

Y chromosome sequence variation and the history of human populations

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Binary polymorphisms associated with the non-recombining region of the human Y chromosome (NRY) preserve the paternal genetic legacy of our species that has persisted to the present, permitting inference of human evolution, population affinity and demographic history¹. We used denaturing high-performance liquid chromatography (DHPLC; ref. 2) to identify 160 of the 166 bi-allelic and 1 tri-allelic site that formed a parsimonious genealogy of 116 haplotypes, several of which display distinct population affinities based on the analysis of 1062 globally representative individuals. A minority of contemporary East Africans and Khoisan represent the descendants of the most ancestral patrilineages of anatomically modern humans that left Africa between 35,000 and 89,000 years ago. We deduced a phylogenetic tree from 167 NRY polymorphisms on the principle of maximum parsimony (Fig. 1). Of the 167 polymorphisms, 7 had been detected by means other than DHPLC and were taken from the literature. Of the 160 polymorphisms detected by DHPLC, 73 had been reported previously^{3,4}. Of the remaining 87 unreported polymorphisms, 53 were discovered in a set of 53 individuals of diverse geographic origin during the screening of the unique sequences and repeat elements, other than long interspersed elements, contained in 3 overlapping cosmid sequences (GenBank accession numbers AC003032, AC003095, AC003097) and a few small fragments scattered throughout the NRY. Finally, we detected 34 during genotyping. In total, the marker panel is composed of 91 transitions, 53 transversions, 22 small insertions or deletions, and 1 *Alu* insertion. All polymorphisms are bi-allelic, except a double transversion (M116) that has three alleles, A, C or T, defining different haplotypes. Two non-CpG associated transitions (M64 and M108) show evidence of recurrence, but generate no ambiguities when considered in the context of other markers. We placed the root of the phylogeny using sequence information generated from the three great ape species. The sequential succession of mutational events is unequivocal, except for those appearing in the same tree segment (for example, M42, M94, M139). The phylogeny is composed of 116 haplotypes and their frequencies in 21 general populations are given (Table 1). Forty-two haplotypes (36.2%) are represented by just one individual. Several haplotypes, however, have higher frequencies and/or geographic associations that dis-

close patterns of population affinities apparent from a maximum likelihood analysis (Fig. 2) performed on the haplotype frequencies (Table 1). To facilitate presentation, we grouped the 116 haplotypes into 10 haplogroups as defined by either the presence or the absence of mutations occupying strategic internal positions in the phylogeny. Haplogroups VI, VIII and X, although polyphyletic, are distinguished by criteria (Table 2).

Three mutually reinforcing mutations, M42, M94 and M139 (two transversions and a 1-bp deletion), distinguish haplogroup I, which is represented today by a minority of Africans—mainly Sudanese, Ethiopians and Khoisans (Table 1). All non-Africans, except a single Sardinian, and most African males sampled carry only the derived alleles at the three sites. This implies that modern extant human Y chromosomes trace ancestry to Africa and that the descendants of the derived lineage left Africa and eventually replaced archaic human Y chromosomes in Eurasia⁵.

An important property of a phylogeny is the randomness of number of mutations per segment of the tree. Of the 166 segments, 41 carry no mutation, whereas 98, 16, 8, 2 and 1 segment have 1, 2, 3, 4 and 8 mutations, respectively. The mean number of mutations per segment is 1.024 with a variance of 0.945. Applying the G-test for goodness of fit and Williams' correction to the observed G, the data do not fit a Poisson distribution ($G_{adj}=34.98$, d.f.=3, $P=10^{-7}$). This is due to an excess of segments with one mutation, as expected in an exponentially growing population. Similar results were obtained recently for the separate analysis of four Y chromosome genes⁴. Further support that the human population has undergone a major expansion comes from the consistently negative values of Tajima's D (ref. 6) for not only the Y chromosome, but also for mitochondrial DNA, X-chromosomal and autosomal genes⁴. Notably, NRY shows evidence of significantly reduced variability to the other genetic systems⁴, confirming a similar comparison of a smaller number of polymorphisms on previously reported NRY sequences with 8 X-linked^{7,8} and 16 autosomal human genes⁴. Possible explanations include positive selection on NRY (ref. 9) and a difference between male and female effective population sizes¹⁰.

Assuming expansion, the age of the most recent common ancestor (T_{MRCA}) was previously estimated at 59,000 years, with a 95% probability interval of 40,000–140,000 years¹¹. This value is similar

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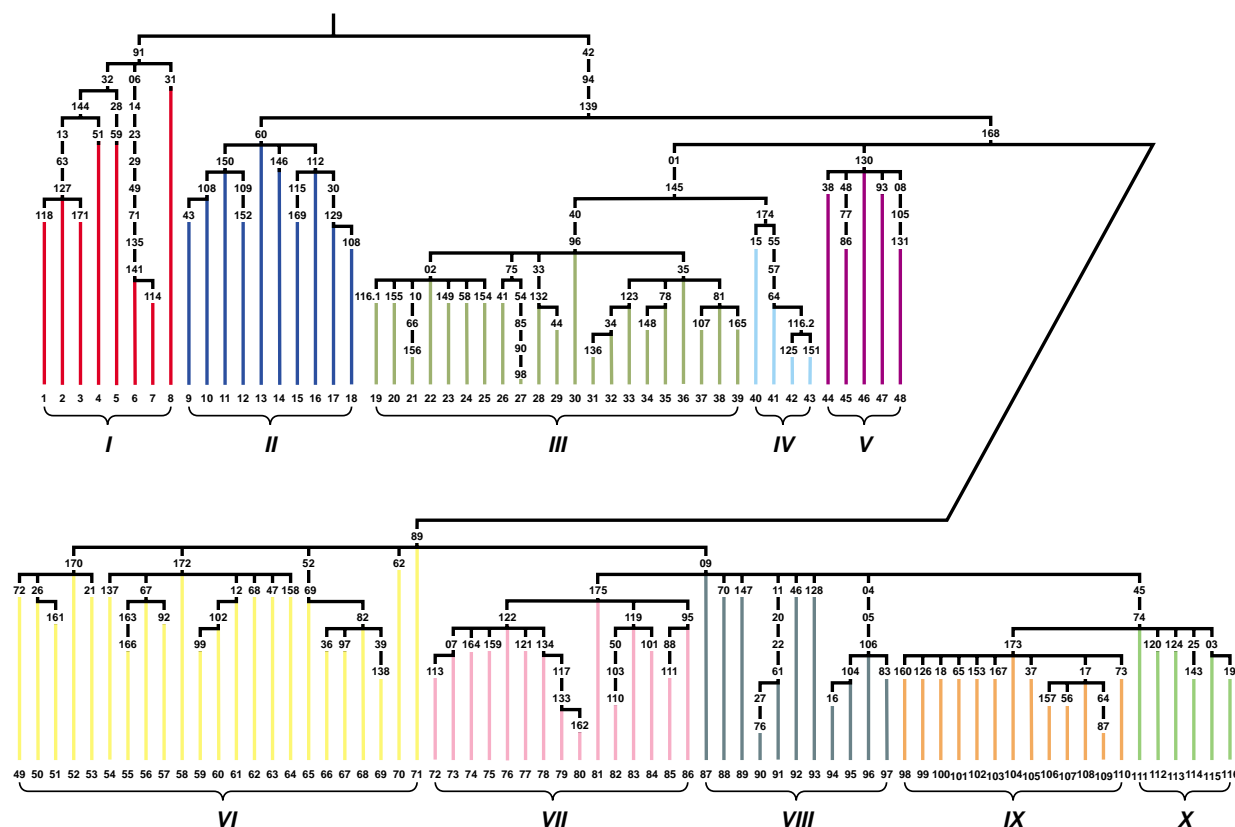


Fig. 1 Maximum parsimony phylogeny of human NRY chromosome bi-allelic variation. The tree is rooted with respect to non-human primate sequences. The 116 numbered compound haplotypes were constructed from 167 mutations, of which 160 were discovered by DHPLC. The remaining seven were taken from the literature and included YAP (M1)¹⁷, DYS271 (M2)¹⁸, PN3 (M29)¹⁹, SRY 4064 (M40)⁵, TAT (M46)²⁰, RPS4YC711T (M130)²¹ and SRY 2627 (M167)²². Marker numbers indicated on the segments are discontinuous because of the removal of all but one polymorphism associated with tandem repeats and homopolymer tracts whose ancestral state is uncertain. Haplotypes are assorted into 10 haplogroups (I–X) using criteria given in Table 2. Haplogroup I members, ancestral for M42, M94 and M139, also share the only homopolymer-associated marker M91. All haplogroup I individuals have an 8-T length variant, whereas 1,009 men in haplogroups II–X have 9 and in 2 cases 10-T length variants (not shown). Only one inconsistent haplogroup X individual had an 8-T length variant (not shown). Haplogroups I and II, both of which are almost exclusively represented in Africa, share the ancestral allele of M168. Haplogroup III is generally the most frequent one in Africa. Its frequency decreases with increasing distance from Africa, from 27% in the Mid-East to a few per cent in Northern Europe and South and Central Asia. Haplogroup IV, related to the former through M1 and M145, is found mainly in Japan. Haplogroups V and VIII are prevalent in New Guinea and Australia, but they are also found at varying though smaller frequencies throughout Asia. Haplogroup VIII represents the relevant source of Haplogroups VII, IX and X. Haplogroups VI and IX are found mostly in Europe and the Indus Valley. They are not observed in East Asia, where haplogroup VII dominates, suggesting that this part of the world where agriculture developed independently resisted effectively subsequent gene flow²³. The distinction between Eurasians and East Asians was also observed with mtDNA (ref. 24) and autosomal genes²⁵. Haplogroup X is common in the Americas, although its origin may have been in Central Asia where traces of it persist (Table 1).

to an estimate of 46,000–91,000 years based on 8 Y chromosome microsatellites¹² and, therefore, is considerably less than estimates of greater than 100,000 years obtained previously⁵. Of course, this assumes that selection or population structure has not had a major effect on NRY diversity, an assumption that may be wrong in light of our findings of significantly reduced variability on NRY. As the average number of mutations of all segments departing from the root is 8.60 (Table 2), and with a T_{MRCA} value of 59,000 years, the average time for adding a new mutation to the tree is approximately 6,900 years. This puts the age of M168, which marks the expansion of anatomically modern humans out of Africa, at approximately 44,000 years, in agreement with a previous estimate of 47,000 years with 95% probability intervals of 35,000–89,000 years using the program GENETREE (ref. 11). This concurs with recent archeological¹³ and mtDNA data¹⁴, and is also consistent, though at a compressed time scale, with the weak Garden-of-Eden hypothesis¹⁵. Under this hypothesis, a small subgroup of behaviourally modern humans¹³ left Africa and separated into several fairly isolated groups represented today by the major haplogroups III–X. Those groups remained small throughout the last glaciation before they underwent roughly simultaneous expansions in size as suggested by a star-like genealogy (Fig. 1).

The new levels of bi-allelic variation revealed here indicate a recent ancestry of the paternal lineages of our species from Africa and testify to the informativeness of the Y chromosome in deciphering the evolution of humankind.

Methods

DNA samples. The ascertainment set consisted of the following 53 samples with their subsequently determined haplogroup designations: Africa: 3 Central African Republic Biaka II, III (1); 2 Zaire Mbuti II, III; 2 Lissongo II, III; 2 Khoisan I, III; 1 Berta VI; 1 Surma I; 1 Mali Tuareg III; 1 Mali Bozo III; Europe: 1 Sardinian VI; 2 Italian VI IX; 1 German VI; 3 Basque VI, IX (2); Asia: 3 Japanese IV, V, VII; 2 Han Chinese VII, 1 Taiwan Atayal VII, 1 Taiwan Ami, VII, 2 Cambodian VI, VII; Pakistan: 2 Hunza VI, IX; 2 Pathan VI, VII; 1 Brahui VIII; 1 Baloochi VI; 3 Sindhi III, VI, VIII; Central Asia: 2 Arab IX; 1 Uzbek IX; 1 Kazak V; MidEast: 1 Druze VI; Pacific: 2 New Guinean V, VIII; 2 Bougainville Islanders VIII; 2 Australian VI, X; America: 1 Brazil Surui, 1 Brazil Karatina, 1 Columbian, 1 Mayan all X. We genotyped an additional 1,009 chromosomes, representing 21 geographic regions, by DHPLC for all markers other than those on the terminal branches of the phylogeny. We genotyped the latter only in individuals from the haplogroup to which those markers belonged. This hierarchic genotyping protocol was necessitated by the limited amounts of genomic DNA available for most samples.

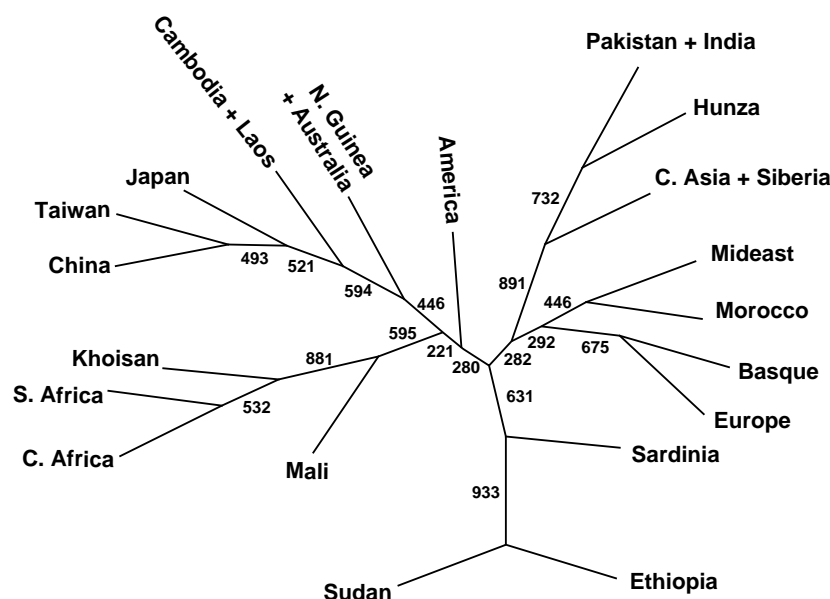


Fig. 2 Maximum likelihood network inferred from the haplotype frequencies reported in Table 1. The gene frequencies of New Guineans and Australian aborigines were grouped together because of the small sample size of the latter. Values at nodes indicate number of 1,000 bootstrap trees presenting cluster distal of node. Sudanese and Ethiopians are distinct from the other Africans and appear to be more associated with samples from the Mediterranean basin. This may reflect either repeated genetic contact between Arabia and East Africa during the last 5,000–6,000 years or a Middle Eastern origin with subsequent acquisition of African alleles on the way southwest with agricultural expansion²⁶. The Moroccan samples are under-represented with respect to Group III (J.B., unpublished data). Native Americans are located between Eurasians and East Asian indicating common ancestry with both. This network is consistent with the first two principal components capturing 18% of the variation present in the 116 haplotypes.

terminator reaction mix and 0.8 μ l primer (5 μ M). Cycle sequencing was started at 94 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 2 s and 60 °C for 4 min. We purified the cycle sequencing reactions using Centrifex gel filtration cartridges (Edge Biosystems), which were then analysed on a PE Biosystems 373A sequencer.

Statistical analysis. We used the program CONTML in PHYLIP, version 3.57c, to construct a frequency based maximum likelihood network.

Accession numbers. Most of the NRY sequence surveyed was derived from 5 cosmid sequences retrievable from GenBank using the accession numbers AC003031, AC003032, AC003094, AC003095, and AC003097. Six polymorphisms were affiliated with genomic regions for DFFRY (AC002531), one

each for DBY (AC004474) and UTU1 (AC006376), 3 for SRY (NM003140), and 15 for random genomic STSs reported by Vollrath and collaborators¹⁶.

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