13 April 2006 | www.nature.com/nature | £10

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature

nature 440, 845–968 13 April 2006

www.nature.com/nature

ULTRACOLD PHYSICS
A quantum Newton's cradle

ANTIMALARIAL DRUGS
Tackling the artemisinin shortage

SAN FRANCISCO 1906
The earthquake that shook the world

CHANGE FOR THE BITTER

Taste sensitivity in chimpanzees and humans

NATUREJOBS
Match me a mentor

no.7086 npg

Vol 440|13 April 2006|doi:10.1038/nature04655

LETTERS

Independent evolution of bitter-taste sensitivity in humans and chimpanzees

Stephen Wooding[1], Bernd Bufe[2], Christina Grassi[3], Michael T. Howard[1], Anne C. Stone[4], Maribel Vazquez[3], Diane M. Dunn[1], Wolfgang Meyerhof[2], Robert B. Weiss[1] & Michael J. Bamshad[1]†

It was reported over 65 years ago that chimpanzees, like humans, vary in taste sensitivity to the bitter compound phenylthiocarbamide (PTC)[1]. This was suggested to be the result of a shared balanced polymorphism, defining the first, and now classic, example of the effects of balancing selection in great apes. In humans, variable PTC sensitivity is largely controlled by the segregation of two common alleles at the *TAS2R38* locus, which encode receptor variants with different ligand affinities[2–4]. Here we show that PTC taste sensitivity in chimpanzees is also controlled by two common alleles of *TAS2R38*; however, neither of these alleles is shared with humans. Instead, a mutation of the initiation codon results in the use of an alternative downstream start codon and production of a truncated receptor variant that fails to respond to PTC *in vitro*. Association testing of PTC sensitivity in a cohort of captive chimpanzees confirmed that chimpanzee *TAS2R38* genotype accurately predicts taster status *in vivo*. Therefore, although Fisher *et al.*'s observations[1] were accurate, their explanation was wrong. Humans and chimpanzees share variable taste sensitivity to bitter compounds mediated by PTC receptor variants, but the molecular basis of this variation has arisen twice, independently, in the two species.

Sensitivity to bitter tastes provides an important means for animals to interact with their environment[5–7]. By allowing the detection of various toxic compounds in food, especially noxious compounds produced by plants as a means of defence against herbivores, bitter-taste sensitivity enables animals to regulate their intake of toxins[8,9]. Consequently, bitter-taste perception allows animals to exploit nutritious but toxic food sources by monitoring the consumption of compounds that might cause sickness or death. For humans today, bitter-taste sensitivity is probably less important for avoiding poisoning, but might still influence health through its effects on diet choice[9] and other behaviours, such as smoking[10,11].

In mammals, the ability to taste bitter compounds is mediated principally by a series of small G-protein-coupled receptors encoded by the *TAS2R* gene family that are expressed in specialized taste bud cells in the lingual epithelium[7]. Inter-individual differences in bitter-taste sensitivity arise by a variety of mechanisms, including substantial intra- and inter-genic variation in the *TAS2R* genes[12–14]. For example, genetic variants that code for functionally distinct receptor types contribute to variation in bitter-taste sensitivity in both humans and mice[2,4,15,16]. Several *T2R* variants seem to have experienced selective pressures as humans moved into new environments during their dispersal from sub-Saharan Africa about 100,000 years ago[14,17,18]. Other primate species show variation in their taste sensitivity to bitter compounds as well[1], suggesting that natural selection might have affected patterns of variation in *T2R* genes even earlier in hominid evolution.

In 1939, Fisher *et al.* reported that chimpanzees and humans both harbour apparently dominant and recessive 'taster' and 'nontaster' alleles at roughly equal frequencies. They argued that the best explanation for this finding is that humans and chimpanzees share anciently derived alleles that evolved before the human–chimpanzee divergence (Fig. 1a) and have since been maintained at equilibrium as the result of heterozygote advantage: "Wherein the selective advantages lie, it would at present be useless to conjecture, but of the existence of a stably balanced and enduring dimorphism determined by this gene there can be no room for doubt."[1]

It has recently been reported[2] that 50–85% of the phenotypic variance in PTC sensitivity in humans is attributable to polymorphisms at the *TAS2R38* locus, in h*TAS2R38*, a ~1-kb single-exon gene on chromosome 7. Most of this variance is explained by amino acid polymorphisms at positions 49 (encoding proline or alanine), 262 (encoding alanine or valine) and 296 (encoding valine or isoleucine) that give rise to two common isoforms, denoted PAV and AVI (haplotypes A and G in Fig. 2a)[2,3]. h*TAS2R38*(PAV) and h*TAS2R38*(AVI) are strongly associated with PTC sensitivity in human subjects[3]. *In vitro* stimulation of cells expressing h*TAS2R38*(PAV) with PTC increases cytosolic Ca^{2+} concentrations, whereas stimulation of cells expressing h*TAS2R38*(AVI) does not[4]. To test the hypothesis that PTC sensitivity in chimpanzees is controlled by the

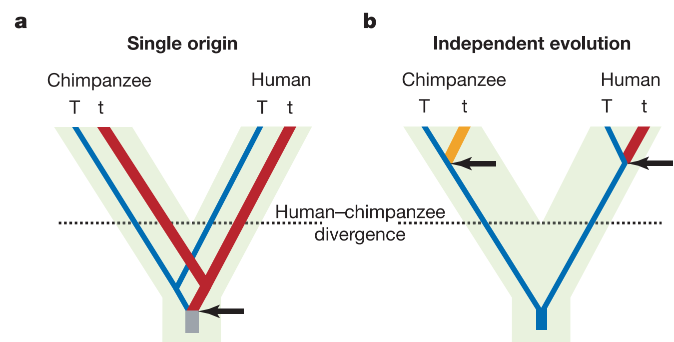

Figure 1 | Hypothetical origins of PTC taster and nontaster alleles in humans and chimpanzees. a, Divergence of taster (T) and nontaster (t) alleles before the human–chimpanzee divergence, with T and t alleles subsequently maintained within each species by balancing natural selection. **b**, Independent origin of t alleles from T alleles in humans and chimpanzees, after the human–chimpanzee divergence.

[1]Department of Human Genetics, University of Utah, 15 North 2030 East, Salt Lake City, Utah 84112-5330, USA. [2]German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, Nuthetal 14558, Germany. [3]Department of Comparative Medicine, Southwest Foundation for Biomedical Research, San Antonio, Texas 78245-0549, USA. [4]School of Human Evolution and Social Change, Arizona State University, Tempe, Arizona 85287-2402, USA. †Present address: Departments of Pediatrics and Genome Sciences, University of Washington, Seattle, Washington 98195, USA.

© 2006 Nature Publishing Group

same taster and nontaster alleles found in humans[1], we re-sequenced the h*TAS2R38* orthologue in chimpanzees[19] (ch*TAS2R38*) in 37 wild-born animals representing three subspecies (26 *Pan troglodytes verus*, 9 *P. t. troglodytes* and 2 *P. t. schweinfurthii*) and 49 captive-born animals (*P. t. verus*), and compared the molecular and functional characteristics of human and chimpanzee alleles.

We found seven variable nucleotide positions in ch*TAS2R38* (Fig. 2a). These did not occur at any of the nucleotide or amino acid positions variable in humans, including the positions distinguishing the h*TAS2R38*(PAV) and h*TAS2R38*(AVI) isoforms. However, one site—a T→G substitution found at a frequency of 0.52 in the second position of the initiation codon (that is, ATG→AGG)—is noteworthy because it is predicted to attenuate ch*TAS2R38* translation. On the basis of this finding, we hypothesized that the ch*TAS2R38* haplotypes harbouring the AGG variant were chimpanzee PTC nontaster alleles (Fig. 2a).

A maximum-likelihood phylogeny revealed that *TAS2R38* haplotypes in humans and chimpanzees form separate clades, and that the putative nontaster allele within each species is evolutionarily derived from an ancestral taster allele (Fig. 2b). In addition, the application of Tajima's D test to the data from wild-born chimpanzees failed to reject the hypothesis of evolutionary neutrality ($D = -0.59$, $P < 0.40$), yielding a D value well within the range of D values reported for 59 noncoding regions thought to be evolving neutrally in chimpanzees (-0.39 to -1.94; refs 20–22). This result stands in contrast to findings for *TAS2R38* in humans, for which the value of Tajima's D is 1.55. This value is (1) significantly greater than expected ($P < 0.01$), (2) greater than 47 of 50 D values in noncoding regions, and (3) greater than 99.5% of D values previously reported for more than 1,600 genes[23]. The high value of D in humans has been interpreted as evidence for balancing selection acting on h*TAS2R38* (ref. 17). These findings suggest that not only have nontaster alleles been independently derived in humans and chimpanzees, but they seem to have arisen under different selective regimes. Thus, we reject Fisher *et al.*'s hypothesis that humans and chimpanzees share anciently derived taster and nontaster alleles maintained by balancing natural selection.

To further explore the evolutionary implications of the ATG→AGG mutation in ch*TAS2R38*, we investigated its functional consequences for translation initiation and receptor activity. *In vitro* transcription and translation of a ch*TAS2R38*–reporter fusion construct confirmed that a full-length TAS2R38 protein (chTAS2R38$_{WT}$–R) was produced by the wild-type ch*TAS2R38*(ATG) allele, but not by the ch*TAS2R38*(AGG) allele (Fig. 3a and Supplementary Fig. S1). Instead, the main product of the ch*TAS2R38*(AGG) allele was a truncated polypeptide, chTAS2R38$_{TR}$–R, resulting from translation initiation at a downstream ATG codon (M97), the expression of which could be abolished by substituting a leucine (CTG) codon at that position (Fig. 3a). chTAS2R38$_{TR}$–R is predicted to lack 96 amino-terminal residues of the chTAS2R38$_{WT}$–R receptor, suggesting that the ATG→AGG mutation severely reduces or abolishes the production of functional PTC receptor in chimpanzees.

To test the functional properties of the chTAS2R38–R receptor variants, we cloned the ATG and AGG alleles of ch*TAS2R38* from the genomic DNA of two homozygous chimpanzees as fusion proteins with an N-terminal SST-tag (which facilitates cell-surface expression) and a C-terminal Flag tag to allow immunological detection of the recombinantly expressed fusion proteins[24] (Supplementary Fig. S2). These constructs were then transiently transfected into HEK-293T cells that stably express the chimaeric G-protein subunit $G_{\alpha16gust44}$, which has been shown to efficiently couple TAS2R receptors to the release of intracellular calcium[25]. Immunohistochemical analysis revealed that all receptor variants were expressed, and no obvious differences in cell-surface expression levels were detected (Fig. 3b and Supplementary Figs S3, S4).

Fluorescence imaging plate reader recordings of calcium transients in cells expressing ch*TAS2R38* receptor variants showed that micromolar concentrations of PTC increased cytosolic Ca^{2+} in cells transfected with ch*TAS2R38*(ATG) in a concentration-dependent manner. These analyses showed that although the dose–response curve of the predicted chimpanzee taster allele, ch*TAS2R38*(ATG), was similar to that of the human taster allele, h*TAS2R38*(PAV), cells expressing ch*TAS2R38*(AGG) failed to respond to PTC even at concentrations as high as 100 μM (Fig. 3c and Supplementary Fig. S4). Engineering an initiation codon upstream of the native start site in the ch*TAS2R38*(AGG) allele resulted in complete rescue of receptor function (Fig. 3b, c). These results confirm that ch*TAS2R38*(ATG) produces a functional PTC receptor, but the ch*TAS2R38*(AGG) allele does not.

To determine whether ch*TAS2R38*(AGG) is the nontaster allele

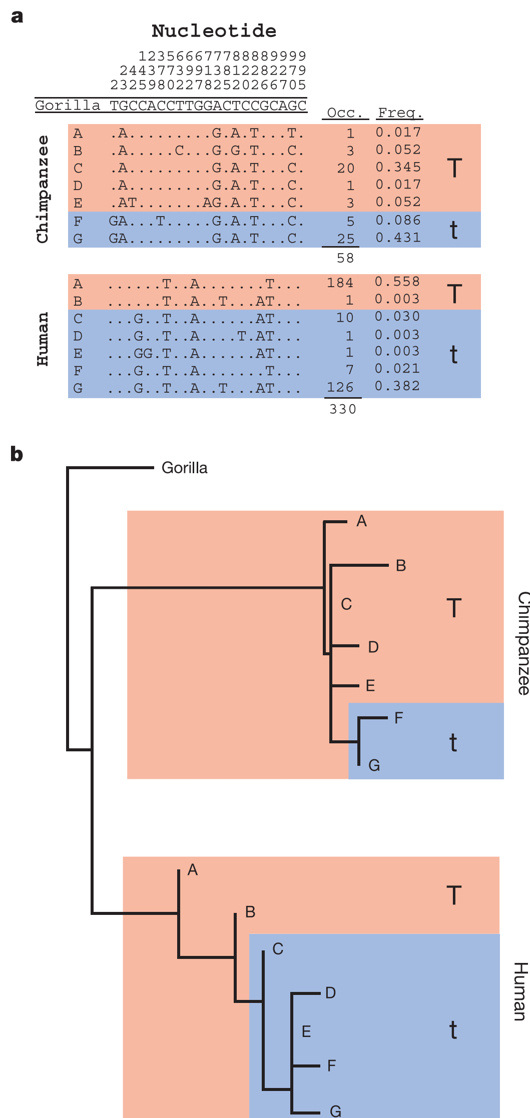

Figure 2 | Patterns of genetic variation in *TAS2R38* in human, chimpanzee and gorilla. a, The nucleotide sequence of each *TAS2R38* haplotype compared to that of gorilla (top). Dots indicate sequence identity and letters indicate differences. Human and chimpanzee *TAS2R38* haplotypes differed by a minimum of six nucleotides. No nucleotide position was variable in both humans and chimpanzees. T and t denote 'taster' and 'nontaster' alleles, respectively. **b**, Maximum-likelihood phylogeny of human and chimpanzee haplotypes generated using PHYLIP and using the gorilla as an outgroup. Linkage disequilibrium was high ($D' \sim 1.0$) across the length of the gene (~1 kb). The human and chimpanzee *TAS2R38* alleles form two separate clades.

© 2006 Nature Publishing Group

in vivo, we tested whether ch*TAS2R38* genotype predicted PTC sensitivity in chimpanzees. PTC sensitivity was measured in 42 unrelated captive-born chimpanzees by presenting each animal with apple slices soaked in control and test solutions (water and 4.0 mM PTC in water, respectively). Apples were chosen because they are a typical enrichment food for chimpanzees and are easily saturated with PTC. The response of each chimpanzee was then scored on a categorical scale from 1 (ready acceptance) to 5 (strong rejection) by a primate behavioural specialist who directs the enrichment program for these chimpanzees, has day-to-day contact with the animals and is familiar with their dietary habits (see Methods for details). The scorer was blinded to chimpanzee genotype.

Among the 39 chimpanzees for which complete genotype and phenotype data were available, 11 had an ATG/ATG genotype, 19 had ATG/AGG and 9 had AGG/AGG (Supplementary Notes S1 and S2). Preliminary association analyses comparing only the ATG/ATG and ATG/AGG animals showed that these genotypes responded similarly to the PTC treatment, suggesting that ATG is dominant or nearly dominant to AGG (Supplementary Note S3). This observation is consistent with the results of our *in vitro* translation experiments, which suggest that ch*TAS2R38*(AGG) is effectively a null allele. For this reason, we grouped the ATG/ATG and ATG/AGG animals as predicted tasters in all subsequent analyses, and AGG/AGG animals were predicted nontasters.

Of the 30 predicted tasters, 18 showed the taster phenotype (that is, responses ranging from 3 to 5 on our scale) (Fig. 4). Of the 9 chimpanzees predicted to be PTC nontasters, all showed the non-taster phenotype (that is, responses of 1 or 2 on our scale) (Fig. 4 and Supplementary Note S4). A one-sided Fisher's exact test revealed a significant association between ch*TAS2R38* genotype and response to the test (PTC) solution ($P < 0.015$), but not response to the control (water) solution ($P > 0.75$). When the experiment was repeated, 23 out of 30 predicted tasters showed the taster phenotype, and 8 of 9 predicted nontasters showed the nontaster phenotype (Fig. 4). These results confirm a significant association between genotype and response to the PTC solution ($P < 0.01$) but not the control solution ($P > 0.75$).

A number of predicted tasters did not reject PTC-treated apples in one or both tests. We hypothesize that these animals either have PTC taste thresholds higher than that tested in this study (4.0 mM, which is less than half of the maximum concentration of 8.5 mM previously tested in humans[2,3]), or that they could taste the compound but were not noticeably disturbed by it. The assignment of these predicted tasters to the nontaster outcome group makes our association tests statistically more conservative. However, to verify the association test, we compared the responses of predicted tasters and nontasters separately. These tests showed that although predicted tasters showed a strong difference in response between the test and control

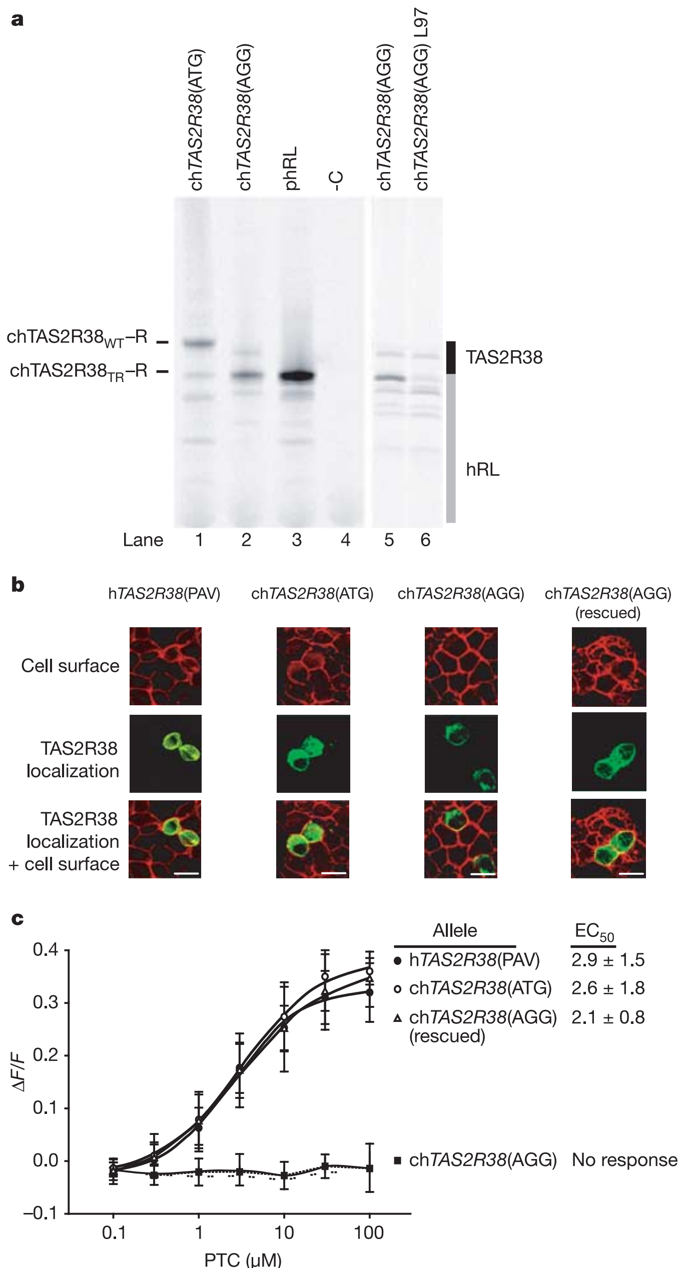

Figure 3 | Translation, membrane localization and functional characteristics of ch*TAS2R38* alleles. **a**, Lane 1, full-length product ($chTAS2R38_{WT}$) of ch*TAS2R38*(ATG). Lanes 2 and 5, truncated product ($chTAS2R38_{TR}$) of ch*TAS2R38*(AGG). Lane 3, vector. Lane 4, negative control. Lane 6, product of ch*TAS2R38*(AGG) with ATG at position 97 changed to CTG. Predicted products of TAS2R38 (black bar) and *Renilla* luciferase (grey bar). **b**, Confocal fluorescence images of HEK-293T cells transfected with *TAS2R38* allele. Cell-surface staining is red, PTC receptor is green, overlay of PTC receptor on the cell surface is yellow. Scale bar, 10 μm. **c**, Dose–response curves of increasing PTC concentration on intracellular Ca^{+2} levels (measured as change in fluorescence, $\Delta F/F$) in cells expressing TAS2R38. Each point represents mean ± s.e.m. of three independent replicates.

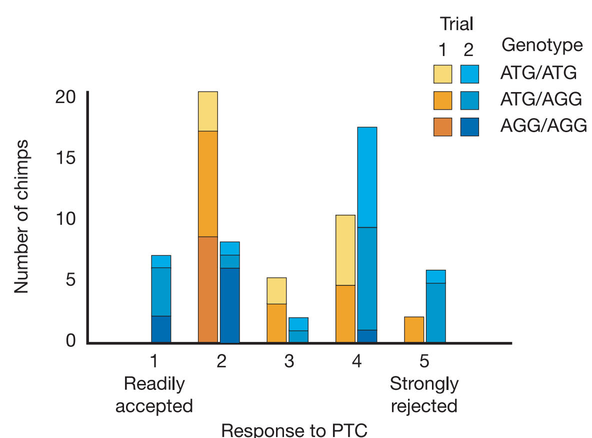

Figure 4 | Chimpanzee responses to PTC. Distribution of scored responses to apple slices soaked in 4.0 mM PTC solution, by genotype. Responses in tests 1 and 2 are shown in yellow and blue columns, respectively. Genotype is indicated by shading: light, ATG/ATG; intermediate, ATG/AGG; dark, AGG/AGG.

© 2006 Nature Publishing Group

treatments (Fisher's exact test; $P < 10^{-5}$ for both the first and second trials), nontasters showed no difference ($P > 0.45$ for both the first and second trials) (Supplementary Note S5). Thus, *TAS2R38* genotype is highly predictive of PTC taste sensitivity in chimpanzees.

On the basis of our results, we reject Fisher *et al.*'s long-standing hypothesis that the variation in PTC taste sensitivity shared between humans and chimpanzees is due to shared ancient genetic polymorphisms maintained by balancing natural selection. Phylogenetic relationships among chimpanzee and human haplotypes are more consistent with the hypothesis that nontaster alleles have evolved at least twice, independently, over the course of hominid evolution (Fig. 1b). Whether this finding is specific to *TAS2R38* or is a more generalized trend among bitter taste receptors remains to be determined. Characterizing such patterns could facilitate the discovery of compounds with particularly important effects on nutrition and health. Access to the recently sequenced chimpanzee genome and a captive cohort of healthy chimpanzees offers the opportunity to explore this issue specifically, and more broadly paves the way for future comparative functional genomic studies with our sibling species.

METHODS

DNA sequence analysis. Haplotypes were inferred from unphased genotypes in wild-born chimpanzees using the PHASE 2.1 computer program[26]. Tajima's *D* test of evolutionary neutrality[27] was performed using the DFSC computer program[17]. This test was performed under the assumption that the effective population size of *P. t. verus*, which represents the majority of the wild-born sample, has remained constant. This assumption is based on several studies suggesting that population sizes in western, but not eastern, chimpanzee subspecies have remained stable for an extended period[20,28–30]. Tajima's *D* value at ch*TAS2R38* was also compared with values calculated for 50 noncoding regions, each more than 5 kb from any known coding region, which were assumed to be evolving neutrally[22].

Expression studies. The last 88 nucleotides of the 5′ untranslated region and first 318 coding nucleotides of ch*TAS2R38* alleles were subcloned into the *Nhe*I and *Ava*I restriction sites of the phRL-CMV plasmid vector (Promega). These constructs and the parent vector (phRL) were transcribed and translated *in vitro* using a coupled transcription and translation system (T7 TNT, Promega) in the presence of ^{35}S-methionine. ^{35}S-labelled protein products were separated by SDS polyacrylamide gel electrophoresis.

Functional assay. The allele encoding chTAS2R38$_{TR}$–R was amplified from genomic DNA of a chimpanzee homozygous for the ch*TAS2R38*(AGG) (nontaster) allele, and the chTAS2R38$_{WT}$–R receptor was amplified from a ch*TAS2R38*(ATG) homozygote using standard polymerase chain reaction (PCR) conditions and primer sets directed against the coding region of the respective receptor variants. A primer-encoded *Eco*RI site in front of the start codon and a primer-encoded *Not*I site in front of the stop codon were used to subclone the PCR products into a pCDNA5 expression vector containing an N-terminal SST and a C-terminal Flag tag (Supplementary Fig. 2). All cloned receptors were sequenced to exclude amplification errors during the PCR reaction.

Functional assays were carried out essentially as described in ref. 18. Plasmids containing the receptor variants were transiently transfected into HEK-293T cells stably expressing the chimaeric G-protein subunit $G_{\alpha 16gust44}$ using Lipofectamine 2000 (Invitrogen). Calcium mobilization of Fluo-4-AM loaded cells upon stimulation was monitored with an automated fluorometric imaging plate reader (FLIPR, Molecular Devices). The obtained calcium signals were corrected for background fluorescence and the response of mock-transfected cells. Concentration–response curves and EC_{50} values were averaged over three independent experiments and were calculated in Sigma Plot by nonlinear regression using the function $f(x) = [(a-d)/(1+(x/EC_{50})^{nh})+d]$.

Immunocytochemistry. Immunocytochemistry was carried out as described in ref. 18. HEK-293T cells expressing $G_{\alpha 16gust44}$ were seeded on coverslips transfected with plasmids containing the different TAS2R38 receptor variants. Twenty-four hours later, the cells were permeabilized using a 1:1 acetone:methanol solution. The receptor variants were detected with a primary monoclonal mouse anti Flag-antibody (1:1,000; Sigma) and a secondary Alexa-488-conjugated goat antiserum against mouse IgG (1:1,000; Molecular Probes). Expression levels were determined on three independent days, analysing three independent visual fields per variant using a fluorescence microscope (Zeiss Axioplan) and a cooled CCD camera (Visitron Systems). To assess cell-surface expression, cells were additionally incubated on ice with $5\,\mu g\,ml^{-1}$ biotin-labelled concanavalin A (Sigma) for 1 h before permeablization. The cell surface was visualized with streptavidin-Alexa-633 (1:1,000; Molecular Probes) (Fig. 3b). Colocalization between the receptor and cell surface was analysed using a Leica TCS SP2 confocal microscope.

Phenotyping. Apples were cored, separated into 14 slices of equal proportion, and soaked in either 2 l of water (control solution) or 2 l of a 4.0 mM PTC solution for 10–12 h overnight before testing. Solutions were prepared with different utensils and in different work areas to minimize cross-contamination. On each test day, the excess solution was drained and individual apple slices were offered to chimpanzee test subjects. This procedure recapitulated the manner in which enrichment foods (for example, grapes and apples) were typically provided to the chimpanzees. Each chimpanzee was monitored by a trained observer until either the apple slice was consumed or rejected, for a maximum period of ~10 min. The response of each chimpanzee was then scored on a categorical scale from 1 (ready acceptance) to 5 (strong rejection) based on whether the apple slice was consumed, the time taken to consume the apple slice, the presence of excessive salivation, and the extent of facial grimacing. Testing of the control and test solutions was alternately performed on different days, each separated by breaks of ~48 h. Immediately after testing with either solution, a second enrichment food (dried apricot without PTC) was provided to each test subject.

Received 17 November 2005; accepted 16 February 2006.

1. Fisher, R. A., Ford, E. B. & Huxley, J. Taste-testing the anthropoid apes. *Nature* **144**, 750 (1939).
2. Kim, U. K. *et al.* Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science* **299**, 1221–1225 (2003).
3. Drayna, D. *et al.* Genetic analysis of a complex trait in the Utah Genetic Reference Project: a major locus for PTC taste ability on chromosome 7q and a secondary locus on chromosome 16p. *Hum. Genet.* **112**, 567–572 (2003).
4. Bufe, B. *et al.* The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr. Biol.* **15**, 322–327 (2005).
5. Meunier, N., Marion-Poll, F., Rospars, J. P. & Tanimura, T. Peripheral coding of bitter taste in *Drosophila*. *J. Neurobiol.* **56**, 139–152 (2003).
6. Hilliard, M. A., Bergamasco, C., Arbucci, S., Plasterk, R. H. & Bazzicalupo, P. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. *EMBO J.* **23**, 1101–1111 (2004).
7. Hoon, M. A. *et al.* Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* **96**, 541–551 (1999).
8. Glendinning, J. I. Is the bitter rejection response always adaptive? *Physiol. Behav.* **56**, 1217–1227 (1994).
9. Drewnowski, A. & Rock, C. L. The influence of genetic taste markers on food acceptance. *Am. J. Clin. Nutr.* **62**, 506–511 (1995).
10. Kaplan, A. R., Glanville, E. V. & Fischer, R. Taste thresholds for bitterness and cigarette smoking. *Nature* **202**, 1366 (1964).
11. Enoch, M. A., Harris, C. R. & Goldman, D. Does a reduced sensitivity to bitter taste increase the risk of becoming nicotine addicted? *Addict. Behav.* **26**, 399–404 (2001).
12. Conte, C., Ebeling, M., Marcuz, A., Nef, P. & Andres-Barquin, P. J. Evolutionary relationships of the Tas2r receptor gene families in mouse and human. *Physiol. Genomics* **14**, 73–82 (2003).
13. Parry, C. M., Erkner, A. & le Coutre, J. Divergence of T2R chemosensory receptor families in humans, bonobos, and chimpanzees. *Proc. Natl Acad. Sci. USA* **101**, 14830–14834 (2004).
14. Kim, U., Wooding, S., Ricci, D., Jorde, L. B. & Drayna, D. Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci. *Hum. Mutat.* **26**, 199–204 (2005).
15. Adler, E. *et al.* A novel family of mammalian taste receptors. *Cell* **100**, 693–702 (2000).
16. Chandrashekar, J. *et al.* T2Rs function as bitter taste receptors. *Cell* **100**, 703–711 (2000).
17. Wooding, S. *et al.* Natural selection and molecular evolution in *PTC*, a bitter-taste receptor gene. *Am. J. Hum. Genet.* **74**, 637–646 (2004).
18. Soranzo, N. *et al.* Positive selection on a high-sensitivity allele of the human bitter-taste receptor TAS2R16. *Curr. Biol.* **15**, 1257–1265 (2005).
19. Wang, X., Thomas, S. D. & Zhang, J. Relaxation of selective constraint and loss of function in the evolution of human bitter taste receptor genes. *Hum. Mol. Genet.* **13**, 2671–2678 (2004).
20. Fischer, A., Wiebe, V., Paabo, S. & Przeworski, M. Evidence for a complex demographic history of chimpanzees. *Mol. Biol. Evol.* **21**, 799–808 (2004).
21. Gilad, Y., Bustamante, C. D., Lancet, D. & Paabo, S. Natural selection on the olfactory receptor gene family in humans and chimpanzees. *Am. J. Hum. Genet.* **73**, 489–501 (2003).
22. Yu, N. *et al.* Low nucleotide diversity in chimpanzees and bonobos. *Genetics* **164**, 1511–1518 (2003).
23. Schneider, J. A. *et al.* DNA variability of human genes. *Mech. Ageing Dev.* **124**, 17–25 (2003).

© 2006 Nature Publishing Group

24. Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J. D. & Meyerhof, W. The human TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides. *Nature Genet.* **32,** 397–401 (2002).
25. Ueda, T., Ugawa, S., Yamamura, H., Imaizumi, Y. & Shimada, S. Functional interaction between T2R taste receptors and G-protein α subunits expressed in taste receptor cells. *J. Neurosci.* **23,** 7376–7380 (2003).
26. Stephens, M., Smith, N. J. & Donnelly, P. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* **68,** 978–989 (2001).
27. Tajima, F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123,** 585–595 (1989).
28. Gagneux, P. *et al.* Mitochondrial sequences show diverse evolutionary histories of African hominoids. *Proc. Natl Acad. Sci. USA* **96,** 5077–5082 (1999).
29. Goldberg, T. L. & Ruvolo, M. The geographic apportionment of mitochondrial genetic diversity in east African chimpanzees, *Pan troglodytes schweinfurthii*. *Mol. Biol. Evol.* **14,** 976–984 (1997).
30. Kaessmann, H., Wiebe, V., Weiss, G. & Paabo, S. Great ape DNA sequences reveal a reduced diversity and an expansion in humans. *Nature Genet.* **27,** 155–156 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements Chimpanzee samples were contributed by Sunset Zoo, Riverside Zoo, Kitwe Point Sanctuary, the Jane Goodall Institute, J. Wickings, the Centre International de Recherches Medicales, the Primate Foundation of Arizona, the New Iberia Primate Center and the Southwest Foundation for Biomedical Research. Chimpanzee samples were imported under a CITES permit. Comments and technical assistance were provided by C. Anderson, D. Drayna, L. Jorde, U-k. Kim, M. Pyrski, A. Rogers, J. Seger and E. Wooding. Funding was provided by grants from the NIH, the Centers for Disease Control, the Muscular Dystrophy Association, the Wenner-Gren Foundation, the National Science Foundation and the German Science Foundation.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to S.W. (swooding@genetics.utah.edu) or M.J.B. (mbamshad@u.washington.edu).

© 2006 Nature Publishing Group