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Haplotypes in the Dystrophin DNA Segment Point to a Mosaic Origin of Modern Human Diversity

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Although Africa has played a central role in human evolutionary history, certain studies have suggested that not all contemporary human genetic diversity is of recent African origin. We investigated 35 simple polymorphic sites and one T_n microsatellite in an 8-kb segment of the dystrophin gene. We found 86 haplotypes in 1,343 chromosomes from around the world. Although a classical out-of-Africa topology was observed in trees based on the variant frequencies, the tree of haplotype sequences reveals three lineages accounting for present-day diversity. The proportion of new recombinants and the diversity of the T_n microsatellite were used to estimate the age of haplotype lineages and the time of colonization events. The lineage that underwent the great expansion originated in Africa prior to the Upper Paleolithic (27,000–56,000 years ago). A second group, of structurally distinct haplotypes that occupy a central position on the tree, has never left Africa. The third lineage is represented by the haplotype that lies closest to the root, is virtually absent in Africa, and appears older than the recent out-of-Africa expansion. We propose that this lineage could have left Africa before the expansion (as early as 160,000 years ago) and admixed, outside of Africa, with the expanding lineage. Contemporary human diversity, although dominated by the recently expanded African lineage, thus represents a mosaic of different contributions.

Introduction

Africa has played a central role in human evolutionary history. The oldest skeletal fossils with modern human characteristics are found at sites dated at 90–120 thousand years ago (kya) that extend from the Klasies River mouth in South Africa up to Qafzeh and Skhul in Israel (Stringer and Andrews 1988; Tattersall 1995; Lahr and Foley 1998; Tattersall and Schwartz 2000) and at sites dated as early as 160 kya from the Awash in Ethiopia (White et al. 2003). Modern human remains found outside Africa and the Levant are much younger and together support the notion that Southeast Asia and Australia were colonized by *Homo sapiens* ~50–60 kya, Europe was colonized 30–40 kya, and the Americas were colonized 12–25 kya (Klein 1999).

The recent African ancestry of modern humans is generally supported by genetic studies, of contemporary populations, that use either classical protein markers (Cavalli-Sforza et al. 1994) or polymorphisms in mtDNA (Cann et al. 1987; Chen et al. 1995), the Y chromosome (Hammer 1995; Underhill et al. 2000), and the recombining portion of the genome (Batzer et al. 1994; Bowcock et al. 1994; Tishkoff et al. 1996; Labuda et al. 2000; Fan et al. 2002; Rosenberg et al. 2002). The majority of studies of human genetic history have been based on the analysis of mitochondrial and Y-chromosome DNA. In spite of the undisputable virtues of these two systems, their value as markers of population history is limited, because (i) each represents but a single locus, (ii) their effective population size is one-fourth that of autosomal segments, and (iii) they reflect the history of either the maternal or paternal lineage only (Seielstad et al. 1998).

In addition to the use of mitochondrial and Y-chromosome DNA, to understand the genetic structure of human populations and to retrace their genetic past, we also need to collect information from autosomal and/or X-chromosome loci. However, such information, which has been collected for different loci and different populations (Batzer et al. 1994; Tishkoff et al. 1996; Harding et al. 1997; Clark et al. 1998; Harris and Hey 1999;

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Jaruzelska et al. 1999; Jin et al. 1999; Kaessmann et al. 1999; Kidd et al. 2000; Labuda et al. 2000; Yu et al. 2001; Fan et al. 2002; Rosenberg et al. 2002), has never achieved either the population depth or the worldwide breadth of mtDNA or Y-chromosome studies.

We have proposed an 8-kb intronic segment of the dystrophin locus, *dys44* on Xp21.3, as a model system to investigate variability in the nuclear non-Y-chromosome DNA (Ziętkiewicz et al. 1997, 1998). The X chromosome is conducive to such studies, since it facilitates analysis by allowing direct haplotype reading and direct assessment of linkage disequilibrium in male samples. Our previous analysis of worldwide *dys44* haplotype diversity indicated the existence of at least two separate founder lineages of modern humans (Labuda et al. 2000). Sub-Saharan Africans, rather than making up a uniform genetic pool, were found to represent two chromosomal lineages with distinct genetic histories that may result from fragmentation of early human population(s) during periods of glaciation. Expansion of one of these lineages led to the global colonization of all presently inhabited continents, whereas the second lineage remained local to Africa. Here, we report an extended survey of *dys44* diversity in a set of 1,343 X chromosomes. Our data, in addition to supporting a split African ancestry, reveal the contribution of another, third lineage, represented by an ancient haplotype found in Eurasia and the Americas that is virtually absent in sub-Saharan Africa. We address the question of whether it represents a lineage that had been lost in Africa following the Upper Paleolithic out-of-Africa expansion or a lineage that could have left Africa much earlier, at the dawn of modern humans, to subsequently contribute to northern human populations.

Material and Methods

Population Samples

All DNA samples were nonnominative, originating either from existing collections or peripheral blood samples donated by consenting informed adults, following the protocol approved by the institutional review boards of Ste.-Justine Hospital (Montréal), Victoria Hospital (Prince Albert), and their collaborating institutions. We analyzed 1,343 chromosomes from 33 human populations representing five continental groups, as detailed in table 1. In addition to the genotypes reported elsewhere (Ziętkiewicz et al. 1997, 1998; Labuda et al. 2000), new data have been obtained for Ashkenazim ($n = 72$ chromosomes), Basques ($n = 20$), North American Natives (NaDene speakers from Saskatchewan, $n = 40$; and Ojibwa from Ontario, $n = 30$), five populations of eastern Indonesians ($n = 91$), and eight additional Mongolian populations ($n = 267$). Supplementary chromosomes have been added to the following previously analyzed populations: Siberian (additional $n = 26$), Polish ($n = 5$), Mayan ($n = 2$), African American ($n = 8$), Mossi ($n = 6$), Rimaibe ($n = 8$), Biaka ($n = 4$) and M'Buti ($n = 2$). In contrast, 106 French Canadian chromosomes reported by Labuda et al. (2000) were not included in the present study. Eleven Nigerian chromosomes, previously assigned with the Burkina Faso populations, were included in the African American group in the present study.

Genotyping

The genomic segment *dys44* consists of exon 44 (148 bp) and its surrounding intronic sequence (positions −2782 to −1 of intron 43 and positions 1 to 4987 of intron 44) of the human dystrophin gene at Xp21 (GenBank accession number U94396) (Ziętkiewicz et al. 1998). Thirty-six simple intronic polymorphisms resulting from 33 nucleotide substitutions (including one three-allele site), two 3-nt deletions, and one 8-nt duplication were previously ascertained by SSCP/heteroduplex analysis of 7,622 bp within *dys44* segment in 250 worldwide-distributed chromosomes (Ziętkiewicz et al. 1997). In addition, this segment contains a poly-T microsatellite, $T_{14–24}$. A 7,917-bp distance between upstream and downstream flanking polymorphic sites determines the length of the haplotype. The newly examined genomic samples were typed by allele-specific oligonucleotide (ASO; see Ziętkiewicz et al. 1997) hybridization to determine alleles at the 36 segregating sites. The T_n polymorphism was typed by standard polyacrylamide gel electrophoresis.

Derivation of Haplotypes

Converting *dys44* genotype data into haplotypes was straightforward, by direct observation, in hemizygous males as well as in homozygous and single-heterozygous females; these unambiguous haplotypes were assigned names starting with an uppercase letter "B." Multiple-heterozygous female genotypes were initially resolved into haplotypes, as described elsewhere (Labuda et al. 2000). In brief, if a genotype represented a unique sum of two previously reported haplotypes, these were accepted. Where more than one solution was possible, the most likely haplotype combination was chosen, taking into account the candidate haplotype frequencies in the respective populations (the most frequent ones being preferred). If only one known haplotype could be recognized within the individual genotype, the novel haplotype was inferred indirectly by subtracting the known one from the genotypes. Such a new haplotype was accepted if it could be derived from the already known haplotypes by a single or double recombination or by a simple mutation (the simple "genealogy" rule) and was assigned a name beginning with a lowercase "b." The

Table 1

Distribution of *dys44* Haplotypes and Their Associated T_n Alleles in all 33 Populations

		No. of Occurrences in[a]																														
		Africa					Europe					Asia												America				Indonesia/PNG						
Haplotype	Total	AA[b]	Mo	Ri	Bi	Mb	Eu	Po	It	Ba	As	Si	Kha	Kho	Ka	Za	Ur	Ol	My	De	Bt	Ch	Ja	Na	Oj	Ma	Kr	Hi	Te	Ro	Fl	Al	PNGc	PNGh
B001 (14)	1								1																								
B001 (15)	397	4	4	3	18	5	8	10	10	3	26	15	12	16	21	14	15	12	12	8	8	38	39	17	8	21	11	6	6	7	9	5	2	3
B001 (16)	3														2										1									
B002 (14)	9				7	2																												
B002 (15)	250	9	1	3	12	2		2	2	1	10	10	7	7	12	8	8	5	7	14	9	25	18		1			9	6	8	8	8	11	27
B002 (16)	19	5	2		11									1																			
B002 (17)	15	6	2	3	1	3																												
B003 (14)	5	3		1						1																								
B003 (15)	123	4			1	3	9	8	7	4	27	5	1	1	3	1	3	3	1	3	1	5	1	10	8	9		1	1		2		1	
B004 (15)	104	1		1			1			1		3	1		1	2		3	1	1		1		3	1	25	55			2			1	
B005 (15)	3												3																					
B005 (21)	3			1						1						1																		
B005 (22)	53	4	2		4		2			1	1	4	2	1	5		3	2	4	2	3	6	2									1	4	
B005 (23)	3														3																			
B006 (15)	17			1					1					1	1	2	1	2	3		3	2												
B006 (16)	74	3					3	3	1	4	3	6		1						1	2			8	8	16	15							
B008 (14)	19	2					3	2		2	2			1	1		3	2		1														
B011 (15)	1	1																																
B011 (22)	6	1						1	2		1	1																						
B013 (22)	5						1	1		1								1			1												
B016 (15)	7	3	1												1										2									
B019 (15)	3													1												2								
B019 (17)	2	1	1																															
B023 (15)	5											1										1									3			
B024 (15)	6	3	1			1																									1			
B025 (15)	3						1								1										1									
B030 (15)	7																							2							1	2		2
B031 (15)	2	1							1																									
B034 (15)	3							1												1											1			
B035 (15)	2	1																									1							
B039 (22)	3		1		1			1																										
b052 (16)	3							1																		2								
B043 (15)	1						1																											
b068 (15)	1								1																									
B082 (16)	1									1																								
b084 (15)	1										1																							
b089 (14)	1										1																							
B104 (15)	1						1																											
B014 (15)	8											2	2								2	2												
B015 (15)	4														3				1															
B026 (15)	2																						2											
B036 (15)	2																						2											
B041 (15)	1																1																	
B048 (15)	1															1																		
B049 (15)	1																		1															
b054 (15)	1																						1											
b100 (15)	1											1																						
b053 (15)	2																									2								
b064 (16)	1																									1								
b066 (15)	1																										1							
B020 (15)	3																																	3
B028 (15)	2																																2	

B029 (15)	5																																1	4
B042 (22)	4																																2	2
B051 (22)	1																													1				
B051 (23)	1																											1						
b087 (15)	1																													1				
B105 (15)	1																											1						
B007 (17)	1					1																												
B007 (19)	1			1																														
B007 (20)	33	3	1	1	2	26																												
B009 (15)	15	2		2	7	4																												
B010 (21)	4	2		2																														
B010 (22)	6	4	2																															
B010 (24)	2	1	1																															
B012 (17)	6	1	2	3																														
B012 (20)	3	2		1																														
B017 (19)	3	2		1																														
B018 (19)	5	2		2	1																													
B021 (20)	3					3																												
B022 (22)	2	1	1																															
B027 (14)	3	2		1																														
B032 (15)	7				6	1																												
B037 (16)	4				4																													
B038 (20)	1		1																															
B040 (19)	1		1																															
B044 (15)	1	1																																
B045 (14)	1	1																																
B046 (19)	1				1																													
B046 (20)	4					4																												
B047 (22)	1		1																															
B050 (17)	1			1																														
b056 (20)	2					2																												
b057 (14)	1	1																																
b058 (15)	1	1																																
b059 (20)	1	1																																
b060 (21)	1	1																																
b061 (19)	1				1																													
b061 (20)	4				4																													
b062 (19)	1				1																													
b063 (16)	1				1																													
b067 (21)	1	1																																
b072 (20)	1		1																															
b088 (21)	1		1																															
b110 (21)	2		1		1																													
b115 (20)	1		1																															
b116 (21)	1		1																															
b117 (21)	2			2																														
b118 (15)	1			1																														
b119 (19)	1				1																													
b120 (19)	1					1																												
b121 (21)	1	1																																
b122 (21)	1	1																																
b123 (15)	1	1																																
b124 (21)	1	1																																
b125 (17)	1	1																																
Total	1,343	86	30	31	85	58	30	30	26	20	72	48	28	30	54	29	34	30	30	31	29	80	65	40	30	78	83	18	13	17	27	16	24	41

[a] Population abbreviations are as follows: AA = African American; Mo = Mossi; Ri = Rimaibe; Bi = Biaka; Mb = Mbuti; Eu = mixed European; Po = Poland; It = Italian; Ba = Basque; As = Ashkenazim; Si = Siberian; Kha = Khalkha; Kho = Khoton; Ka = Kazakh; Za = Zakhchin; Ur = Uriankhai; Ol = Olet; My = Myangad; De = Derbet; Bt = Bait; Ch = Chinese; Ja = Japanese; Na = NaDene; Oj = Ojibwa; Ma = Maya; Kr = Karitiana; Hi = Hiri; Te = Ternate; Ro = Roti; Fl = Flores; Al = Alores; PNGc = PNG coastal; PNGh = PNG highland.

[b] Sample includes 11 Nigerian chromosomes.

only case in which a genotype was solved into two lowercase "b" haplotypes was in the case of a heterozygous individual carrying a singleton variant allele at position 86. According to the above rules, this genotype could have been resolved into one known (B002) and one novel haplotype, made up of the mutated "86" allele assigned to the rare haplotype b124; instead, we chose to assign the singleton variant to the frequent haplotype B002, resulting in the new haplotype b125. The T_n polymorphism was not considered when naming haplotypes, such that a given "B" haplotype may be accompanied by different T_n alleles (e.g., B002 is present as B002_14, B002_15, B002_16, and B002_17). However, because of their linkage with the remaining portion of the haplotype, T_n alleles aided in resolving some of the genotypes. In two cases in which several solutions seemed equally likely, as well as in one case in which no simple sequence of recombination or mutation could be inferred that linked the novel haplotype to the known ones, solutions were accepted on arbitrary bases. A few genotypes that could not be resolved into known haplotypes remained unsolved and were excluded from further analyses; the number of the reported haplotypes is thus conservative. Our inference of haplotype pairs from genotypes was independently assessed by PHASE software (Stephens et al. 2001), which essentially arrived at the same solution. In a few cases, we "disagreed" with PHASE, using the criteria discussed above. However, this does not affect the emerging pattern of *dys44* diversity and bears no consequence for the conclusions of this study.

Distance Trees

Haplotype clustering was performed by the NEIGHBOR program (which implements a neighbor-joining [NJ] algorithm), through use of a distance matrix of haplotype sequences (excluding the T_n polymorphism) obtained by applying equal weighting to all allelic sites. Population trees were obtained by NEIGHBOR, KITCH, and FITCH, using distance matrices obtained by GENDIST. One set of these trees was based on the population frequencies of alleles at individual polymorphic sites (the frequencies of non-new alleles in the hypothetical ancestral population were set to 1.0). A second set of trees was based on the population frequencies of haplotypes (i.e., regarding *dys44* as a single multiallelic site); the haplotype frequencies in the outgroup were set at 0. All programs were from the Phylip package 3.57 (Felsenstein 1993).

Haplotype Diversity Analysis

To test for selective neutrality and population equilibrium, the observed frequency distribution of the haplotypes was compared with that expected under the infinite-allele model (Ewens 1972; Watterson 1978), through use of the Arlequin package, version 1.1 (Schneider et al. 1997). For a detailed description of the test used, please refer to the Arlequin help manual (Arlequin's Home on the Web).

Provided that mutations, occurring at the rate μ, are rare, such that recurrent events can be neglected, the proportion of intact (i.e., nonmutated) chromosomes after one generation is $P_1 = (1 - \mu)$ and after g generations is $P_g = (1 - \mu)^g$, such that

$$-\ln P_g = g \times \mu \ . \tag{1}$$

Similarly, when we consider recombinations rather than mutations in creating haplotype diversity,

$$-\ln P_g = g \times r_{\text{app}} \ , \tag{2}$$

where r_{app} corresponds to the apparent recombination rate—that is, the rate of nonsilent recombinations (those that result in new, observable recombinants). On the basis of the mapping data of Kong et al. (2002), the ratio of genetic to physical distance in the dystrophin exon 44 region is 2 cM/Mb, from which we obtain the estimate of the rate of recombination as $r = 2 \times 10^{-8}$ per bp. If we assume an equal contribution of both sexes to the effective population size, one-third of the X-chromosome population (males) is not involved in meiotic recombinations. Correcting for this, one obtains an estimate of $r = 2/3 \times 2 \times 10^{-8}$ per bp per generation, or 1.06×10^{-4} per 7,917 bp of *dys44* haplotype. Since only ~30% of *dys44* recombinations are informative, leading to newly observable recombinants (data not shown; also see Stephens 1986), the apparent average recombination rate should be lowered to $r_{\text{app}} = 0.32 \times 10^{-4}$ per segment per generation.

To estimate T_n microsatellite diversity, we calculated the variance in the allele length (number of repeats) in a population sample (Di Rienzo et al. 1998):

$$S^2 = \frac{1}{n-1}\sum (x_i - \bar{x})^2 \ , \tag{3}$$

where $x_1, x_2, \ldots, x_n$ denotes the number of repeats in each of the n chromosomes sampled and $\bar{x}$ denotes their average repeat number. Assuming a simple stepwise mutation model (Pritchard and Feldman 1996), we expect, for a constant population size scenario,

$$E(S^2) = N \times \mu \ , \tag{4}$$

where N denotes the constant effective population size expressed in number of chromosomes and μ denotes the

mutation rate per generation. Under a rapid population growth scenario, we expect

$$E(S^2) = g_e \times \mu \ , \qquad (5)$$

where g_e is the number of generations since the rapid expansion started. S^2 was estimated using MSA (microsatellite analyzer) software by Dieringer and Schlötterer (2003).

In a population that experiences a founder effect, a rare mutation (or a rare haplotype) that goes through the bottleneck and spreads rapidly because of the subsequent demographic growth appears younger by

$$g_0 = d^{-1} \ln(mf_d) \qquad (6)$$

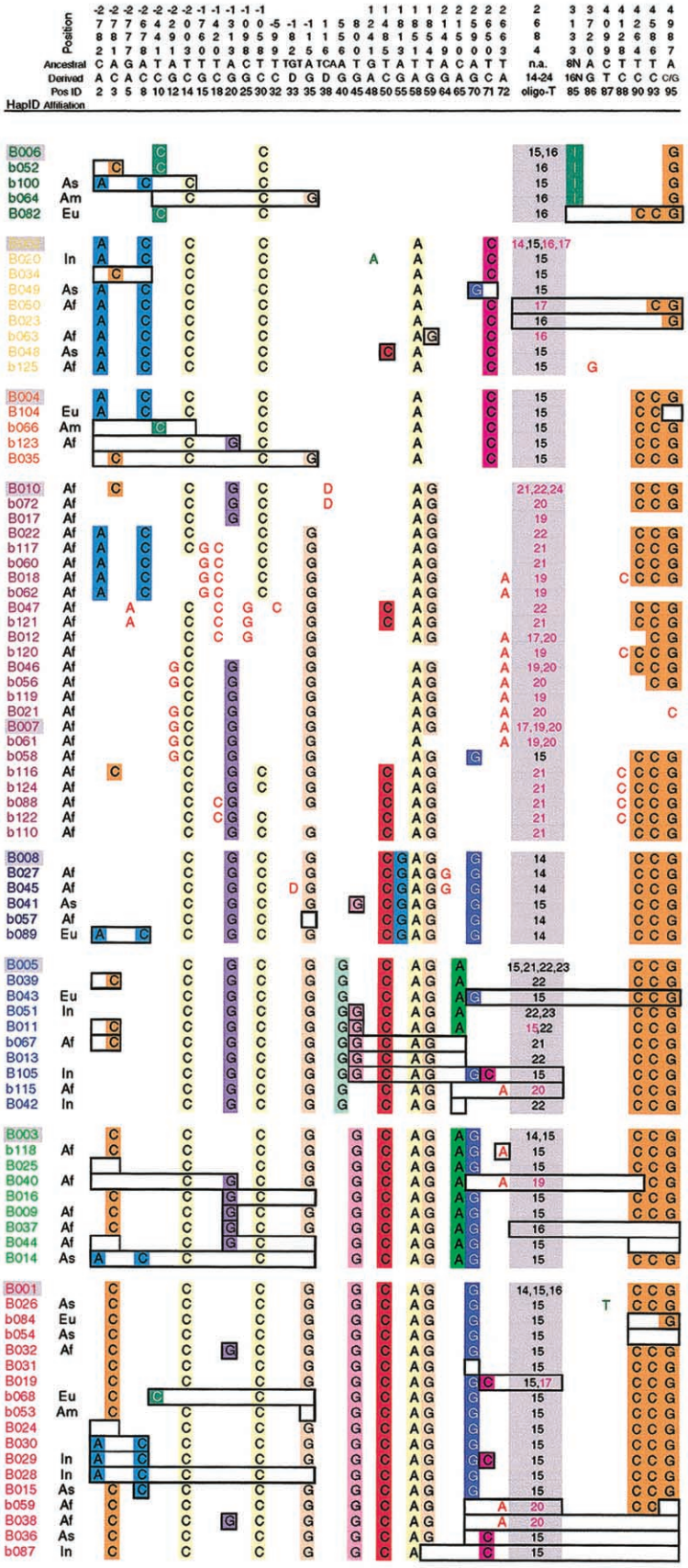
generations, where d is the rate of population growth, m is the rate of genetic events (mutations, recombinations) used to time the founder effect (Luria and Delbrück 1943; Labuda et al. 1997), and $f_d = e^d/(e^d - 1)$; if d is small, then $f_d \sim d^{-1}$.

Results

Allelic Structure and Continental Distribution of dys44 *Haplotypes*

Eighty-six *dys44* haplotypes, composed of the previously ascertained 35 variant sites with 36 derived alleles, were found in the sample of 1,343 X chromosomes from 33 populations (table 1). These haplotypes were either directly observed or inferred by genotype solving, as described in the "Material and Methods" section. Haplotypes are presented as strings of alleles at polymorphic positions (fig. 1), with the new alleles indicated and the ancestral (i.e., not new) ones left blank. Assuming an absence of recurrent mutations, we considered an allele to be ancestral if it was identical by state (IBS) to that found at the orthologous position in at least two ape species (Ziętkiewicz et al. 1998; Labuda et al. 2000). The observed high number of haplotypes and their allelic composition indicate that, in addition to mutations, recombinations (and, presumably, gene conversion events) largely contributed to their evolution.

Haplotype sequence differences were recorded in a simple distance matrix, with each allelic difference counted as one, and haplotypes were subsequently clustered using the NJ algorithm (Saitou and Nei 1987). Although the detailed topology of the resulting tree depended on the number of haplotypes, on their order in the input file, or on the seed number used, we found that the overall clustering pattern remained constant. A representative tree topology is shown in figure 2. Structural similarities within the haplotype clusters can be appreciated in figure 1, where the haplotypes are ordered in accordance with the output of the NJ clustering, with only small modifications to highlight the clustering. Histograms of continental (or regional) frequencies of the corresponding haplotypes, shown next to the NJ tree in figure 2, demonstrate that almost every cluster is dominated by a relatively frequent haplotype. Figure 1 shows that haplotypes within the clusters can be related to the dominating frequent haplotype through a point mutation, recombination, or gene conversion event. For example, a C→T transition at site 87 of a B001 chromosome explains the appearance of haplotype B026. A double recombination or gene conversion event could be inferred to have given rise to haplotype B031, in which allele G at site 70 becomes A on the otherwise intact B001 haplotype background. In haplotype b054, a simple recombination explains the substitution of the rightmost segment of B001 (sites 90–95) from, for example, a B002 haplotype. The same recombination, exchanging the rightmost segments of B002 and B001, could have reciprocally created haplotype B004. However, whereas b054 in our sample was observed only once, in Asia, B004 was seen as >100 copies on different continents. Interestingly, the geographical maxima of B004 and B002, which is the essential ingredient for recreation of B004 by recombination, do not overlap (fig. 2; table 1); in the Americas, where B004 occurs at its highest frequency, haplotype B002 is virtually absent. This strongly suggests that the multiple copies of B004 chromosomes in American populations are identical by descent (IBD), having spread as a result of a founder effect rather than being due to recurrent recombinations. More-detailed analysis of the outcomes of recombination events is presented in appendix A and suggests that most, if not all, of the haplotypes we report are likely to have originated only once. Hence, the present-day population frequencies of *dys44* haplotypes are likely to reflect their times of appearance in a population and the demographic events that followed, such as migrations or founder effects. Thus, to learn about the genetic history of this locus, we have to analyze the evolution of haplotype structure as well as the frequencies of variant haplotypes across populations. In the following, we will refer to a haplotype cluster by the name of its dominating haplotype and to its structurally associated haplotypes as a "haplogroup." The term "haplogroup" was originally coined to denote a monophyletic cluster of mitochondrial sequences (Torroni et al. 1993); the same term has been adopted for the Y-chromosome haplotype lineages (Bianchi et al. 1998). However, in those nonrecombining systems, a haplogroup consists of chromosomes with new polymorphic sites that arose on the ancestral haplotype background. Here, mutation as well as recombination/conversion events participated in the process; assignment of the new recombinant haplotype to a haplogroup is based on the proportion of the parental background in the recombinant's structure.


Position
Ancestral
Derived
Pos ID
HapID Affiliation
B006
b052
b100 As
b064 Am
B082 Eu
B002
B020 In
B034
B049 As
B050 Af
B023
b063 Af
B048 As
b125 Af
B004
B104 Eu
b066 Am
b123 Af
B035
B010 Af
b072 Af
B017 Af
B022 Af
b117 Af
b060 Af
B018 Af
b062 Af
B047 Af
b121 Af
B012 Af
b120 Af
B046 Af
b056 Af
b119 Af
B021 Af
B007 Af
b061 Af
b058 Af
b116 Af
b124 Af
b088 Af
b122 Af
b110 Af
B008
B027 Af
B045 Af
B041 As
b057 Af
b089 Eu
B005
B039
B043 Eu
B051 In
B011
b067 Af
B013
B105 In
b115 Af
B042 In
B003
b118 Af
B025
B040 Af
B016
B009 Af
B037 Af
B044 Af
B014 As
B001
B026 As
b084 Eu
b054 As
B032 Af
B031
B019
b068 Eu
b053 Am
B024
B030
B029 In
B028 In
B015 As
b059 Af
B038 Af
B036 As
b087 In

Clustering of haplotypes on the basis of their sequence similarity (figs. 1 and 2) reflects their "immediate" genealogical relations. Recombinants tend to fall into the haplogroup of their recipient haplotype (i.e., the one of the two recombining haplotypes that constitutes the major portion of the recombinant haplotype). The majority of haplotypes found outside of Africa can easily be connected to the most frequent haplotype of its haplogroup, if we assume simple mutation and/or recombination events (the putative sites of the latter are indicated by boxes in fig. 1). In contrast, the structural/genealogical relations of the haplotypes endemic to sub-Saharan populations are less straightforward and therefore more difficult to trace. Most of the endemic African haplotypes not only form separate clusters but often require multiple genetic events to be mutually reconnected; some intermediate haplotypes may be missing (fig. 1). Taken together, these observations suggest a longer history of the African-only haplogroups. On the other hand, some of the African haplotypes fall within the worldwide-distributed haplogroups, such as B001/B003 or B002/B004, and seem to have simpler genealogical relations within these groups, which might suggest a recent origin.

Sequence Trees versus Population Trees

The NJ tree obtained from the distance matrix of haplotype sequences in figure 2 can be compared with the NJ tree of populations presented in figure 3. The matrices of interpopulation distances used to obtain population trees were based on either allele frequencies or haplotype frequencies. Whereas the sequence tree was rooted with the hypothetical haplotype featuring ancestral (i.e., nonhuman primate) alleles at all sites, the outgroup in population trees was obtained by setting the ancestral allele frequencies at all sites to one or by setting the frequencies of all present-day haplotypes to zero. The topologies of both population trees were very similar; the one shown in figure 3 was obtained using haplotype frequencies. The use of different options of the GENDIST program did not significantly influence tree topology, and the trees preserved all geographical groupings with the exception of the Maya and Karitiana, which were swapped between European and Asian clusters (fig. 3). African populations were always at the root of the tree, separated from non-Africans. The population and regional subdivisions among non-Africans were, almost without exception, very shallow.

It is interesting that, in the haplotype sequence tree in figure 2, the *dys44* haplogroups endemic to sub-Saharan Africa, such as B010, B007, and B012, appeared in the middle of the tree. The haplotype that emerged closest to the root was B006, which differed from the ancestral haplotype by only four new (derived) alleles. Two of these new alleles (sites 10 and 85; see fig. 1) were found only in B006 and related recombinants, which, together with the scarcity of new alleles on the haplotype, made this haplotype structurally distinct from the remaining haplogroups. It is most interesting that B006 was virtually absent in sub-Saharan Africa. Among the African populations studied, it could be inferred only in the Rimaibe from Burkina Faso, in which a single B006 was found in a female sample whose genotype had been resolved into B006 and a new haplotype, b118. This singular occurrence of B006 in the Rimaibe might not, in fact, be exceptional. A partial haplotype (which is thus not reported in table 1) in another Rimaibe sample represented a mosaic of an African-specific haplotype (from site 58 to 72) and B006 (from site 85 to 95). B006 was also found in three African American chromosomes, but we considered this finding as representing the result of Amerindian admixture. What points towards an Amerindian rather than an African origin of B006 among African Americans is the fact that B006 is preponderant in Amerindians and that the B006 chromosomes in our African American samples were found associated with an Amerindian variant of a very informative compound-microsatellite marker located 5 kb downstream of *dys44* (V.Y., T.W., and D.L, unpublished data). Thus, if the presence of B006 in Rimaibe is not accidental (e.g., if it is not due to recent gene flow), they remain unique as the sole carriers of B006 in our sub-Saharan African sample.

Distribution of dys44 *Haplotypes across Continents and Population Expansion*

The occurrence of the 86 *dys44* haplotypes across populations is reported in table 1. Nineteen of these haplotypes were shared by at least two continental

Figure 1 Allelic structure of *dys44* haplotypes. Position IDs and haplotype names are arbitrary (consistent with Ziętkiewicz et al. 1997, 1998; Labuda et al. 2000). For clarity, only new (derived) alleles are shown, and an empty space implies identity with the ancestral allele (the same as that found in nonhuman primates), as shown at the top of the figure. The new alleles of the worldwide polymorphic loci (21 sites) are highlighted in color; those of the continentally restricted polymorphisms (15 sites) are indicated by bold letters, whereas the numbers in the column separating sites 72 and 85 indicate the associated length alleles of the T_n microsatellite. Geographic affiliations are indicated on the left (Af = African; Am = American; As = Asiatic; Eu = European; In = Indonesian/PNG; continentally shared haplotypes are left blank); haplotype names are color coded to indicate their structural clustering into haplogroups (see text and fig. 2), reflected by similarity to the dominant most frequent haplotype; and boxes indicate the shortest putative recombination/conversion sites relating the rare recombinant to the dominant haplotype of the group (only for non-African haplotypes).

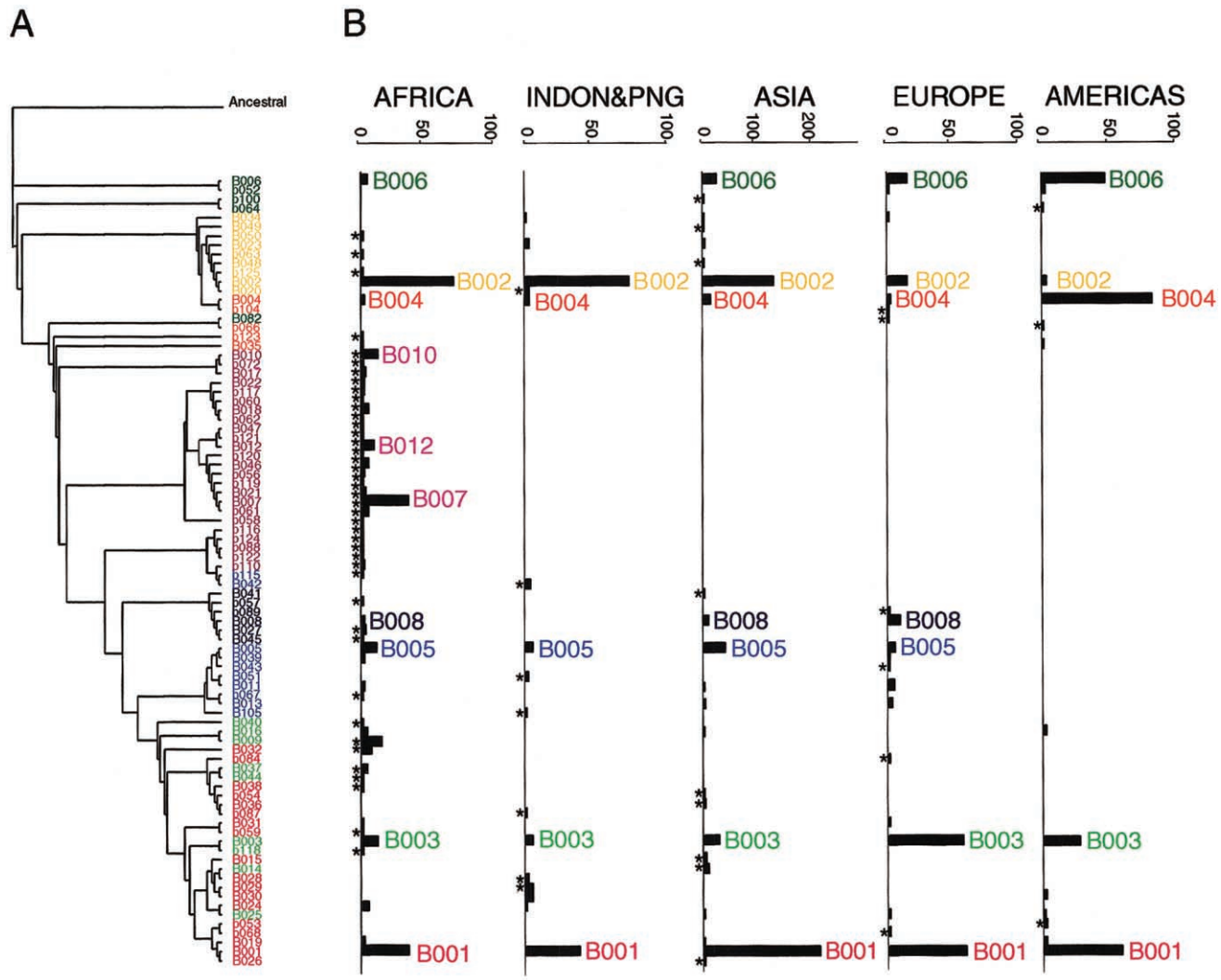


Figure 2 NJ tree (*A*) of haplotype sequences and the corresponding haplotype frequencies (counts) (*B*) in five continental regions. Structural affiliations of haplotypes use the same color code as in figure 1. Asterisks (*) indicate haplotypes that occur in a single continental group.

groups, whereas 67 had a limited distribution: 6 were restricted to Europe, 9 to Asia, 8 to the Indonesian Islands and Papua New Guinea (PNG), 3 to the Americas, and as many as 41 to sub-Saharan Africa. However, it was not only the number of continent-specific haplotypes that distinguished Africa from the other continents. Continentally restricted haplotypes were carried by only 5% of all non-African chromosomes ($n = 50$ out of 1,053), whereas, among African chromosomes, the African-specific haplotypes represented 48% of the sample ($n = 139$ out of 290) (see table 1). Thus, the majority (95%) of chromosomes outside of Africa carried frequent, continentally shared haplotypes, as shown in figure 4. Such a frequency distribution, dominated by a few frequent haplotypes followed by a tail of rare ones, is suggestive of a population expansion. Indeed, both in the total sample and in both groups when subdivided into African and non-African samples, the distribution deviated significantly from neutral expectations under the infinite allele model (Ewens 1972).

Assuming a bottleneck scenario (Tishkoff et al. 1996), we may consider continentally shared haplotypes to represent the ancestral population before expansion and consider rare, region-specific recombinant haplotypes (table 1) to represent the diversity acquired after the range expansion. We used equation (2) (with P_g corresponding to the proportion of continentally shared haplotypes in a given population cluster) to estimate the time elapsed since these proposed colonization events occurred (table 2). Thus, the time of separation between non-Africans and sub-Saharan Africans is estimated at ~55 kya, the colonization of Americas at ~13 kya, and the colonization of

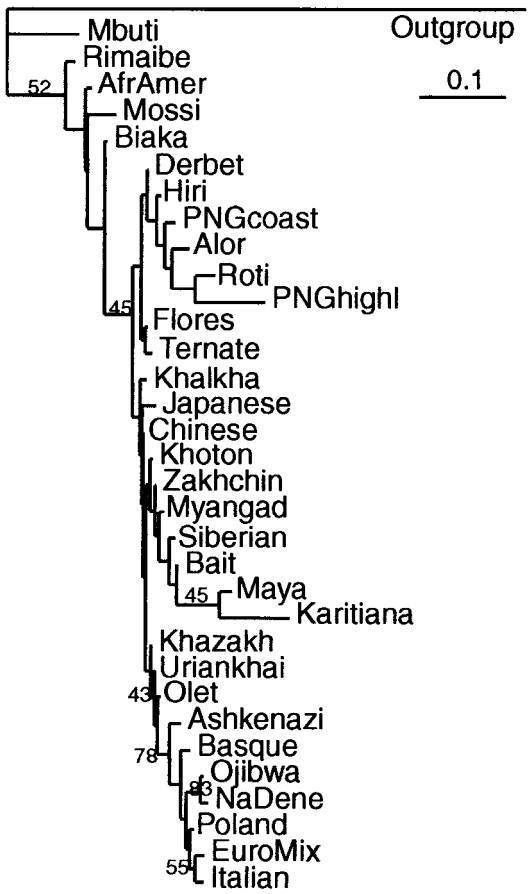


Figure 3 NJ tree of populations from the distance matrix calculated from haplotype frequencies. The bootstrapping values show how many times in 100 runs the cluster to the right was observed.

Europe at ~27 kya. The time estimate for the colonization of the Indonesian Islands and PNG combined is much greater (~145 kya), whereas the Indonesian samples alone yield estimates comparable to those of the Asiatic sample (~33–35 kya). The latter difference reflects the greater proportion of recombinants in the PNG sample. This proportion, augmented by the presence of multiple, presumably IBD copies of the recombinants (table 1), reflects a greater departure from the starlike phylogeny model in PNG than in the other population groups studied and may also account for the much greater variance associated with the corresponding time estimate (Di Rienzo et al. 1998; Kimmel et al. 1998).

Although, given the large uncertainty associated with the obtained values, the results have to be treated with caution, they do provide a reasonable insight into past events. An alternative method of obtaining independent estimates of the timing of past events is the assessment of the *dys44* T_n allele distribution, as considered in the next section.

T_n—*Microsatellite Variance in Populations*

Frequency distributions of the T_n alleles (with length ranging from T_{14} to T_{24}) are shown in figure 5. In the world sample (fig. 5*A*), the distribution is bimodal, with the larger peak at T_{15} and a smaller peak at T_{22}; T_{18} was not observed. Outside of Africa (fig. 5*B*), the picture is even simpler: the major peak at T_{15} is flanked by a few chromosomes carrying alleles T_{14} and T_{16}; the smaller peak, T_{22}, is likewise accompanied by its neighboring alleles, T_{21} and T_{23}. In the African sample (fig. 5*C*), both length modes are present, but the second peak is at T_{20} rather than T_{22}. Furthermore, the T_{15} mode in Africa is preferentially associated with the chromosomes carrying continentally shared haplotypes (fig. 5*E*), whereas the T_{20} mode is found with the subset of exclusively African chromosomes (fig. 5*D*).

In the T_n distribution in figure 5*B* (non-African chromosomes), the flanking alleles differ from the major alleles (T_{15} or T_{22}) by one length unit only and thus appear to have been derived from the major allele by a simple addition or deletion of one T unit at a time. The distribution of the T_n alleles considered in association with individual B haplotypes (table 1) is also consistent with such a mechanism; there is no evidence of mutations occurring in jumps greater than one (three chromosomes carrying haplotype B005 associated with T_{15} are most likely explained by a recombination rather than T_n mutation). Therefore, it appears that this system conforms to a simple version of the stepwise mutation model (Ohta and Kimura 1973). Accordingly, T_n diversity can be described by the variance S^2 in the number of repeats (eq. [3]). The results obtained for our population samples are summarized in table 3. When all samples are considered together, the estimated S^2 value of 4.3 is lower than the estimated value of 7.2 for sub-Saharan Africa and slightly higher than the estimated values of 3.3–3.9 for the other continents (disregarding the exceptionally low S^2 value of 0.2 in the Americas).

Under the constant-population-size model, S^2 is expected to correspond to the product of mutation rate μ and population size N (eq. [4]), whereas, in the case of rapid population growth, it is expected to correspond to the product of the mutation rate and the time since expansion, g_e (eq. [5]). Using the T_n world variance estimated above and assuming the constant effective population size of 11,200—that is, 33,600 X chromosomes (Ziętkiewicz et al. 1998)—one would estimate the T_n mutation rate, from equation (4), at $\mu = 1.3 \times 10^{-4}$ per generation. This estimate, although falling within the usual range of microsatellite mutation rates, does not fit the rest of our data. In particular, the S^2 values estimated separately within the abundantly represented haplotype lineages, such as B001, B002, B003, or B004, do not exceed 0.01 in non-Africans (table 4). Substituting these

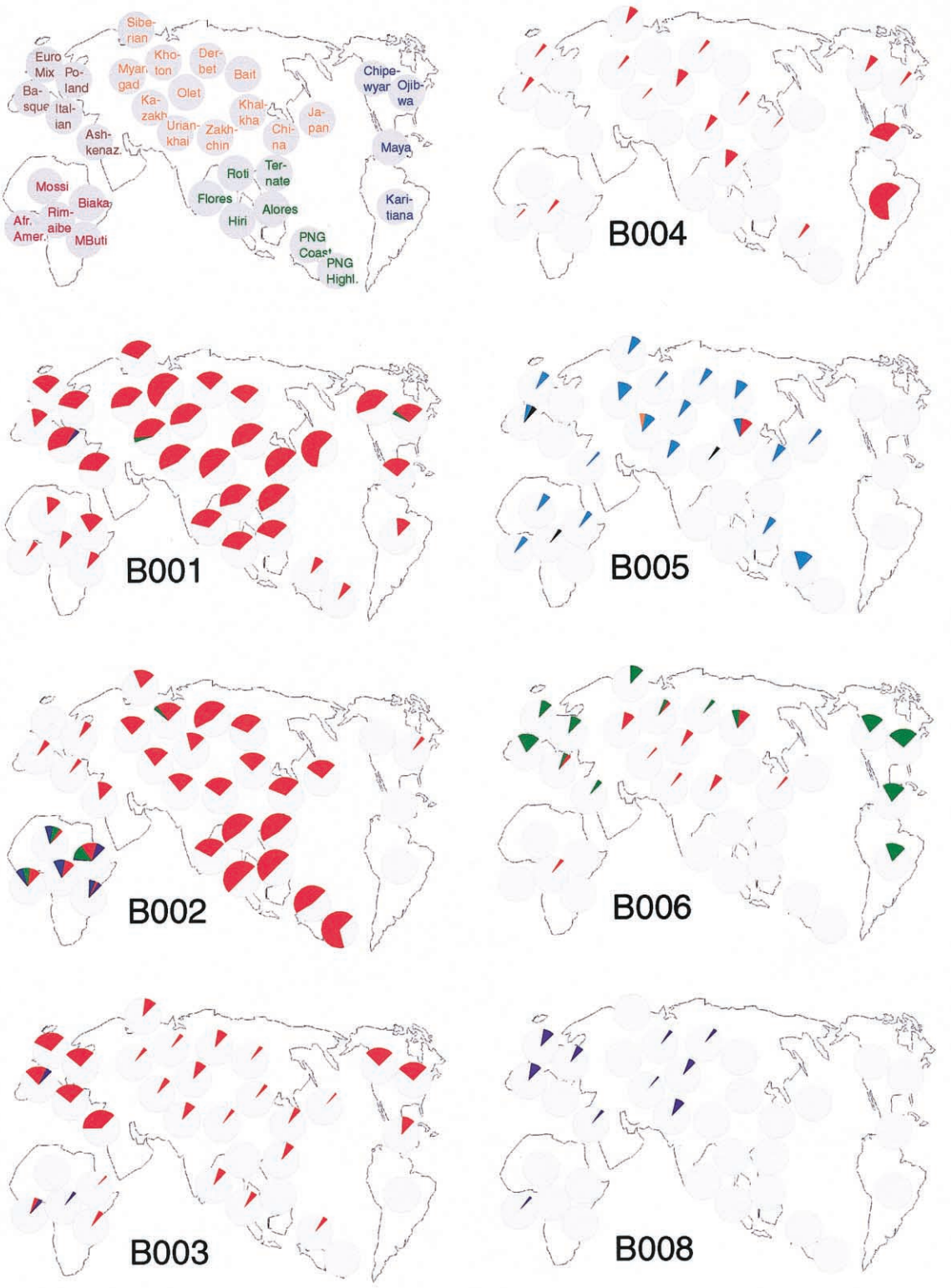


Figure 4 Population frequencies of the frequent, continentally shared haplotypes. Different colors, if present, indicate different T_n alleles shared by the same B haplotype. Note that B006 is not shown in the African American sample, because of the strong evidence that it is a result of recent admixture with the Amerindian population (see text).

Table 2

Estimates of the Time Since Expansion for Each Continent Based on Recombination within the *dys44* Segment

Area/Group	No. of Chromosomes	No. of Region-Specific Recombinants	$-\ln P$	Putative Time Since Expansion[a] ($g \times 25$) (years)
Americas	231	4	.017	13,300
Asia	488	20	.042	32,700
Europe	178	6	.034	27,300
Indonesia/PNG	156	15	.101	79,000
Indonesia	91	4	.045	35,100
PNG	65	11	.185	144,800
Non-Africans	1,053	45	.044	34,100
Non-Africans versus Africans	1,053	72	.073	55,300

[a] The putative expansion time was calculated from equation (5), using $r_{app} = .32 \times 10^{-4}$ (see the "Material and Methods" section) and an arbitrary 25 years as generation length. Note that, given the possible variation in local recombination rates and the sampling error and simplifying assumptions of the underlying demographic model, the variance associated with these estimates may be very large.

S^2 values and the mutation rate above into equation (2) would lead to the unrealistically small population size of $\leqslant$100 chromosomes carrying each of these frequent haplotypes. Using a more appropriate scenario of rapid population growth (i.e., using eq. [5] rather than eq. [4]) would lead, in turn, to a time estimate of $\leqslant$100 generations (2,500–3,000 years) since the expansion of these lineages, which is equally untenable. The source of the incongruity seems to lie in the above estimate of the T_n mutation rate. Given the bimodal distributions of allele length (fig. 5), it is likely that the overall estimates of S^2 are inflated and that we should rather obtain S^2 separately for the short (T_{15}) and long ($T_{20/22}$) modes, which are naturally demarcated by the nonexistent allele T_{18} . From the resulting values of $S^2_{(15)}$ and $S^2_{(20/22)}$ (table 3) and using equation (4), we obtained mutation rate estimates of 0.6 and 3.9×10^{-5} (average $\mu = 2.3 \times 10^{-5}$) for the worldwide sample, and 2.2 and 3.5×10^{-5} (average $\mu = 2.8 \times 10^{-5}$) for the African sample alone. In the above, equation (4) was used because we considered that the impact of the stationary phase, extended over a long evolutionary time period and described by a constant-population-size model, would prevail in these two samples, in spite of any subsequent population expansion. However, if population growth was preceded by a bottleneck (Tishkoff et al. 1996), one would expect to observe a substantial loss of diversity. Indeed, the estimates of $S^2_{(15)}$ and $S^2_{(20/22)}$ (0.098 and 0.093, respectively) in non-Africans were smaller by one order of magnitude (table 3), consistent with a bottleneck scenario and suggesting that equation (5) may be more appropriate for non-Africans. Accordingly, assuming the mutation rate $\mu = 2.3 \times 10^{-5}$, we estimated the time of the non-African lineage expansion (g_e from eq. [5]) to be 4,040–4,260 generations, or 101–107 kya.

Chromosomal Lineages as Markers of Population Expansion

The expansion time estimation can be performed separately for each of the distinct B haplotype lineages, for which the issue of distinct modes of allele lengths no longer exists. The S^2-based age estimates can be obtained from equation (5), as discussed above. Under the plausible assumption that the most frequent T_n allele is the ancestral one, one can apply equation (1) to assess the age of a haplotype lineage from $-\ln P$ (where P is the proportion of haplotypes carrying the ancestral T_n allele; also note that g from equation [1] is equivalent to g_e from equation [5]). The results for all B haplotypes associated with more than one T_n allele are presented in table 4; haplotype B004 is included as well, to emphasize its lack of T_n allele diversity. The values of S^2 and $-\ln P$ are generally very similar; at low variance, the results are identical. Their differences at higher values can be ascribed to the fact that the legitimacy of equation (1) was stretched when used for data with a level of divergence from the ancestral state of >10%. Table 4 demonstrates that, outside of Africa, there is little or no variance in the T_n allele length associated with either haplotype B002 and its recombinant product B004 or with B003. In contrast, among the African chromosomes, there is a large variance associated with B002 and B003. This discrepancy suggests that the founding event of the B002/B004 and B003 haplotypes outside Africa was relatively recent and was accompanied by a severe bottleneck. Such a bottleneck presumably reduced the originally high repeat-length variance, as observed today only in African B002/B004 and B003 chromosomes. In Africa, a large variance also accumulated in a number of other, continent-specific haplotypes, which

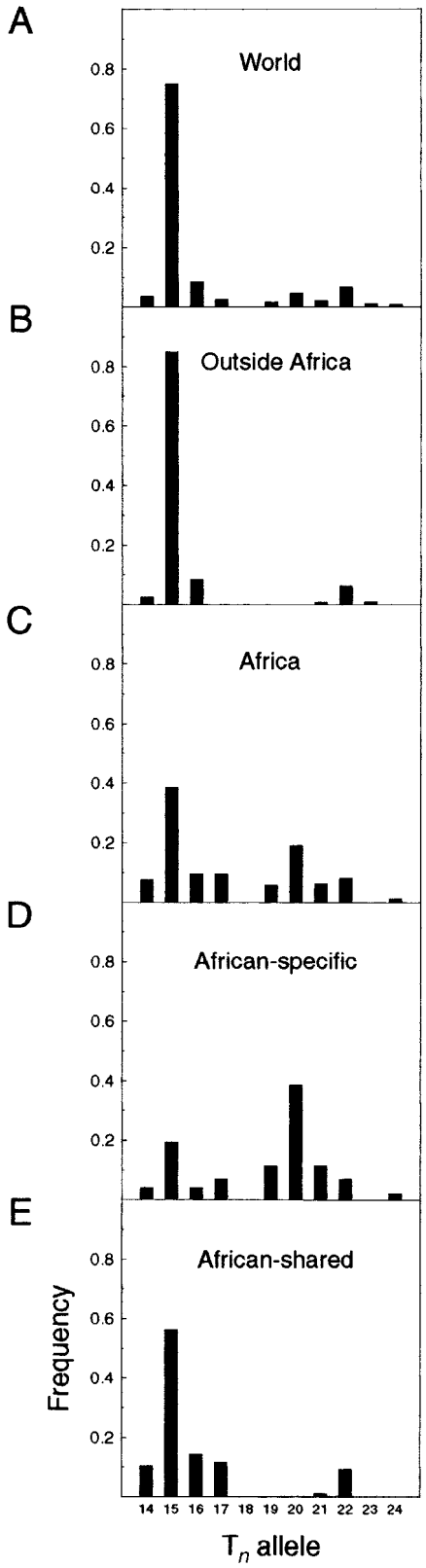


Figure 5 Frequency distribution of length alleles of the T_n microsatellite worldwide (*A*), in non-Africans (*B*), and in sub-Saharan Africans (*C*), subdivided further into chromosomes carrying African-specific haplotypes (*D*) and those shared with other continents (*E*).

never left the continent (table 4), either because they did not pass through the bottleneck or because they belonged to the local, nonexpanding lineage(s) (Labuda et al. 2000). The ubiquitous, young B001 haplotype, which is monomorphic at the T_n site on African chromosomes and is associated with a small variance in T_n length in non-Africans, appears to be a marker of the expanding lineage that spread both outside and within Africa (fig. 4).

Substituting the non-African S^2 or $-\ln P$ values for the above haplotypes (B001, B002/B004, and B003; table 4) in equation (5) or equation (2), we arrived at out-of-Africa bottleneck time estimates of ~5 and 12 kya. These dates appear too recent when compared with the historically documented Upper Paleolithic expansions dated at 35–50 kya. However, under the model of a bottleneck followed by population expansion (i.e., a founder effect), the time of the founding event estimated from genetic data is expected to appear younger than it actually is (Luria and Delbrück 1943; Hastbacka et al. 1992; Labuda et al. 1996, 1997). This can be corrected using the heuristic approach of Luria and Delbrück (1943), as described elsewhere (Labuda et al. 1997; Colombo 2000; Slatkin and Rannala 2000). Such a correction of the age of the founder effect is based on demographic growth and the rate of either recombination or mutation, whichever causes the decay of the ancestral haplotype/allele with time. Given a demographic growth rate of 0.002–0.005 per generation and given the above mutation rate, the correction (from eq. [6]) is very substantial (in the range of 27–56 ky), placing the age estimates for these out-of-Africa lineages within a plausible time range.

The non-African ancient haplotype B006 displays a relatively large T_n variance, an order of magnitude greater than in B001, B003, and B002/B004, although smaller than that of the African-only haplotypes (table 4). A similarly moderate level of T_n length variance char-

Table 3

Variance in Allele Length of the T_n Microsatellite across Continents for the Entire Range of Allele Lengths (S^2), for Modal Length 15, Comprising Alleles 14–17 ($S^2_{(15)}$), and for Modal Length 22, Comprising Alleles 19–24 ($S^2_{(22)}$)

Area/Group	*N*	S^2	$S^2_{(15)}$	$S^2_{(22)}$
Americas	231	.173	.173	...
Asia	488	3.870	.047	.097
Europe	178	3.403	.170	.077
Indonesia/PNG	156	3.323	0	.091
Non-Africans	1,053	2.913[a]	.098	.093
Sub-Saharan Africa	290	7.161	.727	1.168
Shared	151	4.323	.674	.066
Specific	139	6.139	.862	.966
Overall	1,343	4.338	.204	1.316

[a] When the Americas were excluded, the total variance (n = 822) was S^2 = 3.658.

Table 4

Variance in Allele Length of the T_n Microsatellite for the World, Non-Africans, and Africans, by Haplotype

	World			Non-Africans			Africans		
Haplotype	N	S^2	$-\ln P$	n	S^2	$-\ln P$	n	S^2	$-\ln P$
B001	401	.010	.010	366	.011	.011	34	.0	.0
B002	292	.284	.159	222	.005	.004	69	.955	.938
B003	128	.038	.040	116	.009	.009	12	.242	.410
B004	104	.0	.0	102	.0	.0	2	.0	.0
B005[a]	59	.103	.107	48	.106	.110	11	.091	.095
B006[b]	91	.154	.207	87	.152	.203	1	.0	.0
B019	5	1.200	.511	3	.0	.0	2	.0	.0
B051	2	.500	NA	2	.500	NA	...	...	...
B007	35	.281	.06	...	...	...	35	.281	.59
B010	12	1.091	.69	...	...	...	12	1.091	.693
B012	9	2.250	.401	...	...	...	9	2.250	.401
B046	5	.200	.223	...	...	...	5	.200	.223
b061	5	.200	.223	...	...	...	5	.200	.223

Note.—Only haplotypes showing variable T_n are shown. In calculating the proportion of intact ancestral alleles, *P,* the major allele was assumed to be ancestral, which is a fragile assumption when made with a low number of chromosomes. The $-\ln P$ values were nevertheless calculated, for comparison with S^2. NA = not applicable.

[a] Three B005 chromosomes with T_n allele 15 were not considered in the calculation. We assumed that this length allele was acquired in B005 by recombination rather than by mutation (see text).

[b] Because of the likely origin of three B006 haplotypes in African Americans by admixture (see text), only B006 found in the Rimaibe was retained in the African sample.

acterizes haplotype B005, found in both Eurasia and Africa. B006 apparently did not go through the out-of-Africa bottleneck, since it is found only outside Africa; the same may be true for B005, for which the associated T_n variance in Eurasia is equal to if not higher than that in Africa. The estimated times of expansion of the B006 and B005 lineages—165 kya and 115 kya, respectively (obtained using eq. [5])—are older than the time of the out-of-Africa bottleneck estimated above (100 kya), suggesting that B006 and, possibly, B005 might have originated outside Africa.

Discussion

Human genetic history and the partitioning of genetic diversity across populations have been extensively studied through the analysis of mitochondrial and Y-chromosome DNA. The worldwide breadth and population depth of such studies have never been matched by investigations employing nuclear, non-Y DNA polymorphisms. However, to truly understand the genetic structure of human populations, we have to collect information from loci other than those representing the maternal or paternal lineages only. Towards this goal, we analyzed genetic diversity in the X-chromosome segment *dys44* (Ziętkiewicz et al. 1997, 1998; Labuda et al. 2000), in 33 populations worldwide. This system provides a genomic record of a variety of mutations, including nucleotide substitutions, small insertions, deletions, and changes in a T_n repeat-length polymorphism, as well as a record of informative recombinations, as reflected in the structural diversity of *dys44* haplotypes. The interpretation of this genomic record should take into account the temporal frame within which the underlying genetic events have occurred, the demographic histories of the analyzed populations, and the sampling scheme applied by the investigator.

The initial ascertainment of polymorphisms within the *dys44* segment was limited to a sample of 250 chromosomes (Ziętkiewicz et al. 1997). Genotyping of the enlarged population sample in this study was performed using the ASO-hybridization technique, aimed at the already detected polymorphisms. Therefore, no new nucleotide variants were expected to show up. This limitation did not extend, however, to the ascertainment of new *dys44* haplotypes or to the T_n alleles reported here. The proportion of non-African chromosomes with non-African continental-specific haplotypes was 5%, whereas 48% of African chromosomes carried African-specific haplotypes. Among the 35 polymorphic sites of the *dys44* segment, there were 14 new SNP-like alleles endemic to Africa, versus 2 private alleles found outside Africa (fig. 1). The variance in T_n allele repeat length for both length modes ($S^2_{(15)}$ and $S^2_{(20/22)}$) was ~1 in Africa versus ~0.1 in non-Africans. The comparisons above indicate a ratio of ~10:1 for the different measures of *dys44* variability between Africans and non-Africans. This observation is not new: the greater diversity in sub-Saharan Africans can be expected on the basis of previous studies (Cann et al.

1987; Batzer et al. 1994; Bowcock et al. 1994; Tishkoff et al. 1996; Jorde et al. 1997; Stoneking et al. 1997; Clark et al. 1998; Hammer et al. 1998; Ziętkiewicz et al. 1998; Kaessmann et al. 1999; Kidd et al. 2000; Yu et al. 2001).

The excess of African genetic variation has been taken as evidence of an older age of African populations (Cann et al. 1987; Chen et al. 1995), a greater long-term effective population size in Africa (Relethford 1995), or a severe out-of-Africa demographic bottleneck (Tishkoff et al. 1996). Each of these models, which perceive the partitioning of contemporary genetic diversity as resulting from a prehistoric rift between sub-Saharan Africa and the rest of the world, can provide a nonexclusive explanation of the historical conditions that led to contemporary population characteristics. On the basis of our earlier analysis of *dys44* haplotypes (also see Baird et al. 2000; Labuda et al. 2000), we proposed a more subtle and complex scenario that can unite the above aspects. In our model, the genetic pool of sub-Saharan Africans reflects the contribution of at least two lineages, which had evolved separately for some period of time and eventually hybridized. One of these lineages existed in the population(s) that underwent an out-of-Africa expansion, whereas the other lineage(s) were present in groups that remained in Africa and/or contributed locally to the diversity of the emerging worldwide population. The former lineage is represented by the frequent, continentally shared haplotypes and their structurally related variants, and the latter is represented by diverse haplotypes, most of them carrying several of the 14 new alleles not found on chromosomes from outside Africa (fig. 1).

The NJ trees of populations (as in fig. 3) illustrate a "classical" out-of-Africa topology, as previously observed in population trees from other genetic systems (e.g., Bowcock et al. 1994; Cavalli-Sforza et al. 1994; Fan et al. 2002). Most published trees of sequences (or haplotypes) have also presented an out-of-Africa topology, with Sub-Saharan, African-only haplotypes emerging closest to, or even containing, the ancestral sequence. This has been observed for mitochondrial (Cann et al. 1987; Chen et al. 1995) and Y-chromosome (Hammer 1995; Underhill et al. 2000) haplotype trees, as well as for the trees of many non-Y-chromosome nuclear DNA haplotypes (e.g., Harding et al. 1997; Harris and Hey 1999; Jaruzelska et al. 1999; Kaessmann et al. 1999; Verrelli et al. 2002). However, the topology of the haplotype (sequence) tree based on NJ clustering of *dys44* haplotypes (fig. 2) did not match the pattern typical for other DNA segments. The B006 haplotype, which is virtually absent in sub-Saharan Africa, was closest to the root, carrying only four new alleles on the ancestral background (as defined by the ancestral haplotype). The haplogroups endemic to sub-Saharan Africa, such as B010, B007, and B012, appeared in the middle of the tree. Although structurally diverse, these haplotypes carrying African-specific alleles are sufficiently distinct from the non-African-specific haplotypes to form a relatively well-defined cluster. The fact that the new, African-specific alleles are distributed on a variety of poorly related and structurally diverse haplotypes (fig. 1) indicates the contribution of historical recombination. This suggests the relatively old age of the African-specific haplotypes (by the same token, the contributing mutations must also be old, in spite of their low new-allele frequencies).

African-specific haplotypes are almost exclusively associated with T_n alleles representing the long $T_{20/22}$ mode. This further distinguishes them from the non-African-specific haplotypes, which, except for the haplotype/haplogroup B005, are associated with T_n alleles representing the short T_{15} mode. It is among the African-specific haplotypes, in spite of their small number in the analyzed sample, that the S^2 variance of the T_n allele length is the highest, indicating again the relatively old age of these haplotypes (table 4). On the other hand, the frequent, continentally shared haplotypes, such as B001, B002, B003, and B005, are also well represented in sub-Saharan Africa, thus supporting the split ancestry of modern African populations, as previously suggested by Labuda et al. (2000).

Detailed inspection of the individual haplotype lineages can reveal information concerning their origins. There is little doubt about the African origin of B002 and B003. On the basis of the variance in their short-mode T_n repeat, these haplotypes appear very old when analyzed in Africa but not when studied outside of Africa (in contrast to the African B002 and B003 haplotypes, their frequent non-African copies are almost monomorphic for the allele T_{15}). Conspicuously, even in Africa, there are no B002 or B003 chromosomes found associated with the long-mode T_n alleles, although such alleles could have been acquired by recombination with the numerous $T_{20/22}$-length-mode haplotypes found in the same sub-Saharan populations. The study of recombination within the haplotype lineages can also aid in the search for their origins. For example, recombination is a plausible mechanism by which the T_{15}-associated B005 (from the otherwise T_{22}-linked haplotype) was created in Asia (tables 1 and 4); in addition, a recombination event exchanging the 3′ end of B002 was also responsible for creating B004, which is very rare in Africa, frequent outside of Africa, and completely monomorphic for T_{15}. The virtual absence of 3′ terminal crossovers between B002 and B003, on the one hand, and the exclusively African haplotypes associated with the $T_{20/22}$ length mode, on the other, suggest that their cohabitation started only recently and that they evolved separately for a significant period of time. B005, with S^2 variance at similarly moderate levels within and outside Africa, seems to represent an old lineage, which

did not necessarily go through the bottleneck. Although its position within the haplotype tree in figure 2, as well as its association with the $T_{20/22}$ length mode, suggests a structural connection of B005 with the African-specific lineage, its geographic origin remains elusive.

In contrast to B002/B004 and B003, the ubiquitous B001 haplotype seems to be young both within and outside of Africa (table 4). B001 is almost exclusively associated with the predominant allele T_{15}, as is observed in all continental populations, including Africa. B001 could have been recently derived from B003 by elimination of the new allele A at site 65 (fig. 1); if such an event happened recently, it would explain the low associated T_n diversity. Its time of origin, however, had to precede the out-of-Africa expansion (since it was the B001-carrying population that spread most widely, as is reflected in the observation that a quarter of all chromosomes observed worldwide are of type B001), and the out-of-Africa spread of B002 and B003 haplotypes appears concomitant with B001 expansion, thus providing good evidence for the African origin of the latter lineage. The fact that B001 constitutes 10% of present-day sub-Saharan African chromosomes suggests that expansion not only was directed out of Africa but also occurred within Africa itself; understandably, the "expanding" chromosomes on this continent were diluted within the existing gene pool and, hence, did not reach the high frequencies observed outside Africa.

Evidence for an ancient within-Africa expansion has also been observed in mtDNA (Watson et al. 1997). Watson et al. (1997) proposed that most of the African mitochondrial sequences originated from demographic expansions that started ~60–80 kya, the earliest of which led to the colonization of Eurasia. The same expansion presumably involved haplotypes B002 and B003. An expansion signature, reflected by the lack of the B002 and B003 T_n variability, would only be visible outside of Africa because of a bottleneck and the subsequent population growth, whereas any similar effect within Africa would likely have been masked by the presence of endemic B002 and B003 copies polymorphic at the T_n site. By the same token, the fact that the old, African-specific alleles (characteristic of African-specific haplotypes) occur at a relatively low frequency could be explained by their "dilution" as a result of hybridization with the expanding lineage. This, again, is in agreement with earlier observations by Watson et al. (1997), who noted that a minority of mtDNA sequences (13%) fell outside the presumed out-of-Africa expansion clusters. It was postulated that these sequences echoed the time before the out-of-Africa expansions, when the human mitochondrial gene pool was more diverse. In our data, this "minority" is reflected by the local African-specific lineages.

Although B001 appears to be the best marker of the recently expanded lineage, it does not aid our understanding of the expansion route(s) out of Africa. The clues here may come from the analysis of other frequent *dys44* haplotypes, which together suggest that the impact of modern human expansion on human diversity in Africa and other continents does not reflect a single demographic event. The frequency distributions of B002 and B003 outside Africa are skewed: B002 is poorly represented in Europe and is relatively abundant in Southeast Asia, in contrast to B003, which is frequent in Europe and relatively rare among Asiatic populations. Do these distributions mark two separate expansion events, or do they result from genetic drift (due to the initial population bottleneck) and subsequent reinforcement as a result of their geographic separation? Although there are insufficient data to examine possible temporal differences in the time of expansion of the two lineages, the geographic separation of the haplotype frequency gradients suggests independent expansions, towards Europe and towards Southeast Asia, that could correspond to the northern and southern routes referred to in the literature (Lahr and Foley 1994; Kivisild et al. 1999; Quintana-Murci et al. 1999).

In our data, the northern route seems to be locally enriched by the addition of another, very ancient and distinct lineage, represented by a single haplotype, B006. This haplotype is not only structurally the simplest but is also the one that lies closest to the root, as defined by the hypothetical haplotype with ancestral alleles at all positions (fig. 2). Two of B006's four derived alleles, at sites 10 and 85, are private (restricted to B006 and its closest recombinants), whereas the remaining two, with worldwide frequencies of 0.7–0.9 at sites 30 and 95, are among the oldest polymorphisms of the *dys44* segment (Ziętkiewicz et al. 1998; Ziętkiewicz and Labuda 2001). The T_n allele variance associated with B006 independently suggests the haplotype's relatively ancient origin. In spite of the otherwise substantial role of recombination in the *dys44* segment, there is no record of extensive B006 interaction with haplotypes from other lineages, and only a few recent recombinant haplotypes are observed (fig. 1). These characteristics are reminiscent of the African-specific haplotypes that are structurally distinct, old, continentally restricted, and that carry private polymorphisms. B006 is practically absent in Africa and is poorly represented in Southeast Asia, in contrast to its relative abundance in Europe, central Asia and the Americas. Where, then, could haplotype B006 and the lineage it represents have originated?

Given its structural uniqueness and its large T_n allele variance, B006 is unlike any of the haplotypes associated with the main, out-of-Africa expanding lineages (i.e., B002/B004 and B003/B001). We can therefore postulate two scenarios regarding the origin of B006. First, it may represent an ancient African lineage that made

its way out of Africa, spreading within the newly colonized regions and becoming extinct in its place of origin. The arid climatic conditions (due to the glacial maxima) between 50 and 100 kya were likely to have induced population fragmentation (Lahr and Foley 1994), thereby allowing sister populations of modern *H. sapiens* to evolve in Africa prior to the Upper Paleolithic expansion. Therefore, in a way similar to the structurally unique African-specific lineage(s), B006 could represent a vestige of an independent northern lineage. Alternatively, B006 may represent an as-yet-unknown non-African contribution to the human gene pool. Such a "northern" contribution to the contemporary human gene pool could have escaped detection in studies of mitochondrial or Y lineages, because of the shorter "evolutionary memory" of these systems as compared with nuclear loci. It is notable, however, that B006 has been found at very low frequency in one sub-Saharan population, the West African Rimaibe. The question of whether this occurrence is (i) suggestive of the haplotype's ancient African roots, (ii) a record of back migration from Eurasia, as postulated by a number of authors (Hammer et al. 1997; Harding et al. 1997; Cruciani et al. 2002), or (iii) a result of more recent admixture with northern populations across the Sahara remains unanswered. However, the presence of another, essentially non-African haplotype (B004) in the Rimaibe would lend support to the back migration and/or recent admixture scenario(s). On the other hand, we have to emphasize that our results were obtained with a limited sub-Saharan African sample, mostly representative for West Africans and Pygmies. Among other important groups, such as the Khoisan from South Africa and/or the East Africans, which were not included in our sample, the latter could be of particular interest in this context. For example, mtDNA haplogroup M was long considered typical of Asians, until it was observed that a specific and very limited subset of it (now termed "M1") was present in East Africans (but not in other African groups); this lead to the final conclusion that M was indeed among the mtDNA haplogroups that were involved in the out-of-Africa exit (Quintana-Murci et al. 1999). It is therefore necessary to extend the analysis of the dystrophin locus to include other African populations. It is possible that such studies will not only provide evidence for the origin of B006 but also shed more light on a number of ancient lineages that contributed to the human gene pool (also see Watson et al. 1997).

A similar scenario, of the hybridization of distinct lineages ultimately giving rise to modern *H. sapiens,* was previously suggested by Baird et al. (2000), to explain the coexistence of deeply diverged haplotypes adjacent to the Xp/Yp and 2q telomeres in humans. The study suggested that the lineages could have remained separate for as long as 1.9 million years, although the time of their fusion might have been as recent as the out-of-Africa expansion of modern humans. The results of Baird et al. (2000) parallel those from *dys44*. Although estimating the time to the most recent common ancestor of the *dys44* haplotypes is complicated by a number of historical recombination and gene conversion events, the divergent structure of the African-specific and continentally shared haplotypes argues for their independent evolution for a significant period of time (Labuda et al. 2000). A similar conclusion can be reached for the lineage represented by B006. Taken together, these data suggest a mosaic origin of modern human populations; this amalgamation of lineages did not precede but rather accompanied the great expansion of one of these lineages represented by haplotypes B003/B001 and B002/B004.

We estimated the time since the bottleneck that accompanied the out-of-Africa expansion by two independent approaches: one based on population frequencies of *dys44* recombinants, and the other based on variance in the associated T_n microsatellite, both of which provided relatively consistent results. The recombination-based estimates presented in table 2 were obtained under the assumption that continentally shared haplotypes represented the ancestral population before expansion, whereas region-specific recombinants were acquired after the initial expansion. There are a few factors that may render these estimates uncertain. The first of these is the recombination rate, which was determined at a "macro" scale from mapping data (Kong et al. 2002) but may differ locally because of micro-scale fluctuations (such as the presence of recombination hotspots, as reported by Harding et al. [1997]). The second is that some of the region-specific haplotypes, rather than being the result of postexpansion recombination, might in fact represent old but rare sequences; such a phenomenon would lead to an overestimation of the time since expansion. However, this can be at least partly controlled by inspecting the structure of the new haplotypes and ensuring that they are genealogically simply related to their assumed ancestral haplotypes (i.e., those associated with the expansion), as is the case in *dys44*. Third, the overall proportion of informative recombinants, estimated from our total data at 30%, actually fluctuates regionally, depending on the local haplotype diversity and structural complexity. Because of this, the level of informative recombinations within *dys44* is notably higher in Africa than outside of Africa. However, African samples were not analyzed using this method, because of difficulties in assigning cohorts of ancestral haplotypes. Finally, the variance associated with the estimated time since expansion is affected by population history; strong expansion re-

duces variance and will be discussed further, in the case of PNG populations.

The second approach we took to estimating the time since expansion utilized the variance at the T_n microsatellite. Its mutation rate, 0.23×10^{-4}, was assessed from the observed variance of two T_n length modes and from the effective population size (estimated previously by Ziętkiewicz et al. 1998). We justified the separate analysis for each T_n length mode because we observed (i) the persistent association of a single allele length mode with a particular B haplotype and (ii) a simple mutation mechanism that changed the allele length by one repeat unit at a time (table 1; figs. 4 and 5). The two length modes could have been created either by a rare mutational jump, over many repeat units, or by a duplication of the short ancestral allele (11–13 units); once formed, the two modes could have coexisted and evolved independently. Alternatively, the much greater effective population size of ancient hominids (Chen and Li 2001) might have resulted in a broader spread of T_n allele lengths in the ancestral human population; only the lineages representing the short and long alleles could have persisted after population fragmentation and size reduction (e.g., Takahata 1994; Stiner et al. 1999; Satta and Takahata 2002). However, regardless of the underlying model, the estimation of variance (S^2) for each length mode proved reasonable and provided consistent results.

The separation time between Africans and non-Africans, estimated from T_n variance in non-Africans, was 101–107 kya. This likely represents an overall figure summarizing the contributions from both the older (B005 and B006) and younger (B001, B003, and B002/B004) haplotypes. Using the T_n variance estimated from non-African populations for these haplotype lineages, we arrived at the out-of-Africa bottleneck time estimates of 5–12 kya. After applying a correction for the effect of a growing population, the estimated time since the out-of-Africa expansion increases to 27–56 kya, placing it within a historically plausible time range that corresponds to the documented Upper Paleolithic expansions of 35–50 kya. However, the above estimates have to be treated with caution, because of the large variance inherently associated with microsatellite markers. The use of the g_o correction, which depends on the rate of population growth rate d, brings an additional source of uncertainty to the above estimates, since the variance within different haplogroups could be also explained by different values of d. For example, had B001 and B003 been equally represented in the original expanding population, then their present-day occurrence would have indicated different growth dynamics (B001 having expanded to its present-day frequency of 0.25, and B003 having expanded to a frequency just under 0.1). Accordingly, the population growth rate d and, hence, the derived g_o correction, would have been different for each of the two haplotypes, possibly explaining the lower variability associated with the less frequent haplotype (see eq. [6]). These considerations also suggest that, under the assumption of a founder effect followed by rapid population growth, the time-since-expansion values in table 2 are also underestimates. Although easily applicable in the cases of B001, B002/B004, and B003, this model is less suitable when used with B005, B006, and other haplotypes.

The estimates of time since expansion from the two independent methods discussed above provide a reasonable time frame of past events that compares well with other estimates from the literature (Rogers and Harpending 1992; Torroni et al. 1998; Richards et al. 2000). They both indicate that major migrations and population expansions coincided with the demographic events of the Upper Paleolithic. Although the absolute time estimates need necessarily be treated with caution, the relative values are illuminating. They indicate that a mosaic of lineages, of different time depths and likely of different geographic provenience, within Africa and perhaps including western Eurasia, contributed to contemporary human diversity. Although it is likely that the origin of haplotype B006 is in Africa, the question remains as to whether its frequent, non-African presence dates from the time of the great expansion, or whether it left Africa earlier and was then admixed into the major expanding lineage on its northern route into Eurasia. To further address this question, we will need to expand our sample pool and study further polymorphisms, adjacent to *dys44,* to increase temporal resolution and accuracy.

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Appendix A

Fraction of Informative Recombinations

The extent of genome diversity is due to the cumulative effect of mutations and recombinations. Mutations such

as nucleotide substitutions almost always lead to new variants, since, given a mutation rate on the order of 10^{-8} per generation, recurrence is extremely rare. In contrast, a significant fraction of recombination events have no effect on haplotype diversity; they occur but do not result in an outcome that can be perceived as a new recombinant. This is the case of recombinations occurring between homologous genomic segments that are identical or that differ at a single site only (i.e., in homozygous or single-site heterozygous individuals). The fraction of such "silent"—or noninformative—recombinations depends on the density of sequence polymorphisms (Hudson and Kaplan 1985; Stephens 1986). Indeed, an informative (i.e., potentially detectable) recombination has to occur within a segment delimited by heterozygous sites, such that the resulting recombinant haplotype will differ from the parental ones.

The fraction of informative recombinations, F_{IR}, for a segment with K haplotypes in a population, can be written as

$$F_{IR} = \sum_{ij=1}^{K} \frac{f_i f_j d_{\max,ij}}{L} ,$$

where $f_i f_j$ is the population frequency of a diploid genotype combining haplotype variants i and j, such that $\sum_{i,j=1}^{K} f_i f_j = 1$ represents the sum of all possible genotypes; $d_{\max,ij}$ represents the distance (in number of nucleotides) between maximally separated pairs of heterozygous sites for a combination of the ij haplotypes (note that if the combination ij has less than two heterozygous sites, $d_{\max,ij} = 0$, such that noninformative recombinations do not contribute to F_{IR}); and L denotes haplotype length, which corresponds to the distance between maximally separated, potentially informative positions (for the *dys44* haplotype, L corresponds to 7,917 bp between sites "2" and "96").

F_{IR} represents the fraction of all recombinations leading to haplotypes that differ from the parental haplotypes. Such recombinants will always be detected in family studies, when both parents and their child are analyzed. In population studies, however, only the recombinants that represent structurally new, distinct haplotypes will be discerned. Recombinants IBS with the haplotypes already observed in the analyzed population will be perceived as IBD with the latter rather than as being due to a separate crossover event. Therefore, among potentially informative recombinations, F_{IR}, we need to distinguish the fraction of events leading to new recombinants, F_{NR}, from that of back recombinations "recreating" already observed haplotypes, F_{BR} ($F_{IR} = F_{BR} + F_{NR}$). F_{NR} and F_{BR} can be calculated by simulating recombination between the set of observed haplotypes and cataloguing all outcomes of informative recombinations as either new or already observed haplotypes, respectively. The apparent recombination rate of the haplotype, r_{app}, to be used in a population study for time and/or recombinational divergence estimations is therefore $r_{app} = rF_{NR}$, where r represents the recombination rate of the haplotype estimated from the genetic map.

Using our sample of 1,343 chromosomes from around the world and assuming panmixia, we obtained an F_{IR} of 0.62 ($F_{BR} = 0.21$ and $F_{NR} = 0.41$). The histogram in figure A1 shows the observed haplotype frequencies (fig. A1*A*) compared with the contribution of each of the haplotypes to the overall F_{BR} (fig. A1*B*). Figure A1*C* depicts the distribution of the probabilities of occurrence of 6,857 distinct, novel recombinants that could arise through single crossovers from all pairwise combinations of the 86 observed haplotypes (i.e., individual contributions of these new haplotypes to the overall F_{NR}). The cumulative probability of the recombinations that would result in 86 (i.e., equivalent to the number of the observed haplotypes) most probable new recombinants corresponds to two-thirds of the overall F_{NR} (0.26 out of 0.41). The overall F_{NR} values vary among populations, from 0.54 in Africa to 0.26 in Indonesia and PNG; nevertheless, using $F_{NR} = 0.3$ to calculate the apparent recombination rate for non-African populations is a good approximation. With <30 novel haplotypes observed outside of Africa, a similar count of back recombinants might be expected (F_{BR} and F_{NR} are similar), and, therefore, even haplotypes with individual contributions to F_{BR} as high as 1% have little chance to reoccur through back recombination. For most of the existing haplotypes, the individual probability of their reappearance resulting from back recombination is lower than the collective probability of the appearance of the potential newcomers (fig. A1*B* and A1*C;* note different scaling of the *Y*-axes). Furthermore, there is no correlation between population frequency and the recurrence rate recreating a particular haplotype through independent crossovers, suggesting that new recombinants originate from high and low probability values of the recombinations' probability spectrum, illustrated in figure A1*C*.

The population frequency of haplotype B004 exceeds the probability of its reappearance through a recurrent recombination. However, a reciprocal solution of this recombination leading to B004 and engaging the most frequent combination of ubiquitous haplotypes B001 and B002 is the haplotype b054, which, conspicuously, was observed only once. Haplotypes B030 and B034 appear to have the highest rate of recurrence through back recombinations (fig. A1*B*), yet only few copies of these haplotypes were seen in our sample. Therefore, although the rate of back recombinations is not negligible, their confounding effect on interpreting the geographic distribution and frequencies of haplotype variants can be largely neglected for the *dys44* data set. A few haplotypes especially likely to be recreated through

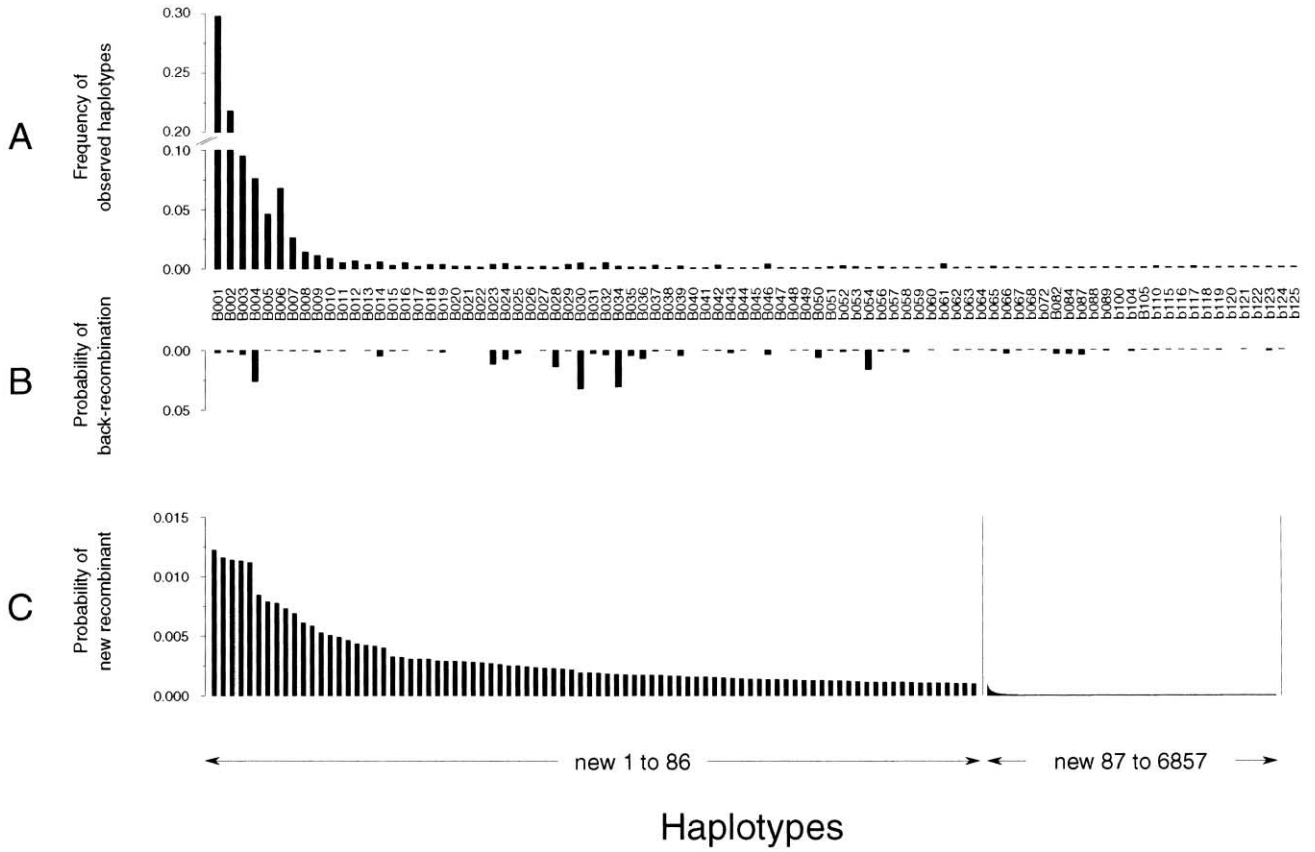

Figure A1 Frequencies of 86 observed haplotypes (*A*) compared with their individual probabilities of back recombination (*B*). *C*, Probability distribution of new recombinants resulting from pairwise recombination of the observed haplotypes from the whole data set. Note that the scale of the ordinate in *C* is reduced by a factor of 10 compared with *A* and *B*, that the total count of new haplotypes is 6,857 (*C*), and that the left and right parts of chart *C* depict the 86 most frequent novel haplotypes versus the remaining 6,771 relatively infrequent novel haplotypes.

back recombinations are easily identifiable through the analysis of possible recombination outcomes, as shown in fig. A1*B*, which may assist data interpretation. For example, the individual probability of reappearance of B004 through back recombination was found to be 2.6% worldwide, 2.4% in Africa, 1.5% in Europe, 3.5% in Asia, and 4.6% in Indonesia and PNG, whereas, in the Americas, where 80% of B004 chromosomes reside, the probability was only 0.05%. This strongly suggests that all Amerindian copies of B004 are IBD. Their high frequency on this continent is most probably due to a local founder effect, which, at the same time, presumably eliminated haplotype B002, an essential ingredient needed to recreate B004 by de novo recombination.

The program used to evaluate F_{NR} and F_{BR} is available upon request from D.G. (Dominik.Gehl@UMontreal.ca).

Electronic-Database Information

The accession number and URL for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for the human dystrophin gene [accession number U94396])

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