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Inter- and intrapopulation studies of ancient humans

W. W. Hauswirth*, C. D. Dickel, D. J. Rowold and M. A. Hauswirth

Department of Immunology and Medical Microbiology, College of Medicine, University of Florida, Gainesville (Florida 32610-0266, USA)

Abstract. For a genetic analysis of ancient human populations to be useful, it must be demonstrated that the DNA samples under investigation represent a single human population. Toward that end, we have analyzed human DNA from the Windover site (7000–8000 BP). MHC-I analysis, using allele-specific oligonucleotide hybridization to PCR amplified Windover DNA, microsatellite anaysis by PCR of the APO-A2 repeat and mtD-loop 3′ region sequencing on multiple individuals spanning nearly the full range of estimated burial dates all confirm the hypothesis that there is a persistence of both nuclear and mitochondrial haplotypes at Windover throughout its entire period of use. Thus, Windover can be considered a single population. Neighbor-joining tree analysis of mtDNA sequences suggests that some mitochondrial types are clearly related to extant Amerind types, whereas others, more distantly related, may reflect genetically distinct origins. A more complete sequence analysis will be required to firmly resolve this issue. Calibrating genetic relationships deduced by tree analysis, radiocarbon dates and burial position, yields a human mtD-loop DNA rate of evolution of 3700 to 14,000 years per percent change. Both values are within the range of recent, independently calculated values using estimates of evolutionary divergence or theoretical population genetics. Thus we are beginning to realize the promise of ancient DNA analysis to experimentally answer heretofore unapproachable questions regarding human prehistory and genetic change.

Key words. Amerindian; mitochondrial DNA; human; microsatellite; HLA; DNA evolution.

Introduction

Analysis of molecular genetic information from past populations offers unique opportunities to learn about human history unavailable with any other method. However, the technical difficulties and challenges present in these endeavors are substantial (see this issue). With the advent of PCR, retrieval of DNA sequence data from the low quantity of DNA present in ancient DNA samples has been possible. It has also become clear that each ancient source of DNA is unique and the ease or difficulty of extracting useful information varies widely, both with the type of preservation, as well as among samples from the same context.

A range of samples has been used as sources of ancient human DNA, including bone, mummified tissue, and wet-site preserved tissue[5, 6, 11]. Our work has focused on the recovery and analysis of DNA from soft tissue preserved in the latter environment at the Windover site, a shallow pond located in the central coastal region of Florida[5]. The site was radiocarbon-dated at between 7000 and 8100 years before the present (BP). In contrast to most other comparably ancient sites, a large number of individuals – at least 176 – was recovered at Windover. Of that number, 91 crania were recovered containing preserved soft tissue[7]. The site appears to possess other well-preserved organic material as well, including fabric, wood artifacts, and human tissue. Reasons for the quality of preservation are conjectural but are likely to include key properties of the pond water/peat: among which are its relatively neutral pH 6.5–6.9, the highly mineralized content of the water-saturated peat matrix, and the cultural burial practice of rapid interment[7].

Previous studies using gross visual examination, light and electron microscopy, X-ray analysis, and magnetic resonance imaging of the preserved human soft tissue within intact crania showed that anatomical and cellular features of brain tissue still remained[5]. A Southern blot of DNA extracted in this initial study from one Windover brain was done using a human-specific mitochondrial DNA (mtDNA) probe. The results demonstrated the presence of human DNA in the sample, although at about 1% of the levels expected in an equivalent amount of modern human brain tissue. This suggested that much of the isolated DNA was plant-derived from the surrounding peat, consistent with magnetic resonance image analysis and gross visual examination showing peat material surrounding, and embedded within, human brain material. Nevertheless, these early studies clearly demonstrated the possibility of using Windover soft tissue as a DNA source for genetic studies even before targeted PCR-mediated approaches became widely available in the late 1980s.

Genetic analyses of ancient human remains are now technically feasible. But, given the many inherent uncertainties present, are they scientifically worthwhile? A

universal problem will be to establish that a specific assemblage of DNA samples represents a true prehistoric population in the genetic sense. For contemporary humans, it is usually possible through careful examination of oral and written histories to establish with reasonable certainty that a given group represents a genetically related population. This allows inferences regarding relationships to other populations, interbreeding frequency and migration patterns to be made, precisely the types of information most valuable from ancient populations. When only a few individuals from one or more ancient populations are analyzed, more limited but suggestive conclusions can be reached (see this issue). In this case, the risk is always present that the limited sample size may not accurately represent the true genetic make-up of the ancient people studied. To resolve this problem, ancient populations – like Windover – which have a large sample size are required. However, a new problem will frequently arise. Ancient cemeteries often represent an extended period of interment and may, in theory, be comprised of genetically distinct, simultaneously or successively interred populations. For Windover, where human burials span more than 1000 years, the problem is obvious and, we suspect, not unique. To treat Windover individuals as a genetically defined group useful for population analysis, it is necessary to first establish an intrapopulation genetic continuity. That is, using informative genetic loci, we need to determine whether certain alleles are present in samples spanning large segments of the 1000-year history of Windover. This, therefore, is one goal of the work presented here. Because of the uniparental maternal inheritance of mitochondrial genes and the biparental inheritance of most nuclear genes, we analyzed both hypervariable mitochondrial and nuclear loci to that end. The results demonstrate the genetic continuity of Windover. They also highlight some types of genetic inferences that are likely to be unique to this sort of intrapopulation analysis of ancient DNA. Pre-eminent among these is the opportunity to estimate the rate of domain-specific mtDNA sequence change based on a radiocarbon time scale. An accurate estimate of this rate is at the heart of many controversies in North American molecular archeology.

Materials and methods

DNA isolation and pre-amplification

DNA was extracted from 5–10 grams of preserved cranial material as described in Lawler et al.[10], except that after CsCl banding, the DNA was resuspended in 250 µl of 10 mg/ml ethidium bromide and 140 µl of 7.5 M ammonium acetate, extracted with 420 µl of phenol:chloroform (1:1) and centrifuged at 12,000 × *g* for 2 min[14]. The upper aqueous solution was ethanol-precipitated, washed with 70% ethanol, and resuspended in 500 µl of 10 mM Tris pH 8.0, 1 mM EDTA. Negative controls were extracted simultaneously and carried through all subsequent analysis. In order to produce undamaged templates for PCR with gene-specific primers, an aliquot of old brain DNA was amplified using random 15-mer oligonucleotides[22] in a 50 µl volume containing 10 mM tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 175 µM of dNTPs, 1% Triton X-100, 2 mg/ml of acetylated BSA (5′-3′, Inc), 1 µM random primer mix and 2 units of Taq polymerase (BRL, Inc.). Fifty primer-extension cycles were carried out at 92 °C for 1 min, 37 °C for 2 min, a 3 min ramp time to 55 °C, and 55 °C for 4 min using the Perkin-Elmer 9600 thermocycler. Again, negative controls were performed simultaneously with old brain samples.

PCR of a mtDNA D-loop segment

A nested primer strategy was required for production of specific gene products. The outer primer pair were: 16091-5′-ATT-TCGTACATTACTG-3′-16106 and 16340-5′-TGTGCTATGTACGGTAAATG-3′-16321. The inner primer pair were: 16104-5′-CTGCCAGC-CACCATGAATAT-3′-16123 and 16336-5′-CTATG-TACGGTAAATGG-CT-3′-16318. The first round of PCR was done with a 3–5 µl aliquot from the pre-amplification sample for 40 cycles at 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 2 min. PCR buffers were the same as above, except that BSA was omitted and gene-specific primers were used at a concentration of 0.2 µM. The second PCR was done with a 3–5 µl aliquot from the first round with the same PCR parameters for 25 cycles. Samples from the final round of PCR were analyzed on 2% agarose gels and positive samples in which control reactions were negative, isopropanol-precipitated, and sequenced by the cycle-sequencing method (BRL, Inc.). The sequence from 16151 to 16317 was analyzed.

PCR of a dinucleotide repeat microsatellite

A similar strategy as above was followed for microsatellite PCR with the following changes. A 3–5 µl aliquot of the pre-amplification reaction was used for PCR using a 5′ end-labeled primer from the APO-A2 locus (chromosome 1). The primers were 5′-GATTCACT-GCTGTGGACCCA and 5′-GGTCTGGAAGTACT-GAGAAA. Reagent concentrations were as above except that the primer concentration was 0.4 uM and the PCR parameters were 94 °C for 10 min, followed by 40 cycles of 94 °C for 1 min, 60 °C for 2 min, and 72 °C for 2 min. Samples were analyzed on a 6% denaturing polyacrylamide gel.

PCR of MHC Class I genes and allele-specific oligonucleotide (ASO) analysis

PCR amplification of a hypervariable region of exon 3 of MHC Class I genes has been previously described[10].

For ASO analysis, PCR products (10 µl of a 50 µl reaction) were blotted onto Nytran membrane using a Millipore dot blot manifold. Strips containing one sample for each Windover individual to be analyzed were then hybridized to radiolabeled, allele-specific oligonucleotide sense strand probes. The sequence of each probe was designed to have a minimum of three mismatches with all other alleles known to be present at Windover in an earlier study[10]. Probe sequences are: for the HLA-G allele, 5′ AATGTGGCTGAACAAAGGAG; for the HLA-DAN4 allele, 5′ CGGACAATACTCAGAGCAG; for the HLA-B37 alleles, 5′ GCGGAGCAGGACAGAGCCTA; and for HLA-A19 alleles, 5′ ACCTGCCGCTCTTGGACCGCG. Ten ng of each probe was labeled with ^{32}P at its 5′ end using polynucleotide kinase and $\gamma^{32}P$-ATP. Specific activities were approximately equivalent. Hybridization was carried out at 50 °C for 3 hr in 2 × SSC with three 30 min washes in 0.1 × SSC at 50 °C. Hybridization intensities were scanned and quantitated on a Molecular Dynamics Phosphorimager.

Contamination controls

Standard precautions were followed to limit cross-over and modern DNA contamination[9]. As described above, extraction controls and duplicate PCR reactions of the extraction controls were performed. PCR reactions were assembled in a Baker Bioguard safety hood, reagents and tubes were autoclaved for 1 h, reagents were aliquoted and dated, tube openers were used, surfaces were decontaminated with 10% Clorox, and disposable gloves, filter pipet tips, and dedicated pipets were also used. It appeared to us that aerosols and splashes occurred when opening PCR tubes even with tube openers. This could be minimized if the tubes were opened while holding the tubes at a slight downwash angle with the hinge of the tube pointing upward. Finally, PCR tubes themselves appeared to also be a source of sporadic contamination. To help avoid this problem, tubes were soaked in 10% Clorox for 30 min before rinsing in distilled water and autoclaving for 1 hour.

Time-of-burial estimates

From a series of 14 radiocarbon dates of human bone, artifacts, and peat closely associated with human bone and brain matter[4], it has been possible to estimate that Windover was used as a cemetery between about 6980 and 8120 ± 100 years BP. The physical location of these accurately-dated items revealed a burial pattern within the Windover pond in which the oldest human bone exists nearest the pond center, and the most recent nearest the current pond edge. Using these dates and locations as a reference, it is possible to estimate the interment date of all other human bone, including crania-encased brain from which DNA was extracted.

Results and discussion

MHC-I analysis

Initial PCR-cloning-DNA sequence analysis on one Windover individual identified six class I MHC alleles[10]. Of those, two are related to known, expressed genes in the A19 and B37 families. In order to more rapidly screen multiple individuals, we designed a set of diagnostic oligonucleotide probes that would determine by dot-blot analysis the frequency of these alleles (as well as two non-classical, non-expressed alleles, G and Dan4). A sample experimental result is shown in figure 1, in which PCR amplifications of six Windover individuals were hybridized with each of the allele-specific oligonucleotide (ASO) probes. Quantitative analysis of hybridization signal intensity was determined relative to negative control target DNA (plasmid vector DNA) and positive controls (cloned, allele-specific DNA). Individuals were scored as containing an allele if a signal was more than five times the highest negative control value in the experiment. However, a negative value in

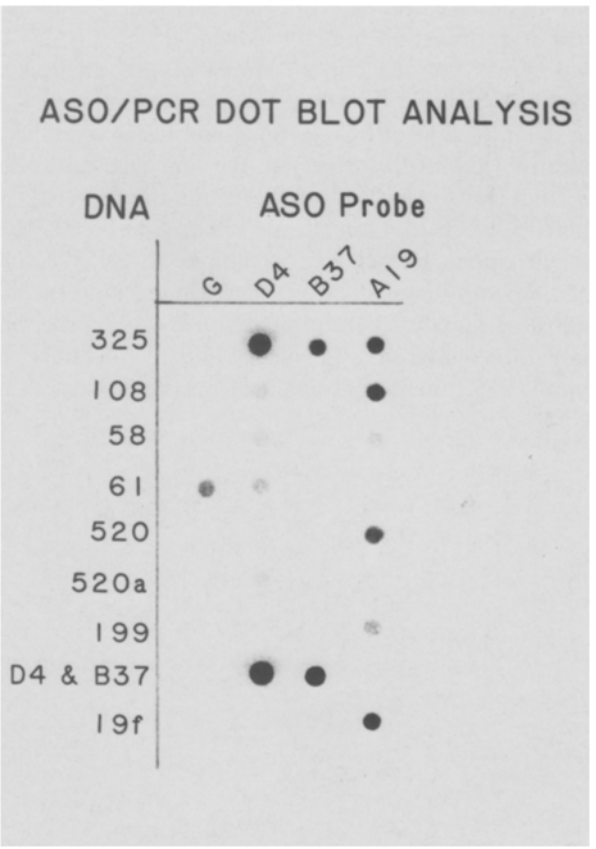

Figure 1. Allele-specific oligonucleotide (ASO) analysis of MHC Class I genes in 14 Windover brain DNAs. Example of an ASO probed dot blot of PCR amplified DNAs. A strip containing a DNA dot of each sample (#325, etc.) was probed with each radiolabeled oligo-probe (G, HLA-G; D4, HLA-DAN4; B37, HLA-B37 family; A19, HLA-A19 family) visualized and quantitated by autoradiographic imaging. D4, B37, and 19f DNA samples are cloned DNA controls demonstrating the specificity of the ASO hybridization.

Table 1. MHC-I ASO analysis

Brain	Probes	*Age BP	G	D4	B37	A19
325	8	7300	+	+	+	+
58	3	8100	0	+	0	+
61	3	7850	+	+	+	+
64	3	7750	+	0	+	+
67	2	7400	+	+	0	+
108	3	7800	0	+	0	+
127	1	7600	+	+	0	+
195	4	7900	0	+	0	+
199	3	7800	0	0	0	+
217	4	7700	+	+	0	+
278	3	8000	0	0	0	0
444	1	7400	+	+	0	+
459	4	7800	+	+	0	+
471	1	7400	+	+	0	+
520	4	7300	+	+	+	+

*estimated from burial position, see 'Materials and methods'.

one experiment (e.g. fig. 1) is insufficient evidence to exclude that allele from that individual because PCR analysis of small amounts of ancient DNA may not always amplify all alleles present, especially if the DNA is highly damaged. Hence, multiple independent amplifications are necessary to firmly establish the absence of a given target allele in an individual.

A summary of the current status of this analysis is shown in table 1. Fourteen individuals were surveyed. Sample 278 did not PCR-amplify and hence serves as a negative control. As expected, the non-classical alleles G and Dan4 which should exist in the majority of humans are indeed usually detected. Additional PCR amplifications, particularly on samples 58, 64, 108, and 199, are still required. More significantly, however, all individuals analyzed are positive for the A19 allele, but only three have the B37 allele initially identified in sample 325. Interestingly, this is consistent with recent studies of MHC-I alleles in contemporary South American Indians that found a surprisingly high diversity of B-alleles[3,20]. It was suggested that HLA-B diversity may be due to a pressure to maintain resistance to a broad range of tropical diseases encountered during the initial southward migration in the Americas. Clearly, at the B locus Windover is multi-allelic, suggesting that this diversity may indeed be a characteristic of the early migrants into the Western Hemisphere. However, the view that maintenance of diverse B-locus alleles is related primarily to tropical pathogen-driven mechanisms is not fully supported by the Windover study since this North American population presumably had not yet migrated through the tropics 8000 years ago. However, insofar as determining the presence of a single genetic continuum at Windover, the universality of A19 in all samples is supportive, but variability at the B locus leaves the question somewhat open.

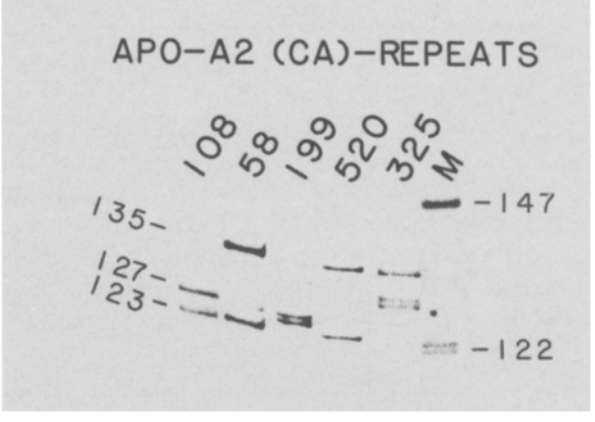

Figure 2. Microsatellite analysis of six Windover brain DNA samples (#325, etc.) at the Apo-A2 dinucleotide repeat locus. Gel autoradiograph of the PCR products demonstrate the 2-nucleotide resolution (sample #199) and the clear resolution of heterozygotes. The numbers for each allele refer to the length of the PCR product (123 bp, etc.). Lane M is a size marker as indicated in bp.

Microsatellite analysis

Microsatellite sequences are short (usually di-, tri-, or tetranucleotide) repeats which occur more than 100,000 times in the human genome on every chromosome. Repeat units are highly polymorphic but relatively stable in the human population and therefore provide excellent loci for chromosome identification. In order to assess the prevalence of this nuclear sequence at Windover, we made APO-A2 locus (chromosome 1) single-copy sequence primers[21] and screened Windover samples by PCR amplification using one ^{32}P-end labeled primer and sizing the products on high resolution sequencing gels (fig. 2). Pairs of predominant bands, indicative of heterozygotes, are seen for each sample, demonstrating that PCR amplification does not generate artifactually lengthened or shortened products due to slippage or jumping. Thus, we were able to rapidly type multiple individuals for a chromosome 1 marker. Five length alleles were found among the six individuals tested, with four of six individuals sharing one or both of the two most common alleles of 123 bp and 135 bp (table 2). These lengths of PCR products correspond to a CA repeat lengths of 9 and 15, respectively. Examination of the estimated burial dates for individuals containing either or both of these alleles demonstrates that

Table 2. APO-A2 CA-repeat analysis of Windover DNA

DNA	*Age BP	Repeat number						
		9	10	11	12	13	14	15
325	7300	–	–	–	129	–	–	135
58	8100	123	–	–	–	–	–	135
61	7850	123	–	–	–	–	–	135
108	7800	123	–	127	–	–	–	–
199	7800	–	125	127	–	–	–	–
520	7300	123	–	–	–	–	–	135

*estimated from burial position, see 'Materials and methods'.

both span nearly the entire period of Windover. Hence, as with the MHC-I analysis, the persistence of hypervariable nuclear alleles is consistent with the view that the burials at Windover were sampled from a population of related individuals.

Mitochondrial DNA analysis

The initial focus of our mtDNA sequence analysis has been on a region near the 3'-end of the D-loop region (nucleotides 16,151 to 16,317). This segment was selected because it accumulates substitutions more rapidly than other regions of the genome[1,15], it targets most of the informative positions noted by Ward et al.[18] in previous sequence studies of contemporary Amerinds, and because this distance between PCR primers represents nearly the largest reproducibly amplifiable segment that we can obtain from Windover DNA, presumably due to the number of damaged sites when primer pairs are spaced further apart. Currently the sequence of the target domain has been determined for fourteen individuals (table 3). The initial question is whether evidence exists within this set of mitochondrial sequences to confirm the tentative conclusion based on nuclear DNA that Windover represents burials from one population. Of the fourteen sequences, five (from individuals 28, 61, 199, 325, and 471) are related by a single base transition (T→C at nucleotide 16,193). Given the high rate of change estimated for this portion of the mitochondrial D-loop (see below), it is reasonable to assume that these five individuals are maternally related. The approximate burial dates for these samples span the entire range of dates within the site (see fig. 2), hence, at two nuclear and one mitochondrial locus, haplotype persistence is entirely consistent with treating Windover burials as being sampled from a genetically continuous human group. This being the case, there is justification for viewing Windover as a population equivalent to extant groups whose genetic affiliations can be established by more traditional anthropological methods.

Inter- and intrapopulation inference

Given the limited number of Windover samples currently analyzed and the restricted length of mtDNA sequence analyzed (approximately half of the 3'-D-loop domain normally used) any inference regarding Windover structure or its relationship to contemporary Amerind groups is necessarily tentative. With that caveat, we will present one example each of inter- and intrapopulation analyses, with the aim of demonstrating the type of information uniquely available from ancient human populations, once they have been established as a single genetic group by multiple genetic criteria.

Several independent studies have established that the mtDNA sequences of modern Amerinds can be related to four founding haplotypes either by analysis of multiple loci throughout the mitochondrial genome[2,12,16,17] or by sequence analysis at the mtDNA D-loop 3' end[8,13,18,19] (see also this issue). For comparison among different labs, groups I, II, III, and IV of Ward et al.[18] correspond to groups B, D, A, and C, respectively, of others[8,12,16]. Since previous analyses on modern samples were based on a mt D-loop region approximately twice the size as we have determined for Windover, we first needed to establish that the comparably restricted sequence domain from contemporary samples was sufficient for resolving the established four genetic types. A neighbor-joining tree analysis is shown in figure 3 in

Table 3.

	Nucleotide Position (16,151–16,317)																			
	16168	17172	19192	19213	21218	21219	22223	22224	23239	24245	24247	25256	26264	26265	27270	27278	29290	29293	29298	31311
DNA	T	T	C	C	C	A	C	T	C	C	A	C	C	A	C	C	C	A	T	T
61	–	–	–	T	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–
28	–	–	–	T	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–
108	–	–	–	–	–	–	–	–	–	–	–	T	–	–	T	–	–	–	–	–
520	–	–	–	–	–	–	–	C	–	T	–	–	–	T	–	–	–	–	–	–
94	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	T	–	–	–	–
471	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–
325	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–
199	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–
159	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C
64	–	–	–	–	T	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–
67	–	C	–	–	T	–	–	–	A	–	–	–	–	–	–	–	–	–	C	–
373	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–
278	–	–	T	–	–	–	–	C	–	–	–	–	–	–	–	–	–	G	–	C
489	–	–	–	–	T	–	–	–	–	–	–	–	T	–	–	T	T	–	–	C
DR	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CD	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

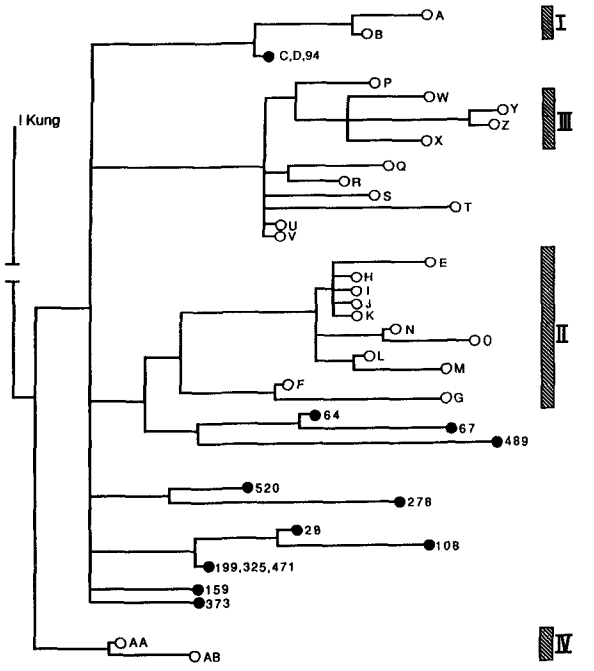

Figure 3. Neighbor-Joining/UPGMA (PAUP, version 3.52C) analysis of the Windover mtDNA D-loop sequences (filled circles) (table 2) and the corresponding region in a contemporary Amerind group, the Nuu-Chuh-Nulth (open circles)[18]. Numbers and letters refer to specific samples in each data set. Note that the sequence of individual 28 is identical to individual 61 (not shown). Roman numerals refer to the deduced four groups of haplotypes currently found in the Western hemisphere[18].

which the data of Ward et al.[18] for the Nuu-Chuh-Nulth tribe from nucleotides 16,151 to 16,317 have been included with the Windover data. The results show that the four haplotypes are still distinguished even when only half the sequence is used. However, to achieve this, one branch node with only a 24% bootstrap confidence level must be retained. This, therefore, is the minimum confidence level of any node included in the tree.

The interpopulation analysis of ancient and contemporary groups in figure 3 reveals several aspects of Windover mtDNA haplotypes relative to modern haplotypes. First, all Windover sequences are intermediate between the extremes of the Nuu-Chuh-Nulth, suggesting that there are no non-Amerind contaminants present. Second, approximately 1/3 of the Windover individuals are related to two of the four known sequence groups (I and II), confirming the antiquity of the haplotype groups. Third, more than half, although related to groups (I, II, and III) do not fall clearly into any modern class. Does this suggest that additional haplotypes, now perhaps extinct in modern groups, were present at Windover? Given our current limited confidence levels due to the restricted region sequenced, such a conclusion is premature but provocative. Resolution will require that significantly larger numbers of individuals and sequence lengths be completed for Windover. Nevertheless, this example serves to illuminate the unique type of interpopulation information available in ancient samples, in this case the possibility of deducing extinct haplotypes, perhaps reflecting a post-Columbian genetic bottleneck.

One aspect of many ancient cemeteries which makes them potentially unique resources for intrapopulation analysis is the extended burial period – for Windover, more than 1000 years. If independent determinations of burial dates are available, as we can derive from radiocarbon data at Windover, sufficiently detailed mtDNA sequence analysis may allow both temporal resolution of genetic contact between ancient groups and, more directly, estimates of rates of genetic change. One example of the latter will be given. In order to estimate a rate of mtDNA change, we need a set of identical or closely related haplotypes which can be reasonably assumed to be maternally related, either because no sequence differences exist, or a single base change links haplotypes. From the data in table 3 two haplotypes (A and B) are shared by groups of three and two individuals, respectively (individuals 199, 325, and 471 have type A, and individuals 28 and 61 have type B). The haplotypes are related by a single C→T transition at 16,193. We can use the estimated times of burial based on radiocarbon dates as a temporal scale (see 'Materials and methods'). There remain a number of possible scenarios for recreating the observed distribution of sequence types A and B at Windover. For simplicity we will first assume that the most abundant type is the founding mtDNA (type A). We also assume that type A existed at 8100 years BP, the oldest date at Windover. It is highly likely that the presumptive 16,193 C→T transition creating type B from type A occurred only once, hence the two type B individuals must be maternally related. Rates of change can then be determined by assuming that all type A individuals are maternally related, the most likely scenario and one that will give the most rapid rate. Alternatively, we can assume that no type A individuals are maternally related back to 8100 years BP, a less likely scenario leading to a slower rate of change. These two extremes are 3700 years per percent change and 14,000 years per percent change, respectively.

How do these rates compare to estimates based on less precise measurements of time? Recent sequence analysis of a somewhat larger but overlapping D-loop region in modern Amerind groups has been used to calculate rates of mtD-loop DNA change based on estimates of either primate/human divergence or population theory[13]. These calculated rates are 30,000 and 8000 years per percent change, respectively. Intrapopulation analysis of Windover yields comparable values, closest to the more rapid evolutionary rate, thus demonstrating the power of ancient DNA sequence analysis for this sort of highly-focused evolutionary rate calculation.

In summary, the potential of ancient human DNA analysis to reveal population behavior during prehistory

and to allow calculation of rates of chemical change at selected genetic loci are exciting. Current studies at Windover, although still in their early stages, are beginning to provide glimpses of that potential.

* To whom correspondence should be addressed.

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