SENSORY EVOLUTION

Early origin of sweet perception in the songbird radiation

Yasuka Toda[1,2,3], Meng-Ching Ko[4], Qiaoyi Liang[4], Eliot T. Miller[5], Alejandro Rico-Guevara[6,7], Tomoya Nakagita[8], Ayano Sakakibara[9], Kana Uemura[9], Timothy Sackton[10], Takashi Hayakawa[11,12], Simon Yung Wa Sin[13,14], Yoshiro Ishimaru[2], Takumi Misaka[1], Pablo Oteiza[15], James Crall[14,16], Scott V. Edwards[14], William Buttemer[17,18], Shuichi Matsumura[9], Maude W. Baldwin[4,14]*

Early events in the evolutionary history of a clade can shape the sensory systems of descendant lineages. Although the avian ancestor may not have had a sweet receptor, the widespread incidence of nectar-feeding birds suggests multiple acquisitions of sugar detection. In this study, we identify a single early sensory shift of the umami receptor (the T1R1-T1R3 heterodimer) that conferred sweet-sensing abilities in songbirds, a large evolutionary radiation containing nearly half of all living birds. We demonstrate sugar responses across species with diverse diets, uncover critical sites underlying carbohydrate detection, and identify the molecular basis of sensory convergence between songbirds and nectar-specialist hummingbirds. This early shift shaped the sensory biology of an entire radiation, emphasizing the role of contingency and providing an example of the genetic basis of convergence in avian evolution.

Sensory systems evolve and adapt, allowing animals to perceive the species-specific cues relevant for survival. Sensory receptor modifications can have profound ecological consequences, affecting behaviors such as foraging (1) and mate choice (2) and even driving speciation (3). Evolution of novel sensory adaptations enables organisms to exploit extreme environments and new niches (4–6). Determining the timing of sensory changes is essential for understanding the underlying ecological causes and consequences of shifts in perception, as sensory differences may reflect not only adaptation to current lifestyles but also persistence of traits established earlier in the evolutionary history of a clade.

Taste is an important sense used to discriminate between nutrient-rich and toxic food items. Most basic taste categories, such as bitter (eliciting aversion) and umami (the appetitive taste of amino acids), are conserved in mammals and fish (7, 8). An appetitive taste for sugars (conferred by the T1R2-T1R3 sweet receptor heterodimer) is widespread in mammals (9, 10), but T1R2 was lost early in bird evolution (11). Despite this loss, divergent lineages of birds (including hummingbirds, parrots, and honeyeaters) consume sugar-rich nectar and fruit. Hummingbirds, a large radiation of nectarivores, acquired the ability to detect sugars through modifications to the ancestral savory receptor heterodimer (T1R1-T1R3) after divergence from their close relatives, swifts (11). Whether the myriad other frugivorous and nectarivorous birds can taste sweet is currently unknown.

To understand the origins of avian sweet taste, we examined nectar consumption patterns across the phylogeny. Unexpectedly, we observed a marked enrichment of nectar-taking behavior in songbirds from a variety of dietary guilds [Fig. 1, fig. S1, and table S1; diet data from (12, 13)]. Ancestral state reconstructions created with a hidden Markov model (14) suggest infrequent gains and losses of sweet taste but frequent transitions to and from nectar-feeding once sweet taste had been gained (fig. S2 and tables S3 and S4). We therefore wondered whether sweet taste was gained early in the songbird radiation and subsequently retained, even in species for which nectar is not a major dietary component.

To understand the mechanism underlying the possible gain of sweet taste in songbirds, we first conducted brief-access taste trials on New Holland honeyeaters, which are specialized flower visitors (movie S1). In our two-choice assay, honeyeaters exhibited a clear preference for sugars (Fig. 2A, fig S3, and table S5) over water controls. To assess whether a sugar preference also exists in non-nectarivorous songbirds, we performed brief-access sucrose

tests with canaries, granivorous finches distantly related to honeyeaters. The canaries' responses to sucrose (Fig. 2B and table S5) suggest that the ability to taste sugar may have persisted in members of the songbird radiation, regardless of diet.

To examine whether songbirds, like hummingbirds, evolved a mechanism to taste sugars that involved changes to the savory receptor, we cloned and functionally profiled T1R1-T1R3 receptors from honeyeaters and canaries. We also tested receptors from representatives of different dietary guilds (Fig. 2C and fig. S4). We observed a strong response to carbohydrates in the savory receptors of the honeyeater, white-eye, and bulbul— species that consume large amounts of fruit and nectar (Fig. 2C and fig. S5). Surprisingly, receptors from the canary and great tit, two non-nectar specialists, also showed significant sugar responses (Fig. 2C and table S6).

Next, we cloned taste receptors from the barred antshrike (*Thamnophilus doliatus*) and the rusty-margined flycatcher (*Myiozetetes cayanensis*), members of the sister group of songbirds (suboscines). Receptors from both species exhibited strong responses to amino acids but did not respond to sugars (Fig. 2C), suggesting that the sugar response seen in songbirds evolved after these two passerine clades diverged. Receptors from the brown treecreeper (*Climacteris picumnus*), an early-diverging Australian songbird that is primarily insectivorous but occasionally takes nectar, exhibited a strong response to amino acids but also a small response to sugars (fig. S4), implying an early origin of sugar perception in songbirds.

To investigate whether songbird receptors employed a shared mechanism to respond to sugars, we examined responses of cross-species T1R1-T1R3 pairs. Our study of hummingbird receptors indicated that sugar detection required coordinated functioning of both members of the heterodimer (T1R1 and T1R3). We therefore hypothesized that if songbirds had evolved a response to sugars early in their evolutionary history, a response that was retained by later lineages rather than evolving multiple times independently, then cross-species heterodimers may still respond to sucrose. First, we examined mixed pairs of hummingbird and honeyeater receptors and observed responses to amino acids but not to sugars, confirming that receptor heterodimers
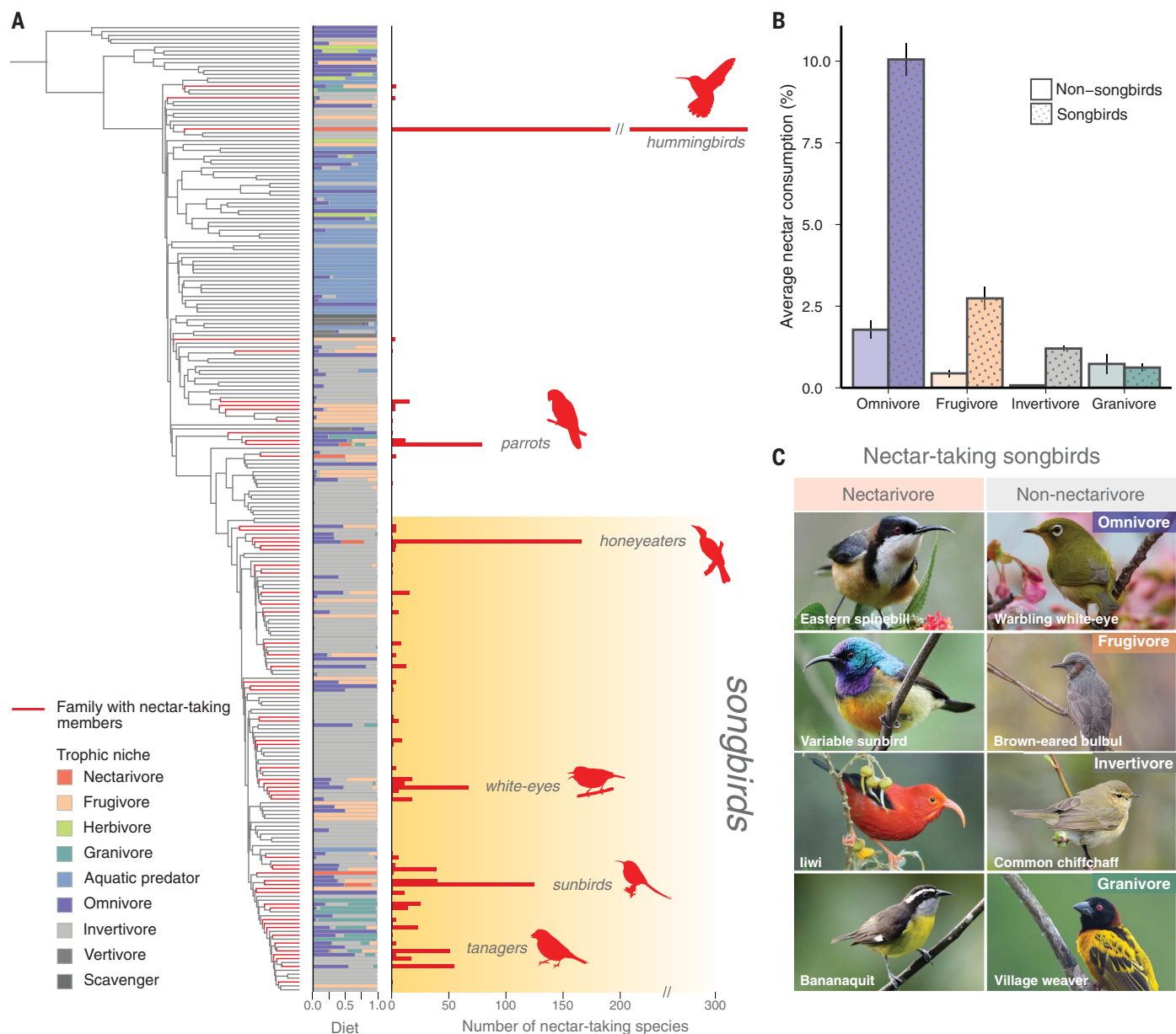
Fig. 1. Widespread nectar consumption across songbirds. (**A**) Songbirds (yellow shading) display extensive nectar-taking behavior. Proportion of species per trophic niche and number of nectar-taking species are plotted per family; see fig. S1. Red branches: >1% of species take nectar. (**B**) Average nectar consumption (percent of diet) from species occupying diverse trophic niches is higher in songbirds compared with other birds (see table S1 and fig. S1). Error bars indicate standard error of the mean. (**C**) Examples of specialized songbird nectarivores, as well as nectar-consuming songbird species from other trophic groups [diet classified in accordance with (13)]; (see table S2 for photo credits).

were still functional and suggesting a distinct mechanism for sugar detection in each radiation (Fig. 3, A and B, and fig. S6). We next tested mixed pairs between songbirds and close relatives. Responses to sugars were seen in honeyeater receptors coexpressed with corresponding receptors from the canary, great tit, bulbul, and white-eye, but not with those of the antshrike or lyrebird (Fig. 3C), even though these pairs displayed robust amino acid responses (fig. S6).

Next, we synthesized and tested ancestral receptors (see supplementary materials) to confirm that the receptors of early songbirds could respond to sugars. Receptors from the ancestor of honeyeaters and all other songbirds (Anc2), but not from the earlier ancestor (Anc1), responded strongly to carbohydrates (Fig. 4A). Changes in both T1R1 and T1R3 were necessary for this response (fig. S7). By creating chimeric receptors, we identified two critical regions involved in carbohydrate detection (Fig. 4B);

responses were observed only when both regions were present (Fig. 4B and fig. S8). We further narrowed the region to seven residues in T1R1 and nine in T1R3, which together elicited a strong sucrose response when inserted in the background of Anc1 receptors (Fig. 4, B and C, and fig. S9). Surprisingly, most of the identified residues in the ligand-binding region are in T1R1 rather than in T1R3, as in hummingbirds, suggesting that convergence between these lineages occurs at the level of the tertiary structure
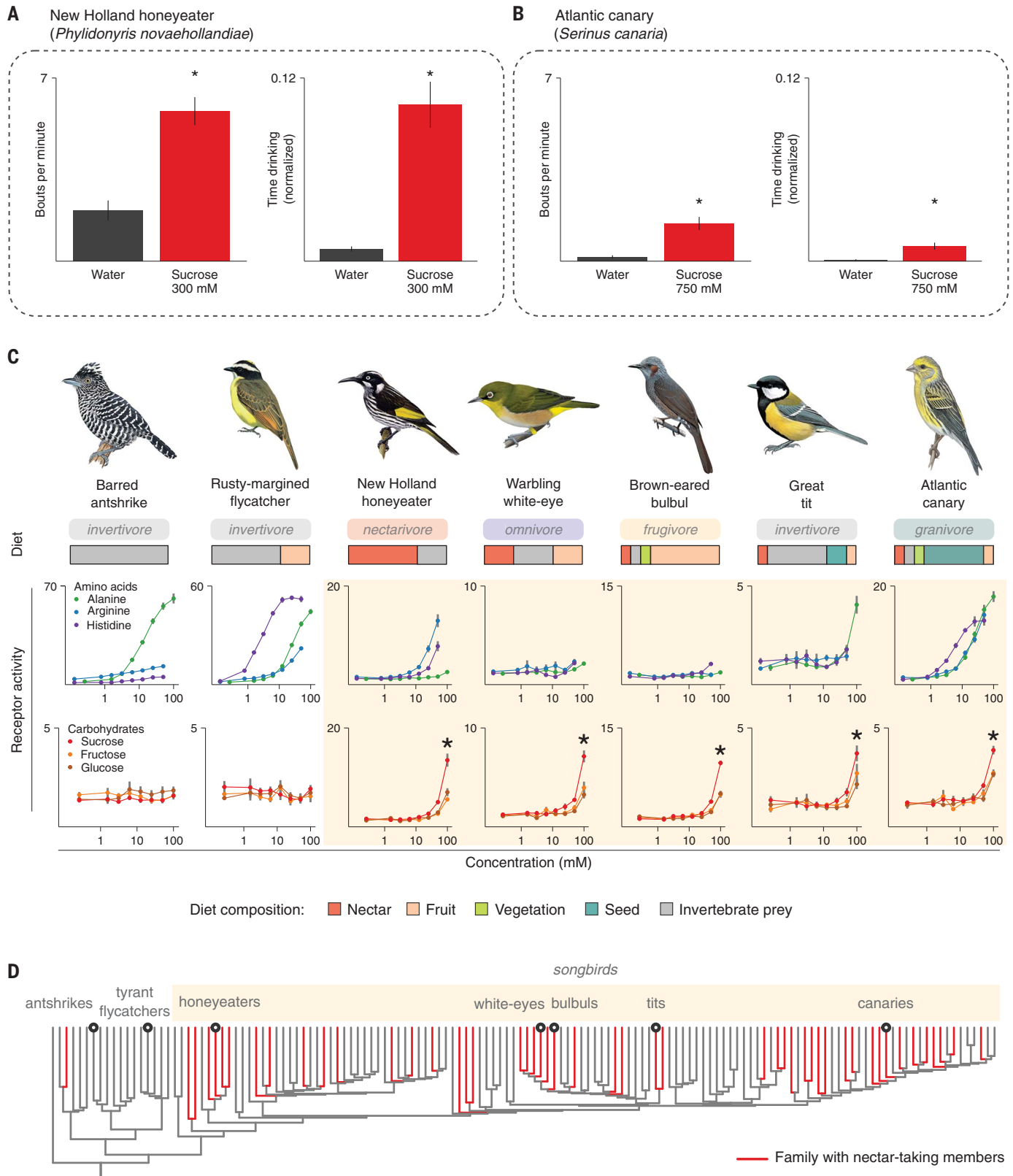
Fig. 2. Songbirds with diverse diets respond to sugars. Honeyeaters (**A**) ($n = 10$) and canaries (**B**) ($n = 8$) display higher drinking rates and spend more time per trial drinking sucrose than water (mean ± SE, *$P < 0.001$; table S5). (**C**) T1R1-T1R3 from five songbird species (yellow shading) responds to carbohydrates; receptors from both suboscines respond only to amino acids ($n = 6$ to 8, mean ± SE; *$P < 0.01$ shown for sucrose; for other stimuli, see table S6). Illustrations reproduced with permission of Lynx Edicions. (**D**) Phylogenetic position of tested songbirds (red: families with >1% nectar-taking species).
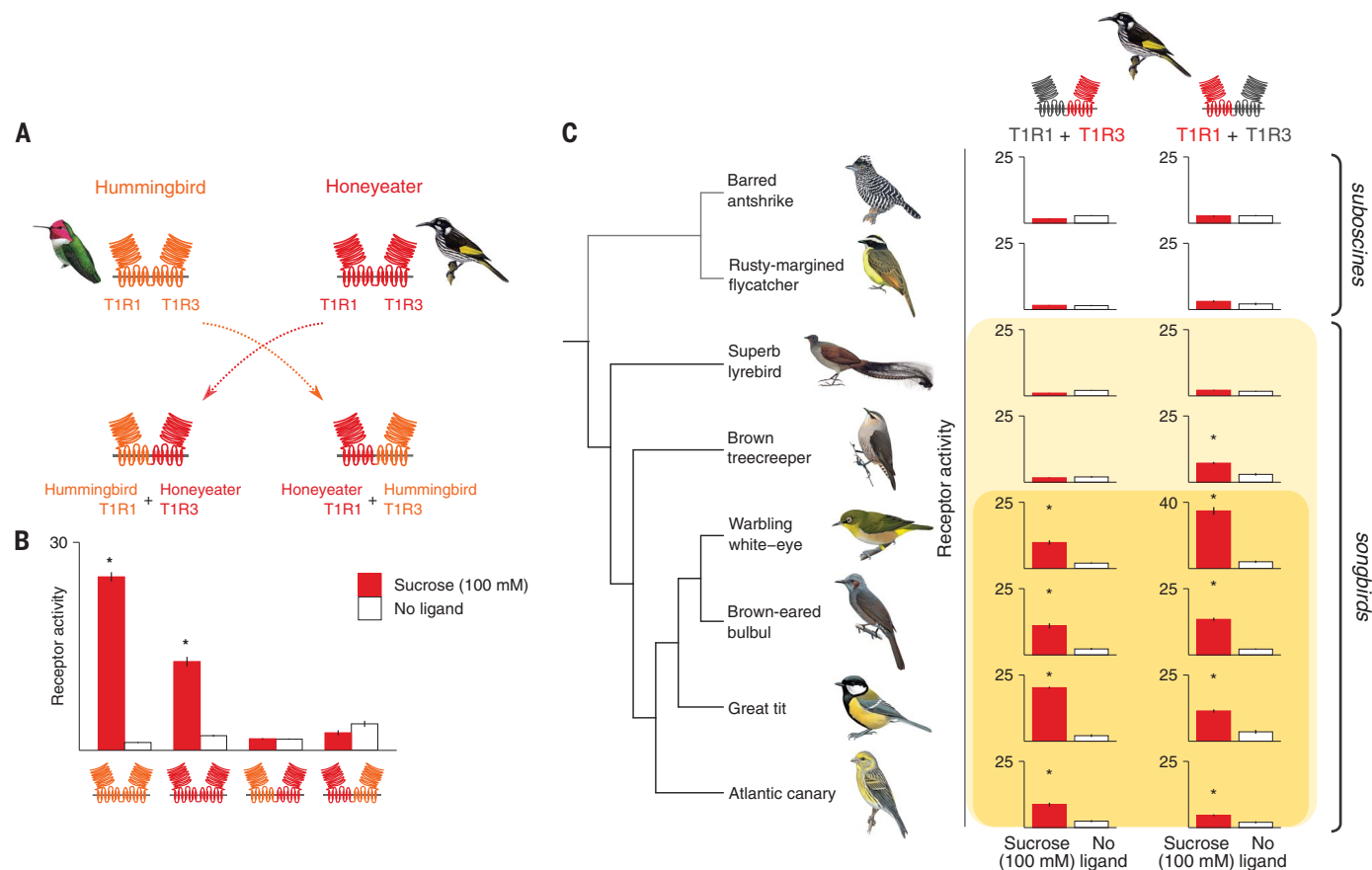
Fig. 3. Unique sensory shift evolves early in songbirds. (**A** and **B**) Honeyeater receptors coexpressed with the corresponding T1R from hummingbirds (mixed pairs) (A) reveal the lack of response to sugars (B) (n = 3 to 6, mean ± SE, *P < 0.01). (**C**) Mixed pairs between taste receptors from honeyeaters and other species (yellow shading, songbirds). (Left) Honeyeater T1R3; (right) honeyeater T1R1 (n = 6, mean ± SE, *P < 0.01). Responses from both pairs (dark yellow) suggest a shared mechanism that has evolved in songbirds. Bird illustrations in (A) and (C) reproduced with permission of Lynx Edicions.

of the protein rather than via identical residues. In T1R3, functionally important sites are located across the protein, including in the transmembrane domain (TM) and in the cysteine-rich domain (CRD), which connects the extracellular Venus flytrap (VFT) domain to the transmembrane domain (Fig. 4D and fig. S10). Notably, homology modeling revealed that T1R1 sites are located on the surface of the ligand-binding region facing T1R3, with most residues located in helices at the dimer interface involved in receptor activation [15] (Fig. 4, D and E, and fig. S11). Two songbird residues (Arg[139] and Thr[162]) are adjacent to the orthosteric binding pocket (Fig. 4E and fig. S11), and one (Thr[162]) occurs at the same location as a critical site in the hummingbird binding pocket of T1R3 (Ile[167]) (Fig. 4F). Thus, songbirds and hummingbirds both independently modified the ligand-binding region of the umami taste receptors. However, these changes occurred in alternate heterodimeric partners in each radiation (Fig. 4G) and, with the exception of a single identical site, involve distinct subsets of residues within this domain.

The changes in ancestral songbird taste receptors imply a complex set of modifications in regions involved in ligand binding, signal transmission, and receptor activation. These suggest coordinated changes, both between the different domains and between the two members of the heterodimer (consistent with some selection tests; fig. S13). Moreover, examining the responses of single residues also reveals extensive inter- [16] and intramolecular epistasis (fig. S14). Compared with shifts in visual pigment tuning, which can be caused by small numbers of substitutions and can therefore occur frequently across the phylogeny [17], the molecular basis of the acquisition of carbohydrate detection appears more complex; this complexity may lessen the likelihood that sweet taste has repeatedly evolved.

The ecology driving the initial acquisition of sugar sensing in songbirds is enigmatic. Many extant Australian birds rely heavily on additional and unique sources of sugar. By the Oligocene, Australia had become increasingly arid and was dominated by eucalypts [18], which can produce large quantities of a sugary

exudate known as manna. Both manna and insect secretions (called lerp and honeydew) make up substantial components of the diets of many Australian songbirds [19]. The divergence times of the ancestral nodes reconstructed here appear to predate the period of Australian aridification [20]. However, because eucalypts, which originated in Gondwana, may have been present in Australia during this period [21], it is unclear whether sweet taste originated in birds with diets favoring nectar or fruit, or lerp and/or manna. Our results indicate that Australasian treecreepers, the sister group to the frugivorous bowerbirds [20], use a mechanism that differs from but is potentially related to that described here. This suggests a scenario in which initial, permissive changes [22] may have evolved earlier than the ancestral receptors that we characterize, facilitating later functional change (Fig. 4G).

Our results reveal an unexpected early event with widespread consequences for the diets and ecologies of later lineages. Although subsequent loss may also have occurred in some species, songbirds appear to have broadly
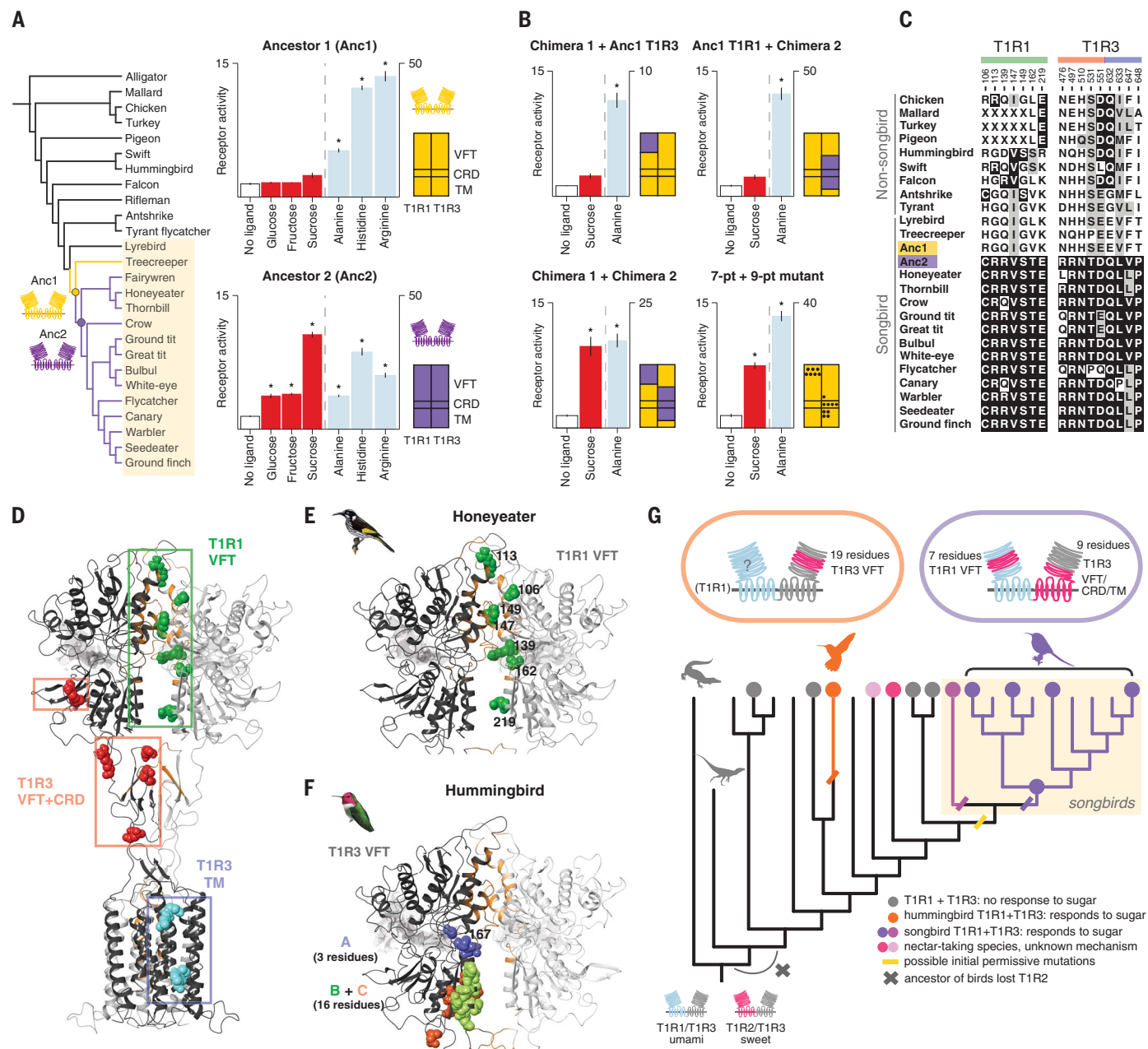
Fig. 4. Molecular basis of songbird sweet perception. (**A**) Anc2 (purple) but not Anc1 (yellow) T1R1-T1R3 responds to sugars (n = 6; mean ± SE; *P < 0.01). (**B**) Residues from two domains confer sugar responsiveness (n = 6; mean ± SE; *P < 0.01). (**C**) The 16 residues are largely conserved across songbirds. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**D**) Homology model of T1R1-T1R3 showing residues located across domains. (**E**) T1R1 VFT residues face T1R3 (yellow, within 4 Å of other T1Rs; binding pocket shaded gray). Bird illustrations in (D) and (E) reproduced with permission of Lynx Edicions. (**F**) Location of hummingbird VFT residues in T1R3 [in accordance with (11)]. (**G**) Model showing convergent evolution of sweet perception: Songbirds and hummingbirds independently recruit distinct regions of the ancestral savory receptor.

retained an ancestral sensory ability as they radiated out of Australia and colonized diverse habitats across the planet. This sensory shift enabled non-nectar specialists to opportunistically exploit novel or seasonally varying food sources, such as the nectar consumed by many insectivorous species during migration (23). The series of molecular changes that conferred the ability to sense sugars in the Australian ancestors of songbirds shaped the sensory *umwelt*

and subsequent evolution of nearly half of the world's birds.

REFERENCES AND NOTES

1. T. O. Auer et al., Nature **579**, 402–408 (2020).
2. Y. Grosjean et al., Nature **478**, 236–240 (2011).
3. O. Seehausen et al., Nature **455**, 620–626 (2008).
4. V. Matos-Cruz et al., Cell Rep. **21**, 3329–3337 (2017).
5. O. Eigenbrod et al., Science **364**, 852–859 (2019).
6. Z. Musilová et al., Science **364**, 588–592 (2019).
7. G. Nelson et al., Nature **416**, 199–202 (2002).
8. H. Oike et al., J. Neurosci. **27**, 5584–5592 (2007).
9. G. Nelson et al., Cell **106**, 381–390 (2001).
10. D. Glaser, Pure Appl. Chem. **74**, 1153–1158 (2002).
11. M. W. Baldwin et al., Science **345**, 929–933 (2014).
12. H. Wilman et al., Ecology **95**, 2027 (2014).
13. A. L. Pigot et al., Nat. Ecol. Evol. **4**, 230–239 (2020).
14. J. D. Boyko, J. M. Beaulieu, Methods Ecol. Evol. **12**, 468–478 (2021).
15. A. Koehl et al., Nature **566**, 79–84 (2019).
16. D. W. Anderson, A. N. McKeown, J. W. Thornton, eLife **4**, e07864 (2015).
17. A. Ödeen, O. Håstad, BMC Evol. Biol. **13**, 36 (2013).
18. G. H. Orians, A. V. Milewski, Biol. Rev. **82**, 393–423 (2007).
19. D. C. Paton, Emu **80**, 213–226 (1980).

20. C. H. Oliveros et al., Proc. Natl. Acad. Sci. U.S.A. 116, 7916–7925 (2019).
21. A. H. Thornhill et al., Aust. Syst. Bot. 32, 29–48 (2019).
22. E. A. Ortlund, J. T. Bridgham, M. R. Redinbo, J. W. Thornton, Science 317, 1544–1548 (2007).
23. J. G. Cecere, F. Spina, S. Jenni-Eiermann, L. Boitani, J. Ornithol. 152, 923–931 (2011).
24. Y. Toda et al., Data from "Early origin of sweet perception in the songbird radiation," Dryad (2021); https://doi.org/10.5061/dryad.xksn02vfz.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S14
Tables S1 to S7
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MDAR Reproducibility Checklist
Movie S1

Science

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From savory to sweet
Seeing a bird eat nectar from a flower is a common sight in our world. The ability to detect sugars, however, is not ancestral in the bird lineage, where most species were carnivorous. Toda *et al.* looked at receptors within the largest group of birds, the passerines or songbirds, and found that the emergence of sweet detection involved a single shift in a receptor for umami (see the Perspective by Barker). This ancient change facilitated sugar detection not just in nectar feeding birds, but also across the songbird group, and in a way that was different from, though convergent with, that in hummingbirds.
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