

Out of Africa again and again

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The publication of a haplotype tree of human mitochondrial DNA variation in 1987 provoked a controversy about the details of recent human evolution that continues to this day. Now many haplotype trees are available, and new analytical techniques exist for testing hypotheses about recent evolutionary history using haplotype trees. Here I present formal statistical analysis of human haplotype trees for mitochondrial DNA, Y-chromosomal DNA, two X-linked regions and six autosomal regions. A coherent picture of recent human evolution emerges with two major themes. First is the dominant role that Africa has played in shaping the modern human gene pool through at least two—not one—major expansions after the original range extension of *Homo erectus* out of Africa. Second is the ubiquity of genetic interchange between human populations, both in terms of recurrent gene flow constrained by geographical distance and of major population expansion events resulting in interbreeding, not replacement.

Recent human evolution is an area of great controversy¹. There is general agreement that the human lineage evolved in Africa, and then spread to southern Eurasia as *Homo erectus*. After *Homo erectus* spread out of Africa, the out-of-Africa replacement model² posits that populations in Africa, Europe and Asia had little genetic contact and evolved independently, with anatomically modern humans evolving only in Africa. After the evolution of modern humans in Africa, a second expansion occurred out of Africa about 100,000 years ago that resulted in the global replacement and genetic extinction of nonmodern human populations by anatomically modern humans. Under the multiregional trellis model^{3,4} genetic contact between African and non-African *Homo erectus* populations was maintained although restricted by isolation by distance. Isolation by distance allowed local populations to become differentiated from one another, but gene flow prevented long-term independent evolution such that humanity evolved into modernity as a single evolutionary lineage.

Much of this controversy started with the publication of evolutionary trees of haplotype variation in human mitochondrial DNA (mtDNA)^{5,6}. The estimation of these initial mtDNA haplotype trees was flawed^{7,8}, but many subsequent studies have generated more reliable haplotype trees for mtDNA^{9–12}, Y-chromosomal DNA regions (Y-DNA)¹³, X-linked DNA regions^{14,15} and several autosomal DNA regions^{16–20}. Not all of these haplotype trees have been analysed with respect to models of recent human evolution. The purpose of this paper is to analyse several recently published haplotype trees and combine these new analyses with older analyses.

Reconstructing human evolution without previous models

Instead of testing *a priori* hypotheses of recent human evolution, nested clade phylogeographic analyses²¹ as implemented with the program GEODIS²² are used to infer both historical events (such as range expansions) and recurrent events (such as gene flow) without regard to any prior model. Although it is relatively new²¹, nested clade analysis has been validated by applying it to data sets with known *a priori* information and has been found to be accurate and not prone to false positives, although it fails to detect all known events²³. This methodology has now become a standard tool in phylogeographic analyses (for example, see refs 24–27).

The nested clade analysis first tests the null hypotheses of no association between geography and the haplotype tree. Only when this null hypothesis is rejected at the 5% level of significance is there an attempt to interpret the pattern biologically. Inferences are thereby limited to patterns that are based upon sufficient sampling and genetic resolution to have a significant phylogeographic signal. The biological meaning of these significant signals is interpreted by using an inference key²¹. This key can lead to the conclusion that the

sampling design was inadequate to make clear biological inferences. Therefore, only a subset of the statistically significant results lead to a biological interpretation. The use of an *a priori* inference key published well before the production of all the data sets to be analysed here prevents me from making *post hoc* explanations or trying to fit the results to a favoured hypotheses. The GEODIS program and the inference key are available at http://biog.byu.edu/zoology/crandall_lab/geodis.htm. The key, the GEODIS program documentation, and a fuller description of the nested clade methodology are available as Supplementary Information.

In choosing data for analyses from the recent literature, four criteria were used: (1) a minimum of four populations had to be sampled with at least one each in Europe, Asia and Africa; (2) the minimum sample size was 35 individuals; (3) haplotypes were determined, and the frequencies of these haplotypes within each sampled population were available; and (4) there was little or no recombination within the DNA region being investigated, allowing the estimation of a biologically meaningful haplotype tree. The data sets satisfying these criteria include a recent survey of complete mtDNA genome variation in humans⁹, the X-linked gene coding for the pyruvate dehydrogenase E1 α -subunit (*PDHA1*)¹⁴, and five autosomal regions: these are the *MX1* locus on chromosome 21 (ref. 17), the gene coding for eosinophil-derived neurotoxin (*EDN*) on chromosome 14 (ref. 20), the gene coding for eosinophil cationic protein (*ECP*) on chromosome 14 (ref. 20), the gene coding for the melanocortin 1 receptor (*MC1R*) on chromosome 16 (ref. 18), and compound haplotypes generated in the MS205 hypervariable minisatellite region on chromosome 16 (ref. 19). The whole genome mtDNA survey estimated a haplotype tree that excluded the sites in the D-loop region to focus upon the older phylogenetic signal in the mtDNA⁹, and the current analysis also excludes the D-loop. Haplotype trees were estimated by statistical parsimony²⁸ using the program TCS²⁹. In all cases, the statistical parsimony tree is either identical to or is a topologically compatible but more resolved version of the haplotype tree estimated by the original authors, who usually used either maximum parsimony or neighbour-joining. In some cases, tree ambiguities were further resolved using coalescent criteria³⁰.

The results obtained from these DNA regions are combined with previously published nested clade analyses^{13,23,31–33} of mtDNA data^{10–12,34,35}, the *SRY* and *YAP* regions on the Y chromosome¹³, a non-coding, low-recombination region on the X chromosome called Xq13.3 (ref. 15), and the autosomal haemoglobin β -chain locus¹⁶.

Results

The biological inferences made from previously published nested

Table 1 Previously published inferences using the GEODIS program

DNA region	Mitochondrial DNA			SRY and YAP	Xq13.3	Haemoglobin β
Reference Range	36*, 37*, 33† Global	11*, 12*, 23† NE Asia and America	10*, 23† SE Asia and Pacific	13* Global	15*, 35† Global	16*, 34† Global
	IBD	IBD, LDC to America	IBD, LDD	RE out of Africa		RE out of Africa
	IBD	IBD, RE to Siberia	LDC to Pacific	IBD, RE out of Africa	IBD	IBD
		F (Asia versus America), IBD	IBD, RE and/or LDC to Pacific			
	IBD; RE to N Eurasia	IBD, LDD, RE within America	IBD, RE to Pacific	IBD, RE out of Asia	IBD	RE out of Asia

Inferences are shown from oldest (top) to most recent (bottom). IBD, recurrent gene flow restricted by isolation by distance; LDD, recurrent gene flow with some long-distance dispersal; RE, range expansion of populations to geographically contiguous areas; LDC, range expansion of populations through long-distance colonization; F, genetic fragmentation into two or more allopatric populations.

* Reference for the data.

† Reference for the analysis (if different).

clade analyses are summarized in Table 1. The geographical extent of sampling is indicated, with 'global' meaning that human populations in Africa, Europe, Asia and America were included. Only statistically significant (5% level) inferences are shown that also resulted in an unambiguous biological interpretation. The inferences are ordered from the highest clade level in the nested analysis (the oldest contrasts in the haplotype tree) to the lowest clade level (the most recent contrasts). When no inference is indicated on the first inference line in Table 1, either no statistically significant associations were detected at the highest nesting level or their biological meaning was ambiguous. Similarly, if no entry occurs on the bottom line, no statistically significant, biologically unambiguous inference occurred at the most recent level of analysis. Inferences are ordered solely with respect to the nesting structure for a specific gene or DNA region. Table 1 does not give a temporal ordering of inferences across genes.

The output from even a single GEODIS analysis is lengthy, so the results of the new analyses are not given here but are available in the Supplementary Information. Table 2 presents the unambiguous biological inferences associated with statistically significant phylogeographic associations, with relative temporal ranks within each gene.

Integrating inferences across loci

There is no expectation that every haplotype tree should yield the same inferences. The inferences obtained from any one tree depend upon several sampling features, which are summarized in Table 3 for the data sets analysed. Table 3 reflects the numbers used in the actual GEODIS analyses, which may differ from the numbers given in the original references. For example, the genetic survey of a non-coding region on the X chromosome was performed on 69 individuals, each from a different location¹⁵. Having a sample size of one at every location precludes any assessment of within-population variation versus between-populations variation, so several nearby populations were pooled to reduce the total number of geographical sites to 29 for the purposes of the nested phylogeographic analysis³³.

One sampling constraint on inference is the spatial spread of the

samples relative to the distribution of the species. Not all data sets covered the same geographic areas, and the inferences from any one data set are confined to the area sampled. Another constraint is the number of locations sampled, which varies from 4 to 35 (Table 3). For example, the analysis of the mtDNA of Torroni *et al.*^{11,12} was unique in detecting range expansion, long-distance dispersal, and isolation by distance within the Americas. Most of the other data sets had only one native American population, thereby making inferences within the American continents impossible. As the number of locations within an area increases, the spatial scale of inference becomes finer.

A third aspect of sampling is sample size, which varies from 35 to 1,544 (Table 3). Biological inference is confined to statistically significant associations, so the number of individuals sampled is important. In general, the larger the sample size, the more power in making inferences. Also, there is generally more power to detect older events or processes that influence many populations and haplotypes than more recent events or forces. For example, the smallest sample in Table 3 (the X-linked *PDHA1* locus) could only detect a statistically significant signal at the highest level of nesting, a contrast that makes use of all 35 individuals sampled.

The DNA region sampled also determines inference properties. For example, only mtDNA detected the fragmentation event between native American populations and Old World (Asian) populations (Table 1). This event was too recent (perhaps only 14,000 years ago) to have much chance of being marked by mutations in the other genetic systems, owing to their slower rates of overall evolution. Moreover, major range expansions can occur that are not detected by a particular locus simply because mutations did not occur at the right time and place to mark the event²³. Thus, although the nested analysis is not prone to false positives, any one haplotype tree may not detect certain events or processes owing to inadequate genetic resolution²³. Genetic resolution also constrains what is meant by a 'recurrent' evolutionary process²¹. For example, when gene flow is inferred, it does not necessarily mean that gene flow occurred every generation, but rather that gene flow occurred recurrently relative to the mutation

Table 2 New inferences using the GEODIS program

DNA region	mtDNA	PDHA1	MS205	MC1R	MX1	EDN	ECP
Reference*	9	14	19	18	17	20	20
Range	Global	Africa and Eurasia	Global	Global	Global	Global	Global
	RE out of Africa	IBD	RE, origin ambiguous RE to Pacific and America	RE out of Africa	IBD RE to Pacific	IBD	IBD
	F (America)		IBD, RE to Pacific	RE to N Eurasia	IBD, RE to America	RE to America	

Inference abbreviations are the same as given in Table 1. Inferences are shown from oldest (top) to most recent (bottom).

* Reference for the data being analysed.

Table 3 Sampling properties of the data sets analysed in Tables 1 and 2

Locus or DNA region and reference	Pattern of inheritance	Number of individuals	Number of locations	Number of haplotypes	TMRCA* (Myr ago)
mtDNA ^{36,37}	Maternal haploid	1,389	12	77	0.24
mtDNA ^{11,12}	Maternal haploid	532	21	109	0.24
mtDNA ¹⁰	Maternal haploid	1,178	14	127	0.24
mtDNA ⁹	Maternal haploid	53	16	51	0.24
SRY and YAP ¹³	Paternal haploid	1,544	35	10	0.23
X non-coding ¹⁵	Haplo-diploid	69	29	33	0.67
PDHA1 ¹⁴	Haplo-diploid	35	8	11	1.91
Haemoglobin β ¹⁶	Diploid	160	9	14	1.63
EDN ²⁰	Diploid	67	4	9	1.15
ECP ²⁰	Diploid	67	4	8	1.09
MC1R ¹⁸	Diploid	121	6	7	0.85
MX1 ¹⁷	Diploid	354	21	10	8.5
MS205 ¹⁹	Diploid	389	15	15	1.25

*Time to the most recent common ancestral haplotype.

rate at that locus. Even for the rapidly evolving mtDNA, populations isolated from one another for a few thousand years would not be detected as fragmented in a nested clade analysis.

The temporal sampling period also varies across loci. A haplotype tree contains information up to but excluding the final coalescent event to a common ancestral molecule. Different genes have different expected coalescent times as a function of their pattern of inheritance (Table 4). Hence, the unisexual haploid elements (mtDNA and Y-DNA) are expected to detect only relatively recent events, whereas the bisexual X-linked and autosomal genes are expected to detect far older events. There is also a large variance in coalescent times across loci sharing a common pattern of inheritance³⁶. For example, the expected coalescent time for a large sample at an autosomal locus under neutrality is $4N_{ef}$ and the variance is about $4.64N_{ef}$ (ref. 2), where N_{ef} is the long-term inbreeding effective size of the population (see Table 4). Thus, different DNA regions sharing a common pattern of inheritance are expected to show considerable variation in their temporal depths, as is indeed found in the DNA regions analysed (Table 3).

The dates in Table 3 were obtained using the method of ref. 37 that estimates the time to the most recent common ancestral haplotype (TMRCA) by calculating the ratio of the average nucleotide differences within the human sample to one-half the average nucleotide difference between chimpanzees and humans and multiplying the ratio by an estimate of the divergence time between humans and chimpanzees. Takahata *et al.*³⁷ used 5 million years for this divergence time, but recent fossil finds^{38,39} indicate that it is more likely to be at least 6 million years, a figure still compatible with the molecular data⁴⁰. Hence, the estimates³⁷ for PDHA1, Xq13.3, the β -globin locus, mtDNA and Y-DNA are recalculated with a calibration point of 6 million years. This same estimator is applied to the remaining loci used in this study, with the results shown in Table 3. Two loci require special consideration. The first is MX1, which has an estimated TMRCA of 8.5 million years ago, a date older than the chimpanzee–human divergence of 6 million years ago. A coalescent time older than the species is a true biological possibility known as trans-specific polymorphism. The TMRCA for MS205 had already been estimated at 1 million years⁴¹ with the calibration date of 5 million years, but is given as 1.2 million years in

Table 3 using the 6 million year calibration date.

Rannala and Bertorelle⁴² argue that phylogenetic dating approaches like that of ref. 37 are also appropriate for dating the ages of the older clades within a haplotype tree but are unreliable for the more recent clades. In particular, the procedure of ref. 37 ignores the sampling error in estimating the average nucleotide differences both within and between species and clades of haplotypes, and the error in the coalescent process itself due to genetic drift⁴⁰. These errors can be quite large for recent haplotypes⁴². Therefore, the procedure³⁷ is used only to date the inferences at the higher (older) nesting levels in the GEODIS analyses. The age of an inferred process or event is estimated as the age of the youngest monophyletic clade that contributed in a statistically significant fashion to the inference. This represents the time in the population's history during which all haplotype lineages affected by the event were present but more derived haplotype lineages not affected by the event had not yet evolved. This method of ageing reflects the fact that the mutations that occur during the event or process provide the strongest signal for biological inference under a nested clade analysis. For example, a mutation that occurs in a population in the act of expanding into a new geographical area provides the signal used by the nested clade analysis to infer range-expansion events^{21,23}. The age of the youngest clade marking an event or process is therefore expected to be largely coincident with the age of the event itself in most cases. However, there are exceptions. For example, a mutation occurring in a population shortly before a range expansion will have a large geographical range for its frequency once the expansion has occurred—another signal for range expansion in the nested clade analysis^{21,23}. Alternatively, a mutation occurring in the new geographical area shortly after a range expansion could create a new haplotype with a limited geographical range that is located far from the geographical centre of its ancestral haplotype—yet another signal for range expansion in the nested clade analysis^{21,23}. Thus, the estimated age of an event could sometimes be older or younger than the actual event.

Because the procedure of ref. 37 does not take into account the potentially large error associated with the coalescent process, I used my method⁴⁰ to fit a gamma distribution with a mean equal to the date estimated³⁷ and with a variance given by a neutral coalescent process given the amount of pairwise diversity observed within the monophyletic clade being dated. The ages of the inferred events and processes are therefore treated as random variables rather than constants. Because the dating of an event from any one gene could be either younger or older than the actual event and the error associated with any one gene can be large, a conservative approach is taken in reserving hard inference only for those events for which there is significant overlap (5% or more) in the probability distributions of the inferred ages from more than one gene. By requiring cross-validation over genes, a parsimonious interpretation of the number of events is obtained, and the dating of these events can be averaged over the genes to minimize errors associated

Table 4 Expected coalescent times for different inheritance patterns

DNA region	Pattern of inheritance	Expected coalescence time in generations
mtDNA	Maternal haploid	$2N_{ef} \approx N_{ef}^*$
Y-Chromosomal DNA	Paternal haploid	$2N_{efm} \approx N_{ef}$
X-linked DNA	Bisexual haplo-diploid, equal sex ratio	$3N_{ef}$
Autosomal DNA	Bisexual diploid	$4N_{ef}$

* N_{ef} is the long-term inbreeding effective size of the population, N_{efm} the inbreeding effective size of females, and N_{efm} the inbreeding effective size of males. The approximations to the expected coalescence time are made under the assumption that $N_{eff} = N_{efm} = N_{ef}/2$.

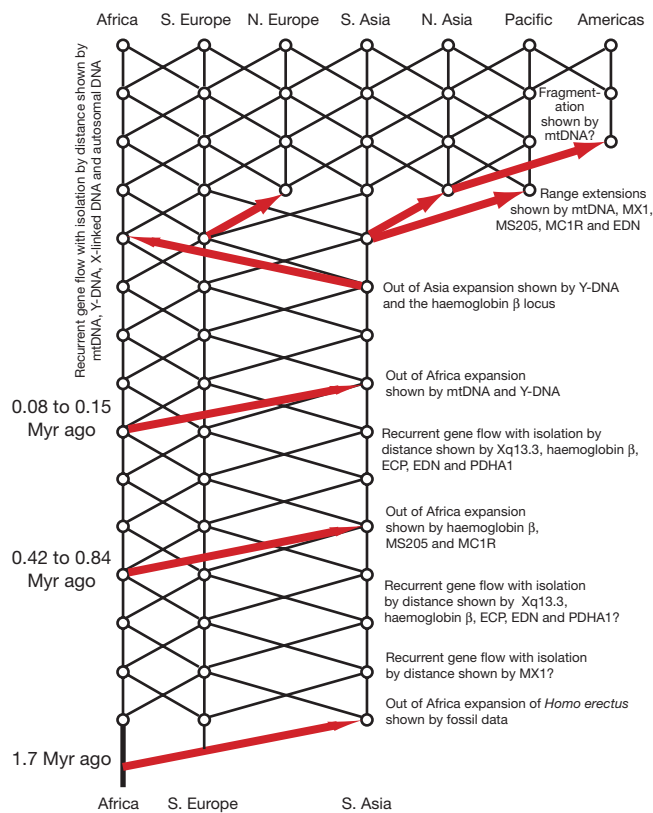


Figure 1 A new model of recent human evolution. All statistically significant inferences in Tables 1 and 2 are incorporated into this single model. Major expansions of human populations are indicated by red arrows. Genetic descent is indicated by vertical lines, and gene flow by diagonal lines. The timing of inferences lacking resolution at the 5% level and/or not validated by more than one locus are indicated by question marks.

with any one gene. Any event that is not cross-validated is regarded as tentative and questionable. There is no biological reason why dates of inferences of recurrent processes such as gene flow should be concordant across loci. However, a conservative approach is taken here as well in which those time intervals indicating gene flow that are not cross-validated at the 95% level are regarded as tentative and questionable.

Inferences on recent human evolution

All the inferences in Tables 1 and 2 can be placed into a single, internally consistent model of recent human evolution (Fig. 1). This model accepts the fossil evidence that the human lineage started in Africa and initially spread out of Africa about 1.7 million years ago⁴³. The oldest inferences for four genes (Table 2) are recurrent gene flow constrained by isolation by distance at the *MX1* locus (3.41 million years ago or Myr ago), *PDHA1* (0.86 Myr ago), *EDN* (0.69 Myr ago) and *ECP* (0.58 Myr ago). Two other genes yield the same inference in middle clades (Table 1) that date to a similar time period (β -globin, 0.49 Myr ago; and Xq 13.3, 0.48 Myr ago). Figure 2 shows the distributions for these ages. All of these six inferences of isolation by distance have broadly overlapping time intervals with the exception of *MX1*, the locus that samples the oldest time period (Table 3). The 95% confidence interval for the inference of isolation by distance from *MX1* ranges from 8.5 Myr ago to 0.61 Myr ago, indicating a low confidence in the timing of this gene flow. The spatial pattern of gene flow detected with *MX1* could not have existed until after there were human populations in Eurasia, so the fossil date of 1.7 Myr ago places a biological bound on the older limit of the age interval. This confidence interval is consistent with recurrent gene flow constrained by isolation by

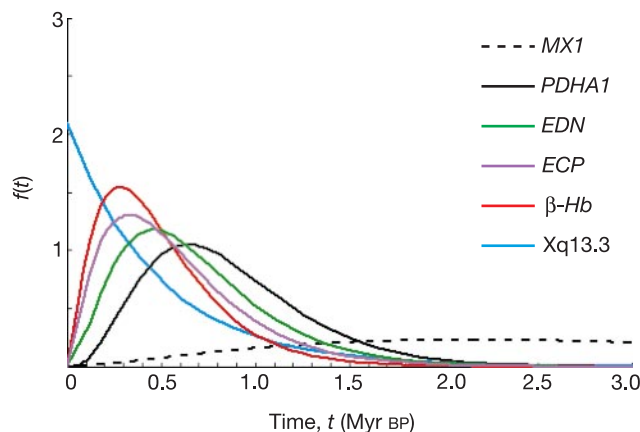


Figure 2 The distributions for the ages of the youngest clade contributing to a significant inference of gene flow constrained by isolation by distance at the highest nesting level for the genes *MX1*, *PDHA1*, *EDN* and *ECP*, and at intermediate nesting levels for the β -globin (β -*Hb*) locus and the Xq13.3 region. The x axis gives the time in millions of years before present (BP), and the y axis gives the gamma probability distribution, $f(t)$, that was fitted to the clade data.

distance shortly after the original expansion out of Africa because 21% of the probability mass of this gamma distribution is associated with times less than 1.7 Myr ago. However, no other interference of isolation by distance has 5% or more of its probability mass extending that far back in time. Moreover, *MX1* is an outlier in TMRCA (Table 3), which raises the possibility that natural selection has shaped the coalescent process at this locus. Therefore, the evidence for recurrent gene flow among the *Homo erectus* populations shortly after they first came out of Africa is tentative and of questionable validity.

Figure 2 shows that as time progresses, the evidence for gene flow with isolation by distance among Old World human populations is increasingly cross-validated even when *MX1* is excluded. For example, all five remaining inferences of restricted gene flow have more than 5% of their probability mass on times 1 Myr ago and older: *PDHA1* ($P = 0.32$), *EDN* ($P = 0.19$), *ECP* ($P = 0.13$), β -globin (0.08), and Xq13.3 (0.12) and the probability that one or more of these loci is detecting recurrent gene flow 1 Myr ago or older is 0.61. This probability increases to 0.95 by 0.61 Myr ago or older. These results indicate that recurrent gene flow occurred among Old World human populations from the present back to at least 600,000 years ago, and probably even further into the past.

The highest clade level inferences for five genes are range expansions out of Africa (mtDNA at 0.13 Myr ago; Y-DNA at 0.09 Myr ago; *MC1R* at 0.64 Myr ago; and β -globin at 0.82 Myr ago) and a range expansion of ambiguous origin (*MS205* at 0.63 Myr ago). Figure 3 shows the gamma distributions associated with the ages of these expansion events. The β -globin inference has 5.6% of its probability mass at 1.7 Myr ago or older, but all the other inferred range expansions have much less probability mass at 1.7 Myr ago or older. Hence, there is no cross-validated inference of marking the original expansion of *Homo erectus* out of Africa. Figure 3 shows that the five inferences fall into two broadly overlapping classes; the mtDNA and Y-DNA that are tightly clustered around an expansion event out of Africa at about 100,000 years ago, and the three autosomal loci with means between 0.64 to 0.82 Myr ago. To test the null hypothesis that all five loci are compatible with a single range expansion event, the lower age bound was found such that 0.1% of the β -globin probability distribution was above this age (this locus has the oldest inferred expansion event) and the upper age bound was found such that 0.1% of the Y-DNA probability distribution was below this age (this locus has the youngest inferred expansion event). Any age outside of this interval would be strongly

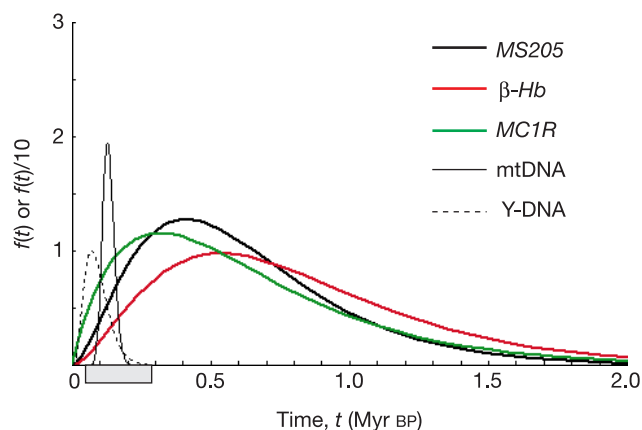


Figure 3 The distributions for the ages of the youngest clade contributing to a significant inference of a population range expansion for mtDNA, Y-DNA, *MC1R*, *MS205*, and the β -globin (*β -Hb*) locus. The y -axis gives the gamma probability distribution, $f(t)$, that was fitted to the clade data. Because the probability mass of the gamma distributions for mtDNA and Y-DNA are heavily concentrated into a small, recent, time interval, their probability distributions divided by ten, $f(t)/10$, are plotted instead. The grey area on the time axis indicates the interval of most likely overlap among these five distributions.

rejected (at the 0.1% level) on this basis of Y-DNA or β -globin alone. This interval spans between 0.0474 to 0.2906 Myr ago and is shown in grey in Fig. 3. The probability that all five loci are detecting an event in this age interval is 0.003. Because the expansion event detected with *MS205* is of ambiguous geographical origin, the calculation was repeated excluding that locus to focus specifically upon out-of-Africa expansion events. With this exclusion, the hypothesis of a single out-of-Africa expansion event is rejected with $P = 0.018$. The broad overlap of the mtDNA and Y-DNA distributions (Fig. 3) indicates that the null hypothesis of these two genetic elements marking the same out-of-Africa range-expansion event cannot be rejected, and similarly the null hypothesis that the three nuclear markers in Fig. 3 are marking the same expansion event cannot be rejected. The parsimonious explanation of Fig. 3 is therefore a minimum of two out-of-Africa expansion events, both of which are cross-validated by more than one locus. Combining the three nuclear genes, the 95% confidence interval for the older out-of-Africa range-expansion event is 0.42 to 0.84 Myr ago. Combining the mtDNA and Y-DNA distributions, the 95% confidence interval for the more recent out-of-Africa expansion event is 0.08 to 0.15 Myr ago.

The GEODIS analyses indicate that the most recent out-of-Africa expansion event was not a replacement event. If it had been, the three significant genetic signatures of the older expansion event (Fig. 3) and the six significant genetic signatures of older recurrent gene flow (Fig. 2) would have been wiped away. Although there is considerable error in dating any single inference from only one gene, an out-of-Africa replacement event would require that all nine significant inferences found in all eight bisexually inherited nuclear loci examined would have to be in error simultaneously. Moreover, the dating errors would have to be large in all nine cases and in the same direction. The hypothesis of a recent out-of-Africa replacement event is therefore strongly rejected. Indeed, this is the most highly cross-validated conclusion of the entire analysis because every gene region that is expected to contain information about events or processes substantially older than 0.15 Myr ago leads to a rejection of recent replacement. Hence, this is a genome-wide conclusion and not a locus-specific conclusion. The out-of-Africa expansion that took place between 0.08 to 0.15 Myr ago therefore represented a major movement of peoples characterized by interbreeding and not replacement.

It is likely that the earlier out-of-Africa expansion event between

0.42 to 0.84 Myr ago was also characterized by interbreeding and not replacement. Even excluding the *MX1* result, the other distributions shown in Fig. 2 jointly define a probability of 0.78 of recurrent gene flow before 0.84 Myr ago, a signal that would have been erased by replacement. Hence, the evidence suggests that the intermediate out-of-Africa expansion event shown in Fig. 1 was also not a replacement event, but this suggestion must be regarded as tentative because the probability of such ancient gene flow is less than 0.95.

Proceeding downwards in the nested inferences shown in Tables 1 and 2, several recent interferences are encountered of recurrent but restricted gene flow and of population expansions. Rannala and Bertorelle⁴² warned that recent events cannot be reliably dated phylogenetically. Their warning is confirmed by this analysis. The youngest clade marking many of these recent inferences is a tip haplotype that has no mutational derivatives, resulting in an average within-nucleotide difference of zero. It is impossible to use any method of phylogenetic dating in these cases. Even when the average within-nucleotide difference is greater than zero, the estimate is likely to be unreliable because the number of informative mutations for recent events is so small⁴². For example, the expansion of humans into the Americas as detected by *MS205* (Table 2) is dated phylogenetically at 449,000 years ago, with a 95% confidence interval of 1.18 Myr ago to 68,000 years ago. Even 68,000 years ago appears to be too old for this event¹⁷. The time estimate in this case depends upon just two mutational events, and as predicted⁴², the resulting estimate seems to be unreliable. Rannala and Bertorelle⁴² suggest that recent haplotypes should be dated instead through a population genetic approach that uses models of demography, mutation, and/or recombination to estimate allele age. However, the population genetic approach in turn is limited by the reliability of these underlying models. In light of these considerations, the recent inferences found in the lower hierarchical levels shown in Tables 1 and 2 will undoubtedly be more accurately dated by palaeontological and archaeological evidence than by genetic data. Therefore, no estimates of dates of these inferences will be given here.

Most of these recent interferences come from Y-DNA or mtDNA (Tables 1 and 2), and their nesting position means that they must be more recent than the most recent out-of-Africa expansion event found at the highest (oldest) nesting levels for Y-DNA and mtDNA. Many of these recent interferences are expansions of humans into areas not formerly occupied; such as northern Eurasia, the Pacific (including Australia), and the Americas. However, one of these recent expansion events did occur within the range of previous human occupation. Both the Y-DNA and β -globin locus detect a recent out-of-Asia expansion event that did not erase earlier genetic signals (Table 1) and therefore was an expansion/interbreeding event rather than an expansion/replacement event. Indeed, the failure to detect this recent out-of-Asia expansion event with mtDNA despite good sample sizes and genetic resolution may indicate that this recent out-of-Asia expansion event was primarily male-mediated, and therefore had to be characterized by interbreeding in order to leave a genetic signature.

Genetic contact among human populations after the latest out-of-Africa expansion is also indicated by several inferences at the lower clade levels of recurrent gene flow constrained by isolation by distance with occasional long-distance dispersal (Tables 1 and 2), a conclusion confirmed by other genetic analyses³². The only evidence for significant fragmentation (long-term genetic isolation) among the human populations who have left living descendants is the recent genetic isolation between Amerindians and the rest of humanity that followed the colonization of the Americas (Table 1). All of these inferences are summarized in Fig. 1.

Discussion

The model of recent human evolution shown in Fig. 1 is dominated

by genetic interchange and a special role for Africa. I consider first genetic interchange. African and Eurasian populations were linked by recurrent gene flow, certainly over the last half a million years, and probably longer. Overlaid upon this gene-flow trellis are occasional major movements out of Africa and out of Asia that enhanced gene interchange through interbreeding. More recently, population expansions acted to extend the geographical range of the human species and to establish additional areas linked by gene flow. This model emphasizes that genetic interchange among human populations, facilitated both by gene flow and range expansions coupled with interbreeding, has been a major force in shaping the human species and its spatial pattern of genetic diversity.

Second, Fig. 1 reveals the special role that African populations have played in human evolution. There were at least two major movements of peoples out of Africa after the original spread of *Homo erectus*. This inference is consistent with the archaeological record of cultural expansions out of Africa (Acheulean) in the middle Pleistocene^{44–48}. These Acheulean cultural expansions broadly overlap the time frame of the middle out-of-Africa expansion event shown in Fig. 1, indicating that this expansion involved both people and ideas coming out of Africa and interacting with local populations in Eurasia. This expansion is also compatible with the fossil data. After the initial expansion of *Homo erectus* out of Africa about 1.7 Myr ago, there was little change in average brain size up to 700,000 years ago¹. By 400,000 to 500,000 years ago, average cranial capacities had shown a substantial increase¹. The time period of this transition in cranial capacity overlaps extensively with the time period for the older out-of-Africa expansion event shown in Fig. 3.

The most recent out-of-Africa expansion event shown in Figs 1 and 3 is also compatible with fossil evidence. Many 'modern' traits (such as high, rounded skulls; small brow ridges; a vertical forehead; and a noticeable chin) first appear in Africa about 130,000 years ago, followed by an expansion out-of-Africa more than 90,000 years ago¹. This time frame overlaps extensively with the out-of-Africa expansion marked by the mtDNA and Y-DNA distributions in Fig. 3, implying that many of these traits could have been carried into Eurasia by this African population range expansion. Other traits, however, do not display any significant changes before, during or after this most recent expansion out of Africa¹. This later set of traits is difficult to reconcile with a population replacement, but is compatible with this most recent out-of-Africa expansion event being characterized by interbreeding. With interbreeding, mendelian inheritance allows some traits to spread while others do not. Moreover, living humans are still polymorphic for 'modern' traits, and the frequencies of different 'modern' traits show heterogeneity in their present geographical distributions¹. The current spatial and frequency heterogeneity in 'modern' traits undercuts the idea of a global replacement of an 'archaic' type by a 'modern' type but is consistent with a trait-based evolution of humans that is allowed under expansion with interbreeding. The model in Fig. 1 indicates the recent fossil evidence should be interpreted in terms of traits and not population types.

The genetic impacts of Africa upon the entire human species is large because of at least three major expansions out of Africa, although the genetic impact is not as complete as it would be under total replacement. This model is similar to earlier models that have emphasized the role of out-of-Africa population expansion coupled with gene flow and not replacement, such as the assimilation model of Smith *et al.*⁴⁹, the multiregional model with expansions followed by admixture of Wolpoff *et al.*⁵⁰, and the 'mostly out of Africa' model of Relethford¹.

The predicted large genetic impact of African populations explains the results of Takahata *et al.*³⁷ that about 90% of the haplotype trees in the nuclear genome appear to be rooted in Africa. These results³⁷ also falsify a total replacement hypothesis, which predicts that all haplotype trees with coalescent times greater

than 100,000 years must be rooted in Africa. All of the haplotype trees considered³⁷ have expected coalescent times greater than 100,000 years, so 100% of such old trees should have African roots under complete replacement, and not the observed 90%.

The results given here show the importance of examining many DNA regions with a common analytical technique in making phylogeographic inferences. Indeed, the clearest result from Tables 1 and 2 is how incomplete our view of human evolution would be if it were based upon just one locus or DNA region. As more DNA regions are examined, additional insights into human evolution are sure to follow. However, this current analysis already demonstrates the inadequacies of both the out-of-Africa replacement model and of a simple trellis model. Humans expanded again and again out of Africa, but these expansions resulted in interbreeding, not replacement, and thereby strengthened the genetic ties between human populations throughout the world. □

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1. Relethford, J. H. *Genetics and the Search for Modern Human Origins* (Wiley, New York, 2001).
2. Stoneking, M. & Soodyall, H. Human evolution and the mitochondrial genome. *Curr. Opin. Genet. Dev.* **6**, 731–736 (1996).
3. Wolpoff, M. H., Hawks, J. & Caspari, R. Multiregional, not multiple origins. *Am. J. Phys. Anthropol.* **112**, 129–136 (2000).
4. Weidenreich, F. *Apes, Giants, and Man* (Univ. Chicago Press, Chicago, 1946).
5. Cann, R. L., Stoneking, M. & Wilson, A. C. Mitochondrial DNA and human evolution. *Nature* **325**, 31–36 (1987).
6. Vigilant, L., Stoneking, M., Harpending, H., Hawkes, K. & Wilson, A. C. African populations and the evolution of human mitochondrial DNA. *Science* **253**, 1503–1507 (1991).
7. Maddison, D. R. African origin of human mitochondrial DNA reexamined. *Syst. Zool.* **40**, 355–363 (1991).
8. Templeton, A. R. Human origins and analysis of mitochondrial DNA sequences. *Science* **255**, 737 (1992).
9. Ingman, M., Kaessmann, H., Pääbo, S. & Gyllenstein, U. Mitochondrial genome variation and the origin of modern humans. *Nature* **408**, 708–713 (2000).
10. Sykes, B., Leiboff, A., Low-Beer, J., Tetzner, S. & Richards, M. The origins of the Polynesians: an interpretation from mitochondrial lineage analysis. *Am. J. Hum. Genet.* **57**, 1463–1475 (1995).
11. Torroni, A. *et al.* Asian affinities and continental radiation of the four founding native American mtDNAs. *Am. J. Hum. Genet.* **53**, 563–590 (1993).
12. Torroni, A. *et al.* mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with Native Americans. *Am. J. Hum. Genet.* **53**, 591–608 (1993).
13. Hammer, M. F. *et al.* Out of Africa and back again: Nested cladistic analysis of human Y chromosome variation. *Mol. Biol. Evol.* **15**, 427–441 (1998).
14. Harris, E. E. & Hey, J. X. Chromosome evidence for ancient human histories. *Proc. Natl Acad. Sci. USA* **96**, 3320–3324 (1999).
15. Kaessman, H., Heißig, F., Haeseler, A. V. & Pääbo, S. DNA sequence variation in a non-coding region of low recombination on the human X chromosome. *Nature Genet.* **22**, 78–81 (1999).
16. Harding, R. M. *et al.* Archaic African and Asian lineages in the genetic ancestry of modern humans. *Am. J. Hum. Genet.* **60** 772–789 (1997).
17. Jin, L. *et al.* Distribution of haplotypes from a chromosome 21 region distinguishes multiple prehistoric human migrations. *Proc. Natl Acad. Sci. USA* **96**, 3796–3800 (1999).
18. Rana, B. K. *et al.* High polymorphism at the human melanocortin 1 receptor locus. *Genetics* **151**, 1547–1557 (1999).
19. Rogers, E. J., Shone, A. C., Alonso, S., May, C. A. & Armour, J. A. L. Integrated analysis of sequence evolution and population history using hypervariable compound haplotypes. *Hum. Mol. Genet.* **9**, 2675–2681 (2000).
20. Zhang, J. & Rosenberg, H. F. Sequence variation at two eosinophil-associated ribonuclease loci in humans. *Genetics* **156**, 1949–1958 (2000).
21. Templeton, A. R., Routman, E. & Phillips, C. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* **140**, 767–782 (1995).
22. Posada, D., Crandall, K. A. & Templeton, A. R. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Mol. Ecol.* **9**, 487–488 (2000).
23. Templeton, A. R. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol.* **7**, 381–397 (1998).
24. Bernatchez, L. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* **55**, 351–379 (2001).
25. Gomez, A., Carvalho, G. R. & Hunt, D. H. Phylogeography and regional endemism of a passively dispersing zooplankton: mitochondrial DNA variation in rotifer resting egg banks. *Proc. R. Soc. Lond. B* **267**, 2189–2197 (2000).
26. Nielson, M., Lohman, K. & Sullivan, J. Phylogeography of the tailed frog (*Ascaphus truei*): Implications for the biogeography of the Pacific Northwest. *Evol.* **55**, 147–160 (2001).
27. Turner, T. F., Trexler, J. C., Harris, J. L. & Haynes, J. L. Nested cladistic analysis indicates population fragmentation shapes genetic diversity in a freshwater mussel. *Genetics* **154**, 777–785 (2000).
28. Templeton, A. R., Crandall, K. A. & Sing, C. F. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**, 619–633 (1992).
29. Posada, D. TCS 1.06 (Provo, Utah, 2000); available at http://bioag.byu.edu/zoology/crandall_lab/tcs.htm.
30. Crandall, K. A. & Templeton, A. R. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**, 959–969 (1993).

31. Templeton, A. R. in *Conceptual Issues in Modern Human Origins Research* (eds Clark, G. A. & Willermet, C. M.) 329–360 (Aldine de Gruyter, New York, 1997).
32. Templeton, A. R. Human races: A genetic and evolutionary perspective. *Am. Anthropol.* **100**, 632–650 (1998).
33. Templeton, A. R. Using phylogeographic analyses of gene trees to test species status and processes. *Mol. Ecol.* **10** 779–791 (2001).
34. Excoffier, L. & Langaney, A. Origin and differentiation of human mitochondrial DNA. *Am. J. Human Genet.* **44**, 73–85 (1989).
35. Excoffier, L. Evolution of human mitochondrial DNA: evidence for departure from a pure neutral model of populations at equilibrium. *J. Mol. Evol.* **30**, 125–139 (1990).
36. Donnelly, P. & Tavaré, S. Coalescents and genealogical structure under neutrality. *Annu. Rev. Genet.* **29**, 401–421 (1995).
37. Takahata, N., Lee, S.-H. & Satta, Y. Testing multiregionality of modern human origins. *Mol. Biol. Evol.* **18**, 172–183 (2001).
38. Haile-Selassie, Y. Late Miocene hominids from the Middle Awash, Ethiopia. *Nature* **412**, 178–181 (2001).
39. Pickford, M. & Senut, B. The geological and faunal context of the Late Miocene hominid remains from Lukeino, Kenya. *C.R. Acad. Sci. IIA* **332**, 145–152 (2001).
40. Templeton, A. R. The “Eve” hypothesis: a genetic critique and reanalysis. *Am. Anthropol.* **95**, 51–72 (1993).
41. Alonso, S. & Armour, J. A. L. A highly variable segment of human subterminal 16p reveals a history of population growth for modern humans outside Africa. *Proc. Natl. Acad. Sci. USA* **98**, 864–869 (2001).
42. Rannala, B. & Bertorelle, G. Using linked markers to infer the age of a mutation. *Hum. Mutat.* **18**, 87–100 (2001).
43. Gabunia, L. *et al.* Earliest Pleistocene hominid cranial remains from Dmanisi, Republic of Georgia: Taxonomy, geological setting, and age. *Science* **288**, 1019–1025 (2000).
44. Aguirre, E. & Carbonell, E. Early human expansions into Eurasia: The Atapuerca evidence. *Quat. Int.* **75**, 11–18 (2001).
45. Bar-Yosef, O. & Belfer-Cohen, A. From Africa to Eurasia—early dispersals. *Quat. Int.* **75**, 19–28 (2001).
46. Hou, Y. M. *et al.* Mid-Pleistocene Acheulean-like stone technology of the Bose basin, South China. *Science* **287**, 1622–1626 (2000).
47. Saragusti, I. & Goren-Inbar, N. The biface assemblage from Gesher Benot Ya’aqov, Israel: illuminating patterns in “Out of Africa” dispersal. *Quat. Int.* **75**, 85–89 (2001).
48. Otte, M. in *Archaeogenetics: DNA and the Population Prehistory of Europe* (eds Renfrew, C. & Boyle, K.) 41–44 (Univ. Cambridge, Cambridge, 1999).
49. Smith, F. H., Falsetti, A. B. & Donnelly, S. M. Modern human origins. *Yb. Physical Anthropol.* **32**, 35–68 (1989).
50. Wolpoff, M., Thorne, A. G., Smith, F. H., Frayer, D. W. & Pope, G. G. in *Origins of Anatomically Modern Humans* (eds Nitecki, M. H. & Nitecki, D. V.) 175–200 (Plenum, New York, 1994).

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