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## Study of the Human Mitochondrial DNA Polymorphism

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# Study of the Human Mitochondrial DNA Polymorphism

Catherine Willis

## Abstract

Traces of Paleolithic exodus routes of modern humans from Africa to Europe have been studied genetically using female specific ancestral lineages via mitochondrial DNA (mtDNA). We suggest modern humans entered Europe near the Carpathian Mountains and Hungarian Valley. If so, isolated populations of highlanders in Carpathian Europe may carry a genetic fingerprint of the ancient European predecessors and illustrate the migration routes of modern humans into Europe. Lineage-specific polymorphic sites on mtDNA were analyzed in the Carpathian Highlander population. The Carpathian Highlanders genetically resemble modern Europeans when analyzed by a hierarchical haplotyping scheme; however more studies need to be undertaken to fully understand the Carpathian Highlander's relation to the modern European population. Further analysis of European-specific subhaplogroups in Carpathian Highlanders will further clarify the relationship between this group and the rest of European inhabitants.

**KEYWORDS:** mtDNA, Mitochondrial DNA, Genetic Fingerprint, Migration Routes of Modern Humans

# Study of the human mitochondrial DNA polymorphism



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## ABSTRACT

*Traces of Paleolithic exodus routes of modern humans from Africa to Europe have been studied genetically using female specific ancestral lineages via mitochondrial DNA (mtDNA). We suggest modern humans entered Europe near the Carpathian Mountains and Hungarian Valley. If so, isolated populations of highlanders in Carpathian Europe may carry a genetic fingerprint of the ancient European predecessors and illustrate the migration routes of modern humans into Europe. Lineage-specific polymorphic sites on mtDNA were analyzed in the Carpathian Highlander population. The Carpathian Highlanders genetically resemble modern Europeans when analyzed by a hierarchical haplotyping scheme; however more studies need to be undertaken to fully understand the Carpathian Highlander's relation to the modern European population. Further analysis of European-specific subhaplogroups in Carpathian Highlanders will further clarify the relationship between this group and the rest of European inhabitants.*



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## Introduction

Multidisciplinary theories suggest that modern humans (*Homo sapiens*) originated in Sub-Saharan Africa about 200,000-100,000 years ago (Nei 1995). The modern human race is all, in fact, related. In this study the goal is to find a trace of a specific lineage of modern humans that in Paleolithic times came out of Africa and into Europe. We will be looking for the lineage tracings by studying modern human DNA. The focus will be on a small portion of the human genome, which is mitochondrial DNA (mtDNA) and how it can be used as a tool to trace the links between modern humans.

Mitochondrial DNA is found in the mitochondria of all eukaryotic cells. Mitochondria are the powerhouses of the cell. These are the organelles that produce energy for cellular function (Ingman and Gyllensten 2001). Mitochondria are abundant in each eukaryotic cell, hence the high copy number of mtDNA. mtDNA is a 16Kb molecule represented by hundreds to thousands of double stranded, circular copies per cell (Torrioni 2006). The mtDNA genome is maternally inherited and is known to have a much higher evolutionary rate than nuclear DNA (Torrioni 2006). Ancestral maternal lineages can be traced by the sequence variation—the mutations that have taken place in the molecule since the first modern human. The sequence variation can be noted by neutral differences that are found in the mtDNA called Single Nucleotide Polymorphisms or SNPs.

All modern humans have a certain type of mtDNA. The types of mtDNA are associated with the SNP pattern within the mitochondrial genome. mtDNA types are called haplogroups, so each person belongs to a certain haplogroup. Haplogroups are notated by letters of the alphabet, and there are a little over a dozen of them. Many major haplogroups are further subdivided into subhaplogroups.

Belonging to a particular haplogroup can display a person's common female specific place of origin (Torroni 2006). Haplogroup analysis is also used to trace population migration patterns (Santos 2004). The relative frequency of a haplogroup in a certain area determines its geographical origin.

The haplogroup L was the very first haplogroup of modern humans—originating in Africa (Torroni 2006). All the haplogroups that are known today stem from this single predecessor haplogroup. N, the earliest European haplogroup, stemmed from L about 65,000 years ago. About 60,000 years ago the R group was derived from N. The derivatives of R represent the early European haplogroups that are still the majority of the haplotypes in this area of the world. These are U/K (established about 50,000 years ago), H/V (established about 45,000 years ago), and J/T (established about 20,000 years ago). Stemming from the Paleolithic H haplogroup are two relatively recent sub-groups; H1 and H3. These sub-groups are European specific and are thought to originate in Southern France about 16,000 years ago.

The populating of Europe took place about 50,000 years ago (Torroni 2006), when most of Europe was covered by a thick sheet of ice (Adams 1997). These people were not spread all throughout Europe however; they initially resided in small glacial refuges.

In this study we addressed the question of how modern humans traveled out of Africa to Europe—what exodus routes were taken and what haplogroups can be used as clues to answer the question. We are suggesting an exodus of people traveling northeast out of the Horn of Africa then west into Europe. The movement out of Africa took place about 100,000 years ago by a small group of the first modern humans (Nei 1995). These people traveled east into the Fertile Crescent

and may have established small colonies there. Because the people were in new environmental conditions and given the fast evolutionary pace of mtDNA, new mutations may have started to emerge from the predecessor haplogroup. Some of the population continued on their move out of the Fertile Crescent following the Mediterranean Sea and then into the area of the Black Sea. At this time, the Black Sea was thought to be a freshwater lake (Ballard 2000). Small human populations might have been established in this area. This is also the area that we are suggesting the Paleolithic H haplogroup was conceived. From there, further expansion into Europe most likely took place via major European waterways. One of such waterways might have been the Danube River. People followed this river northwest, and some may have settled in the Carpathian Mountains and the Hungarian Valley.

The rest of the story of how people migrated across Europe is quite clear. Those whose haplogroup is the Paleolithic H spread throughout Europe after the ice shield partially retreated. They continued into Southern France where the haplogroup H was firmly established and then spread across Europe. This haplogroup then split into subgroups H1 and H3, which are European specific sub-groups (these sub-groups are the most frequent haplotypes of those living in modern Europe; representing about 46% (Santos 2004)).

By looking at the mtDNA genetics of an isolated population in the Carpathian Mountains we could test our Out-of-Africa exodus hypothesis. The isolated population is of the Carpathian Highlanders—the Lemko, Hutsul, and Boyko people. These people claim to have been Carpathian Mountains since the dawn of time. The Highlanders' genetics have never been previously studied. By studying their mtDNA genetics we were hoping to address

if these people are relatives of those who first populated Europe or if they are actually more closely related to the modern European population.

If, in fact, the Carpathian Highlanders are representatives from a geographical reserve for ancient haplogroups it is expected that they will have a higher proportion of the Paleolithic H in their mtDNA genetic make-up rather than the more recent European specific sub-group types, H1 and H3. This is because of their proximity to the genetic cradle. If the Carpathian Highlanders are representatives of a more modern European population then, we would expect to see a majority of the common Eurasian haplogroups in the sample. We would especially expect a majority of modern European specific H subhaplogroups H1 and H3 in the Carpathian Highlanders.

#### Materials and Methods

DNA samples were collected using Epicentre® buccal swabs. One hundred-fifteen Carpathian Highlanders consented to donate a sample. The DNA was extracted from the swabs using a QIAgen QIAamp DNA Mini Kit® following the protocol for buccal swab spin procedure extraction. The DNA sample extractions were eluted in one hundred micro liters of sterile water. An aliquot of fifty micro liters of the DNA was stored at -80°C, and the remaining fifty micro liters of DNA were stored at 4°C as a working stock for haplotyping usage.

The samples were haplotyped according to a hierarchical approach (Figure 1) using Polymerase Chain Reaction (PCR) and restriction enzyme digests. (Specifications for PCRs and restriction digests on Table 1.) (The haplotyping approach and primer sequences were adapted from Santos 2004).

Each sample went through the same basic PCR procedure. The PCR

consisted of a 50 micro liter mix. The reagents used were one unit of *Taq* DNA Polymerase with Standard *Taq* Buffer from New England Biolabs Inc.®, one unit of Deoxynucleotide Solution Mix from New England Biolabs Inc.®, and 10 pmols of each primer per reaction. The final volume was 50 micro liters filled with sterile water. The PCR thermocycler set-up was an initial 5 minute 95°C denaturing period, 95°C cycle denaturing period of 1 minute, 50 second primer annealing time, a 1 minute period of polymerization extension at 72°C, and a final extension of 5 minutes at 72°C. The PCR was run for 39 cycles. After each PCR was completed it was run on a 1.0% agarose gel to verify a successful reaction.

Each digestion used 8 micro liters of PCR product, enzyme, and enzyme buffer. The amounts and reaction temperature of the enzyme and enzyme buffer were followed according to the manufacturers (New England Biolabs Inc.®) specifications. Each reaction ran for 2-4 hours. The RFLP analysis were done on 2.0-2.5% agarose gels using the New England Biolabs Inc.® Low Molecular Weight DNA Ladder and 50bp DNA Ladder.

The sequencing for the 12705 *C/T* region was sent to the University of Michigan Sequencing Core. The preparation was followed according to the sequencing core instructions. The PCR product of the 12705 *C/T* amplification was cleaned up using a Qiagen MinElute PCR Purification Kit® for sequencing purposes.

## Results

From haplotyping the Carpathian Highlander mtDNA in the method shown on Figure 1, the majority of the haplotypes found in this population are representative of common European background. Most of the haplotypes found in the Carpathian Highlander population are Western-Eurasian. The

haplogroup of each sample can be found in Table 2.

A statistical comparison made between the haplogroup percentages represented by the Carpathian Highlanders and the modern European population was performed. This was to identify whether the Carpathian Highlanders provide a different proportion of haplogroups than modern Europeans. The modern European population haplogroup percentages used in this analysis were from the Santos (2004) study. The comparison was between haplogroup representation percentages of U/K, H/V, J/T, and other (refers to haplogroups represented in a non-significant amount) in both the Carpathian Highlanders and modern Europeans.

The Carpathian Highlander population is not different from the modern European population according to a chi<sup>2</sup> test. The chi<sup>2</sup> statistic is 0.414519 with a p-value of 0.93. For the Carpathian Highlanders to be a significantly different population than modern Europeans the chi<sup>2</sup> statistic would have to be greater than 7.81.

The statistical analysis shows that the sampled Carpathian Highlanders are most likely closely related to modern Europeans. The haplogroup representations in both groups are very similar.

## Discussion

The data show that by using the hierarchical approach represented on Figure 1 that the population sample of Carpathian Highlanders look similar to those of modern Europeans. This could mean that, in fact, the Carpathian Highlanders are more closely related to those people who populated Europe at a later time than what was hypothesized. (The Carpathian Highlanders may represent a population more closely related to those who first populated Europe out of Africa considering their isolation and proximity to the primary genetic cradle.)

To further check the results we wish to analyze more polymorphic sites in the sample mtDNA. These sites would be of the recently found subhaplogroups of H, H1 and H3. These subhaplogroups are European specific, and they are relatively recent developments in the Eurasian haplogroups (Torroni 2006). The classification and origin of the Eurasian haplogroups can be seen in Figure 2. If the subhaplogroups H1 and H3 are taken into consideration, more genetic history could be discovered about the Carpathian Highlanders. Determining this could also provide us with a clearer picture of exodus routes of modern humans from Africa. If the Carpathian Highlanders are, in fact, a population that is related to modern Europeans then we would expect to see a majority of those who belong to the H haplogroup to have the polymorphisms for H1 or H3. This is because the H1 and H3 subgroups are a more modern type of the H haplogroup (Torroni 2006). These subhaplogroups represent the majority of modern Europeans.

However, if these polymorphisms are not present at a high frequency in the Carpathian Highlanders, then it is probable that these people are actually relatives of those who first populated Europe from Africa—the European predecessors. The high representation of the haplogroup H in the Carpathian Highlanders would be of the ancient Paleolithic H that originated on an exodus route from Africa.



**Table 1.** Primers used with the polymorphic sites and sequences, the annealing temperature, the restriction enzyme used to digest PCR fragment and temperature, and what fragments to expect from digestion.

PCR	Digestion					
Polymorphic Site	Primer Sequence	Annealing Temperature (°C)	PCR Product Size	Enzyme	Digestion Temperature (°C)	SNP Digestion Fragments
MseI 14766	MseI4603L- CTAAACCCCATAAATAGGAG	54	226	<i>MseI</i>	65	Nonpolymorphic: 201bp; 21bp; 4bp
	MseI4791H- AGGTCGATGAATGAGTGG					Polymorphic: 184bp; 21bp; 17bp; 4bp
AluI 7025 (7028C)	Alu6949L- CCGTAGGTGGCCTGACTGGC	58	123	<i>AluI</i>	37	Nonpolymorphic: 108bp; 15bp
	Alu7025R- TGATGGCAAATA CAGCTCCT					Polymorphic: 78bp; 30bp; 15bp
NlaIII 4216	NlaIII4538- CACTCATCACAGCGCTAAGC	60	266	<i>NlaIII</i>	37	Nonpolymorphic: 266bp
	NlaIII4621- TGGCAGCTTCTGTGGAAC					Polymorphic: 177bp; 89bp
DdeI 10394	DdeI10252L- TTGATCTAGAAATTGCCCTC	48.2	208	<i>DdeI</i>	37	Nonpolymorphic: 208bp
	DdeI10527R- GTATTCCTAGAAGTGAGATG					Polymorphic: 143bp; 65bp
AluI 15606	AluI15503- AGACCCCTCTAGGCGACC	53	154	<i>AluI</i>	37	Nonpolymorphic: 154bp
	AluI15620- GATGGATAGTAATAGGGAAG					Polymorphic: 121bp; 33bp
BstOI 13704	BstOIL13627- TAGAATAATTCTTCTCACCC	53	137	<i>BstOI</i>	60	Nonpolymorphic: 137bp
	BstOIH13725- TAGTAATGAGAAATCCTCCG					Polymorphic: 98bp; 39bp
HinfI 12308	HinfI2216L- CACAAGAAGTCTAACTCATGC	58	123	<i>HinfI</i>	37	Nonpolymorphic: 123bp
	HinfI2338H- ATTACTTTATTGGAGTTGCACCAAGATT					Polymorphic: 93bp; 30bp
HaeIII 8994	Hae8880L- ACAGTGATTATAGGCTTTCGC	56	200	<i>HaeIII</i>	37	Nonpolymorphic: 167bp; 33bp
	Hae9042H- GTGCTTCCAATTAGG					Polymorphic: 136bp; 33bp; 31bp
HaeII 9052	HaeIIL8997- AACCAATAGCCCTGGCC	52	160	<i>HaeII</i>	37	Nonpolymorphic: 160bp
	HaeIHH9121- GCGATTCTAGGATAGTCAG					Polymorphic: 84bp; 76bp
MnlI 10871	MnlI10727L- CTCAATCTCCAACACATATGGC	58	232	<i>MnlI</i>	37	Nonpolymorphic: 232bp
	MnlI10920R- GGTCGGAGGAAAAGTTG					Polymorphic: 176bp; 56bp
Seq. 12705 C/T	CTL12553- ACAACCCAGCTCTCCCTAAG	58	635			Sequence results C: R*
	CTH13127- TGGAAGCGGATGAGTAAGAA					Sequence results T: N*

**Figure 2.** Breakdown and conception of European haplogroups from the first haplogroup, L that first originated in Africa [(Torrioni 2006) and (Santos 2004)].

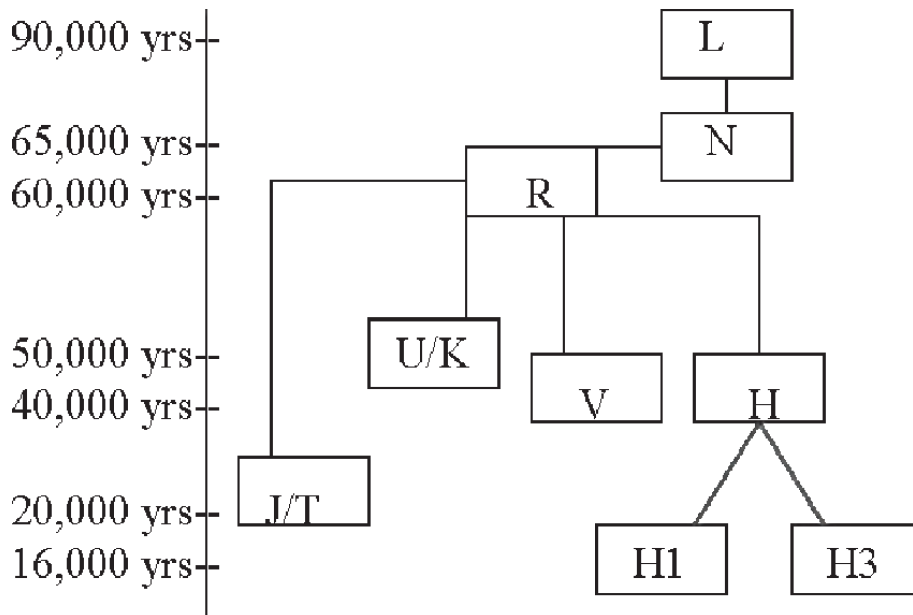


Table 2. Carpathian Highlander sample population haplotyping results.

Sample	Haplotype	Sample	Haplotype	Sample	Haplotype
A1	U	C4	J	E9	H
A2	H	C5	R*	E10	J
A3	U	C6	U	E11	U
A4	H	C7	H	E12	U
A5	T	C8	T	E13	H
A6	H	C9	T	E14	U
A7	R*	C10	H	E15	K
A8	U	D1	H	F1	U
A9	H	D2	H	F2	H
A10	HV, PRE*V, V	D3	W	F3	T
B1	H	D4	N*	F4	OTHER
B2	H	D5	H	F5	H
B3	H	D6	U	F6	J
B4	T	D7	R*	F7	H
B5	K	E1	J	L1M2	U
B6	W	E2	H	Z3L1	T
B7	H	E3	U	15C59	H
B8	H	E4	H	2MC65	HV, PRE*V, V
B9	H	E5	H	3L458	T
C1	H	E6	U	5M3L8	H
C2	X	E7	J	70936	H
C3	U	E8	J	856E3	H

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