Supplementary Material for

**Functional divergence of bitter taste receptors in a nectar-feeding bird**

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Supplementary Methods:

Functional assays:
All of the Tas2r coding sequences for our functional assays were codon-optimized and synthesized. The coding sequences were inserted into the expression vector pCDNA3.1(+), with the first 45 amino acid residues of the rat somatostatin receptor 3 as the signal peptide at the 5'-end of the Tas2r receptors.

The bitter taste chemicals tested in our study included 13 natural and 11 synthetic compounds. Most of the naturally occurring compounds could be isolated from nectars, such as amygdalin, arbutin, caffeine, camphor, taurine and thiamine (Haydak et al. 1942; Lynn 2001; London-Shafir et al. 2003; Jerković and Kuć 2014; Nepi 2014); Some other bitter compounds were synthesized or distributed in plant tissues other than pollen, such as colchicine, salicin, picrotoxinin and yohimbine (Bader et al. 1954; Julkunen-Tiitto 1989; Finkelstein et al. 2010; Gössinger 2010). Additionally, chloramphenicol is a kind of bacteriostatic antibiotic isolated from soil bacterium (Fernandez-Martinez et al. 2014). Detailed information on the chemicals used in this study is presented in supplementary table S1. Our cell-based functional assays were carried out as previously described (Lei et al. 2015; Jiao et al. 2018). The concentrations of bitter compounds were followed according to a previous study (Meyerhof et al. 2010). Calcium mobilization was expressed as the percentage of fluorescence changes (ΔF) relative to the baseline (F); ΔF was quantified as the peak fluorescence minus the baseline. All experiments were run in triplicate.

Literature cited:
Table S1. Information about 24 bitter compounds and the maximum concentrations used in our assays.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Manufacturer</th>
<th>Catalog</th>
<th>(mM)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame K</td>
<td>Sigma</td>
<td>4054</td>
<td>10</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>Sigma</td>
<td>A6005</td>
<td>30</td>
<td>Occurs naturally in almond nectar, distributed in Asia, Africa, Europe and America</td>
</tr>
<tr>
<td>Arbutin</td>
<td>Sigma</td>
<td>A4256</td>
<td>30</td>
<td>Isolated from the nectar of <em>Arbutus unedo</em> (Ericaceae), which has a wide distribution</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Sigma</td>
<td>C0750</td>
<td>0.3</td>
<td>Occurs naturally in nectar of <em>Coffea</em> and <em>Citrus</em> species, in Central and South America</td>
</tr>
<tr>
<td>Camphor</td>
<td>Sigma</td>
<td>I48075</td>
<td>1</td>
<td>Occurs naturally in floral components from <em>Achillea millefolium</em>.</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Sigma</td>
<td>C0378</td>
<td>1</td>
<td>Chloramphenicol is an antibiotic isolated from <em>Streptomyces venezuelae</em></td>
</tr>
<tr>
<td>Chloroquine diphosphate salt</td>
<td>Sigma</td>
<td>C6628</td>
<td>10</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>Sigma</td>
<td>C3025</td>
<td>0.1</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Colchicine</td>
<td>Sigma</td>
<td>C3915</td>
<td>3</td>
<td>Extracted from two flowering plant: <em>Colchicum autumnale</em> and <em>Gloriosa superba</em></td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Sigma</td>
<td>1810</td>
<td>1</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Denatonium benzoate</td>
<td>Sigma</td>
<td>D5765</td>
<td>10</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Methimazole</td>
<td>Sigma</td>
<td>M8506</td>
<td>5</td>
<td>Synthetic</td>
</tr>
<tr>
<td>6-n-propythiouracil</td>
<td>Sigma</td>
<td>P3755</td>
<td>1</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Papaverine hydrochloride</td>
<td>Sigma</td>
<td>P3510</td>
<td>0.01</td>
<td>Mainly naturally exists in the mature capsule shell of <em>Papaver somniferum</em></td>
</tr>
<tr>
<td>Phenanthroline</td>
<td>Sigma</td>
<td>131377</td>
<td>1</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Phenylthiocarbamide</td>
<td>Sigma</td>
<td>P7629</td>
<td>1</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Picrotoxinin</td>
<td>Sigma</td>
<td>P8390</td>
<td>1</td>
<td>Isolated from <em>Menisperum cocculus</em> in India and Southeast;</td>
</tr>
<tr>
<td>Quinine</td>
<td>Sigma</td>
<td>Q1125</td>
<td>0.01</td>
<td>First isolated from the bark of <em>cinchona</em> tree, which are native to western South America.</td>
</tr>
<tr>
<td>Ranitidine hydrochloride</td>
<td>Sigma</td>
<td>R101</td>
<td>10</td>
<td>Synthetic</td>
</tr>
<tr>
<td>D-Salicin</td>
<td>Sigma</td>
<td>S0625</td>
<td>3</td>
<td>A glucoside for the whole genus of <em>Salix</em>, usually exists in flower bud and leaf bud</td>
</tr>
<tr>
<td>Sodium thiocyanate</td>
<td>Sigma</td>
<td>S7757</td>
<td>3</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Taurine</td>
<td>Sigma</td>
<td>T0625</td>
<td>1</td>
<td>A kind of non-protein amino acid, which exists in animal tissues abundantly, and also found in some insects and nectar</td>
</tr>
<tr>
<td>Substance</td>
<td>Supplier</td>
<td>Code</td>
<td>Amount</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Thiamine</td>
<td>Sigma</td>
<td>T4625</td>
<td>1</td>
<td>Also called vitamin B1, naturally exists in nectar of many native American flowers</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>Sigma</td>
<td>Y3125</td>
<td>0.15</td>
<td>An indole alkaloid mainly from African yohimbe tree, but is also found in some other plants, such as <em>Rauvolfia tetraphylla</em>, which is native to America</td>
</tr>
</tbody>
</table>
Table S2. Selective pressure analysis of *Tas2r1* genes in the Anna’s hummingbird using the improved branch-site model.

<table>
<thead>
<tr>
<th>Models</th>
<th>np</th>
<th>$^\omega_0$ (%)</th>
<th>$^\omega_1$ (%)</th>
<th>$^\omega_2$ (%)</th>
<th>lnL$^b$</th>
<th>$2\Delta$(ln L)$^c$</th>
<th>P-value$^d$</th>
<th>Positively selected sites$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data set: <em>Tas2r1</em> genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null Model</td>
<td>125</td>
<td>0.200 (50.5%)</td>
<td>1 (49.5%)</td>
<td></td>
<td>-21945.60</td>
<td>23.079</td>
<td>1.55E-06</td>
<td></td>
</tr>
<tr>
<td>Alternative Model</td>
<td>126</td>
<td>0.201 (46.9%)</td>
<td>1 (47.4%)</td>
<td>6.226 (5.7%)</td>
<td>-21934.06</td>
<td></td>
<td></td>
<td><strong>921 (0.979), 181L (0.912), 262S (0.819), 266S (0.983), 267L (0.906), 271K (0.845), 274M (0.923)</strong></td>
</tr>
</tbody>
</table>

$^a$ The $\omega$ values of each site class ($\omega_0$, $\omega_1$ and $\omega_2$) are shown as percentages in parentheses.

$^b$ The natural logarithm of the likelihood value.

$^c$ Twice the difference in ln L between the two models compared.

$^d$ P-values were generated by comparing the two models with a chi-square test.

$^e$ Positively selected sites with the posterior probabilities >0.8 were listed based on bayes empirical bayes (BEB) analysis. Sites in bold were also detected by site models (see the table S3 below).
Table S3. Analysis of positively selected sites of the five hummingbird *Tas2r1* genes using site models.

<table>
<thead>
<tr>
<th>Site models</th>
<th>np&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ln L&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Model compared</th>
<th>p value&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Positively selected sites&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1a</td>
<td>10</td>
<td>-3410.822</td>
<td>M1a vs M2a</td>
<td>3.40E-13</td>
<td>91K (0.984), 92I (0.986), 94I (0.962), 96S (0.975), 154V (0.971), 181L (0.993), 266S (1.000), 267L (0.961), 271K (0.985), 274M (0.989).</td>
</tr>
<tr>
<td>M2a</td>
<td>12</td>
<td>-3382.112</td>
<td></td>
<td></td>
<td>181L (0.992), 266S (1.000), 267L (0.961), 271K (0.985), 274M (0.989).</td>
</tr>
<tr>
<td>M8a</td>
<td>11</td>
<td>-3410.823</td>
<td>M8a vs M8</td>
<td>6.84E-14</td>
<td>91K (0.992), 92I (0.993), 94I (0.982), 96S (0.985), 154V (0.985), 1601 (0.967), 1621 (0.971), 181L (0.992), 187L (0.974), 266S (0.999), 267L (0.984), 271K (0.993), 274M (0.992).</td>
</tr>
<tr>
<td>M8</td>
<td>12</td>
<td>-3382.765</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Numbers of parameters.

<sup>b</sup>The natural logarithm of the likelihood value.

<sup>c</sup>P values were generated by comparing the two models with a chi-square test.

<sup>d</sup>Positively selected sites with the posterior probabilities >0.95 were listed based on bayes empirical bayes (BEB) analysis. Sites in bold were identified by both site models M2a and M8.
Table S4. Response profiles of the nine bird Tas2r1 receptors stimulated by 24 bitter compounds

<table>
<thead>
<tr>
<th>Bitter Compounds</th>
<th>CalAnn_Tas2r1a</th>
<th>CalAnn_Tas2r1b</th>
<th>CalAnn_Tas2r1c</th>
<th>CalAnn_Tas2r1d</th>
<th>CalAnn_Tas2r1e</th>
<th>ChaPel_Tas2r1a</th>
<th>ChaPel_Tas2r1b</th>
<th>ChaPel_Tas2r1c</th>
<th>CapCar_Tas2r1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame K</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td>Arbutin</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<td>n</td>
</tr>
<tr>
<td>Caffeine</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td>Camphor</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>n</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>Chloramphenicol</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Chloroquine diphosphate salt</td>
<td>n</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>n</td>
<td>Y</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>Y</td>
</tr>
<tr>
<td>Colchicine</td>
<td>Y</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>Y</td>
</tr>
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<td>Cycloheximide</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Denatonium benzoate</td>
<td>Y</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>Y</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td>Methimazole</td>
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<td>n</td>
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<td>n</td>
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<tr>
<td>6-n-propyliouracil</td>
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<td>n</td>
<td>n</td>
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<td>n</td>
<td>n</td>
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<tr>
<td>Papaverine hydrochloride</td>
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<td>Quinine</td>
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<td>Ranitidine hydrochloride</td>
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<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td>D-Salicin</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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</tr>
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<td>n</td>
<td>n</td>
<td>n</td>
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<td>n</td>
<td>n</td>
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</tr>
<tr>
<td>Thiamine</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<td>n</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>Y</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>Y</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
</tbody>
</table>

Y = Has response, n = Has no response; CalAnn = Anna's hummingbird, ChaPel = chimney swift, CapCar = chuck-will's widow
Data set S1. Tas2r gene sequences used in our cell-based functional assays. We renamed their gene names and provided their previous names in Wang and Zhao 2015 GBE.

>CalAnn_Tas2r1a (previously Anna’s Hummingbird_Tas2r1a)
ATGGAAGCTTGCTCCTCTCAAGGGAAAATTAAATGTGACACGTCACCATGACATGGCGATAT
GGCCATCATCACCCTCAAGGAAAATGCTGCCAGATGGATGTCAGATGCAGATGGGAGCATCAG
GTCTGGTGTGTTCAGGCTGAAGGTATGTTAGCAGTGGCAGTGGGAGCATCAGATGGGAGCATCAG
CTCTGGGATGCTCCCGGTTGGGTACTTGACATGGCGAGGTGTTCTCTATAATG
>CalAnn_Tas2r1b (previously Anna’s Hummingbird_Tas2r1b)
ATGGAAGCTTGCTCCTCTCAAGGGAAAATTAAATGTGACACGTCACCATGACATGGCGATAT
GGCCATCATCACCCTCAAGGAAAATGCTGCCAGATGGATGTCAGATGCAGATGGGAGCATCAG
GTCTGGTGTGTTCAGGCTGAAGGTATGTTAGCAGTGGCAGTGGGAGCATCAGATGGGAGCATCAG
CTCTGGGATGCTCCCGGTTGGGTACTTGACATGGCGAGGTGTTCTCTATAATG
>CalAnn_Tas2r1c (previously Anna’s Hummingbird_Tas2r1c)
ATGGAAGCTTGCTCCTCTCAAGGGAAAATTAAATGTGACACGTCACCATGACATGGCGATAT
GGCCATCATCACCCTCAAGGAAAATGCTGCCAGATGGATGTCAGATGCAGATGGGAGCATCAG
GTCTGGTGTGTTCAGGCTGAAGGTATGTTAGCAGTGGCAGTGGGAGCATCAGATGGGAGCATCAG
CTCTGGGATGCTCCCGGTTGGGTACTTGACATGGCGAGGTGTTCTCTATAATG
ACTGGAAAAGACACTGCTAAGGACCCTACTCTGGTCTGTAAGTGCAAATTTTGCATGAAGTAG

>CalAnn_Tas2r1d (previously Anna’s Hummingbird_Tas2r5)
ATGGAAAGCTTGACCCTCTCAAGGAAATTTAATGTGACCACGTCAATGTCCTGGCAAT
GGCCATCATCACCCCTGaAGAATTTGTCGCAGTTGGAATATGTGACCTTTCTGCTG
GCTTTTGTGTGACTGGATGGGTTTTAAAATATTTGTTAAATATTTCTTGCATATTGTTT
TCTCTGCAATGGGGCTTCTTCTTTTTTTTTTTTTTTTGAA

>CalAnn_Tas2r1e (previously Anna’s Hummingbird_Tas2r2)
ATGGAAAGCTTGACCCTCTCAAGGAAATTTAATGTGACCACGTCAATGTCCTGGCAAT
GGCCATCATCACCCCTGaAGAATTTGTCGCAGTTGGAATATGTGACCTTTCTGCTG
GCTTTTGTGTGACTGGATGGGTTTTAAAATATTTGTTAAATATTTCTTGCATATTGTTT
TCTCTGCAATGGGGCTTCTTCTTTTTTTTTTTTTTTTGAA

>CalAnn_Tas2r3 (previously Anna’s Hummingbird_Tas2r4)
ATGGAAAGCTTGACCCTCTCAAGGAAATTTAATGTGACCACGTCAATGTCCTGGCAAT
GGCCATCATCACCCCTGaAGAATTTGTCGCAGTTGGAATATGTGACCTTTCTGCTG
GCTTTTGTGTGACTGGATGGGTTTTAAAATATTTGTTAAATATTTCTTGCATATTGTTT
TCTCTGCAATGGGGCTTCTTCTTTTTTTTTTTTTTTTGAA

TAGGAGGAAACACTGCTGCAAGGACCCTACTCTGGTCTGTAAGTGCAAATTTTGCATGAAGTAG
CCAGCATCTTAGTGCCATTTGAAAATATGA

>ChaPel_Tas2r1a (previously Chimney Swift_Tas2r1)
ATGGAAGCTTGTTCACCTCAACACACATAATGATGTCACACATCAACAATGCTTTGGCAATT
TTCCCTCTGTCCTGACAGATTTGTCAGTGGATATACACGCTTTCAATGGTTCTTGT
GCTTTGCAATGTGCATTGGGTGATCTACACCAAGAAGCTCTAACTCCAATGAGAAGA
TCTTGGTCATTTCTGGGATGCTCTTGGGTGAAATCTCCTTTTA
CCCTGAGCTTTCGGCA

>ChaPel_Tas2r1b (previously Chimney Swift_Tas2r3)
ATGGAAGCTTGTTACTCTCATTTGAAAATATGA
TTCCCTCTGTCCTGACAGATTTGTCAGTGGATATACACGCTTTCAATGGTTCTTGT
GCTTTGCAATGTGCATTGGGTGATCTACACCAAGAAGCTCTAACTCCAATGAGAAGA
TCTTGGTCATTTCTGGGATGCTCTTGGGTGAAATCTCCTTTTA
CCCTGAGCTTTCGGCA

>ChaPel_Tas2r1c (previously Chimney Swift_Tas2r2)
ATGGAAGCTTGTTCACCTCAACACACATAATGATGTCACACATCAACAATGCTTTGGCAATT
TTCCCTCTGTCCTGACAGATTTGTCAGTGGATATACACGCTTTCAATGGTTCTTGT
GCTTTGCAATGTGCATTGGGTGATCTACACCAAGAAGCTCTAACTCCAATGAGAAGA
TCTTGGTCATTTCTGGGATGCTCTTGGGTGAAATCTCCTTTTA
CCCTGAGCTTTCGGCA

>CapCar_Tas2r3 (previously Chuck-will’s widow_Tas2r3)

ATGTTGCCACCCAGTCTTTTATTTGCTAATTAGGTCCGTAGCTTGAAGTTCTGAATGGCTTGG
GTTGGAAATGGATTTATTTACAGCTGTTAATTATTAACATGCTCGATTGCAACGGTTCTC
ACGTTACTGATGCACATTGACTCTTGTTTTAATGGAAGTTTGTGGTTCAGTGAC
TGTTGGCTCTATGACTGTGGCTCTTGTAATAATATCAACATCAACCTTAGGGATGGCTGCTGC
AACTCAAAGCTCAGATAGCAGGCTGATGGTCCCATGGCTGCTTCTAGGATCACTGGTGATC
TCTCTGCACTTTCTTCTTTTCTTTATGGGATTACCAACCGACACTACTCTGCACTCA
ACGTTGAACACTGAGAAATAGCAGCACATATCAGCTAGGGAACAGTGCTCAGT
TCTACGCTCAGTTTTGATGGTGGTTTTTCTCTTAATTACTCCTGATAAACCT
TCAGCTCTATTTATTATCTCTCTATGGGAAACACACAAAAAGAAAATGCAATGTTATGCA
ACTTTCAAGGATTTCTTTAAGTAGGCTGTACCTAAACTCCATTAAATCAATTATTTCTTCT
TGATCCTACTACCTCACTGTTTTGTAGCTAAAATCTGTGATATGCTGACCTCTCAAAG
GTAAAGACGATGTGAAAGTGGCAGACTATCTTAGTGGTATCTGGCATTGCTCTTAC
ACTCTATATCCTGACTAGTCAAATCAACTGAAATTGGCATTTCTGGCTTTGGTC
AGCATCTTAAAGCACCATTGGAAAAATATGACTCTGTGCTTTATAGCCTAA
Supplementary figure captions

Figure S1. Positive selected sites in hummingbird Tas2r1 genes. (a) Alignment of the amino acid sequences of five hummingbird Tas2r1s. Positively selected sites identified by both site models were indicated with red asterisks; positively selected sites detected by both site models and the branch-site model were shown in blue. (b) Snake plot of hummingbird Tas2r1s based on the alignment of the amino acid of five hummingbird Tas2r1 receptors. The positively selected sites identified by site models were indicated with an open circle and an amino acid position, while the sites detected by both site models and the branch-site model were indicated by blue circles with amino acid positions. The seven membrane domains were predicted using the TMHMM methods.

Figure S2. Responses of avian Tas2r1 receptors to bitter compounds. HEK293 cells transfected with one bird Tas2r1 construct along with Ga16-gust44 were assayed for their responses to 24 bitter compounds. Black traces, calcium mobilization of the nine Tas2r1 receptors to bitter compounds; grey traces, mock-transfected cells.

Figure S3. Quantitative analysis of responses of bird Tas2r1 receptors to bitter compounds. Responses of HEK293 cells transiently transfected with one bird Tas2r1 receptor and Ga16-gust44 to bitter compounds were quantified by the expression of bar graph. Black bar, responsiveness of Tas2r1-transfected cells; grey bar: mock-transfected cells. Data are expressed as mean ± SE percent change in fluorescence (ΔF, peak fluorescence-baseline fluorescence) compared with baseline fluorescence (F) from three independent wells. Two-tailed Student’s t-test were performed to confirm whether responses from Tas2r-transfected cells were significantly different from that of mock-transfected cells (p<0.05). All the bitter compound-elicited responses were significantly higher than mock-transfected cell baseline, with the exception of CalAnn_Tas2r1c toward camphor (c).

Figure S4. Functional responses of bird Tas2r2 and Tas2r3 receptors. Tas2r constructs were expressed along with Ga16-gust44 in HEK293 cells to examine their responses to two bitter compounds. Black bar, responsiveness of Tas2r-transfected cells; grey bar: mock-transfected cells. (a, c) Quantitative analysis of responses of bird Tas2rs to denatonium benzoate (10 mM) and yohimbine (0.15 mM). (b, d) Dose-dependent responses of bird Tas2r receptors to denatonium benzoate and yohimbine. GraphPad Prism 7 was used to fit the curves.
**Figure S1**

CalAnn_Tas2r1a

MEACSSQGKF NVTTYNAVM AIITLQTTPAG MHNAFIVSV LCVSNWKKKS FNTEKILLLL LCQRBFVYGSC TAMVSSFLEI YSFCVTVYQQ RQIQISSALLS

CalAnn_Tas2r1b

MEACSSQGKF NVTTYNASM AIITLQTTPAG MHNAFIVSV LCVSNWKKKS FNSNEKILLF LCQVRFCYFIC ITWYSLFLKLI YQCRFYAHM LAGLFAIQAQS

CalAnn_Tas2r1c

MEACTSQGKF NVTTYNTVSM AIITLQTTPAG MHNAFIVSV LCGNWKKKS FNSNEKILFL LCQSRFCYLIF ITWYSSLFLFAY IYFCHDYVFY ATULLGQTQN

CalAnn_Tas2r1d

MEACTSQGKF NVTTYNAVM AIITLQTTPAG MHNAFIVSV LCVSNWKKKS FNSNEKILLF LCQSRSCYLF ITWYSSLFFEN IYFLCNVYVS FTLFLPGIQS

CalAnn_Tas2r1e

MEACTSQGKF NVTTYNAVM AIITLQTTPAG MHNAFIVSV LCVSNWKKKS FNTNEKILLL LCQRBFWYSC ITWYSSLFLFEN IYFLCQYVSF TQLTLFQIGS

FFNIFSFLWIS ALLEVFYCYC ILANFQNTFPI YLKVRIDRV PMLWETSSVPV SSLYSFVFFY1 VIDEARCDNN NCTTSGYLYH LTVRRFHLFF PLYFTTGFYY

FFSFSNLSMV APLSEVFYCYC ILANFQNTTPSI YLKVRIDRV PMLWASLAVL SLSFSLYFGD IADEALNNWH NEAPGNNWH HNINMDRFF PIFISFGFYE

FFVSSELSV WIS ALLEVFYCYC ILANFQNTSPI YLKVRIDRV PMLWASLVL SSLGSSSSSYK VLSESCINNT NCTSAEYLMK CSHHRKDELL LFLFLGISVII

FFVNSVMSVWIS ALLEVFYCYC ILANFQNTSPI YLKVRIDRV PMLWASLVL SSLGSSSSSYK VVSDKCINNT NCTSAEYLMK HNINMDRFF PIFISFGFYE

FFNIFSFLWIS ALLEVFYCYC ILANFQNTFPI YLKVRIDRV PMLWASLVL SLSY graffiti VMEEVULCEH NCTTSGYNYH RQMICNTY TFQTLFLGSFY

100

200

300

320

KTTLLRTLSL KCKICMKHRT

ETLILRTLSL KCKICMKHRT

KTTLRTLSL KCKICMKHRT

KTTLRTLSL KCKICMKHRT

AIAFAVICTS ALLLLFLSLWR HKHRQKXSQ KNVSVDAHKL AMKSIISFFV IYVSIFCILE HELSVATKSA TILLTVISLFP KHEFFSYEL IYISFNPKRLE

KTTLRTLSL KCKICMKHRT

ETLILRTLSL KCKICMKHRT

KTTLRTLSL KCKICMKHRT

KTTLRTLSL KCKICMKHRT

AIAFAVICTS ALLLLFLSLWR HKHRQKXSQ KNVSVDAHKL AMKSIISFFV IYVSIFCILE HELSVATKSA TILLTVISLFP KHEFFSYEL IYISFNPKRLE

AIAFAVICTS ALLLLFLSLWR HKHRQKXSQ KNVSVDAHKL AMKSIISFFV IYVSIFCILE HELSVATKSA TILLTVISLFP KHEFFSYEL IYISFNPKRLE
Figure S3
Figure S4