Supporting Information Materials and Methods

Population Samples

Institutional Review Board approval for this project was obtained from the University of Maryland at College Park and the University of Pennsylvania and written informed consent was obtained from all African participants. Research/ethics approval and permits were obtained from the following institutions prior to sample collection: COSTECH and NIMR in Dar es Salaam, Tanzania; KEMRI in Nairobi, Kenya; the Ministry of Health and National Committee of Ethics, Cameroon. White cells from diverse Africans were isolated in the field from whole blood using a modified salting-out procedure, and DNA was subsequently extracted in the laboratory using a Purgene DNA extraction kit (Gentra) as previously described (Tishkoff et al. 2009). We also included 132 non-African samples from the Centre d'Etude du Polymorphisme Humain (CEPH) human genome diversity panel (HGDP) (Cann et al. 2002) , as well as samples provided by Mark Shriver (Department of Anthropology, Pennsylvania State University), and Ángel Carracedo (Instituto de Medicina Legal, Universidad de Santiago Compostela) for comparative analysis (Table S1).

Phenotype Determination

Bitter taste phenotypes were measured for 463 individuals from Cameroon and Kenya using a modification of the classic method described by Harris and Kalmus (Harris and Kalmus 1951). A PTC test was administered to subjects using serial dilutions of PTC in vials labeled 13, 11, 9, 7, 5, 3, and 1, with vial 13 containing the most dilute solution of PTC and vial 1 containing the most concentrated solution. Individuals would taste successive dilutions of PTC, beginning with the most dilute concentration and stopping when they detected the bitter taste of PTC at a particular vial number which would then become their raw PTC score. Raw PTC scores were also adjusted for age and sex using the following formula: $n + [(a - a_M)/20] - 0.73\text{female}$, where n is the raw PTC score, a is the age of a given individual and a_M is the average age of all individuals in the study. The value of 0.73 was deducted in females since women are reported to be 0.73 dilution steps more sensitive than men regardless of age (Harris and Kalmus 1951; Prodi et al. 2004).

Polymerase Chain Reaction Protocol

Genomic DNA samples were obtained from white blood cells and subjected to Polymerase Chain Reaction (PCR), using the following concentrations of reagents: 10x HIFI PCR Buffer (Invitrogen), 50 mM $MgSO_4$, 10 mM dNTP blend for HIFI Taq Polymerase, 10 µM Forward Primer, 10 µM Reverse Primer, 1 unit per reaction of Platinum HIFI Taq Polymerase and 100 ng/µl of genomic DNA in a 50 µl reaction.

PCR conditions (for the Eppendorf Mastercycler) were as follows: the reactions were heated to an activation temperature of 95° for 1 minute, followed by a melting temperature of 94° for 30 seconds, annealing at 60° for 25 seconds and extension at 68° for 3 minutes. The melting to extension steps were repeated 35 times.

Sequencing Reaction Protocol

PCR products were separated and visualized on a 1% agarose gel; 15 µl of PCR product was then purified using 1 unit/ µl of shrimp alkaline phosphatase and 10 units/ul of exonuclease I at 37°C for 1 hour, followed by an incubation period at 80°C for 15 minutes.

Sequencing reactions were performed in both directions on an Eppendorf Mastercycler using purified PCR products in reactions containing 10 μM primer, 1 μl Big Dye Terminator Ready Reaction Mix (PE Biosystems), and 0.5 μl of 5X sequencing buffer and 2.5 μl H_2O. Cycling conditions were 96°C for 20 seconds, 50°C for 5 seconds, 61°C for 4 minutes, repeated 24 times. Sequencing reaction products were ethanol precipitated, and the pellets were resuspended in 10 μl of formamide loading dye. Sequencing primers were designed to anneal approximately every 500 bp across the amplified region in both directions. All nucleotide sequence data were generated using the 3730xl automated sequencer (Applied Biosystems), and resulting chromatograms were exported to Sequencher 4.0 (Gene Codes Corporation) for assembly into contigs. Sequence files were aligned, and SNPs were subsequently identified using the Sequencher and MEGA software. A visual analysis of the trace files was also conducted to confirm identified polymorphisms.

PCR and Sequencing Primers

Primer Name	Primer Sequence
TAS PCRF	GAAAGTATCGAAAAACACCAATGGG
TAS PCRR	ATCTATTAAGACCAAGTGCC
PTC_540R	CTCATCTGTGAAGTAATGACTC
PTC_1212R	CAGGAACAGCAGTCCATGC
PTC_1860R	GCTGCCATTATCCCAACACA
PTC_2547R	GAAGTTATGTGGTACCTCTTG
PTC_1860F	TGTGTTGGGATAATGGCAGC
PTC_2416F	TCGGAACTTGTGCTAGCATC
PTC_540F	GAGTCATTACTTCACAGATGA G
PTC_1212F	GCATGGACTGCTGTTCCTG

Coalescent simulations of population demography

Using the ms program (Hudson 1990), statistical significance of observed D_T values was determined by simulating a range of demographic scenarios of population growth. In Africans, we simulated a 10-fold increase in population size of Africans (from an initial population size of 10,000 individuals) starting at 70 kya until the present; a 15-fold increase in population growth starting 70 kya until; a 20-fold increase in population size (with an initial N_e = 10,000) at 70 kya until the present; and a 40-fold increase in population size (from an initial population size of 10,000 individuals). In non-Africans, we simulated several demographic models involving a population bottleneck (from an initial population size of 10,000 individuals and decreasing to 3,000 individuals) at 60,000 years ago, corresponding to the approximate time modern humans

migrated out of Africa, followed by a 10-fold, 20-fold, and 40-fold increase in population size starting at 50 kya until the present (Table S13).

All simulations were performed by simulating 1000 gene genealogies (i.e 1000 replicates). The p-values are the proportion of simulated statistics that were higher than the observed D_T statistic. The p value of 0.025 or less (a two-tailed p value cutoff of 0.025) indicates that the observed statistic is greater than expected based on demographic factors.

Age Estimates of Mutations

The GENETREE program (Griffith 1999) was used to estimate the expected ages of mutations and the expected time since the most recent common ancestor (TMRCA) for the coding region polymorphisms. This method is based on the coalescent process and assumes an infinite alleles model and no recombination. Haplotypes and sites that were not compatible with the infinite-sites model (which were 6 singleton haplotypes) were removed from the analysis. Each simulation produced an ordering of coalescent and mutation events. The age of mutations and the TMRCA of the gene tree were also estimated for a given run (Griffith 1999). The expected age was estimated from the weighted average of the simulated ages over different independent runs (in this case, 10,000 runs). The mutation rate, μ, was calculated based on the average number of differences between human sequences and the estimated divergence time between chimp and human (5,500,000 years ago). Using this μ (based on human/chimp comparison) and the estimated maximum likelihood of θ (θ_{ML}), we calculated the effective population size parameter (N_e) which was ~29,000. Estimated TMRCA are given in units of N_e generations. However, to convert these estimates into years, ages in units of N_e generations were multiplied by the generation time of humans (20 years) and the calculated effective population size (N_e).

Tests of Genotype-Phenotype Association

We also measured the within-locus dominance effect of the PAV haplotype on PTC response in our sample. Dominance is defined as the deviation of the heterozygote phenotype value from the mean value of the two homozygote phenotype values. If d=0 there is no dominance and the locus is said to show additive effects; d is positive if the PAV haplotype is dominant, and negative if the non-PAV haplotype (for example, AAV, AAI or AVI) is dominant.

At a hypothetical single locus with two alleles, A and a, by convention, the A allele contributes to larger phenotypic values and the a allele contributes to smaller phenotypic values. A scale of measurement typically used to represent the genotypic values of the genotypes, AA, Aa and aa are as follows: the AA genotype has a genotypic value of 1, while the genotypic value of aa is -1. The genotypic value of Aa is measured in relation to the midpoint between the above two homozygotes and is 0. For a genetic model with both additive (a) and dominance (d) terms, we assigned genotypic values to genotypes PAV/PAV, PAV/non-PAV, non-PAV/non-PAV below:

Haplotype_1	*Haplotype_2*	*a*	*d*
PAV	PAV	1	0
PAV	non-PAV	0	1
non-PAV	PAV	0	1
non-PAV	non-PAV	-1	0

We then performed a linear regression analysis that incorporated both the additive and dominance parameters into the regression model in SPSS.

$$y_{ij} = \mu + \alpha_i + \alpha_j + d_{ij} + e$$

where y_{ij} is the quantitative PTC phenotype (dependent variable), μ is the population mean PTC score, α_i is the additive effect of the PAV allele, α_j is the additive effect of non-PAV allele, d_{ij} is the dominance deviation for the PAV/non-PAV genotype, and e is error.

Supplemental References

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