Major taste loss in carnivorous mammals

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Mammalian sweet taste is primarily mediated by the type 1 taste receptor Tas1r2/Tas1r3, whereas Tas1r1/Tas1r3 act as the principal umami taste receptor. Bitter taste is mediated by a different group of G protein-coupled receptors, the Tas2rs, numbering 3 to ~66, depending on the species. We showed previously that the behavioral indifference of cats toward sweet-tasting compounds can be explained by the pseudogenization of the *Tas1r2* gene, which encodes the Tas1r2 receptor. To examine the generality of this finding, we sequenced the entire coding region of *Tas1r2* from 12 species in the order Carnivora. Seven of these nonfeline species, all of which are exclusive meat eaters, also have independently pseudogenized *Tas1r2* caused by ORF-disrupting mutations. Fittingly, the purifying selection pressure is markedly relaxed in these species with a pseudogenized *Tas1r2*. In behavioral tests, the Asian otter (defective *Tas1r2*) showed no preference for sweet compounds, but the spectacled bear (intact *Tas1r2*) did. In addition to the inactivation of *Tas1r2*, we found that sea lion *Tas1r1* and *Tas1r3* are also pseudogenized, consistent with their unique feeding behavior, which entails swallowing food whole without chewing. The extensive loss of Tas1r receptor function is not restricted to the sea lion: the bottlenose dolphin, which evolved independently from the sea lion but displays similar feeding behavior, also has all three *Tas1rs* inactivated, and may also lack functional bitter receptors. These data provide strong support for the view that loss of taste receptor function in mammals is widespread and directly related to feeding specializations.

diet | sweetener

It is widely believed that most mammals perceive five basic taste qualities: sweet, umami, bitter, salty, and sour. The receptors for sweet, umami and bitter tastes are G protein-coupled receptors (GPCRs) (1). Sweet taste is mediated largely by a heteromer of two closely related Tas1r (type 1 taste receptor) family GPCRs: Tas1r2 and Tas1r3 (2–5). Tas1r1, another member of the Tas1r family, in combination with Tas1r3, forms an umami taste receptor (6). Tas1r receptors are class C GPCRs. Unlike sweet and umami tastes, bitter taste is mediated by Tas2r family GPCRs, which belong to class A GPCRs and are structurally unrelated to Tas1r family receptors (7, 8). The genes encoding Tas2r receptors, the *Tas2r* genes, differ substantially in gene number and primary sequences among species, most likely reflecting the likelihood that these genes are required for detecting toxic or harmful substances in a species' ecological niche (9–11).

Direct evidence for a close correlation between taste function and feeding ecology comes from work on domestic and wild Felidae. Cats, obligate carnivores, are behaviorally insensitive to sweet-tasting compounds (12, 13). We proposed that this behavioral insensitivity was a consequence of the pseudogenization of *Tas1r2* (14). *Tas1r2* also is known to be pseudogenized in chicken, tongueless Western clawed frogs, and vampire bats (11, 15). The loss of the sweet taste receptor in chicken and vampire bats is consistent with their sweet insensitive behavior (16, 17). It is yet to be established how Western clawed frogs respond to sweeteners. In contrast with the feline, the giant panda lacks a functional umami taste receptor gene (*Tas1r1*) (18) and feeds primarily on bamboo. Nevertheless, the remainder of the taste system in both cats and giant pandas is similar to those of other mammals (12, 13, 18, 19).

Based on anatomical studies, it is likely that some aquatic mammals, such as sea lions (Carnivora) and dolphins (Cetacea)—

species from two lineages that independently "returned" to the sea more than 35 and 50 million years ago, respectively (20)— have lost some taste function. Both animals exhibit an atrophied taste system, exemplified by few taste buds present in their lingual epithelium (21). Consistent with an atrophied taste system, both species exhibit a feeding behavior pattern that suggests that taste may not play a major role in food choice: they swallow their food whole, perhaps minimizing opportunities and needs for taste input (22, 23).

To further elaborate on the idea that taste behavior, taste receptor function, and feeding ecology are intimately interrelated, we have chosen a comparative approach. Specifically, we have tested two hypotheses. First, we hypothesized that mutations rendering sweet taste receptors dysfunctional should be observed in exclusively meat-eating species in addition to the cats. We selected for study a range of species from the order Carnivora to test this hypothesis. This group is particularly useful for testing this hypothesis because it includes species differing significantly in their dietary habits, ranging from obligate carnivores (e.g., domestic and wild cats) to relatively omnivorous species (e.g., bears) to rather strict herbivores (e.g., the giant panda) (24, 25). The second hypothesis tested here involved mammals that were both exclusive carnivores and were known anatomically to have an atrophied taste system. We hypothesized that not only sweet taste receptor function but receptors for other taste qualities, such as umami and bitter, would also be disrupted. To examine this prediction, we evaluated the molecular structure of the other *Tas1rs* in the sea lion and the *Tas1rs* and the *Tas2rs* in bottlenose dolphins from the order Cetacea.

We found that seven of the 12 species examined from the order Carnivora—only those that feed exclusively on meat—had pseudogenized *Tas1r2* genes as predicted. Furthermore, we confirmed our hypothesis that, in addition to the loss of *Tas1r2*, both the sea lion and bottlenose dolphin lack *Tas1r1* and *Tas1r3* receptor genes, suggesting an absence of both sweet and umami taste-quality perception. Additionally, we failed to detect intact bitter receptor genes *Tas2rs* from the dolphin genome, suggesting that this modality may be lost, or its function greatly reduced, in dolphins. Thus, taste loss is much more widespread than previously thought, and such losses are consistent with altered feeding strategies.

Results

Molecular Cloning of *Tas1r2* from Selected Species Within Carnivora.
To determine how widespread the pseudogenization of *Tas1r2* is in the order Carnivora, and to evaluate how this relates to food habits of these species, we fully sequenced all six exons of *Tas1r2* from 12 species within Carnivora using degenerate primers designed from conserved exon-intron boundary sequences among cat, dog, and giant panda *Tas1r2*s, three species with an assembled genome in the order Carnivora. Among these 12 species, we identified five that appear to have an intact *Tas1r2*: the aardwolf, Canadian otter, spectacled bear, raccoon, and red wolf. The intact *Tas1r2* genes had entire complete coding sequences ranging from 2,511 to 2,517 bp.

Pseudogenized Tas1r2 *genes in Pinnipedia.* The sea lion, fur seal, and Pacific harbor seal belong to the Pinnipedia superfamily within the Caniformia suborder of Carnivora. In exon 1 of *Tas1r2*, both the sea lion and fur seal (Otariidae family) have an ATA instead of ATG in the start codon position. This mutation is predicted to prevent *Tas1r2* from being translated (Fig. 1*A* and *B* and *SI Appendix*, Fig. S1*A*). Additionally, we detected a 1-bp deletion between 579 and 580 bp in exon 3 of the sea lion *Tas1r2* (Fig. 1*A* and *SI Appendix*, Fig. S1*B*); 2-bp deletions between 674 and 675 bp in exon 3 of the sea lion *Tas1r2* and between 675 and 676 bp in exon 3 of the fur seal *Tas1r2* (Fig. 1*A* and *SI Appendix*, Fig. S1*C*); and a 1-bp deletion between 802 and 803 bp in exon 6 of both species (Fig. 1*A* and *SI Appendix*, Fig. S1*D*). These defects in the coding sequence predict that the sea lion and fur seal *Tas1r2* genes are pseudogenes.

Unlike in the sea lion and fur seal, *Tas1r2* of the Pacific harbor seal (Phocidae family) has a normal ATG start codon; however, it has a nonsense mutation at 32 bp in exon 6 that leads to a premature stop codon (TAA; Fig. 1 *A* and *B* and *SI Appendix*, Fig. S2*A*). Additionally, it has a 2-bp deletion between 192 and 193 bp in the sixth exon that results in a frame shift and multiple stop codons thereafter (Fig. 1*A* and *SI Appendix*, Fig. S2*B*). These ORF-disrupting mutations would likely also render the Pacific harbor seal *Tas1r2* gene defective.

Pseudogenized Tas1r2 *in Asian small-clawed otter.* The Asian small-clawed otter, Canadian otter, and ferret all belong to the Mustelidae family within the Caniformia. In a previous study, we reported an intact *Tas1r2* sequence in ferret, predicting a functional Tas1r2 receptor (24). Based on our current data, the Canadian otter appears to have an intact *Tas1r2* gene as well. In contrast, we detected a T insertion at 360 bp in exon 3 of the Asian otter *Tas1r2* based on the sequence alignment with the dog ortholog (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S3), predicting a defective *Tas1r2*. The same insertion was found in DNA sample from a second Asian otter.

Pseudogenized Tas1r2 *in spotted hyena.* The spotted hyena and aardwolf belong to the family Hyaenidae within the Feliformia. The aardwolf appears to have an intact *Tas1r2* gene. In contrast, we detected a 1-bp ORF-disrupting deletion between 130 and 131 bp in exon 2 of *Tas1r2* in the spotted hyena (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S4). The same deletion was found in a second spotted hyena. Therefore, the spotted hyena *Tas1r2* is a pseudogene.

Pseudogenized Tas1r2 *in fossa.* Fossa is a species in the family Eupleridae within the Feliformia. Two ORF-disrupting mutations were found in exons of the fossa *Tas1r2*. Specifically, there is a nonsense mutation (A) at 125 bp in exon 3 that results in a stop codon (TAG; Fig. 1 *A* and *B* and *SI Appendix*, Fig. S5*A*). Moreover, in exon 4, we detected a T insertion at 58 bp that creates a stop codon immediately after (TGA, 58–60) (Fig. 1*A* and *SI Appendix*, Fig. S5*B*). The T insertion was found in three additional individuals, indicating that this mutation is fixed in the fossa *Tas1r2* gene. In contrast, the nonsense mutation TAG (125 bp, exon 3) displayed polymorphism (TAG or TGG). Collectively, these ORF-disrupting mutations in the fossa *Tas1r2* predict that the fossa *Tas1r2* is a pseudogene.

Pseudogenized banded linsang Tas1r2. The banded linsang belongs to the family Prionodontidae within the Feliformia. In exon 2, we detected an A insertion at 70 bp (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S6*A*). Furthermore, we detected another 10-bp microdeletion between 274 and 275 bp in the second exon (Fig. 1*A* and *SI Appendix*, Fig. S6*B*), a 14-bp insertion between 78 and 91 bp in exon 4 (Fig. 1*A* and *SI Appendix*, Fig. S6*C*), a 20-bp microdeletion between 27 and 28 bp in exon 5 (Fig. 1*A* and *SI Appendix*, Fig. S6*D*), and another 2-bp deletion between 54 and 55 bp in exon 5 (Fig. 1*A* and *SI Appendix*, Fig. S6*E*). In exon 6, there is a 1-bp deletion between 210 and 211 bp, a 28-bp insertion between 235 and 262 bp, and a 1-bp deletion between 444 and 445 bp (Fig. 1*A* and *SI Appendix*, Fig. S6 *F–H*). With multiple ORF-disrupting mutations in the coding sequence, the banded linsang *Tas1r2* is apparently defective.

In summary, we found that, in addition to cats, seven other species in the order Carnivora have been identified as possessing pseudogenized *Tas1r2* genes. Moreover, the ORF-disrupting mutations that cause a pseudogenized *Tas1r2* differ among species in different families and clades. Within a single family, the pseudogenization of *Tas1r2* occurred in some species, but not others. A common feature shared by those species with pseudogenized *Tas1r2* genes is that they are strict carnivores or piscivores (fish eaters) (25).

Evolutionary Analysis of *Tas1r2* in Carnivora. To gain insight into the evolution of the sweet taste receptor in the order Carnivora, we conducted a detailed evolutionary analysis of *Tas1r2* from 18 species within Carnivora, including eight species (seven determined in this study and the domestic cat) with a pseudogenized *Tas1r2* and 10 species (five determined in this study and five determined previously) with an intact *Tas1r2*. The human *Tas1r2* was used as the outgroup for the analysis. We aligned the entire coding sequence of *Tas1r2* from 18 carnivore species along with the human *Tas1r2* sequence, then removed gaps, indels, and stop codons from the alignment, which results in 2,160-bp aligned nucleotides for phylogenetic analysis. A phylogenetic tree was built using the maximum-likelihood analysis method implemented in MEGA5 (Fig. 2)

Fig. 1. Widespread pseudogenization of the sweet-taste receptor gene *Tas1r2* in 7 species within Carnivora. (*A*) Schematic diagram shows the positions of ORF-disrupting mutations in *Tas1r2* from selected species within Carnivora. The intact dog *Tas1r2* gene structure is shown as a reference. The positions where ORF-disrupting mutations occurred are marked with a red asterisk (*). (*B*) A 42-bp–long nucleotide sequence containing the ORF-disrupting mutation that occurs closest to 5′ end of the gene is shown for each species. The aligned dog sequence is shown above it, and the amino acid sequence deduced from the nucleotide sequence up to the mutation site is shown underneath it. The codon that contains the ORF-disrupting mutation (marked in red and underlined) is indicated by a box.
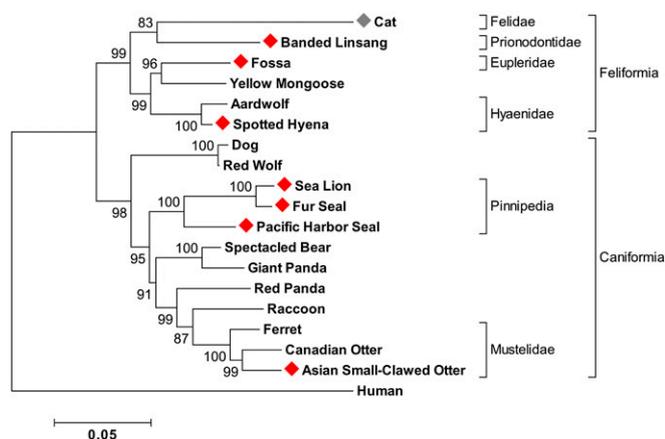
Fig. 2. An evolutionary tree of *Tas1r2* gene from 18 species within Carnivora. The evolutionary history is inferred by using the maximum-likelihood method based on the Tamura–Nei model (37) implemented in MEGA5 (26). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2,000 replicates) is shown next to the branches (38). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Species with a pseudogenized *Tas1r2* are marked with a diamond (red and gray depict species characterized in this study or previously, respectively). The human *Tas1r2* is used as the outgroup for the analysis.

(26). The same tree was derived using the neighbor-joining method implemented in MEGA5 (*SI Appendix,* Fig. S7). We used our tree for further statistical analysis because our phylogenetic tree agrees well with trees proposed previously using other gene sequences or intron sequences (27, 28).

To evaluate whether *Tas1r2* is under strong purifying selection in the order Carnivora, we estimated the ratio (ω) of non-synonymous to synonymous substitution rates by a likelihood method implemented in CODEML (29). The total nucleotides used for analysis were 2,160 bp after removing gaps, indels, and stop codons from the alignment. In model A, we analyzed this dataset of 18 species to evaluate the overall selective constraint on *Tas1r2*. With an assumption of a uniform ω, the average ω across the tree was estimated to be 0.1909, which is significantly <1 (model B), again indicating a strong overall negative selective pressure on *Tas1r2* ($P \sim 0$; Table 1).

To examine whether the selective pressure is somewhat relaxed on species with a pseudogenized *Tas1r2*, we tested a two-ratio model (model C) with the assumption of a uniform ω for branches with a pseudogenized *Tas1r2* (ω2) and for branches with an intact *Tas1r2* (ω1), respectively. In this model, ω1 was estimated to be 0.13656, similar to that estimated in model A; ω2 was estimated to be 0.41974. This two-ratio model C fits significantly better than the above-mentioned one-ratio model A ($P = 1.1 \times 10^{-16}$; Table 1), indicative of a divergence in the selective pressure between the branches with a pseudogenized *Tas1r2* and

the branches with an intact *Tas1r2*. The selective purifying pressure is markedly relaxed in the branches with a pseudogenized *Tas1r2*.

To investigate whether the selective pressure is completely removed from the branches with a pseudogenized *Tas1r2*, we tested another two-ratio model (model D) that allows ω2 (pseudogenized) to be fixed to 1 and a uniform ω1 for the branches with an intact *Tas1r2*. This model D fits significantly less well than the above-mentioned two-ratio model C ($P = 1.2 \times 10^{-11}$; Table 1), suggesting that the selective pressure is partially but not completely relaxed. Finally, we tested an alternative model (E) in which ω is allowed to vary among branches, this model was found not to fit significantly better than a two-ratio model C ($P = 0.05955$).

Behavioral Taste-Testing of the Asian Small-Clawed Otter and Spectacled Bear. To investigate the proposed relationship between *Tas1r2* receptor structure and taste perception and diet, we carried out behavioral tests on two available carnivore species from among those we genotyped. Previously, we had tested sweet preferences in a number of species, including lesser panda, domestic ferret, haussa genet, meerkat, yellow mongoose, and Asiatic lion (24). With the exception of the Asiatic lion, all these species within Carnivora appear to have an intact sweet taste receptor and prefer sweet-tasting compounds. Thus, aside from cats, there have been no previous taste preference studies in carnivorans with a defective *Tas1r2*.

Here, we tested two Asian small-clawed otters, a species with defective Tas1r2, and four spectacled bears, which appear to have an intact sweet-taste receptor, for their preferences for a wide range of sweet-tasting compounds, including both sweet carbohydrates (natural sugars) and noncaloric sweeteners.

We used a two-bowl group test to assess preference for sweet-tasting compounds. When presented with a simultaneous choice between sugar-containing solution and water, the Asian otters showed no clear preference for sugars. The preference ratios range from 35.4% for sucrose (0.5 M) to 56.5% for galactose (0.8 M; Fig. 3). They showed indifference to, or avoidance of, a few nonnutritive sweeteners tested, including indifference to sucralose (45.5% at 5.03 mM) and avoidance to saccharin (20.6% at 6.2 mM; Fig. 3), indicating that the taste-test procedure is capable of demonstrating discrimination among tastants. The spectacled bears showed strong preferences for natural sugars, with preference ratios ranging from 83% for glucose (0.5 M) to 100% for galactose (0.8 M; Fig. 3). They also showed strong preferences for certain noncaloric sweeteners, including 5.0 mM sucralose (86.2%) and 6.2 mM acesulfame-K (92.9%) (Fig. 3). These results are congruent with the molecular data from both species.

Sea Lions and Dolphins Lack All Three *Tas1r* Receptors. Given the atrophied taste system and unique feeding behavior in sea lions, we next investigated what happened to the other two *Tas1r*s in the sea lion. Using degenerate primers (*SI Appendix,* Table S1) and genomic DNA-based PCR, we sequenced the protein-coding

Table 1. Likelihood ratio tests of selective pressures on carnivore *Tas1r2* gene

Models	ω (d_N/d_S)	ln L*	np[†]	Models compared	2Δ(ln L)[‡]	P values
A. All branches have the same ω	$\omega = 0.1909$	−9,840.81	36			
B. All branches have the same ω= 1	$\omega = 1$	−10,203.39	35	B vs. A	725.16	∼0
C. The branches with a pseudogenized	$\omega1 = 0.13656$					
Tas1r2 have the ω2, other branches have ω1	$\omega2 = 0.41974$	−9,806.38	37	A vs. C	68.86	1.1×10^{-16}
D. The branches with a pseudogenized	$\omega1 = 0.13253$					
Tas1r2 have ω2 = 1, other branches have ω1	$\omega2 = 1$	−9,829.34	36	D vs. C	45.92	1.2×10^{-11}
E. Each branch has its own ω	Variable ω	−9,783.72	69	C vs. E	45.32	0.05955

Dataset: 18 species (all species, after removing gaps and nonsense mutations in pseudogenes).
*The natural logarithm of the likelihood value.
[†]No. of parameters.
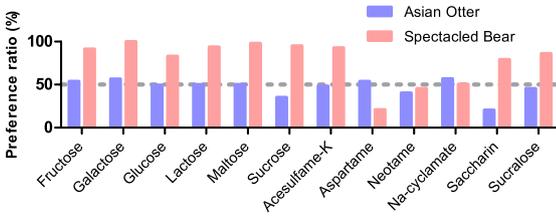[‡]Twice the difference in ln L between the two models compared.

Fig. 3. Sweet-taste preferences of two genotyped species. Two Asian otter and four spectacled bears were tested behaviorally for their preferences for sweeteners using a two-bowl preference setup. One bowl contained sweetener solution and the other contained plain water. Dashed line indicates no preference (50%). Sweeteners were tested at the following concentrations: fructose (0.8 M), galactose (0.8 M), lactose (0.5 M), maltose (0.7 M), sucrose (0.5 M), acesulfame-K (6.0 mM), aspartame (10 mM), neotame (10.5 mM), saccharin (6.2 mM), and sucralose (5.0 mM).

Fig. 4. The sea lion and dolphin *Tas1r* receptor genes are inactivated by pseudogenization. (*A*) Sea lion *Tas1r1* (*Upper*) and *Tas1r3* (*Lower*) gene structures with boxes representing exons, and lines representing introns. Regions where ORF-disrupting mutations were found are marked with an asterisk. Regions with no sequence coverage are labeled with dashed lines. Only three exons have been sequenced for the sea lion *Tas1r3*. (*B*) Schematic drawings of the dolphin *Tas1r1* (*Top*), *Tas1r2* (*Middle*), and *Tas1r3* (*Bottom*) gene structures. The symbols are the same as in *A*. The straight line with slashes indicates the intron is not proportionally scaled.

regions of *Tas1r* exons. By aligning with the dog *Tas1r1* sequence, we found that the sea lion *Tas1r1* has a 1-bp deletion in exon 2 between 53 and 54 bp (Fig. 4*A* and *SI Appendix*, Fig. S8*A*), and an 11-bp deletion in exon 6 between 302 and 303 bp (Fig. 4*A* and *SI Appendix*, Fig. S8*B*). *Tas1r3* has a 1-bp deletion between 72 and 73 bp in exon 4 (Fig. 4*A* and *SI Appendix*, Fig. S8*C*). Only exons 2, 4, and 5 have been sequenced for sea lion *Tas1r3*. As was the case for *Tas1r2*, sea lion *Tas1r1* and *Tas1r3* are also pseudogenes.

Dolphin is an aquatic mammal that evolved independently from the sea lion but displays similar feeding behavior and dramatic loss-of-taste system (23). We searched the draft dolphin genome (2.59X) for *Tas1r* genes using the dog Tas1r amino acid sequences (TBlastN). We found several contigs with sequence similarity to dog Tas1rs (~70–80% amino acid identity). ABRN01270722 contains the first two exons and part of the third exon of dolphin *Tas1r1*, and ABRN01270723 contains exons 4–6 of *Tas1r1*. Dolphin intron 2 has a mutation in the donor splice site at the 5′ end (AT instead of GT; Fig. 4*B*) that would interfere with splicing of the *Tas1r1* mRNA, a 5-bp deletion in exon 4 that disrupts the ORF of dolphin *Tas1r1* (Fig. 4*B* and *SI Appendix*, Fig. S9*A*), and a 2-bp deletion in exon 6 (Fig. 4*B*). These ORF-disrupting mutations render dolphin *Tas1r1* nonfunctional.

Using the dog Tas1r2 amino acid sequence, we retrieved only contig ABRN01341268 and determined potential coding sequences of dolphin *Tas1r2* exons 1–3 and 5–6. Exon 4 appears to have been lost entirely based on using dog, cow, mouse, or human sequences, because queries and in exons 3 and 6 ORF-disrupting mutations were found (Fig. 4*B*). Specifically, there is a 20-bp ORF-disrupting insertion between 488 and 507 bp in exon 3 (Fig. 4*B* and *SI Appendix*, Fig. S9*B*), and there is a 1-bp ORF-disrupting deletion in exon 6 between 378 and 379 bp (Fig. 4*B*). Thus, dolphin *Tas1r2* also is a pseudogene.

Next, we retrieved two contigs ABRN01316859 (exons 1–3) and ABRN01316858 (exons 4–6), covering the entire coding sequences of the dolphin *Tas1r3* gene. Multiple ORF-disrupting mutations were found throughout the coding region (Fig. 4*B*). A 7-bp deletion between 99 and 100 bp in exon 6 is shown in *SI Appendix*, Fig. S9*C*. These data indicate that dolphin *Tas1r3* also is a pseudogene. Like the sea lion, the bottlenose dolphin lacks all three *Tas1r* receptors.

Pseudogenization of Dolphin *Tas2r* Receptor Genes. Because all three *Tas1r* receptor genes are pseudogenized in dolphins, what happened to genes mediating another major taste quality: bitter? To address this question, TBlastN searches were performed on the dolphin genome using 15 dog and 16 cow intact *Tas2r* genes as queries. Because *Tas2r*s are intronless genes, it was relatively easy to identify the dolphin *Tas2r* genes. The query results revealed only 10 pseudogenes, and no intact dolphin *Tas2r* receptor gene was found. The genes are named after their dog or cow orthologs (*Tas2r1p*, *Tas2r2p*, *Tas2r3p*, *Tas2r5p*, *Tas2r16p*, *Tas2r38p*, *Tas2r39p*, *Tas2r60p*, *Tas2r62ap*, and *Tas2r62bp*). Pseudogenization was caused
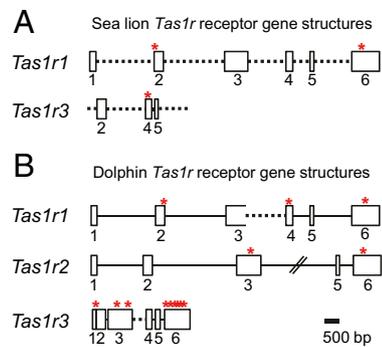
by ORF-disrupting mutations (including nonsense mutations and frameshift mutations; Fig. 5 *A* and *B* and *SI Appendix*, Document 1). Sequence similarities (nucleotide) between dolphin *Tas2r*s and their orthologs in dog and cow are between 75% and 86%. A neighbor-joining tree was constructed by using aligned dolphin, dog, and cow *Tas2r* sequences (*SI Appendix*, Fig. S10) to show the evolutionary relationship among dog, cow, and dolphin bitter receptors. Despite the relatively low coverage of the dolphin genome assembly, the fact that none of the 10 identified *Tas2r* receptors is intact hints that this modality may also be lost, or its function greatly reduced.

Discussion

Widespread Pseudogenization of *Tas1r2* in the Order Carnivora. In the present study, by examining *Tas1r2* receptor genes in additional carnivore species, both closely and distantly related to Felidae, we found that functional loss of Tas1r2 is widespread and independent in Carnivora. ORF-disrupting mutations (e.g., stop codons, insertion or deletion of nontriplet nucleotides) were detected in seven nonfeline carnivore species: the sea lion, fur seal, Pacific harbor seal, Asian small-clawed otter, spotted hyena, fossa, and banded linsang, as well as in one cetacean species: the bottlenose dolphin. Looking specifically at the obvious mutations, with the exception of the sea lion and fur seal (sister species in the family Otariidae), none of the mutations disrupting the ORF of *Tas1r2* were shared between any two species of these seven.

The widespread loss of *Tas1r2* in species of the order Carnivora, the variance in their lineages, and the independent pseudogenizing mutations among species in distinct families strongly support the hypothesis that the loss of *Tas1r2* occurred independently many times during the evolution of the order Carnivora. It seems most likely that changes in dietary behavior in the process of becoming obligate carnivores may favor the loss of a functional *Tas1r2*, arguing for convergent evolution of pseudogenized *Tas1r2*. Another case of convergent evolution of taste was found in the *Tas2r38* receptor gene. Mutations in the *Tas2r38* gene have resulted in independent loss of phenylthiocarbamide sensitivity in some humans, chimpanzees, and macaques (30, 31). Notably, the mutations in chimpanzees and macaques are start codon mutations, resembling the case with sea lion and fur seal *Tas1r2*s (30, 31).

Our evolutionary analysis has provided further evidence to support the view of convergent evolution of pseudogenized *Tas1r2*. Similar to many other vertebrate genes, we found that *Tas1r2* is under an overall strong purifying selection. During
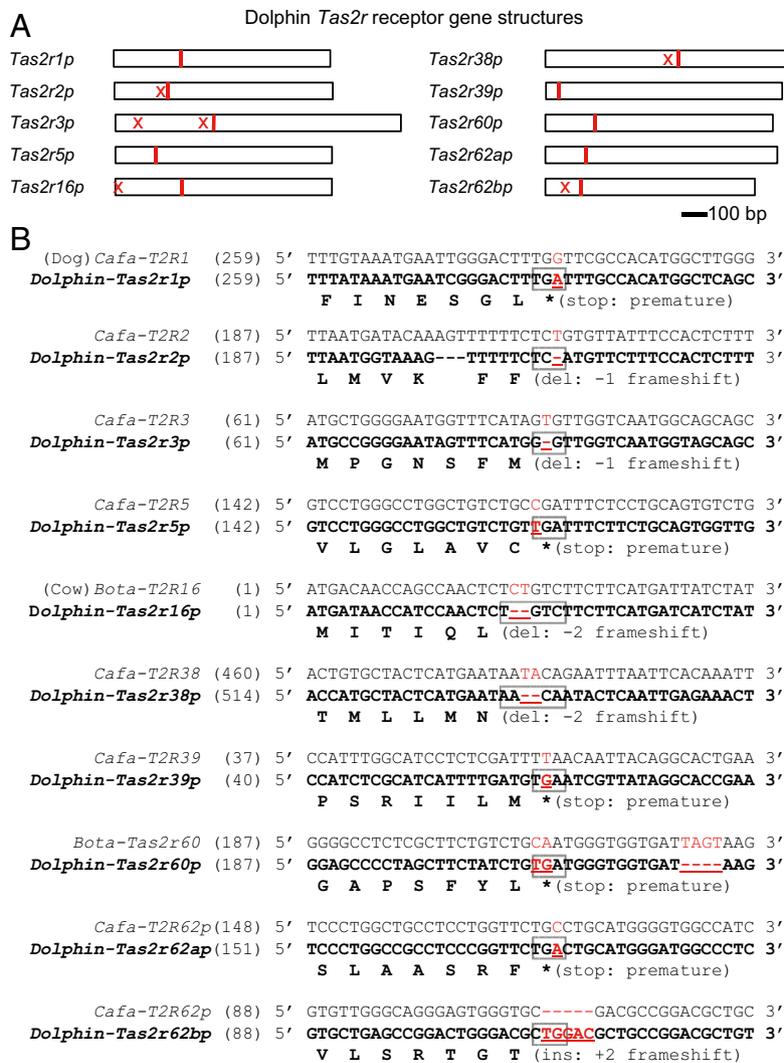
Fig. 5. Dolphin *Tas2r*s are pseudogenes. (*A*) Schematic diagram shows the premature stop codons (either nonsense mutations or stop codons resulting from prior frameshift mutations, depicted with a red line) and only the frame-shift mutations (X) before the premature stop codons in dolphin *Tas2r*s. *Tas2r* receptor genes are shown in scale. No start codon is detected for *Tas2r62bp*. (*B*) A 42-bp–long nucleotide sequence containing the ORF-disrupting mutation that occurs closest to 5′ end of the gene is shown for each of the 10 identified dolphin *Tas2r* receptor genes; the aligned dog sequence is shown above it, and the amino acid sequence deduced from the nucleotide sequence up to the mutation site is shown underneath it. The codon that contains the ORF-disrupting mutation (marked in red and underlined) is indicated by a box.

evolution, several species at separate times may have changed their diet to a more carnivorous one. As the animal moved closer toward obligate carnivore it became increasingly likely that loss of the *Tas1r2* gene would be well tolerated, reflected by the fact that the relaxation of functional constraint occurred in branches with a pseudogenized *Tas1r2*, although not completely.

Sweet Taste Receptor Genes and Taste Behavior. Cats with a defective *Tas1r2* gene do not prefer sweet carbohydrates and noncaloric sweeteners and probably cannot detect them. Is this also true for others? We addressed this question in the current study by behavioral taste-testing of the Asian small-clawed otter. Similar to the cat family Felidae, the two Asian otters we tested did not show a preference for sucrose at a concentration up to 800 mM, or for other sugars or artificial sweeteners. These data are limited in the number of animals tested and the number and kinds of behavioral tests conducted and, consequently, they warrant caution in interpretation. However, a preference for sweet sugars is a powerful and clear phenotype in most mammals studied, so we believe its absence in the Asian otter is striking. Thus, we tentatively conclude that the Asian otter is not capable

of detecting compounds that taste sweet to humans, consistent with their lack of a functional sweet taste receptor Tas1r2/Tas1r3 and their carnivorous diet. In contrast, the spectacled bear, with an intact *Tas1r2* gene, shows strong preferences for sugars and some nonnutritive sweeteners, consistent with the presence of a functional sweet taste receptor and with their omnivorous diet.

Major Taste Loss in Two Lineages of Aquatic Mammals. In the present study, we found that the sweet, umami, and perhaps bitter taste receptor are all pseudogenized in dolphin, and the sweet and umami taste receptors are pseudogenized in sea lions (we have no data on bitter receptor structure in this species). Consistent with the genetic data, anatomical studies of the dolphin taste system revealed that only few taste bud-like structures are present in small pits in the root region of dolphin tongues (32). No buds are found in the canonical taste structures, including fungiform, foliate, and circumvallate papillae (32). Taste bud numbers are greatly reduced in sea lions as well (21). Only a few studies from two groups were conducted to examine the abilities of dolphins and sea lions to detect sour, bitter, salty, and sweet taste stimuli; umami was not examined in these studies (33,

34). Dolphins could not detect sucrose in one study (33) and showed reduced sensitivity in another study (34). Furthermore, dolphins detected only elevated levels of quinine (bitter), and a California sea lion showed no response to quinine sulfate but did respond to elevated levels of quinine monohydrochloride dihydate (0.40 parts per trillion; from 3 to ~4 orders above human detection threshold) (33, 34). However, these data need to be interpreted cautiously because only a limited number of animals were examined. Nevertheless, these behavioral data agree largely with our molecular data. For instance, no sugar detection (or reduced sensitivity to sugar) correlates well with the pseudogenization of *Tas1r2* and *Tas1r3* receptor genes. The remaining response (if any) to sugar could be due to another property of sugar solutions, such as osmolarity or viscosity of sugars (13). Dolphins appeared to maintain some response to quinine at high concentration, but we failed to detect intact *Tas2r* receptor genes from the dolphin genome database. One explanation for this finding is that due to the low coverage, and therefore incompleteness, of the dolphin genome (2.59X), there may exist intact bitter receptor genes in the genome eluding initial genome sequence coverage. An alternative explanation is that quinine may become irritating at such high concentrations, and therefore elicit irritation responses (35). Nevertheless, with the reported greatly elevated threshold to quinine and the abundance of pseudogenized *Tas2r* receptor genes in the available genome database (10 of 10 genes), we predict that bitter taste function of dolphins is also greatly reduced, if not completely absent.

Recently, sweet, umami, and bitter taste receptors have been implicated in several extraoral functions (36). Pseudogenization of *Tas1r* receptor genes in dolphins and sea lions and *Tas2r* receptor genes in dolphin indicates that these receptors cannot be involved in extraoral (e.g., gut, pancreas) chemosensation (36) in these species. Thus, to the extent that these extraoral taste receptors are functionally significant in rodents and humans, these functions must have been assumed by other mechanisms in the species we have identified here with pseudogenized receptors. What these other mechanisms are remains to be determined, and further assessment of the relationships among taste receptor structure, dietary choice, and the associated metabolic pathways will lead to a better understanding of the evolution of diet and food choice as well as their mechanisms.

Materials and Methods

Details about collection of DNA samples, sequencing *Tas1r2* from selected species within Carnivora, sequencing sea lion *Tas1r1* and *Tas1r3*, data mining of the dolphin whole-genome shotgun assembly, evolutionary analysis, and taste testing as well as associated references are described in *SI Appendix, Materials and Methods*. Primers for amplification of taste receptors are listed in Table S1. Dataset for evolutionary analysis is provided in Dataset S1.

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