

1-1-1997

Alu Insertion Polymorphisms and Human Evolution: Evidence for a Larger Population Size in Africa

Mark Stoneking
Pennsylvania State University

Jennifer J. Fontius
Lawrence Livermore National Laboratory

Stephanie L. Clifford
Pennsylvania State University

Himla Soodyall
Pennsylvania State University

Santosh S. Arcot
Lawrence Livermore National Laboratory

See next page for additional authors

Follow this and additional works at: https://repository.lsu.edu/biosci_pubs

Recommended Citation

Stoneking, M., Fontius, J., Clifford, S., Soodyall, H., Arcot, S., Saha, N., Jenkins, T., Tahir, M., Deininger, P., & Batzer, M. (1997). Alu Insertion Polymorphisms and Human Evolution: Evidence for a Larger Population Size in Africa. *Genome Research*, 7(11), 1061-1071. <https://doi.org/10.1101/gr.7.11.1061>

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Scholarly Repository. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Scholarly Repository. For more information, please contact ir@lsu.edu.

Authors

Mark Stoneking, Jennifer J. Fontius, Stephanie L. Clifford, Himla Soodyall, Santosh S. Arcot, Nilmani Saha, Trefor Jenkins, Mohammad A. Tahir, Prescott L. Deininger, and Mark A. Batzer

LETTER

Alu Insertion Polymorphisms and Human Evolution: Evidence for a Larger Population Size in Africa

Mark Stoneking,¹ Jennifer J. Fontius,² Stephanie L. Clifford,¹
Himla Soodyall,^{1,3} Santosh S. Arcot,² Nilmani Saha,⁴ Trefor Jenkins,³
Mohammad A. Tahir,⁵ Prescott L. Deininger,^{6,7} and Mark A. Batzer^{2,8,9}

¹Department of Anthropology, Pennsylvania State University, University Park, Pennsylvania 16802; ²Human Genome Center, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California 94551; ³Department of Human Genetics, The South African Institute for Medical Research, University of the Witwatersrand, Johannesburg 2000, South Africa; ⁴Department of Pediatrics, National University of Singapore, Singapore; ⁵Indianapolis–Marion County Forensic Services Agency, Indianapolis, Indiana 46204; ⁶Department of Biochemistry and Molecular Biology; ⁸Department of Pathology and Department of Biometry and Genetics, Stanley S. Scott Cancer Center, Neuroscience Center of Excellence, Louisiana State University Medical Center, New Orleans, Louisiana 70112; ⁷Laboratory of Molecular Genetics, Alton Ochsner Medical Foundation, New Orleans, Louisiana 70121

Alu insertion polymorphisms (polymorphisms consisting of the presence/absence of an *Alu* element at a particular chromosomal location) offer several advantages over other nuclear DNA polymorphisms for human evolution studies. First, they are typed by rapid, simple, PCR-based assays; second, they are stable polymorphisms—newly inserted *Alu* elements rarely undergo deletion; third, the presence of an *Alu* element represents identity by descent—the probability that different *Alu* elements would independently insert into the exact same chromosomal location is negligible; and fourth, the ancestral state is known with certainty to be the absence of an *Alu* element. We report here a study of 8 loci in 1500 individuals from 34 worldwide populations. African populations exhibit the most between-population differentiation, and the population tree is rooted in Africa; moreover, the estimated effective time of separation of African versus non-African populations is $137,000 \pm 15,000$ years ago, in accordance with other genetic data. However, a principal coordinates analysis indicates that populations from Sahul (Australia and New Guinea) are nearly as close to the hypothetical ancestor as are African populations, suggesting that there was an early expansion of tropical populations of our species. An analysis of heterozygosity versus genetic distance suggests that African populations have had a larger effective population size than non-African populations. Overall, these results support the African origin of modern humans in that an earlier expansion of the ancestors of African populations is indicated.

The *Alu* family of short interspersed elements is one of the most successful mobile genetic elements, having arisen to a copy number in excess of 500,000 within primate genomes in the last 65 million years (for recent reviews, see Okada 1991; Schmid and Maraia 1992; Deininger and Batzer 1993, 1995). *Alu* repeats are thought to be ancestrally derived from the 7SL RNA gene and mobilize through an RNA polymerase III-derived transcript in a process termed retroposition. Each *Alu* sequence is ~300bp

in length; therefore, *Alu* repeats comprise ~5% of the human genome.

Alu sequences can be divided into different subfamilies or clades of related elements based on commonly shared diagnostic mutations. Here, we use the new standardized nomenclature to refer to various *Alu* subfamilies (Batzer et al. 1996a). Two of the most recently formed subfamilies of *Alu* elements within the human genome have been termed Ya5 and Ya8 (Batzer et al. 1990; Batzer and Deininger 1991). Members of the Ya8 *Alu* subfamily are characterized by all five of the Ya5 diagnostic mutations, as well as three additional diagnostic mutations. Be-

⁹Corresponding author.
E-MAIL mbatze@lsumc.edu; FAX (504) 568-6037.

STONEKING ET AL.

cause both the Ya5 and Ya8 *Alu* subfamilies share a number of diagnostic mutations we refer to this lineage collectively as Ya5/8 (Batzer et al. 1996b). The Ya5/8 *Alu* subfamily lineage is comprised of 500–2000 elements that are restricted to the human genome (Arcot et al. 1996), although a few Ya5 *Alu* family members have been found in chimpanzees (for review, see Deininger and Batzer 1995). In parallel, a second subfamily that is an independent derivative of the Y lineage of *Alu* sequences, termed Yb8, has also expanded to ~500 copies within the human genome (Batzer et al. 1995, 1996a).

Some Ya5/8 and Yb8 *Alu* elements have retroposed so recently that they are polymorphic for presence/absence at a specific location within the human genome (Batzer and Deininger 1991; Batzer et al. 1991, 1995; Kass et al. 1994; Hammer 1995). The distribution of these elements varies in different human population groups (Batzer and Deininger 1991; Batzer et al. 1991, 1994, 1995, 1996b; Perna et al. 1992; Kass et al. 1994; Hammer 1995). These polymorphic Ya5/8 and Yb8 *Alu* insertions serve as a unique set of nuclear DNA markers for the study of human evolution, as they are stable polymorphisms that are identical by descent. In addition, the ancestral state of each *Alu* insertion is known, facilitating accurate rooting of population networks (Batzer et al. 1994).

Previously, we have reported on 4 *Alu* insertion polymorphisms in 16 worldwide populations; here, we analyze the distribution of eight polymorphic *Alu* insertions in a survey of 1500 individuals from 34 worldwide population groups. Our results indicate that these polymorphic *Alu* insertions have an African origin, although populations from Sahul (Australia and New Guinea) are also close to the hypothetical ancestral population, possibly indicating an early expansion of human populations in the tropics. Using a model specific for *Alu* insertion polymorphisms, we estimate that the effective separation time between African and non-African populations was relatively recent, in accordance with estimates from other genetic data. We also find that African populations probably have had a larger effective population size than non-African populations; overall, our results are in agreement with an earlier expansion of populations in Africa, which corresponds to an African origin of modern humans.

RESULTS AND DISCUSSION

Population Statistics

An average of 1500 individuals from 34 worldwide

populations (Fig. 1) were typed for each of the 8 *Alu* insertion polymorphisms (Table 1). The allele frequencies and sample sizes for each population are shown in Table 2. All loci were polymorphic in all populations, with the exception of D1 in Nigerians, FXIIB in CAR Pygmies, and A25 in the Moluccans, which were all fixed for the absence of the *Alu* element (Table 2). Significant departures from Hardy–Weinberg equilibrium expectations were observed for 10 of 269 comparisons. Because ~13 comparisons would be expected to be significant at the 5% level by chance alone and because none of the significant departures cluster by locus or by population, we consider these to represent normal statistical fluctuations. The probabilities of these χ^2 outliers range from $Pr = 0.04$ to $Pr = 0.003$.

The average heterozygosity for each locus was substantial, with several values approaching the theoretical maximum heterozygosity of 0.5 for a bi-allelic locus (Table 1a). Although we expected that heterozygosity would be high, because these loci were ascertained on the basis that they were known to be polymorphic, an analysis of the distribution of allele frequencies at loci ascertained in a similar manner indicates that ascertainment bias alone does not completely account for the observed frequency spectrum; the distributions also contain information on the demographic history of human populations (Sherry et al. 1997).

The G_{st} values (a measure of the amount of subpopulation differentiation) were also substantial (Table 1a), and were statistically significant for each locus by a χ^2 contingency analysis (not shown), indicating that there are significant differences in the frequency of the *Alu* element across these human populations at each of these eight loci. The average G_{st} value was 0.128, comparable to other studies of nuclear DNA polymorphisms in human populations (Nei and Roychoudhury 1982; Jorde et al. 1995), and means that ~13% of the total variance in allele frequency differences at these eight loci is found between populations and 87% is found within populations.

For comparing patterns of variation among populations, we grouped the 34 populations into 6 geographic regions, consisting of Africa, Europe, Western Asia, Southeast Asia, Sahul (Australia and New Guinea), and the Americas (Table 1b). The average heterozygosity for each region was substantial, ranging from a low of 0.308 in Sahul to a high of 0.414 in Western Asia (Table 1b). The G_{st} value was calculated for the differentiation among the subpopulations within each region and was greatest in Africa (Table 1b). With respect to these loci, Af-

ALU INSERTION POLYMORPHISMS IN HUMAN POPULATIONS



Figure 1 Map of sample localities.

rican populations show the most between-population diversity of any geographic region of the world, although it should be kept in mind that this finding is based on just eight loci.

Population Relationships

We used both tree reconstruction and principal coordinates (PC) analysis to investigate population relationships. Tree reconstruction methods depict population relationships as a series of bifurcations, which are commonly interpreted as representing population splits; however, it is important to realize that clusters of populations in such trees could arise from migration instead of from shared ancestry. A neighbor-joining tree depicting the population relationships (Fig. 2) shows some concordance with geography. All of the African populations cluster together, as do the Sahulian and the European populations. The Southeast Asian and American populations are intermingled in one cluster. This may re-

flect inadequate sampling of populations from the Americas in this study, as a more extensive study of 5 of these loci in 24 native American populations does show more clustering of native American populations separate from Southeast Asian populations (Novick et al. 1997). The Western Asian populations are not clustered together on the tree, which could reflect either genetic contributions from both eastern Asia and Europe to these geographically intermediate populations, or an inability to accurately resolve population relationships with just eight loci.

To root the tree, we included a hypothetical ancestral population in which the frequency of the *Alu* element at each locus was set to zero. The root of the tree is within the cluster of African populations (Fig. 2), in agreement with an analysis of other gene frequency data that used the chimpanzee as an outgroup (Nei and Takezaki 1996). Apparently the *Alu* insert frequencies in African populations have undergone the least amount of change from the ancestral state. Assuming that allele frequency change at

Table 1. Population Statistics for 8 *Alu* Insertion Polymorphisms in 34 Populations

<i>a. Loci</i>				
Locus	No. of individuals		Heterozygosity (\pm S.E.) ^a	G _{st}
TPA25	1525		0.485 \pm 0.003	0.079
PV92	1432		0.490 \pm 0.003	0.212
APO	1501		0.267 \pm 0.009	0.066
ACE	1528		0.493 \pm 0.002	0.092
FXIIIB	1396		0.494 \pm 0.002	0.282
D1	1434		0.405 \pm 0.007	0.075
A25	1593		0.275 \pm 0.009	0.061
B65	1593		0.497 \pm 0.001	0.089
Average	1500		0.426 \pm 0.035	0.128
<i>b. Geographic regions</i>				
Region ^b	No. of populations	No. of individuals	Heterozygosity (\pm S.E.) ^a	G _{st}
Africa	6	176	0.402 \pm 0.030	0.088
Europe	7	334	0.396 \pm 0.052	0.011
Western Asia	7	262	0.414 \pm 0.041	0.036
Southeast Asia	7	359	0.377 \pm 0.042	0.058
Sahul	3	185	0.308 \pm 0.039	0.001
Americas	4	184	0.381 \pm 0.049	0.026
World	34	1500	0.426 \pm 0.035	0.128

^aMean of the population heterozygosities.
^bAs indicated by color in Fig. 1.

these loci occurs primarily by drift, which in turn is influenced by population size, this suggests that African and non-African populations have a different demographic history. One possible scenario involves an early expansion of the ancestors of African populations, thereby “freezing” allele frequencies when they are more similar to the root, followed by a later expansion of the ancestors of non-African populations, when allele frequencies would have drifted farther from the root. Alternatively, non-African populations may have been derived from African populations by a bottleneck event, which would have accelerated drift in the non-African populations.

Bootstrap resampling (Felsenstein 1985) was used to assess the strength of support of the data for the branching structure of the tree. A total of 500 bootstrap replications were performed, of which 52% placed the root as shown (Fig. 2). An additional 12% of the bootstrap replications placed the root somewhere else within the cluster of African populations, which would still be consistent with an Af-

rican origin. The bootstrap values for various other clusters in the tree ranged from 26% to 58% (Fig. 2). None of these values reach statistical significance, which is not surprising with just eight loci; however, the bootstrap is known to underestimate the true level of statistical support (Sitnikova et al. 1995); hence, there may be more support for the clusters in the tree than the bootstrap values alone would indicate. Overall, these results are concordant with numerous other genetic polymorphisms that appear to have their source in Africa and/or indicate an early, separate expansion of African populations (Stoneking 1993; Bowcock et al. 1994; Goldstein et al. 1995; Armour et al. 1996; Tishkoff et al. 1996) and that have been taken as evidence for an African origin of our species.

A PC analysis (Cavalli-Sforza et al. 1994) of the allele frequencies at the eight polymorphic *Alu* insertion loci was also performed (Fig. 3). The first two coordinates, which provide the most information for a two-dimensional depiction of population relationships, account for 73% of the variance in the

ALU INSERTION POLYMORPHISMS IN HUMAN POPULATIONS

Table 2. *Alu* Insertion Frequencies in 34 Human Populations

	<i>N</i> ^a	ACE	TPA25	PV92	APO	FXIIB	D1	A25	B65
Alaska Natives	41	0.58	0.30	0.62	0.92	0.92	0.42	0.15	0.45
Australia	69	0.91	0.13	0.15	0.87	0.65	0.04	0.35	0.39
Bretons	54	0.48	0.56	0.27	0.90	0.40	0.39	0.16	0.56
China	49	0.67	0.35	0.86	0.82	0.71	0.17	0.10	0.35
European-American	57	0.51	0.56	0.18	0.94	0.47	0.44	0.20	0.56
Filipino	47	0.53	0.63	0.80	0.98	0.72	0.36	0.14	0.57
French	53	0.48	0.56	0.23	0.99	0.42	0.46	0.16	0.57
French Acadian	46	0.51	0.43	0.18	0.92	0.48	0.42	0.12	0.53
Greek Cypriot	48	0.39	0.53	0.25	0.95	0.62	0.27	0.12	0.65
Greenland Natives	41	0.55	0.33	0.61	0.94	0.79	0.45	0.17	0.19
India-Christian	27	0.60	0.57	0.48	0.67	0.61	0.28	0.14	0.31
India-Hindu	28	0.52	0.34	0.52	0.85	0.66	0.10	0.05	0.35
India-Muslim	26	0.52	0.41	0.30	0.86	0.66	0.32	0.12	0.40
Java	32	0.86	0.39	0.84	0.78	0.92	0.42	0.06	0.58
!Kung	40	0.29	0.17	0.20	0.88	0.17	0.16	0.61	0.50
Malaysian	47	0.64	0.50	0.72	0.76	0.73	0.27	0.02	0.42
Mayan	51	0.68	0.65	0.79	0.94	0.90	0.45	0.21	0.27
Moluccan	48	0.67	0.56	0.69	0.76	0.78	0.19	0.00	0.26
Mvskoke	50	0.70	0.49	0.57	0.96	0.79	0.46	0.21	0.48
Nguni	43	0.40	0.21	0.24	0.60	0.12	0.27	0.41	0.60
Nigerian	11	0.27	0.41	0.09	0.50	0.08	0.00	0.22	0.83
Pakistan	42	0.44	0.51	0.30	0.72	0.23	0.17	0.07	0.37
PNG-Coastal	48	0.66	0.16	0.36	0.66	0.30	0.17	0.02	0.27
PNG-Highland	68	0.74	0.16	0.24	0.68	0.30	0.01	0.04	0.18
Pushtoon	50	0.52	0.55	0.33	0.86	0.57	0.27	0.18	0.49
Pygmy-CAR	17	0.12	0.21	0.26	0.74	0.00	0.47	0.35	0.78
Pygmy-Zaire	17	0.32	0.24	0.35	0.85	0.03	0.59	0.53	0.82
Sotho	48	0.38	0.33	0.29	0.68	0.18	0.31	0.39	0.48
Swiss	43	0.37	0.45	0.20	0.94	0.48	0.34	0.12	0.58
Taiwan	46	0.50	0.64	0.90	0.93	0.97	0.38	0.22	0.54
Tamill	47	0.69	0.56	0.56	0.81	0.61	0.34	0.17	0.55
Tenggaras	90	0.64	0.38	0.50	0.78	0.81	0.19	0.05	0.40
Turkish Cypriot	33	0.33	0.58	0.33	0.98	0.39	0.35	0.09	0.64
UAE	42	0.33	0.44	0.30	0.97	0.39	0.08	0.12	0.41

^a(*N*) Number of individuals.

data. The same relationships that were evident in the neighbor-joining tree (Fig. 2) are also present here (Fig. 3); namely, geographic clusters of African and European populations are evident, whereas the New World populations are intermingled with the Southeast Asian populations. Two Sahulian populations cluster together in the analysis while the third is intermingled with the more dispersed Western Asian populations. Furthermore, the spread of populations within each geographic cluster is qualitatively similar to the G_{st} results (Table 1b); African, Southeast Asian, and Western Asian populations have the highest G_{st} values and exhibit the greatest spread. However, whereas the ancestral population

in the tree (lacking the *Alu* element at each locus) is closest to the African populations, the African and Sahulian populations appear to be equally close to the ancestral population in the PC plot. This is because African populations are most similar to the ancestral population in the first PC, but Sahulian populations are most similar in the second PC.

A previous PC analysis of a subset of these data (Batzer et al. 1994) by Harpending et al. (1996) also found African and Sahulian populations to be closest to the root. Harpending et al. suggest this indicates an earlier expansion of the ancestors of tropical populations, followed by a later expansion of the ancestors of more peripheral populations such as

STONEKING ET AL.

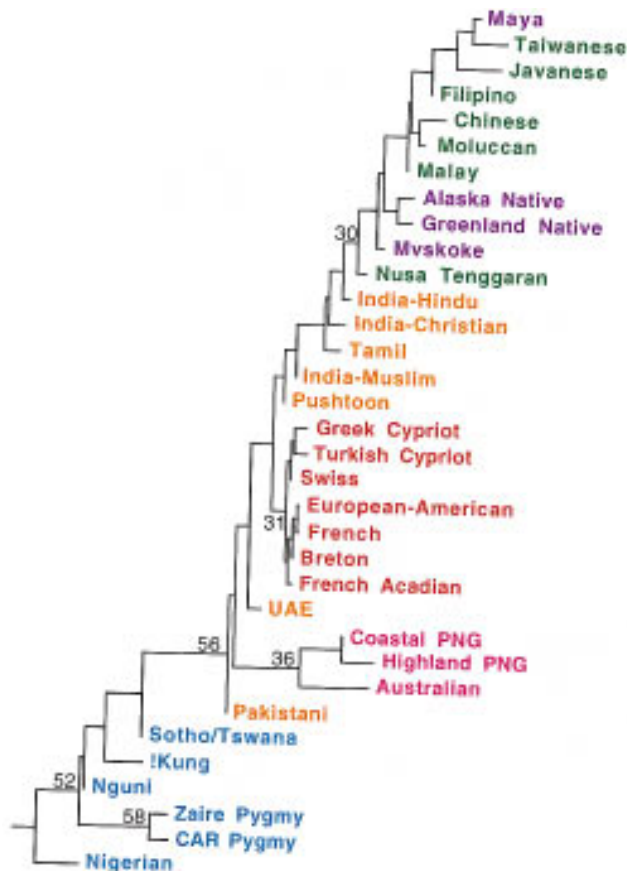


Figure 2 Neighbor-joining tree of population relationships. This tree is rooted where a hypothetical ancestral population—in which the frequency of the *Alu* element at each of the eight loci is set to 0.0—attaches to the unrooted network. Numbers indicate (in per cent) the fraction of 500 bootstrap replicates that supported a particular grouping.

Europeans, Asians, and Amerindians. However, because the African and Sahulian populations are closest to the root along different principal coordinates, which are orthogonal, African and Sahulian populations resemble the root in different ways. One way this situation could arise is if the earliest division of human populations was into a western (African) and eastern (Sahulian) component, with independent expansions following a period of separation.

Most genetic data do not link African and Sahulian populations (Cavalli-Sforza et al. 1994), possibly because the ancestral state for most genetic data is unknown. One notable exception is an analysis of human and chimpanzee restriction fragment length polymorphisms, which identified the most probable ancestral allelic states and demonstrated that African and Sahulian populations had

the highest frequencies of ancestral alleles (Mountain and Cavalli-Sforza 1994). Analyses of morphological variation in skulls (Howells 1973) and teeth (Stringer et al. 1997) also associate African and Sahulian populations, although this is thought to reflect selection acting in similar ways on these tropical populations (Cavalli-Sforza et al. 1994). Analyses of additional genetic loci for which the ancestral state is known are needed to verify this preliminary association of tropical populations based on *Alu* insertion polymorphisms.

Estimated Effective Separation Time of African vs. Non-African Populations

An estimate of the time of separation of African and non-African populations can be obtained from the amount of genetic distance that has accumulated between them, assuming that this genetic distance has accumulated in the absence of any further migration. For human populations this is a dubious assumption at best, and it is not clear what meaning one should attach to “separation times” between human populations. Nevertheless, separation times between African and non-African populations have been estimated from various types of genetic data (Nei and Roychoudhury 1982; Bowcock et al. 1994; Goldstein et al. 1995; Armour et al. 1996; Knight et al. 1996; Tishkoff et al. 1996), and our purpose in doing so here is to see whether the *Alu* insertion polymorphisms are concordant with other nuclear DNA loci. Because we are not attaching any specific meaning to the actual estimated separation time between African and non-African populations, we will henceforth refer to this as the effective separation time, to emphasize that this would be a true separation time only if African and non-African populations had remained completely isolated after this time. We used the model of Tachida and Iizuka (1993), in which the effective separation time is a function of Nei’s genetic distance (estimated for an *Alu* subfamily), the time when the *Alu* subfamily began expanding from a single master copy (t_b), and the time when *Alu* subfamily expansion ended (t_e). For the Ya5/8 *Alu* subfamily, we used Tachida and Iizuka’s estimate (1993) of $t_b = 4.3$ million years ago, and we assumed that $t_e = 0$ (i.e., this subfamily is still expanding) because recent insertion events have been observed (for review, see Deininger and Batzer 1995). The genetic distance estimate must include both polymorphic and monomorphic loci (i.e., loci for which the *Alu* element is present on every chromosome) of the *Alu* subfamily, as an estimate of genetic distance based solely on polymor-

ALU INSERTION POLYMORPHISMS IN HUMAN POPULATIONS

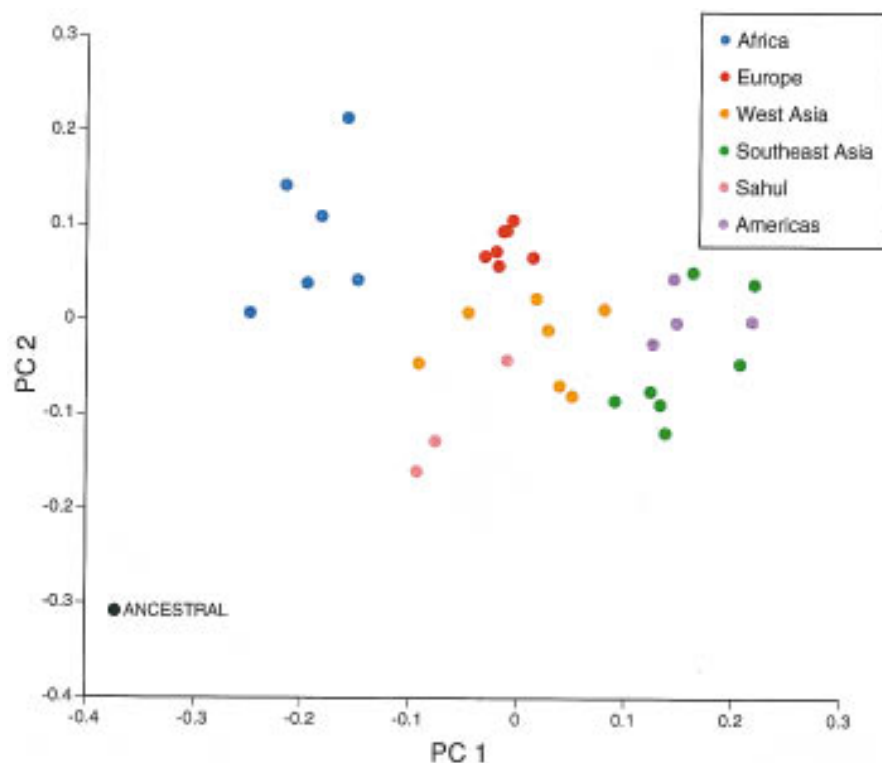


Figure 3 Plot of the first two principal coordinates of the allele frequencies at the eight polymorphic *Alu* insertion loci. The same relative population relationships were observed when the hypothetical ancestral population (consisting of allele frequencies of zero for the presence of the *Alu* element at each locus) was removed from the analysis (data not shown), indicating that the inclusion of the ancestral population is not distorting the population relationships.

phic loci will be biased (Tachida and Iizuka 1993). Random screening of human-specific *Alu* elements indicates that ~20% are polymorphic (Arcot et al. 1996); hence, we estimate that for the 8 polymorphic loci in our study, we need to include 32 monomorphic loci in the genetic distance calculation. The resulting average genetic distance between African and non-African populations is 0.0246, which from equation 33 of Tachida and Iizuka (1993) corresponds to an effective separation time of $137,000 \pm 15,000$ years ago.

The estimate of $t_b = 4.3$ million years to get the above effective separation time between African and non-African populations may be too recent, as a few Ya5/8 elements are known in chimpanzees and gorillas, which diverged from humans 4–6 million years ago. If one uses instead $t_b = 5$ million years, then the separation time becomes 159,000 years ago, whereas $t_b = 6$ million years gives a separation time of 187,000 years ago; these are not substantially different from the above estimate of 137,000 years ago for the effective separation of African and

non-African populations. These estimates of the effective separation time of African and non-African populations, based specifically on a model for polymorphic *Alu* insertion loci, are in good agreement with studies of other nuclear DNA polymorphisms (Nei and Roychoudhury 1982; Bowcock et al. 1994; Goldstein et al. 1995; Armour et al. 1996; Knight et al. 1996; Tishkoff et al. 1996). Thus, we conclude that the *Alu* loci do not differ in this respect from other nuclear loci.

Heterozygosity vs. Distance from the Centroid and Effective Population Size

Previous work has demonstrated that in a structured population a simple linear relationship is expected between the mean heterozygosity of a population and the genetic distance of that population from the centroid (Harpending and Ward 1982), and empirical studies have shown this relationship to hold (Harpending and Ward 1982; Crawford et al. 1989; McComb et al. 1995). A plot of heterozygosity versus distance from the centroid for the 34 populations in this study (Fig. 4) shows a good fit between the observed relationship and that predicted by the model, except that all of the African populations have a greater heterozygosity than predicted. In previous studies of genetic data, populations exhibiting greater heterozygosity than predicted by the model were thought to be experiencing higher rates of gene flow from other populations, which would elevate heterozygosity (Harpending and Ward 1982; Batzer et al. 1996b). However, high rates of gene flow should also reduce population differentiation, but the G_{st} analysis (Table 1b) indicates that African populations show the largest between-population differences.

The model outlined above assumes no systematic bias in the ascertainment of polymorphic loci. Previous studies showing elevated heterozygosities in Europeans because of ascertainment bias should not conform to this model. However, the *Alu* inser-

STONEKING ET AL.

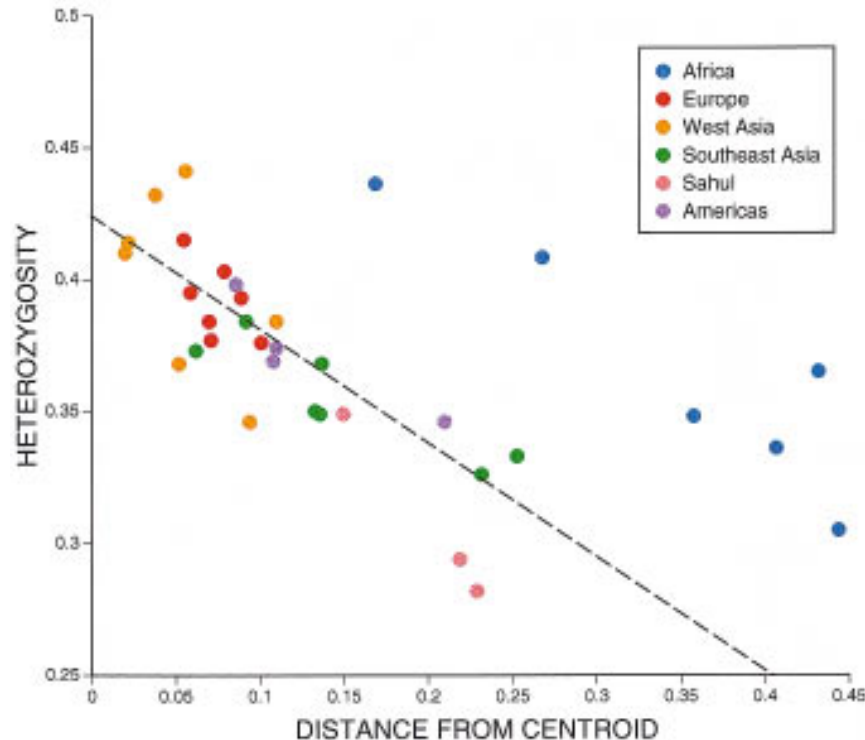


Figure 4 Plot of heterozygosity vs. distance from the centroid. The broken line is the expected relationship predicted by the model of Harpending and Ward (1982), according to the formula $h_i = H(1 - r_i)$, where r_i is the distance from the centroid and h_i and H are the heterozygosities of population i and the total population, respectively.

tion loci analyzed here were either derived from the literature or the genome of an African-American individual. Therefore the ascertainment bias in these polymorphisms is minimal and satisfies the assumptions of the model. We think that the most likely explanation for the elevated heterozygosity in African populations may be that the model depicted by the broken line in Figure 4 assumes that all populations are of the same relative effective size. If African populations have been larger than non-African populations, then the heterozygosity of African populations would also be increased (Relethford and Harpending 1995). We note that this explanation implies a greater effective population size across Africa, as we analyzed a linguistically and geographically diverse sample of African populations, all of which deviated from the model (Fig. 4).

Other groups have previously suggested that their results could be explained by either an African origin or a larger African population size (Armour et al. 1996; Tishkoff et al. 1996); however, our results provide the first direct evidence in support of the

latter hypothesis. A similar analysis of worldwide craniometric variation also found an elevated heterozygosity in African populations when population sizes were assumed to be equal (Relethford and Harpending 1994). This analysis also showed that the difference in size between African and non-African populations could be estimated by manipulating the relative population sizes to give the best fit to the Harpending-Ward model; we are currently exploring how best to do this for the large number of populations in the present analysis.

Previous studies have found greater genetic diversity in African populations for mitochondrial DNA (mtDNA) (Cann et al. 1987; Vigilant et al. 1991), Y-DNA (Hammer 1995), and nuclear DNA (Bowcock et al. 1994; Armour et al. 1996; Tishkoff et al. 1996) and have claimed that such greater African diversity reflects a greater antiquity of African populations and, hence, an African origin of modern humans. Others have pointed out that this greater

African diversity could instead reflect a larger effective size for African populations (Relethford and Harpending 1995; Rogers and Jorde 1995; Armour et al. 1996; Harpending et al. 1996; Tishkoff et al. 1996), and this study supports this argument. A greater genetic diversity in African populations therefore does not necessarily imply an African origin of modern humans, but neither does it contradict this, as African populations could have been both larger than non-African populations and the source of modern humans. Moreover, Harpending et al. (1996) have equated the hypothesis of an African origin of modern humans with a demographic scenario in which the ancestors of African populations expanded earlier than the ancestors of non-African populations. The fact that African populations are closest to the ancestral condition for these *Alu* insertion polymorphisms, and exhibit a greater effective population size, is in excellent agreement with this scenario. Analyses of additional loci for which the ancestral state is known should continue to prove instructive in understanding human origins.

METHODS

Population Samples

The following previously described populations were studied: Nigerian (Batzer et al. 1994), Central African Republic (CAR) Pygmy (Bowcock et al. 1987), Zaire Pygmy (Bowcock et al. 1987), !Kung (Soodyall et al. 1996), Sotho/Tswana (Soodyall et al. 1996), Nguni (Soodyall et al. 1996), European-American (Batzer et al. 1996b), Swiss (Batzer et al. 1996b), Breton (Batzer et al. 1996b), French (Batzer et al. 1996b), French Acadian (Batzer et al. 1996b), Greek Cypriot (Batzer et al. 1994), Turkish Cypriot (Batzer et al. 1994), Pushtoon (Melton et al. 1995), Tamil (Melton et al. 1995), Chinese (Melton et al. 1995), Taiwanese (Melton et al. 1995), Filipino (Melton et al. 1995), Malaysian (Melton et al. 1995), Javanese (Melton et al. 1995), Moluccan (Perna et al. 1992), Nusa Tenggara (Perna et al. 1992), Australian (Perna et al. 1992), Coastal Papua New Guinean (PNG) (Perna et al. 1992), Highland Papua New Guinean (Perna et al. 1992), Alaskan Native (Batzer et al. 1994), Greenland Native (Batzer et al. 1996b), Mvskoke (Weiss et al. 1993), and Mayan (Weiss et al. 1993). United Arab Emirates (UAE) samples were collected in Dubai. Pakistani samples were collected from native Pakistani individuals working in Dubai. Indian-Christian, -Muslim, and -Hindu samples were collected from Madras in southern India. DNA from Pakistani, Indian and UAE samples was prepared from blood stains using an IsoQuick nucleic acid extraction kit (MicroProbe Corporation, Bothell, WA).

Typing of *Alu* Insertion Polymorphisms

PCR amplification conditions, genotyping by agarose gel electrophoresis, and primer sequences for all eight loci (TPA25, PV92, FXIIIIB, APO, ACE, D1, A25 and B65) were described previously (Arcot et al. 1995a,b; Batzer et al. 1996b). The TPA25, FXIIIIB, ACE, and APO loci are also known as PLAT, F13B, APOAI, and DCPI, respectively. All 8 loci are present in humans and absent from orthologous positions within the genomes of (at least) 15 nonhuman primate genomes (Arcot et al. 1995a,b; Batzer et al. 1996b). The observed numbers of each genotype for each locus and population are available upon request from either M. Stoneking or M.A. Batzer.

Data Analysis

Unbiased estimates of heterozygosity (and associated standard error) and G_{st} values, corrected for sample size, were calculated using equations in Nei (1987). The GENDIST program in PHYLIP 3.5 (Felsenstein 1993) was used to calculate Nei's genetic distance (Nei 1972) between each pair of populations for the eight polymorphic loci, and the NEIGHBOR program was used to construct a neighbor-joining tree (Saitou and Nei 1987) from these genetic distances. Programs in PHYLIP 3.5 were also used to perform 500 bootstrap replications of the tree. Principal coordinate analysis was performed using a program provided by H. Harpending (Pennsylvania State University, University Park). Population separation times were estimated using the model of Tachida and Iizuka (1993), as described in more detail above. The standard error of the population separation time estimate was computed by jackknifing (Sokal and Rohlf 1981) Nei's distance, and the result-

ing population separation times, over the eight polymorphic loci. The model of Harpending and Ward (1982) was used to assess the relative amount of gene flow experienced by each population. In this model, which utilizes the standardized variance-covariance (R) matrix of allele frequencies, a simple linear relationship is expected between the heterozygosity of each population and the distance of the population from the centroid (the arithmetic mean of the allele frequencies): $r_i = (p_i - P)^2 / (P(1 - P))$, where r_i is the distance from the centroid and p_i and P are the frequency of the *Alu* insertion in population i and in the total population, respectively. The above equation was used to compute the distance from the centroid for each locus separately, and these values were then averaged over the eight loci.

ACKNOWLEDGMENTS

We thank K. Bhatia, A.S.M. Sofro, K. Weiss, J. Martinson, B.J.B. Keats, P.A. Ioannou, J.-P. Moisan, S.M. Milligan, M. Hochmeister, and W.D. Scheer for samples; L. Ludvico for technical assistance; and H. Harpending for valuable discussion and assistance with the principal coordinates analysis. This work was supported by grants from the National Science Foundation (SBR-9423118 to M.S.), the National Institutes of Health (RO1 HG00770 to P.L.D.), and the U.S. Department of Energy (DOE) (LDRD 94-LW-103 to M.A.B.). Work at Lawrence Livermore National Laboratory was conducted under the auspices of the U.S. DOE (contract W-7405-ENG-48).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

REFERENCES

- Arcot, S.S., J.J. Fontius, P.L. Deininger, and M.A. Batzer. 1995a. Identification and analysis of a "young" polymorphic *Alu* element. *Biochem. Biophys. Acta* 1263: 99-102.
- Arcot, S.S., Z. Wang, J.L. Weber, P.L. Deininger, and M.A. Batzer. 1995b. *Alu* repeats: A source for the genesis of primate microsatellites. *Genomics* 29: 136-144.
- Arcot, S.S., A.W. Adamson, J.E. Lamerdin, B. Kanagy, P.L. Deininger, A.V. Carrano, and M.A. Batzer. 1996. *Alu* fossil relics—Distribution and insertion polymorphism. *Genome Res.* 6: 1084-1092.
- Armour, J.A.L., T. Anttinen, C.A. May, E.E. Vega, A. Sajantila, J.R. Kidd, K.K. Kidd, J. Bertranpetit, S. Paabo, and A.J. Jeffreys. 1996. Minisatellite diversity supports a recent African origin for modern humans. *Nature Genet.* 13: 154-160.
- Batzer, M.A. and P.L. Deininger. 1991. A human-specific subfamily of *Alu* sequences. *Genomics* 9: 481-487.
- Batzer, M.A., G.E. Kilroy, P.E. Richard, T.H. Shaikh, T.D. Desselte, C.L. Hoppens, and P.L. Deininger. 1990. Structure and variability of recently inserted *Alu* family members. *Nucleic Acids Res.* 18: 6793-6798.

STONEKING ET AL.

- Batzer, M.A., V.A. Gudi, J.C. Mena, D.W. Foltz, R.J. Herrera, and P.L. Deininger. 1991. Amplification dynamics of human-specific (HS) Alu family members. *Nucleic Acids Res.* 19: 3619–3623.
- Batzer, M.A., M. Stoneking, M. Alegria-Hartman, H. Bazan, D.H. Kass, T.H. Shaikh, G.E. Novick, P.A. Ioannou, W.D. Scheer, R.J. Herrera, and P.L. Deininger. 1994. African origin of human-specific polymorphic Alu insertions. *Proc. Natl. Acad. Sci.* 91: 12288–12292.
- Batzer, M.A., C.M. Rubin, U. Hellmann-Blumberg, M. Alegria-Hartman, E.P. Leeflang, J.D. Stern, H.A. Bazan, T.H. Shaikh, P.L. Deininger, and C.W. Schmid. 1995. Dispersion and insertion polymorphism in two small subfamilies of recently amplified human Alu repeats. *J. Mol. Biol.* 247: 418–427.
- Batzer, M.A., P.L. Deininger, U. Hellmann-Blumberg, J. Jurka, D. Labuda, C.M. Rubin, C.W. Schmid, E. Zietkiewicz, and E. Zuercher. 1996a. Standardized nomenclature for Alu repeats. *J. Mol. Evol.* 42: 3–6.
- Batzer, M.A., S.S. Arcot, J.W. Phinney, M. Alegria-Hartman, D.H. Kass, S.M. Milligan, C. Kimpton, P. Gill, M. Hochmeister, P.A. Ioannou, R.J. Herrera, D.A. Boudreau, W.D. Scheer, B.J.B. Keats, P.L. Deininger, and M. Stoneking. 1996b. Genetic variation of recent Alu insertions in human populations. *J. Mol. Evol.* 42: 22–29.
- Bowcock, A.M., C. Bucci, J.M. Hebert, J.R. Kidd, K.K. Kidd, J.S. Friedlaender, and L.L. Cavalli-Sforza. 1987. Study of 47 DNA markers in five populations from four continents. *Gene Geogr.* 1: 47–64.
- Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd, and L.L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455–457.
- Cann, R.L., M. Stoneking, and A.C. Wilson. 1987. Mitochondrial DNA and human evolution. *Nature* 325: 31–36.
- Cavalli-Sforza, L.L., P. Menozzi, and A. Piazza. 1994. *The history and geography of human genes*. Princeton University Press, Princeton, N.J.
- Crawford, M.H., D.D. Dykes, and H.F. Polesky. 1989. Genetic structure of Mennonite populations of Kansas and Nebraska. *Hum. Biol.* 61: 493–514.
- Deininger, P.L. and M.A. Batzer. 1993. Evolution of retroposons. *Evol. Biol.* 27: 157–196.
- . 1995. SINE master genes and population biology. In *The impact of short interspersed elements (SINEs) on the host genome* (ed. R.J. Maraia), pp. 43–60. R.G. Landes, Georgetown, TX.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.
- . 1993. PHYLIP, version 3.5c. University of Washington, Seattle, WA.
- Goldstein, D.B., A.R. Linares, L.L. Cavalli-Sforza, and M.W. Feldman. 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. *Proc. Natl. Acad. Sci.* 92: 6723–6727.
- Hammer, M.F. 1995. A recent common ancestry for human Y chromosomes. *Nature* 378: 376–378.
- Harpending, H.C. and R.H. Ward. 1982. Chemical systematics and human populations. In *Biochemical aspects of evolutionary biology* (ed. M. Nitecki), pp. 213–256. University of Chicago Press, Chicago, IL.
- Harpending, H., J. Relethford, and S.T. Sherry. 1996. Methods and models for understanding human diversity. In *Molecular biology and human diversity* (eds. A.J. Boyce and C.G.N. Mascie-Taylor), pp. 283–299. Cambridge University Press, Cambridge, UK.
- Howells, W.W. 1973. Cranial variation in man: A study by multivariate analysis of patterns of difference among recent human populations. *Pap. Peabody Mus. Archaeol. Ethnol. Harv. Univ.* 67: 1–259.
- Jorde, L.B., M.J. Bamshad, W.S. Watkins, R. Zenger, A.E. Fraley, P.A. Krakowiak, K.D. Carpenter, H. Soodyall, T. Jenkins, and A.R. Rogers. 1995. Origins and affinities of modern humans: A comparison of mitochondrial and nuclear genetic data. *Am. J. Hum. Genet.* 57: 523–538.
- Kass, D.H., C. Aleman, M.A. Batzer, and P.L. Deininger. 1994. Identification of a human specific Alu insertion in the factor XIII B gene. *Genetica* 94: 1–8.
- Knight, A., M.A. Batzer, M. Stoneking, H.K. Tiwari, W.D. Scheer, R.J. Herrera, and P.L. Deininger. 1996. DNA sequences of Alu elements indicate a recent replacement of the human autosomal genetic complement. *Proc. Natl. Acad. Sci.* 93: 4360–4364.
- McComb, J., N. Blagitko, A.G. Comuzzie, M.S. Schanfield, R.I. Sukernik, W.R. Leonard, and M.H. Crawford. 1995. VNTR DNA variation in Siberian indigenous populations. *Hum. Biol.* 67: 217–229.
- Melton, T., R. Peterson, A.J. Redd, N. Saha, A.S.M. Sofro, J. Martinson, and M. Stoneking. 1995. Polynesian genetic affinities with Southeast Asian populations as identified by mtDNA analysis. *Am. J. Hum. Genet.* 57: 403–414.
- Mountain, J.L. and L.L. Cavalli-Sforza. 1994. Inference of human evolution through cladistic analysis of nuclear DNA restriction polymorphisms. *Proc. Natl. Acad. Sci.* 91: 6515–6519.
- Nei, M. 1972. Genetic distance between populations. *Am. Nat.* 106: 283–292.
- . 1987. *Molecular evolutionary genetics*. Columbia University Press, New York, NY.

ALU INSERTION POLYMORPHISMS IN HUMAN POPULATIONS

- Nei, M. and A.K. Roychoudhury. 1982. Genetic relationship and evolution of human races. *Evol. Biol.* 14: 1–59.
- Nei, M. and N. Takezaki. 1996. The root of the phylogenetic tree of human populations. *Mol. Biol. Evol.* 13: 170–177.
- Novick, G.E., C.C. Novick, J. Yunis, E. Yunis, P. Antunez de Mayolo, W.D. Scheer, P.L. Deininger, M. Stoneking, D.S. York, M.A. Batzer, and R.J. Herrera. 1997. Polymorphic *Alu* insertions and the Oriental origin of Native American populations. *Hum. Biol.* (in press).
- Okada, N. 1991. SINEs. *Curr. Opin. Genet. Dev.* 1: 498–504.
- Perna, N.T., M.A. Batzer, P.L. Deininger, and M. Stoneking. 1992. *Alu* insertion polymorphism: A new type of marker for human population studies. *Hum. Biol.* 64: 641–648.
- Relethford, J.H. and H.C. Harpending. 1994. Craniometric variation, genetic theory, and modern human origins. *Am. J. Phys. Anthropol.* 95: 249–270.
- . 1995. Ancient differences in population size can mimic a recent African origin of modern humans. *Curr. Anthropol.* 36: 667–674.
- Rogers, A.R. and L.B. Jorde. 1995. Genetic evidence on modern human origins. *Hum. Biol.* 67: 1–36.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.
- Schmid, C.W. and R. Maraia. 1992. Transcriptional regulation and transpositional selection of active SINE sequences. *Curr. Opin. Genet. Dev.* 2: 874–882.
- Sherry, S.T., H.C. Harpending, M.A. Batzer, and M. Stoneking. 1997. *Alu* evolution in human populations: Using the coalescent to estimate effective population size. *Genetics* (in press).
- Sitnikova, T., A. Rzhetsky, and M. Nei. 1995. Interior-branch and bootstrap tests of phylogenetic trees. *Mol. Biol. Evol.* 12: 319–333.
- Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*. W.H. Freeman & Co., New York, NY.
- Soodyall, H., L. Vigilant, A.V. Hill, M. Stoneking, and T. Jenkins. 1996. mtDNA control-region sequence variation suggests multiple independent origins of an “Asian-specific” 9-bp deletion in sub-Saharan Africans. *Am. J. Hum. Genet.* 58: 595–608.
- Stoneking, M. 1993. DNA and recent human evolution. *Evol. Anthropol.* 2: 60–73.
- Stringer, C., L. Humphrey, and T. Compton. 1997. Cladistic analysis of dental traits in recent humans using a fossil outgroup. *J. Hum. Evol.* 32: 389–402.
- Tachida, H. and M. Iizuka. 1993. A population genetic study of the evolution of SINEs. I. Polymorphism with regard to the presence or absence of an element. *Genetics* 133: 1023–1030.
- Tishkoff, S.A., E. Dietzsch, W. Speed, A.J. Pakstis, J.R. Kidd, K. Cheung, B. Bonne-Tamir, A.S. Santachiara-Benerecetti, P. Moral, M. Krings, S. Paabo, E. Watson, N. Risch, T. Jenkins, and K.K. Kidd. 1996. Global patterns of linkage disequilibrium at the CD4 locus and modern human origins. *Science* 271: 1380–1397.
- Vigilant L., M. Stoneking, H. Harpending, K. Hawkes, and A.C. Wilson. 1991. African populations and the evolution of human mitochondrial DNA. *Science* 253: 1503–1507.
- Weiss, K.M., A.V. Buchanan, R. Valdez, J.H. Moore, and J. Campbell. 1993. Amerindians and the price of modernization. In *Urban ecology and health in the third world* (ed. L.M. Schell, M.T. Smith, and A. Bilsborough), pp. 221–243. Cambridge University Press, Cambridge, UK.

Received April 25, 1997; accepted in revised form September 12, 1997.



***Alu* Insertion Polymorphisms and Human Evolution: Evidence for a Larger Population Size in Africa**

Mark Stoneking, Jennifer J. Fontius, Stephanie L. Clifford, et al.

Genome Res. 1997 7: 1061-1071

Access the most recent version at doi:[10.1101/gr.7.11.1061](https://doi.org/10.1101/gr.7.11.1061)

References This article cites 39 articles, 8 of which can be accessed free at:
<http://genome.cshlp.org/content/7/11/1061.full.html#ref-list-1>

License

Email Alerting Service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to *Genome Research* go to:
<https://genome.cshlp.org/subscriptions>