vampire bats, especially bitter taste receptors, to understand the genetic basis of the lack of taste-aversion learning in vampire bats. Vampire bats are the only mammals that feed exclusively on blood. Because of the extreme narrowness in diet, their sense of taste is unlikely to be important. Furthermore, vampire bats use olfaction to find prey (Bahlman and Kelt 2006) and use infrared sensors to locate blood sources (Fenton 1992). These capabilities may have further rendered the taste sensitivity less important, as in several previously proposed cases of sensory tradeoffs (Zhang and Webb 2003; Zhao et al. 2009).

In addition to vampire bats, Tas1r2 is also known to be absent in the cat family Felidae (Li et al. 2005), chicken (Shi and Zhang 2006), and the tongueless western clawed frog (Shi and Zhang 2006) since its origin in the common ancestor of bony fishes (Grus and Zhang 2009). Furthermore, we found that Tas1r2 is missing in the genome sequences of horse, pig, and zebra finch, although its absence in pig may be an artifact of the low sequence coverage. Among the species that clearly lack a functional Tas1r2 gene, vampire bats are sanguivorous, cats are carnivorous, horse is herbivorous, chicken is herbivorous and insectivorous, zebra finch is herbivorous, and western clawed frog is insectivorous. The diverse diets of the Tas1r2-lacking vertebrates suggest no uniform dietary reason for the loss of the gene in these species. It is possible that the loss of Tas1r2 in each species has species-specific reasons, as explained earlier for vampire bats. The complexity of the potential ecological factors impacting Tas1r2 evolution calls for more extensive studies of the physiological roles of sweet perception, which apparently is not as conserved as previously thought.

**Supplementary Material**

Supplementary fig. S1 and table S1 and S2 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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