Mitochondrial DNA of Ancient Cumanians: Culturally Asian Steppe Nomadic Immigrants with Substantially More Western Eurasian Mitochondrial DNA Lineages

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Mitochondrial DNA of Ancient Cumanians: Culturally Asian Steppe Nomadic Immigrants with Substantially More Western Eurasian Mitochondrial DNA Lineages

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Abstract The Cumanians were originally Asian pastoral nomads who in the 13th century migrated to Hungary. We have examined mitochondrial DNA from members of the earliest Cumanian population in Hungary from two archeologically well-documented excavations and from 74 modern Hungarians from different rural locations in Hungary. Haplogroups were defined based on HVS I sequences and examinations of haplogroup-associated polymorphic sites of the protein coding region and of HVS II. To exclude contamination, some ancient DNA samples were cloned. A database was created from previously published mtDNA HVS I sequences (representing 2,615 individuals from different Asian and European populations) and 74 modern Hungarian sequences from the present study. This database was used to determine the relationships between the ancient Cumanians, modern Hungarians, and Eurasian populations and to estimate the genetic distances between these populations. We attempted to deduce the genetic trace of the migration of Cumanians. This study is the first ancient DNA characterization of an eastern pastoral nomad population that migrated into Europe. The results indicate that, while still possessing a Central Asian steppe culture, the Cumanians received a large admixture of maternal genes from more westerly populations before arriving in Hungary. A similar dilution of genetic, but not cultural, factors may have accompanied the settlement of other Asian nomads in Europe.

Driven by the Mongol invasions of the 13th century, a tribal confederation of pastoral nomads known in Europe as Cumanians (called Polovici in Russian, Valben in German, Comains in French, and Quibčãq in Arabic-Persian sources or in their own tongue, Qunok) moved from the Trans-Carpathian steppes into

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the territory of Hungary, which had been devastated by the Mongol hordes (Berend 2001; Marquart 1914; Németh 1941; Selmeczi 1992). Before the Mongol invasion it was the Hungarian king Béla IV (1235–1270) himself who welcomed the Cumanians to the country. The king, in return for their military service, invited the Cumanians to settle in areas of the Great Plain between the Danube and the Theiss Rivers (today's Great Cumania and Little Cumania); this region had become almost uninhabited after the Mongol raids of 1241–1242 (Horváth 2001). The Cumanians lived there until their settlements were destroyed during the Turkish wars (16th–17th centuries) (Szakály 2000). At the beginning of the 18th century the Cumanian territories were resettled by their Hungarian-speaking descendants (Mészáros 2000). In the middle of the 18th century they got their status by becoming free farmers, and no longer serfs (Langó 2000a). They became completely assimilated, both genetically and culturally, with the Hungarians, leaving only the word *Kun* as an element in personal and place names.

Despite their historical status, the ethnic origins of the Cumanians are uncertain (Pálóczi Horváth 1998). They appear in Byzantine history in the 11th century as the westernmost group of the Kipchaks, whose loose and migratory empire extended from the frontier zone of the Byzantine territory to the steppes north of the Aral Sea (Moravcsik 1983; Pletneva 1990). By the end of the 12th century the Cumanians had advanced from the territories north of the Black Sea to east of the Carpathian Mountains (Ferenţ 1981; Kočkarov 2004). Their language, known from a 13th-century trilingual Cumanian-Latin-Persian dictionary, was a form of Turkish and was, until the 14th century, a *lingua franca* over much of the Eurasian steppes (Yule and Cordier 1916).

It is clear from this patchy history that the Cumanians had an ancestry well to the east of their eventual settlement in Hungary.

Analysis of ancient DNA (aDNA) from the bones of such a group of early settlers is the only reliable means to study the genetic affinities and relationships of the Cumanians in Hungary, because their descendants have been completely assimilated into the modern Hungarian population.

Mitochondrial DNA (mtDNA) is commonly used in aDNA studies because of its high copy number (up to 10,000 times that of single-copy nuclear sequences) and the lack of recombination as a result of its exclusively maternal inheritance (Giles et al. 1980). The hypervariable segment I (HVS I) of the mitochondrial control region (D-loop) is routinely used in human phylogeographic studies because it evolves 10 times faster than the protein coding region of mtDNA (Richards and Macaulay 2001; Vigilant et al. 1991). Human mtDNA D-loop mutations have accumulated sequentially along radiating maternal lineages during and after the process of human colonization of different geographic regions of the world (Marjoram and Donnelly 1994). Hence groups of mtDNA types often show geographic specificity (J. Chen et al. 1995; Y. S. Chen et al. 1995; Torroni and Wallace 1995). Analysis of mtDNA in populations therefore allows reconstruction of their maternal lineages, making it possible to study the

genetic traces of migration and admixture of different human communities and helping to estimate the degree of relationships within and between populations.

We have therefore analyzed the aDNA of a set of medieval Cumanian samples. DNA was extracted from excavated bones, and the HVS I was sequenced to determine the haplotypes and the monophyletic clades (haplogroups). The incidences of the different mitochondrial HVS I haplotypes and haplogroups were also investigated in the modern Hungarian gene pool by collecting hairs from different rural locations in Hungary. To classify mtDNAs that did not harbor a diagnostic haplogroup motif in their HVS I sequence, we assayed additional diagnostic markers out of the HVS I after PCR. To confirm the grouping, we also determined the haplogroup-associated polymorphic site at nucleotide position (nt) 10873 in the coding region in all cases.

We compared the mtDNA HVS I sequences of 2,615 samples from 34 European and Asian populations (retrieved from the published data) to HVS I sequences of 74 modern Hungarians and 11 ancient Cumanians from the present study. This data set was used to discover the relationships between Cumanians, Hungarians, and the members of Eurasian populations in order to study the genetic traces of the ancient group migrations and to examine their admixture with different human communities.

Materials and Methods

Populations and Sampling. In the Hungarian village of Csengele, on the borders of what is still called Kiskunság, Little Cumania, an archeological excavation in 1975 revealed the ruins of a medieval church with 38 burials. Several burials had all the characteristics of a Cumanian group: richly jeweled, non-Hungarian, and definitely Cumanian-type costumes; the 12-spiked mace as a weapon; bone girdles; and associated pig bones (Horváth 1978; Kovacs 1971; Sandor 1959). In view of the cultural objects and the historical data, the archeologists concluded that the burials were indeed Cumanian and mid 13th century; hence some of the early settlers in Hungary were from that ethnic group. In 1999 the grave of a high-status Cumanian derived from the same period was discovered about 50 m from the church of Csengele; this was the first anthropologically authenticated grave of a Cumanian chieftain in Hungary (Horváth 2001), and the contents are consistent with the ethnic identity of the excavated remains from the church burials. A separated area of the chieftain grave contained a complete skeleton of a horse.

Bones from 15 skeletons were used in this study, derived from two well-documented excavations at Csengele in 1975 and 1999 (Horváth 1978, 2001). From the earlier excavation it is known that these skeletons belonged to the earliest settlers. According to archeological analysis these 15 skeletons have the greatest probability that they could not mix with local inhabitants.

Table 1. Sample Characteristics

		Anthropological Features	
Sample	*Bone*	*Sex and Age*	*Character*
Cu1	Right humerus	Male, >55 years	Asian
Cu3	Left tibia	Child, 5–6 years	?
Cu4	Right humerus	?	Asian
Cu12	Right femur	Juvenile	?
Cu26	Right tibia	Male, >40 years	Asian
Cu27	Right femur	Child, 10–12 years	?
Cu28	Right tibia	Male, >40 years	Asian
Cu30	Left tibia	Male, >60 years	Asian
Cu31	Left femur	Juvenile (female), 12–13 years	?
Cu33	Right humerus	Juvenile (female), 12–13 years	?
Cu112 (chieftain)	Right femur	Male, >30 years	Euro-Asian

Using polyacrylamide gel after amplification, we detected aDNA in 11 bones. The archeological and anthropological data of these 11 samples are shown in Table 1.

Hair samples were collected from 74 maternally unrelated Hungarian-speaking adults of both sexes from different rural locations within Hungary.

DNA Extraction. DNA was extracted from the root portion of single hairs, using the Chelex-based method (Walsh et al. 1991).

DNA was carefully extracted from bones according to the method of Kalmar et al. (2000). Surface material was removed from the bones by washing them with diluted bleach and distilled water. The surface of the bones was treated with UV-C irradiation at 1 J/cm^2 for 30 min. A 2 cm $\times$ 5 cm portion was cut from each bone diaphysis, and the surface of these portions was removed (at least 2–3 mm deep) with a UV-C treated sterilized sand disk to avoid contamination with modern DNA. The cleaned bone fragments were treated with ultraviolet light at 1.0 J/cm^2 for 30 min and were mechanically ground into a fine powder with a sterile agate mortar. Physically powdered bone (250 mg) was suspended in 500 μl extraction buffer (0.1 *M* EDTA, 0.5% *N*-laurylsarcosine-Na salt, 100 mg/ml proteinase K), vortexed, and incubated overnight at 37°C with continuous vertical rotation. After phase separation by centrifugation at room temperature, 250 μl of supernatant was transferred to a 1.5-ml Eppendorf tube, and 3.5 μl of 1 μg/μl Dextran Blue (Sigma, Budapest, Hungary), 250 μl of 4 *M* NH_4-acetate, and 500 μl of 96% ethanol were added and mixed by vortexing. Because Dextran Blue has a large size with a molecular mass greater than 2 million D, it effectively coprecipitates low concentrations of DNA and colors the pellet. The DNA was precipitated at −70°C for 10 min and centrifuged at 18,000 rpm at 4°C for 15 min. The pellet was redissolved in 20μl deionized, distilled water and stored at −20°C.

Control Region Amplification. PCR amplification of mitochondrial markers was carried out in 40-μl volume reactions containing 1× Ex Taq Buffer (Takara Shuzo Co., Otsu, Japan), 250 μg/ml BSA (Boehringer, Mannheim, Germany), 6 μ*M* of each of the primers, 200 μ*M* of each of the dNTPs (Takara Shuzo Co.), 2.5 m*M* $MgCl_2$ (Takara Shuzo Co.), 7–11 μl bone or 10 μl hair DNA extract, and 1 U Takara Ex Taq Polymerase (Takara Shuzo Co.). The amplification protocol was 90 s at 93°C, followed by 35 cycles of 93°C for 45 s, 56°C for 1 min, and 72°C for 90 s, and a final extension at 72°C for 5 min.

For bone samples two primer pairs were used in separate reactions [L15996-H16221 (5′-TTGATGTGTGATAGTTGAGGG-3′) and L16182-H16401 (L15996 and H16401, Wilson et al. 1995; L16182, Kalmar et al. 2000)]. For hair samples the two outer primers were used. All numbering refers to the Cambridge reference sequence (CRS) (Andrews et al. 1999). Ten microliters of PCR product was run on 8% native polyacrylamide gel and was visualized after ethidium bromide staining by ultraviolet transillumination. The size of the products was determined with a GelBase gel documentation system (UVP).

Examination of Haplogroup-Associated Sequences. To classify mtDNAs that did not harbor a diagnostic haplogroup motif in their HVS I sequence, we assayed additional diagnostic markers from the protein coding region and HVS II (Macaulay et al. 1999; Quintana-Murci et al. 1999; Torroni et al. 1996) after PCR (Table 2). The PCR was carried out as described earlier. Several amplified segments were analyzed by restriction cleavage, and two of them (10400, 12308) were analyzed by direct sequencing to screen haplogroup-specific sites. The status at nt 10873 was checked in all samples to determine the superhaplogroup N (+10871 *Mnl*I). The screen involved haplogroup H (−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I), haplogroup HV (−73 *Apa*LI, +4580 *Nhe*I, +7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I), haplogroup V (−73 *Apa*LI, −4580 *Nhe*I, +10871 *Mnl*I, −14766 *Mse*I), haplogroup U, and haplogroup K (+10871 *Mnl*I; 12308G was determined by direct sequencing).

For ancient samples in which −10871 *Mnl*I and 10400T were detected, we determined the status at nt 5178 to confirm the haplogroup D classification based on HVS I, where −5176 *Alu*I supported the classification.

Postamplification Purification, Cloning, and Sequencing. After amplification 20 μl of the PCR products were run on 1.5% agarose gel at 80 V for 90 min in 1× Tris-acetate-EDTA buffer (40 m*M* Tris, 4 m*M* Na-acetate, 1 m*M* EDTA, pH 7.7–8), and the specific band was excised from the gel when the appropriate controls were negative and the amplified band was in the right site. DNA was recovered from gel using GenElute Agarose Spin Columns (Supelco Inc., Bellefonte, Pennsylvania) following the supplier's instructions. The pellet was resuspended in 20–30 μl deionized, distilled water, and 90–120 ng of PCR product was directly sequenced from both directions with an ABI Prism 310 sequencer (Perkin Elmer, Foster City, California) using the ABI Prism BigDye Terminator

Table 2. Analyzed Fragments of the HVS II and the Protein Coding Region with the Haplogroup-Specific Sites and PCR Primer Sequences[a]

Amplified Region	*Polymorphic Sites*[b]	*Primer Sequences*	*Haplogroup Specificity*
18–274	73 (73 *Apa*LI)	L39: 5′-CCTATTAACCACTCACGGGAGC-3′ H253: 5′-TGTGTGGAAAGTGGCTGTGCAG-3′	HV, H, V
4502–4679	4580 (4580 *Nhe*I)	L4521: 5′-TACCATCTTTGCAGGCACAC-3′ H4660: 5′-AAGGATTATGGATGCGGTTG-3′	V
5124–5273	5178 (5176 *Alu*I)	L5147: 5′-TTATCCTAACTACTACCGCATTCC-3′ H5254: 5′-GTGGCTATGATGGTGGGGATG-3′	D
6943–7146	7028 (7025 *Alu*I)	L6962: 5′-TTTTCACCGTAGGTGGCCTG-3′ H7126: 5′-TGAAATGGATTTTGGCGTAGG-3′	H
10271–10489	10400	L10292: 5′-CCTTTTACCCCTACCATGAGCC-3′ H10466: 5′-TTTATGTAAATGAGGGGCATTTGG-3′	M superhaplogroup
10743–10986	10873 (10871 *Mnl*I)	L10767: 5′-AACCTAAACCTACTCCAATGCTAAA-3′ H10965: 5′-GTGAGGGGTAGGAGTCAGGTAG-3′	N superhaplogroup
12194–12419	12308	L12214: 5′-CCCCTTATTTACCGAGAAAGC-3′ H12398: 5′-TTGTTAGGGTTAACGAGGG-TGG-3′	U
14619–14856	14766 (14766 *Mne*I)	L14638: 5′-ACCCCACAAACCCCATTACT-3′ H14856: 5′-AGGAGTGAGCCGAAGTTTCA-3′	HV, H, V

a. The haplogroup-specific sites were detected by restriction cleavage, except the sites at 10271–10489 and 12194–12419, which were detected by direct sequencing.

b. Numbers in parentheses indicate the first nucleotide of the recognition sequence of the indicated restriction enzyme.

v. 3.0 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California), as suggested by the manufacturer. The primers for the sequencing were the same as those used for amplification.

To minimize the risk of contamination, we cloned four of eleven samples using an Invitrogen PCR 2.1-TOPO cloning kit according to the manufacturer's instructions. From these four bones (samples Cu1, Cu12, Cu26, and Cu112) eight clones were sequenced from the smaller and longer control region fragments, respectively, and direct sequencing was also carried out. Two of the four samples were selected at random for cloning (Cu1 and Cu12). The PCR product of the Cumanian chieftain (sample Cu112) was also cloned, because its haplotype is especially interesting from an archeological aspect. Sample Cu26 was selected for cloning because, according to the direct sequencing of the longer HVS I fragment, it contains a mutation at np 16223, but this nucleotide position cannot be determined from the shorter fragment because it can be found in the target sequence of primer H16221. Furthermore, according to the results of HVS I direct sequencing, out of all the ancient samples, only sample Cu26 carries an Asian mitochondrial haplotype.

The average damage rate was calculated from the cloning results according to the method of Hofreiter et al. (2001). The number of clones carrying a substitution was divided by the total number of clones sequenced from the PCR product, summed over all values, and divided by the total number of positions analyzed to obtain the average damage rate over all positions. The relative damage rate was calculated to 1,000 bp.

Contamination Precautions. During excavation in 1999 and during the subsequent anthropometric analyses, handlers of the bones used latex gloves and mouth masks. To prevent contamination from modern DNA, we used drastic laboratory precautions and systematic controls. We applied rigorous physical separation of modern and ancient DNA laboratories and pre- and post-PCR laboratories. All steps (bone cutting, surface removal, powdering, extraction, and amplification) were carried out in separate places where ultraviolet irradiation was routinely used and no modern DNA work was permitted. All work surfaces and instruments were cleaned with 50% bleach and irradiated with ultraviolet light for 60 min before use. Chemicals for DNA extraction and for precipitation were filtered in a 0.22 μm Millex GS Filter unit (Millipore, Billerica, Massachusetts) and were also irradiated with ultraviolet light before use (apart from proteinase K). The bone powdering and extraction were set up in a laminar airflow box (DEFI, Debrecen, Hungary) supplied with a 254-nm germicidal lamp. An ultraviolet cleaner box, UVC/T (BioSan Ltd., Warren, Michigan), was used to prepare the amplification reaction. UV Air Flow Cleaner-Recirculators UVR-M (BioSan Ltd.) were used to decontaminate the air of the laboratories. Latex gloves, complete protective clothing, mouth masks, and Universal Fit Filter Tips (Corning Inc., New York City, New York) were used for all manipulations.

Table 3. HVS I and RFLP Haplotypes of the Laboratory Personnel

Cluster	*Researcher*	*HVS I Haplotype*	*RFLP Haplotype*[a]
U5b	Archeologist	189 192 270	+73 *Apa*LI, +10871 *Mnl*I, 12308G[b]
J1a	Anthropologist, 1st excavation	69 126 145 231 261	+10871 *Mnl*I
V	Anthropologist, 2nd excavation	240 298	−73 *Apa*LI, −4580 *Nhe*I, +10871 *Mnl*I, −14766 *Mse*I
H	Researcher 1	274	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H	Researcher 2	93 311	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
J	Researcher 3	69 126	+10871 *Mnl*I
K	Technician	176 192 224 311	+73 *Apa*LI, +10871 *Mnl*I, 12308G[b]

Mutations relative to the CRS (Andrews et al. 1999) are transitions unless the base change is specified.

a. Sites are numbered from the first nucleotide of the recognition sequence. A plus sign indicates the presence of a recognition site, and a minus sign indicates the absence of a recognition site.

b. Sites that were detected by direct sequencing.

Negative controls for both extractions and PCR amplifications were used, in which no bone powder was added to the extraction buffer and no template was added to the PCR mix.

To authenticate the results, two different researchers analyzed all bones in independent experiments. One researcher performed at least two extractions and two PCRs per extract to amplify the longer and shorter fragments of mitochondrial HVS I, respectively. The results of the amplifications were accepted as successful and reproducible if three out of the four products of amplification reactions were detectable with ethidium bromide staining in polyacrylamide gel by ultraviolet transillumination and if in the negative controls bands were not detectable. To establish HVS I sequences, the two researchers, individually, directly sequenced one PCR product from both directions. To establish the haplogroup-associated polymorphic sites out of the HVS I region, DNA extractions were performed recurrently. In all cases one PCR product with the appropriate size was analyzed either by restriction cleavage or by direct sequencing of the np 10271–10489 and np 12194–12419 regions.

To detect possible contamination of aDNA, the mtDNA D-loop sequences of all museum and laboratory personnel who handled the bones were determined (Table 3).

To test the molecular preservation of ancient DNA, we also used the outer primers (L15996-H16401) to try to amplify the HVS I of ancient samples.

To obtain information about the DNA contamination and DNA preservation levels of the human remains, we isolated DNA from an ancient horse bone (astragalus) from a Hungarian archeological site from the 10th century. This

bone was selected for examination because it comes from a nearby excavation site (about 30 km away, in Szeged, Hungary) and because the characteristics of the soil at this site are similar to those in Csengele. DNA analyses could not be done for the horse bone from the grave of the Cumanian chieftain at the beginning of the study because its skeleton was exhibited in the local museum in a glass box. After the exhibition we got an astragalus bone from this specimen and performed the DNA analysis. Partially overlapping mtDNA control region sequences between nt 15717 and 15893 and between nt 15539 and 15763 were amplified and sequenced using horse-specific primers (L15736: 5′-ACAGCCCATGTTCCACGAGC-3′ and H15875: 5′-AAAGAATGGGCGAGGTTGG-3′; and L15560: 5′-CACCATACCCACCTGACATGCA-3′ and H15742: 5′-GCTGATTTCCCGCGGCTTGGTG-3′). Nucleotide position numbering follows that of Xu and Arnason (1994). In separate reactions human HVS I-specific primers were also used on the ancient horse samples to study possible human DNA contamination. Human HVS I-specific primers were the same as those used to amplify the HVS I shorter and longer fragments of the human remains.

Statistical Analysis. From the NCBI database we collected 2,615 previously published mtDNA HVS I sequences derived from 34 modern Eurasian populations (10 populations from 3 different Asian geographic regions, 1 European/Asian population, 2 populations from the Uralic region, and 21 populations from 5 different European geographic regions) (Table 4). We edited these sequences to a standard 360-bp length (nt 16024–16383) and used this portion for comparison. In addition, we included 74 modern Hungarian and 11 ancient Cumanian sequences.

We used Arlequin 2.001 software (Schneider et al. 2000) to estimate genetic distances between populations. The statistical significance of F_{ST} values was estimated by permutation analysis using 10,000 permutations. Tamura-Nei distances (Tamura and Nei 1993) and a gamma parameter value of 0.26 were used to construct a distance matrix. This model is the most appropriate to describe the evolutionary process of the human hypervariable region (Meyer et al. 1999).

The resulting matrix of interpopulation pairwise F_{ST} values was summarized in two dimensions by use of multidimensional scaling implemented by the XLSTAT 7.1 program of Microsoft Office.

Results

Sequence Authentication. To prevent contamination from modern DNA, we used drastic laboratory precautions and systematic controls, as described earlier. Negative controls for both extractions and PCR amplifications were used. To authenticate the results, two different researchers analyzed all bones in independent experiments (see “Contamination Precautions” subsection). To detect possible contamination of aDNA, the mtDNA D-loop sequences of all museum and

Table 4. Modern Populations Considered in the Present Study

Region	*Population and Country*	*N*	*Reference*
Eastern Asia	Buryat, Russia	134	Shimada et al.[a]
	Korean, Korea	306	Lee et al. (1997)[b]
	Mongolian, Mongolia	103	Kolman et al. (1996)
Central Asia	Kazakh, Kazakhstan	55	Comas et al. (1998)
	Kirghiz (highland), Kirghizstan	47	Comas et al. (1998)
	Kirghiz (lowland), Kirghizstan	48	Comas et al. (1998)
	Uighur, Kazakhstan	55	Comas et al. (1998)
Western Asia	Georgian, Georgia	45	Comas et al. (2000)
	Kurd, Georgia	29	Comas et al. (2000)
	Turkish, Turkey	74	Calafell et al. (1996); Comas et al. (1996)
Europe/Asia	Russian, Russia	50	Malyarchuk and Derenko (2001)
Uralic	Komi, Russia	16	Voevoda et al.[a]
	Moksha, Russia	21	Sajantila et al. (1995)
Central-Eastern Europe	Bulgarian, Bulgaria	30	Calafell et al. (1996)
	Sekler, Romania	25	Kalmár et al.[a]
	Slovak, Slovakia	129	Metspalu et al.[a]
	Ukrainian, Ukraine	18	Malyarchuk and Derenko (2001)
Western Europe	Austrian, Austria	101	Parson et al. (1998)
	Bosco Gurin, Switzerland	14	Pult et al. (1994)
	Raetoromanian, Switzerland	16	Pult et al. (1994)
Northwest Europe	British, Great Britain	100	Piercy et al. (1993)
Northern Europe	Estonian, Estonia	48	Sajantila et al. (1995, 1996)
	Finnish, Finland	50	Sajantila et al. (1995)
	Icelander, Iceland	39	Sajantila et al. (1995)
	Karelian, Russia	83	Sajantila et al. (1995)
	Saami, Norway	176	Sajantila et al. (1995); Delghandi et al. (1998)
	Swedish, Sweden	32	Sajantila et al. (1996)
Southern Europe	Basque, Spain	151	Bertranpetit et al. (1995); Corte-Real et al. (1996); Pinto et al. (1996)
	Bosnian, Bosnia	180	Harvey et al.[a]
	Canarian, Tenerife, Spain	54	Pinto et al. (1996)
	Croatian, Croatia	64	Harvey et al.[a]
	Greek, Crete	186	Villems et al.[a]
	Sardinian, Italy	69	Di Rienzo and Wilson (1991)
	Serbian, Serbia	67	Harvey et al.[a]

N, number of individuals used in this study.

a. Data available only on GenBank (http://www.ncbi.nlm.nih.gov).

b. Out of the 306 Korean sequences, 290 were used for further analyses. Problematic sequences (Bandelt et al. 2001) were excluded.

laboratory personnel who handled the bones were determined (see Table 3). Haplotype matching was not found among ancient samples and laboratory members. To obtain information about the DNA preservation levels of the human remains, we also used the HVS I outer primers (L15996-H16401) to amplify the ancient samples. The unsuccessful amplification of the 446-bp mtDNA fragments in all cases (data not shown) also supports our contention that the amplified 239-bp and 266-bp fragments derived from the aDNA. Furthermore, DNA from two ancient horse samples, one derived from the grave of the chieftain and one from an excavation site nearby, was isolated. Using horse primers, we were able to acquire sequences; the first sequence was identical with the reference sequence (GenBank X79547) (Xu and Arnason 1994), and the sequences of the horse sample derived from the 10th century differed from that in a C/T transition at nt 15870; with human-specific HVS I primers no PCR product was obtained in either case. Four of the eleven samples were cloned, and the degree of sporadic base changes was determined. The data obtained substantiated the ancient nature of the amplified DNA. Samples possessed the same consensus sequence both in direct sequencing and after the cloning experiments.

Comparing the Result of Direct Sequencing and Sequencing After Cloning. To exclude contamination and test our laboratory safety system, 4 of the 11 samples were cloned and 8 clones were sequenced from each of the 4 samples from the 266-bp-long and 239-bp-long PCR fragments, respectively (Table 5). The direct sequencing and the sequencing after cloning led to the same consensus sequence in all cases. In the bones, which were cloned and sequenced, few altered bases were found but one clone differed from those, found by direct sequencing. But in these questionable positions the remaining seven clones contained the same bases as the sequence derived from direct sequencing. The damage rate of bones was established after cloning: 1.12 substitutions for samples Cu1 and Cu112, 1.4 substitutions for sample Cu12, and 1.68 substitutions for sample Cu26 were presented per 1,000 bp. These figures are similar to those published by Gilbert et al. (2003).

Haplogroup Assignment. For the 11 bone samples two partially overlapping sequences (266 bp and 239 bp long) were aligned to obtain the mitochondrial HVS I sequence between nt 15975 and nt 16420 (446 bp). Sequences represent eight different haplotypes, which belong to six haplogroups according to HVS I sequences and RFLP motifs (Table 6). Five out of the six clades are West Eurasian haplogroups (H, V, U, U3, and JT), which are included in the N macrohaplogroup, and the last one is an East Asian haplogroup (D), which belongs to the M lineage.

Figure 1 shows the mitochondrial phylogenetic network of Cumanian samples.

Mutations in sample Cu26 are characteristic of haplogroup D. This haplogroup occurs with high frequency in Northern Chinese populations (Mongolian,

Table 5. Longer (np 15975–16241) and Shorter (np 16182–16420) Fragment Sequences of Eight Clones of Samples Cu1, Cu12, Cu26, and Cu112 Compared to the Cambridge Reference Sequence (Andrews et al. 1999) and the Results of Direct Sequencing[a]

Sample	*nt*	*CRS*	*Clone 1*	*Clone 2*	*Clone 3*	*Clone 4*	*Clone 5*	*Clone 6*	*Clone 7*	*Clone 8*	*Direct Sequence*
Cu1, nt 15975–16241	16037	A	.	.	G	.	.	.	.	.	.
	16163	A	.	.	.	.	G	.	.	.	.
Cu1, nt 16182–16420	16298	T	C	C	C	C	C	C	C	C	C
	16320	C	.	.	.	T	.	.	.	.	.
	16355	C	.	.	.	.	.	.	.	T	.
Cu12, nt 15975–16241	16013	A	.	.	.	.	.	.	.	G	.
	16090	T	.	.	.	.	.	C	.	.	.
	16134	C	T	.	.	.	.	.	.	.	.
Cu12, nt 16182–16420	16209	T	.	.	.	.	.	C	.	.	.
	16230	A	.	.	.	.	G	.	.	.	.
Cu26, nt 15975–16241	16034	G	.	.	.	.	.	A	.	.	.
	16111	C	.	T	.	.	.	.	.	.	.
	16114	C	.	T	.	.	.	.	.	.	.
	16171	A	G	G	G	G	G	G	G	G	G
Cu26, nt 16182–16420	16223	C	T	T	T	T	T	T	T	T	T
	16276	T	C	.	.	.	.	.	.	.	.
	16311	T	C	C	C	C	C	C	C	C	C
	16336	G	.	.	.	.	.	.	A	.	.
	16362	T	C	C	C	C	C	C	C	C	C
	16363	C	.	.	T	.	.	.	.	.	.
Cu112, nt 15975–16241	16093	T	.	.	C	.	.	.	.	.	.
	16148	C	.	.	.	T	.	.	.	.	.
Cu112, nt 16182–16420	16209	T	.	C	.	.	.	.	.	.	.
	16343	A	G	G	G	G	G	G	G	G	G
	16353	C	.	.	.	.	.	.	T	.	.

a. Only variable sites are shown. Dots mean identical nucleotide with the CRS. Transition at np 16223 is not detectable for shorter fragments because it can be found in the target sequence of primer H16221.

Table 6. HVS I and RFLP Haplotypes with HVS II nt 73 Status of Cumanian and Modern Hungarian Samples

Cluster	*Cumanians*	*Hungarians*	*HVS I Haplotype*	*RFLP Haplotype*[a]
H4		5	CRS	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		4	93 311	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		2	274	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		2	311	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	93Y 169 311[d]	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	129	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	114	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	93 221	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	261	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	60	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	239	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	66 239	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	278 293 311	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	354	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1	51 162	−73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
H		1[b]	CRS	+73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I, 12308A[c]
H		2[b]	51 162 304	+73 *Apa*LI, −7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I, 12308A[c]
HV		1	311	−73 *Apa*LI, +4580 *Nhe*I, +7025*Alu*I, +10871 *Mnl*I, −14766 *Mse*I
HV		1	79 311	−73 *Apa*LI, +4580 *Nhe*I, +7025 *Alu*I, +10871 *Mnl*I, −14766 *Mse*I
V	1	4	298	−73 *Apa*LI, −4580 *Nhe*I, +10871 *Mnl*I, −14766 *Mse*I
V	1		298 311	−73 *Apa*LI, −4580 *Nhe*I, +10871 *Mnl*I, −14766 *Mse*I
V		1	240 298	−73 *Apa*LI, −4580 *Nhe*I, +10871 *Mnl*I, −14766 *Mse*I
U	1		362	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U	1		221 224 362	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U		1	192 311 319	+10871 *Mnl*I, 12308G[c]

Table 6. Continued

Cluster	*Cumanians*	*Hungarians*	*HVS I Haplotype*	*RFLP Haplotype*[a]
U3	1		343	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U4		2	134 356	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5		1	51 270	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5a		1	192 270	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5a1		1	192 256 270	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5a1		1	192 256 270 291	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5a1a		1	256 270	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5a1a		1	256 270 294	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
U5b		1	189 192 270	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
K		2	224 311	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
K		1	93 224 311	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
K		1	214 224 311	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
K		1	176 192 224 311	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
K		1	278 298 311	+73 *Apa*LI, +10871 *Mnl*I, 12308G[c]
JT	1		48 126	+10871 *Mnl*I
JT		1	126 366	+10871 *Mnl*I
JT		1	126 192	+10871 *Mnl*I
J		5	69 126	+10871 *Mnl*I
J1a		1	69 126 145 231 261	+10871 *Mnl*I
T		1	126 294	+10871 *Mnl*I
T		1	126 294 296 362	+10871 *Mnl*I
T1		1	126 163 186 189 274 294	+10871 *Mnl*I
T1		1[b]	126 163 186 189 294C	+10871 *Mnl*I
T2		2	126 294 296 304 305	+10871 *Mnl*I
T2		1	126 294 304	+10871 *Mnl*I
W		2	223 292	+10871 *Mnl*I
W		2	223 292 295	+10871 *Mnl*I
W		1	223 292 295 362	+10871 *Mnl*I, +5176 *Alu*I
W		1	223 292 362	+10871 *Mnl*I, +5176 *Alu*I
F		2	294 304	+73 *Apa*LI, +10871 *Mnl*I, 12308A[c]
F		1	304 355	+73 *Apa*LI, +10871 *Mnl*I, 12308A[c]
D	1		171 223 311 362	10400T,[c] −10871 *Mnl*I, −5176 *Alu*I

Mutations relative to the CRS (Andrews et al. 1999) are transitions unless the base change is specified. Identical haplotypes between Cumanians and Hungarians are highlighted.

a. Sites are numbered from the first nucleotide of the recognition sequence. A plus sign indicates the presence of a recognition site, and a minus sign indicates the absence of a recognition site.

b. Sample appears to have suffered a back mutation.

c. Sites that were detected by direct sequencing.

d. Y means T-C heteroplasmic site.

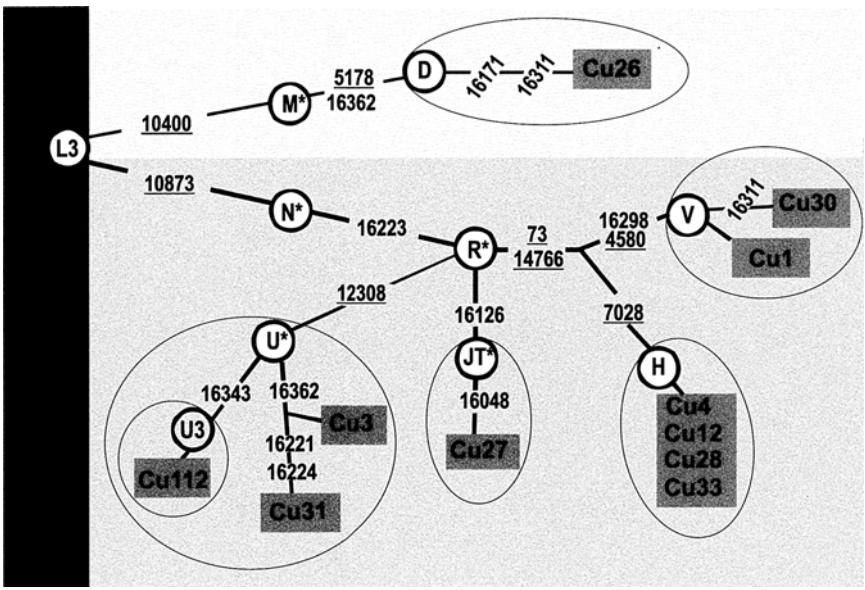

Figure 1. The mitochondrial phylogenetic network of Cumanian samples based on HVS I sequences and the examination of haplogroup-defining sites of the coding region and HVS II. (Haplogroup-defining sites from HVS I sequences are underlined.)

33.3%; Oroqen, 31.8%; Korean, 22.9%; Ewenki, 25.5%; Daur, 15.6%) (Kong et al. 2003), Central Asian populations (Kazakhs, 29%; Kirghiz Highland, 29.7%; Kirghiz Lowland, 41.6%; Uighur, 25.4%) (Comas et al. 1998), and Siberian populations [Sojot, 46.7%; Buryat, 33%) (Derenko et al. 2003); and Nganasan, 37%; Yukagir, 33%; Nivkh, 28%; Eskimo, 20%; Chukchi, 17% (Torroni et al. 1993)], but the occurrence of haplogroup D is very rare among Europeans (e.g., 1.86% in European Russians) (Helgason et al. 2001), except Saamis (5.11%) (Helgason et al. 2001). Among the studied modern Hungarians haplogroup D did not occur.

Comparing the HVS I haplotype of sample Cu26 with the Eurasian modern data set, we found three identical sequences. These three individuals belong to the modern Buryat, Kirghiz Lowland, and Kirghiz Highland populations, respectively. Sequences were taken for comparison from sources presented in Table 4.

Samples Cu1 and Cu30 represent two different haplotypes, which belong to the V haplogroup. Haplogroup V mainly occurs in Mediterranean western and northwestern regions of Europe (Richards et al. 1998), but it is present in almost all European populations, with the highest frequency among the Saamis (39.8%) (Derbeneva et al. 2002). However, the extremely high incidence of haplogroup V among the Saami is probable due to a genetic bottleneck rather than being representative of an early prevalence. Haplogroup V is represented among the

studied Hungarians with two different haplotypes present in five individuals. Comparing the ancient HVS I haplotypes with the modern data set, the haplotype of sample Cu1 occurs at the highest proportion in the Saami population, but it is detectable in four modern Hungarian samples too. The haplotype of sample Cu30 (a T → C transition is present at nt 16311 compared to sample Cu1) has been observed only in one member of the modern Serbian population (see source in Table 4).

The haplotype of sample Cu27 cannot be assigned to one of the major clusters that can be found in Europe. It may belong to the JT* superhaplogroup, characterized by the 16126 transition. This mutation was detected in a central Mediterranean individual and in two western Mediterranean individuals by Richards et al. (1998) and was classified in the JT* cluster. This transition is detectable in our HVS I data set in one person each from the Finn, Karelian, and Mongolian populations (see source of sequences in Table 4) and in two Hungarian samples. The haplotype of sample Cu27 defined by the motif 16048–16126 is unique.

We assigned four samples (Cu4, Cu12, Cu28, and Cu33) that were identical to the CRS to haplogroup H. Haplogroup H is the most frequent cluster in Europe, and it is also common in the Near East (Torroni et al. 1998). Haplogroup H is also the most frequent group among modern Hungarians, occurring at a frequency of 36%. The 27 modern Hungarian individuals belonging to this haplogroup represent 17 different haplotypes. Two haplotypes (represented by three individuals) were found to have a back mutation in HVS II at nt 73. The CRS sequence type occurred in five modern samples.

The haplotypes of samples Cu3 and Cu31 were characteristic of the U* haplogroup. This haplogroup occurs mainly in the southern but also in the eastern (given the high frequency in Bulgaria) region of Europe (Richards et al. 1998); however, it is more frequent in the Near East and North Africa (Richards et al. 2000). The occurrence of the U* haplogroup is detectable among modern Hungarians, but we did not find identical haplotypes between the ancient and the modern samples. In our data set the HVS I haplotype of sample Cu3 occurs at the highest frequency in the Uralic Komi population (source presented in Table 4), but it is detectable in several northern and southern Eurasian populations as well. The haplotype of sample Cu31 was unique in the U* haplogroup.

Sample Cu112 (chieftain) belongs to the U3 haplogroup. This haplogroup can be observed throughout Europe (Macaulay et al. 1999) but cannot be found in the modern Hungarian samples. The HVS I haplotype of sample Cu112 is found most often in the Balkan region, at high frequency among Greeks from Crete, Bulgarians, and Bosnians (source of sequence data presented in Table 4).

For the modern Hungarians the 74 samples represent 51 different haplotypes, which form 15 haplogroups (see Table 6). All but one is a West Eurasian haplogroup [the remaining one is an East Asian haplogroup (F)], but all belong

Table 7. Distribution of mtDNA Haplogroups in the Modern Hungarian Population

Haplogroup	*Number of Samples (Total, 74)*	*Frequency (%)*
H	27	36.5
HV	2	2.7
V	5	6.8
U	1	1.4
U4	2	2.7
U5	1	1.4
U5a	1	1.4
U5a1	2	2.7
U5a1a	2	2.7
U5b	1	1.4
K	6	8.1
JT	2	2.7
J	5	6.8
J1a	1	1.4
T	2	2.7
T1	2	2.7
T2	3	4.1
W	6	8.1
F	3	4.1

to the N lineage. As mentioned earlier, four haplogroups (H, V, U*, JT), represented by the ancient samples, can also be found among the modern Hungarians, but haplogroups U3 and D occur exclusively in the ancient group. We found eleven haplogroups (HV, U4, U5, K, J, J1a, T, T1, T2, W, and F) that occurred only in the modern population. Table 7 shows the haplogroup frequency in the modern Hungarian population. The presence of haplogroup F with 4.1% frequency is interesting, because this haplogroup is almost absent in continental Europe and can reflect some past contribution to the Hungarian population gene pool. Haplogroup F occurs with 5.4% frequency among southern Siberian populations, and it is rare in Buryats, but in Khakassians and Altaians it is found with relatively high frequencies (22.6% and 9.1%, respectively) (Derenko et al. 2003). The frequency of haplogroup F (including its subhaplogroups) is 8.4% among Mongolians in Northern China (Kong et al. 2003).

The haplogroups T, T1, U4, U5, and K, which were present in modern Hungarians, are widespread across Europe. Haplogroup J is widely distributed in the Mediterranean and in Central and Western Europe; haplogroup J1a can be found mainly in Alpine and north-central Europe (Richards et al. 1998). Haplogroup U5a appears to be mainly restricted to Southern Europe, and haplogroup U5a1 is found mainly in northwestern Europe, whereas haplogroup U5a1a occurs mainly in northwestern and north-central Europe (Richards et al. 1998). The haplotype of one individual defined by transitions at 16189, 16192, and 16270

was classified to haplogroup U5b (Richards et al. 1998, 2000). It was previously detected in the Alpine north-central region of Europe and in Finland (Richards et al. 1998). Groups W and T2 are fairly rare in Europe but not among Hungarians. Haplogroup W occurs with a frequency less than 2.5% in Europe (except in populations of Finland and Estonia, which have a 5.45% frequency, and in Bulgaria and Turkey, with 3.92% frequency) (Helgason et al. 2001), but it shows a frequency of 8.1% among Hungarians. For the T2 group the frequency in Europe is less than 1% (except in the populations of European Russia and Iceland, with frequencies of 1.86% and 2.57%, respectively) (Helgason et al. 2001), but it is 4.1% among Hungarians.

Statistical Analysis. The results of the distance matrix are graphically summarized in the multidimensional scaling analyses, where the plotted points correspond to Eurasian populations and the genetic distances among them are proportional to the linear distances between the plotted points (Figure 2). The data points form two distinct clusters. One cluster contains all the Eastern and Central Asian populations and can be divided into two subclusters; one subcluster includes mainly Eastern Asian populations (Buryat, Korean, and Kirghiz Lowland populations), and the other subcluster harbors mainly Central Asian populations (Mongolian, Kazakh, Kirghiz Highland, and Uighur populations). The second cluster contains the remaining populations, except Saamis and Bosco Gurins, which are separated from all the other populations. Inside the second cluster, based on HVS I motifs, a clear structure is not detectable, but almost all European populations assemble in one section with small distances, including the modern Hungarians. Cumanians are outside this section. This ancient population is nearest to the Finnish, Komi, and Turkish populations. In this plot Cumanians can be found above the abscissa; this is the population from the second cluster, which is closest to the East-Central Asian cluster.

Discussion

In this study we have examined a medieval Cumanian group and a modern Hungarian population to determine the genetic origin and relationships of the Cumanian population. We extracted DNA from the human remains of two archeologically well-documented Hungarian excavations, representing members of a local population dating from the late 13th or early 14th century. Eleven of the 15 remains examined contained detectable aDNA. We also examined 74 modern Hungarian DNA extracted from single hairs.

The geographic radiation of the maternal lineages could be followed by determining the mutations of mtDNA, which display a strong geographic specificity (Vigilant et al. 1991). Analyzing the mutation pattern of mitochondria, therefore, gives the possibility of tracing human migrations. Ancient DNA can thus contribute both to the interpretation of individual archeological sites and to

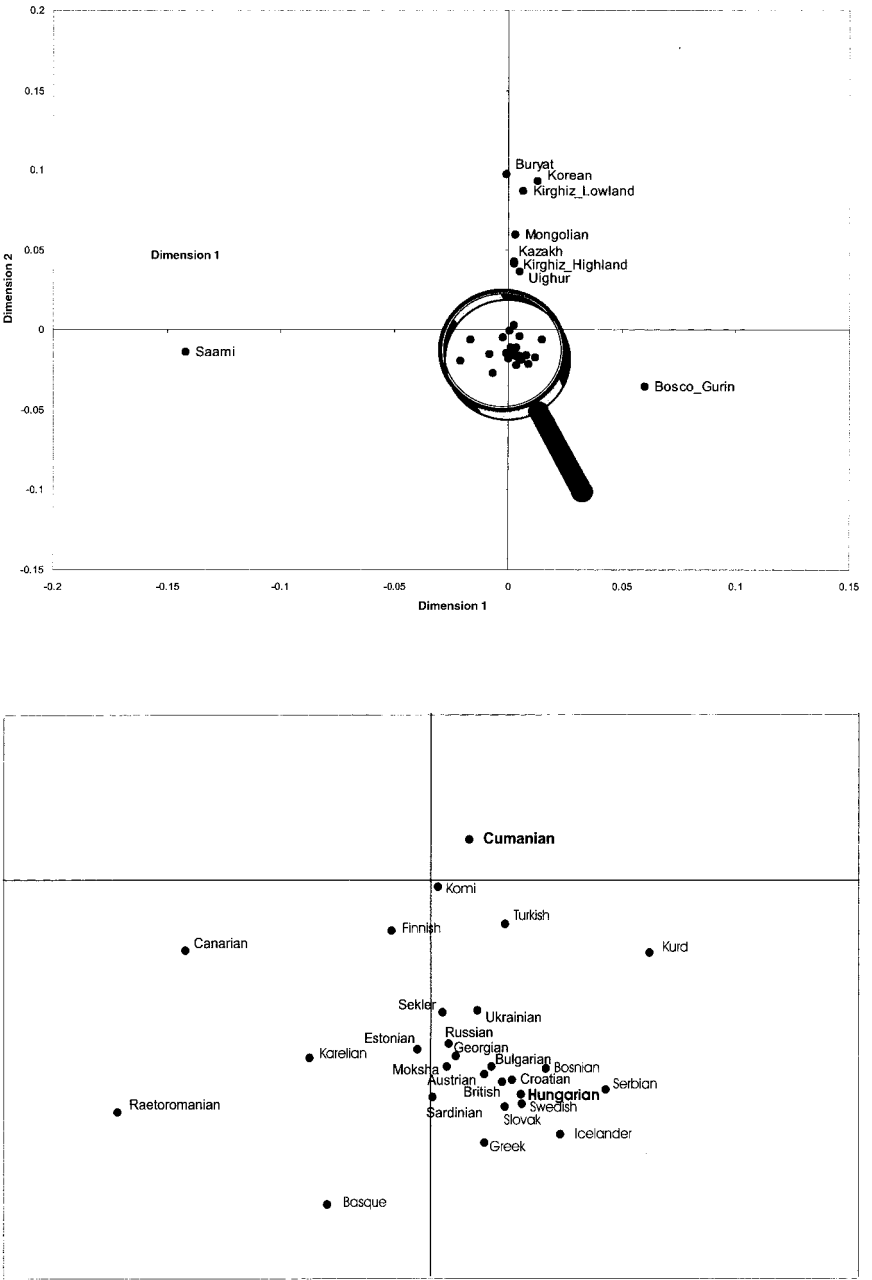

Figure 2. Multidimensional scaling plot of interpopulation pairwise F_{ST} values based on 2,700 HVS I sequence data of 35 modern Eurasian populations and the ancient Cumanians.

testing hypotheses about past populations. Several publications deal with the analysis of mtDNA extracted from archeological remains to investigate the origin and evolution of past populations in the Americas (Gonzalez-Oliver et al. 2001; Kaestle and Smith 2001; Parr et al. 1996; Ribetio-dos-Santos et al. 1996), Australia (Adcock et al. 2001), Asia (Endicott et al. 2003; Lalueza-Fox et al. 2004; Yao et al. 2003), and Europe (Vernesi et al. 2004).

This study is the first aDNA characterization of one of the many historically attested eastern pastoral nomad populations that migrated into Europe—in this case, into the Carpathian basin during the 13th century. These archeological Cumanian samples belong to six haplogroups. One of these haplogroups belongs to the M lineage (haplogroup D) and is characteristic of Eastern Asia, but this is the second most frequent haplogroup in southern Siberia too. All the other haplogroups (H, V, U, U3, and JT) are West Eurasian, belonging to the N macrohaplogroup. Out of the eleven remains, four samples belonged to haplogroup H, two to haplogroup U, two to haplogroup V, and one each to the JT, U3, and D haplogroups. Modern Hungarian samples represent 15 haplogroups. All but one is a West Eurasian haplogroup [the remaining one is East Asian (haplogroup F)], but all belong to the N lineage. Four haplogroups (H, V, U*, JT), present in the ancient samples, can also be found in the modern Hungarians, but only for haplogroups H and V were identical haplotypes found. Haplogroups U3 and D occur exclusively in the ancient group, and 11 haplogroups (HV, U4, U5, K, J, J1a, T, T1, T2, W, and F) occur only in the modern Hungarian population. Haplogroup frequency in the modern Hungarian population is similar to other European populations, although haplogroup F is almost absent in continental Europe; therefore the presence of this haplogroup in the modern Hungarian population can reflect some past contribution.

Our results suggest that the Cumanians, as seen in the excavation at Csengele, were far from genetic homogeneity. Nevertheless, the grave artifacts are typical of the Cumanian steppe culture; and five of the six skeletons that were complete enough for anthropometric analysis appeared Asian rather than European (Horváth 1978, 2001), including two from the mitochondrial haplogroup H, which is typically European. It is interesting that the only skeleton for which anthropological examination indicated a partly European ancestry was that of the chieftain, whose haplotype is most frequently found in the Balkans.

We conclude therefore that the mitochondrial motifs of Cumanians from Csengele show the genetic admixtures of the Cumanians with other populations rather than the ultimate genetic origins of the founders of Cumanian culture. This may be the result of the habits of the Cumanian nomads. Horsemen of the steppes formed a political unit that was independent from their maternal descent or their language and became members of a tribal confederation (Brather 2004). According to legends, Cumanians frequently carried off women from raided territories. So the maternal lineages of a large part of the group would reflect the maternal lineage of those populations that had geographic connection with Cumanians

during their migrations. Nevertheless, the Asian mitochondrial haplotype in sample Cu26 may still reflect the Asian origins of the Cumanians of Csengele. However, by the time the Cumanians left the Trans-Carpathian steppes and settled in Hungary, they had acquired several more westerly genetic elements, probably from the Slavic-, Finno-Ugric-, and Turkic-speaking peoples who inhabited the regions north of the Black and Caspian Seas.

The Cumanian settlement of the Danubian plain is only one of the historically attested arrivals in Europe, some of which led to political dominance, of nomadic peoples with an ultimate origin in the Asian plains: first the Huns, Avars, Bulgarians, then the Hungarians themselves in the 10th century, and later the Pechenegs and Alans (Bachrach 1973; Bálint 1989; Berend 2001; Langó 2000b; Selmeczi 1992). If their travels were like those of the Cumanians studied here, their genetic impact on the European population may have been far less than their cultural impact.

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Electronic-Database Information

The accession numbers from GenBank (http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html) for the Cumanian HVS I sequence data in this paper are AY268482–AY268492.

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