



## Short Communication

## Common ancestors of bats were omnivorous suggested by resurrection of ancestral sweet receptors

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The origins of powered flight and laryngeal echolocation in bats are widely cited as evidence that ancestral bats evolved as insectivores [1]. Indeed, the emergence of major bat lineages in the Eocene has also been linked to insectivory, with bat diversification arising due to the increase in insect abundance, in turn attributed to angiosperm radiation [2]. Further indication that early bats were insectivorous comes from the presence of tympanate moth families in Eocene deposits, which probably evolved simple hearing organs for avoiding echolocating bats [3]. Despite these observations, and the fact that insectivory is the dominant dietary specialisation among modern bat lineages, arguments linking the evolution of echolocation and flight to insectivory are not universally accepted. In particular, others have hypothesized that the first bats were diurnal frugivores, and that insectivory emerged secondarily for protein supplementation [1]. This scenario, if correct, suggests modern frugivorous and nectarivorous bats might have retained ancestral adaptations, rather than undergone derived specializations [4]. Unfortunately, direct evidence relating to the diets of ancestral bats is lacking, reflecting a depauperate fossil record [5]; however, insights may come from studying molecular adaptations in diet-related genes. By conducting the first resurrection and functional assays of sweet receptors in ancestral bat lineages, we found that the ancestral sweet receptor of all extant bats was functionally sensitive to natural sugars, with a lower level of sugar sensitivity than modern pteropodid bats, suggesting that they were omnivorous.

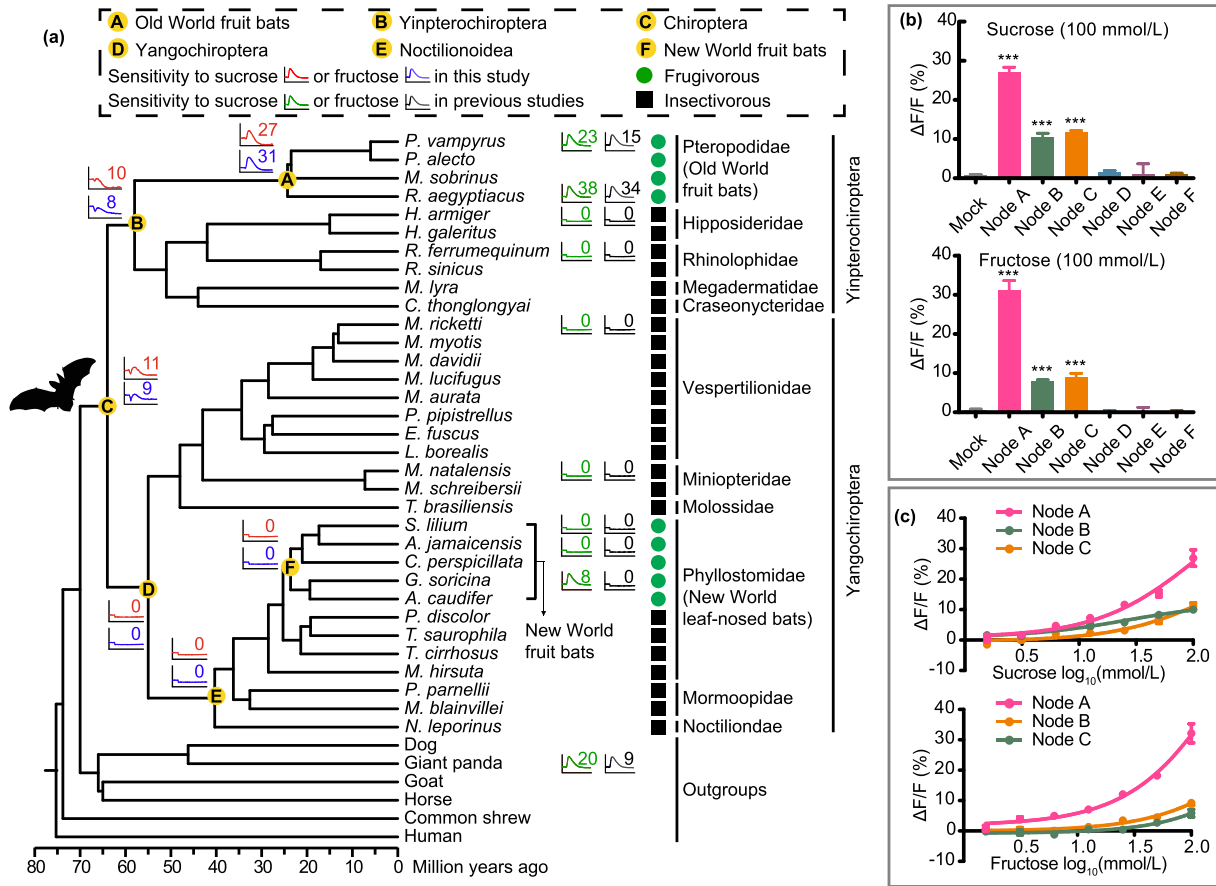
Of the main sensory modalities, taste is especially tied to diet [6]. Mammals possess five basic taste sensations (sweet, umami,

bitter, salt, and sour), of which the perception of sweetness and umami are controlled by a family of type 1 taste receptors. The sweet taste receptor is formed by a dimer of *Tas1r2* and *Tas1r3*, encoded by the genes *Tas1r2* and *Tas1r3*, respectively. Comparative studies of mammalian sweet receptors uncover a close relationship between sweet receptor presence and diet, with multiple gene losses across carnivorous lineages [7]. In bats, protein assays and behavioural studies both indicate that frugivorous species from both suborders can sense natural sugars, whereas insectivorous species cannot [8]. To obtain insights into the early evolution of diet in bats, we performed the first examination of taste receptors in ancestral bat lineages. By resurrecting and measuring the functional properties of ancient proteins from six ancestral taxa, we assess whether ancestral bats were able to sense natural sugars [8].

We used the maximum likelihood (ML) method with the amino acid model to reconstruct the protein sequences of sweet receptors for key ancestral nodes in the bat phylogeny (Dataset S1, Supplementary Methods online). Proteins were resurrected *in vitro*, and their phenotypic responses to natural sugars (sucrose and fructose) measured using calcium mobilization assays. We recorded clear responses to both natural sugars for the sweet receptors of the common ancestor of all extant bats (~10%), the ancestor of the suborder Yinpterochiroptera (~10%), and the ancestor of the family Pteropodidae (i.e., Old World fruit bats), with the latter showing the highest intensity (~30%) (Fig. 1a–c). In contrast, ancestral lineages leading to the suborder Yangochiroptera, and two clades within this suborder, showed no detectable response to the two sugars (Fig. 1a–c). All receptors without response to natural sugars showed clear responses to an artificial sweetener control (NHDC) (Dataset S2 online), confirming that our heterologous expression system worked [8]. Co-expression levels of *Tas1r2* and *Tas1r3* in each species were similar (Fig. S1 and Supplementary Methods

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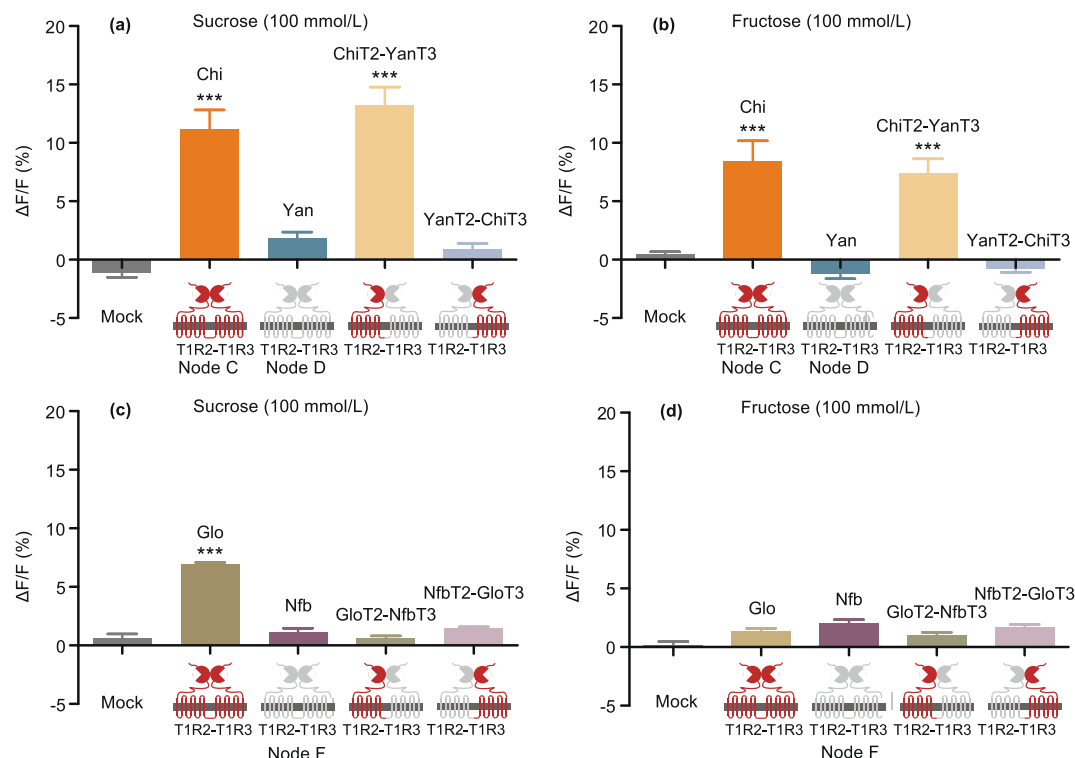
**Fig. 1.** Functional evolution of sweet receptors in bats. (a) Receptor responses to sugars. Response lines with a number indicate the intensity of response to sucrose or fructose. Responses of extant species to sugars were obtained from previous studies [8,12]. (b) Quantitative analysis of responses of ancestral sweet receptors of the six early lineages (mean  $\pm$  standard error of mean (SEM); \*\*\* $P < 0.001$ , one-way ANOVA). (c) Dose-dependent responses of ancestral sweet receptors to sugars. The bat silhouette was taken from PhyloPic.

online), confirming equivalent expression levels of *Tas1r2* and *Tas1r3* for the ancestral protein studies. To assess the robustness of our findings, we repeated the ancestral sequence reconstructions under the codon model, and assessed functional responses of the corresponding synthesized sweet receptors (Datasets S3–S5, Supplementary Methods online). Consistent results were observed across both sets of proteins (Dataset S6 online). We further used the Bayesian inference (BI) and maximum parsimony (MP) methods to re-infer ancestral sequences, both sets of sequences showed a high consistency with those inferred by the ML method with the amino acid model (BI vs. ML: 98.5%; MP vs. ML: 99.3%) and the codon model (BI vs. ML: 98.7%; MP vs. ML: 99.2%) (Datasets S7, S8, Supplementary Methods online).

Since all six resurrected ancestral bat sweet receptors comprised pairs of intact *Tas1r2* and *Tas1r3* subunits (hereafter T2 and T3), the observed losses of the sweet response could not be attributed to known loss-of-function mutations. Therefore, to determine the underlying causes of observed losses of sweet perception in bats, we generated chimeric dimers in which we paired mismatching T2 and T3 subunits. Briefly, we generated four pairs of mismatched sweet receptors: ChiT2-YanT3, YanT2-ChiT3, GloT2-NfbT3, and NfbT2-GloT3 (ChiT2-YanT3 denotes a mismatched receptor of Chiroptera *Tas1r2* and Yangochiroptera *Tas1r3*, and other mismatched receptors are indicated in a similar fashion) (Fig. 2). Our results showed that ChiT2-YanT3 retained clear responses to both sugars, while YanT2-ChiT3 lost such function (Fig. 2a, b), indicating that *Tas1r2* is responsible for the loss of sweet taste in the ancestor of Yangochiroptera. Both GloT2-NfbT3

and NfbT2-GloT3 pairs showed no detectable response to sucrose that can be detected by the nectar-feeding species *Glossophaga soricina* (Fig. 2c, d), suggesting that mutations in both *Tas1r2* and *Tas1r3* have resulted in the regain of the sweet taste in this New World bat (Fig. 1a).

Our experiments provide the first evidence that the ability to sense natural sugars was present in the common ancestor of extant bats (Fig. 1). Based on the correspondence between taste and diet in extant bats [8], we thus suggest that ancestral bats were likely omnivorous, feeding on a mixture of fruits and insects. We also recorded sugar-sensitive taste receptors for the ancestors of the subfamily Yinpterochiroptera and the family Pteropodidae (Fig. 1), implying that the ability to perceive sweetness has been retained throughout the evolutionary history of Old World fruit bats. In contrast, the resurrected receptor of the ancestor of the suborder Yangochiroptera showed no such response to sugars, pointing to an earlier transition to an insectivorous diet in this suborder. Despite this, sensitivity to natural sugars was again present in some New World leaf-nosed bats (Fig. 1a), consistent with an adaptive regain linked to their independent transition to a plant-based diet, as also found in hummingbirds [9]. We note that two New World frugivorous bats (*S. lilium* and *A. jamaicensis*) have not gained sensitivity to natural sugars yet (Fig. 1a), as shown in our earlier work [9], possibly due to the short divergence times within this clade of neotropical bats with exceptional bursts of adaptive radiation [10]. Additionally, downstream genes of sweet taste signaling pathway may also have an impact on sweet taste function, which could be tested in the future.



**Fig. 2.** Responses of mismatched sweet receptors to natural sugars. (a, b) Quantitative analysis of responses of sweet receptors to sucrose and fructose (mean  $\pm$  SEM; \*\*\* $P$  < 0.001, one-way ANOVA). ChiT2-YanT3 denotes a mismatched receptor of Chiroptera Tas1r2 and Yangochiroptera Tas1r3, and other mismatched receptors are indicated in a similar fashion. (c, d) No responses of mismatched sweet receptors to sucrose or fructose. Node C, Chiroptera (Chi); Node D, Yangochiroptera (Yan); Node F, New World fruit bats (Nfb); Glo, *Glossophaga soricina*.

Omnivory occurs in several extant bat lineages [11], and switches between animal- and plant-based diets have occurred multiple times in bats and other mammals, including the giant panda, which has evolved sweet taste perception relating to its bamboo-dominated diet (Fig. 1a) [12]. If the ancestral bat was indeed omnivorous, then this calls into question the common view that bats evolved flight and echolocation for hunting insects. Previously, contrarian speculation that ancestral bats were diurnal frugivores was based on reasoning that, if flight evolved before echolocation, then the small eyes of bats would be ill-adapted to a nocturnal niche [1]. Although this theory predates major phylogenetic revisions of the bat clade, it is nevertheless arguably more credible in light of the discovery of the first transitional fossil bat *Onychonycteris*, an Eocene taxon that is suggested to possess morphological characters consistent with an ability to fly but not echolocate [13,14], but see [15]. While it is thus plausible that the first bats hunted for insects and fruit without echolocation, caveats remain. Notably, approximately ten millions of years separate the origin of bats and the earliest known fossils [5], raising the possibility that sugar sensing in the ancestor of modern bats is itself a derived state. Moreover, recent bat phylogenies place Eocene fossils outside of modern lineages [13], which, if correct, would imply that ancient adaptations inferred from protein reconstructions cannot be directly related to extinct taxa.

### Conflict of interest

The authors declare that they have no conflict of interest.

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### Author contributions

Huabin Zhao designed research. Yingcan Li, Hengwu Jiao, and Ruiqi Wang performed research. Yingcan Li, Hengwu Jiao, Simon Yung Wa Sin, Stephen J Rossiter, and Huabin Zhao analyzed data. Yingcan Li, Stephen J Rossiter, and Huabin Zhao wrote the paper.

### Appendix A. Supplementary materials

Supplementary materials to this short communication can be found online at <https://doi.org/10.1016/j.scib.2023.07.026>.

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