Mitochondrial DNA Sequence Variation in the Boyko, Hutsul, and Lemko Populations of the Carpathian Highlands

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Abstract Genetic studies of the distribution of mitochondrial DNA (mtDNA) haplogroups in human populations residing within the Carpathian Mountain range have been scarce. We present an analysis of mtDNA haplogroup composition of the Boykos, Hutsuls, and Lemkos, three population groups of the Carpathian highlands. In our study Hutsuls had the highest frequency of subhaplogroup H1 in central and eastern Europe. Lemkos shared the highest frequency of haplogroup I ever reported and the highest frequency of haplogroup M* in the region. MtDNA haplogroup frequencies in Boykos were different from most modern European populations. We interpreted these unique mtDNA frequencies to be evidence of diverse and dynamic population histories in the Carpathian highland region.

In the last decade a wealth of information about mitochondrial DNA (mtDNA) polymorphism in European populations has become available, based mostly on samplings of western and to some degree central and eastern European populations across broadly defined nationalities. Numerous studies on the origin and diversity of human mtDNA haplogroups indicate that all modern haplogroups coalesce in Africa around 150,000 years ago (Cann et al. 1987; Forster 2004; Horai et al. 1995; Wallace et al. 1999). The first two branches of the mtDNA tree outside Africa were superclades M* and N*, from which all subsequent non-African world mtDNA haplogroup variety originated (Forster 2004; Mishmar et al. 2003; Quintana-Murci et al. 1999; Wallace et al. 1999). Current genetic evidence suggests that most

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present-day European haplogroups evolved in the Middle East some 40,000 years ago as branches of the N* node and subsequently diffused into Europe through Anatolia and across the Lower Danube basin (Richards et al. 2000; Roostalu et al. 2007 and references therein; Torroni et al. 1998).

Almost all major human mtDNA haplogroups display a region-specific distribution (Torroni et al. 1994 and references therein; Torroni et al. 1996 and references therein; Wallace et al. 1999). Recent examples include studies that identified the H1, H2, H3, and H5a subclades of haplogroup H (Pereira et al. 2005 and references therein), which appeared in the late Pleistocene–early Holocene, as autochthonous European (Achilli et al. 2004). Haplogroups such as T and J are considered to have expanded into Europe from the Near East during the European Neolithic with the advent of farming (Richards 2003 and references therein; Richards et al. 1996, 2000; Serk 2004). It is thus a reasonable assumption that traces of ancestral genetic pools have been preserved in the corresponding modern populations. Therefore mtDNA polymorphism can be used to infer the origins of the founding lineages of human populations currently residing in Europe.

Most studies of human mtDNA haplogroup frequency distribution have focused on broadly defined nationalities without taking into account populational subdivisions. These studies resulted in a relatively homogeneous haplogroup distribution over Europe with some differences, particularly in the frequencies of haplogroup H (Loogväli et al. 2004; Pereira et al. 2005). However, those studies that focused on smaller and relatively more isolated ethnocultural subgroups revealed pronounced peculiarities in haplogroup distributions as well as a more defined prehistoric genetic fingerprint (González et al. 2006; Izagirre and de la Rúa 1999).

In the current study we focus on three population groups that inhabit the highlands of the western and northeastern Carpathian Mountains: the Boykos, the Hutsuls, and the Lemkos (Figure 1). Lemkos are the westernmost group, historically residing within the territory called Lemkivschyna, including the Prešov region in eastern Slovakia as well as the Lesser Poland and Subcarpathian Provinces of southern Poland. In 1944–1947 Lemkos were forcibly relocated and dispersed within Poland and western Ukraine (Misiło 1993). Boykos inhabit the mountain valleys of the Ukrainian Carpathians to the east of Lemkivschyna, with some overlap in distribution in eastern Poland, stretching through the western parts of the Ivano-Frankivsk Province in Ukraine, within the ethnic territory called Boykivschyna. Hutsuls are found within the ethnic territory of Hutsulschyna, southeast of Boykivschyna, and occupy the southeast part of the Ivano-Frankivsk Province, most of the Trans-Carpathian Province, and the southwestern part of the Chernivetska Province of Ukraine as well as the northernmost parts of Romania. The historical center of Hutsulschyna is in the Hutsul Beskyds, around the sources of the Prut, Cheremosh, and Seret rivers.

Anthropologically, Lemkos, Boykos, and Hutsuls belong to the Carpathian group, with Hutsuls forming a discrete subcluster (Dyachenko 1987). Although the Carpathian anthropological type shares many characteristics with the Slavic

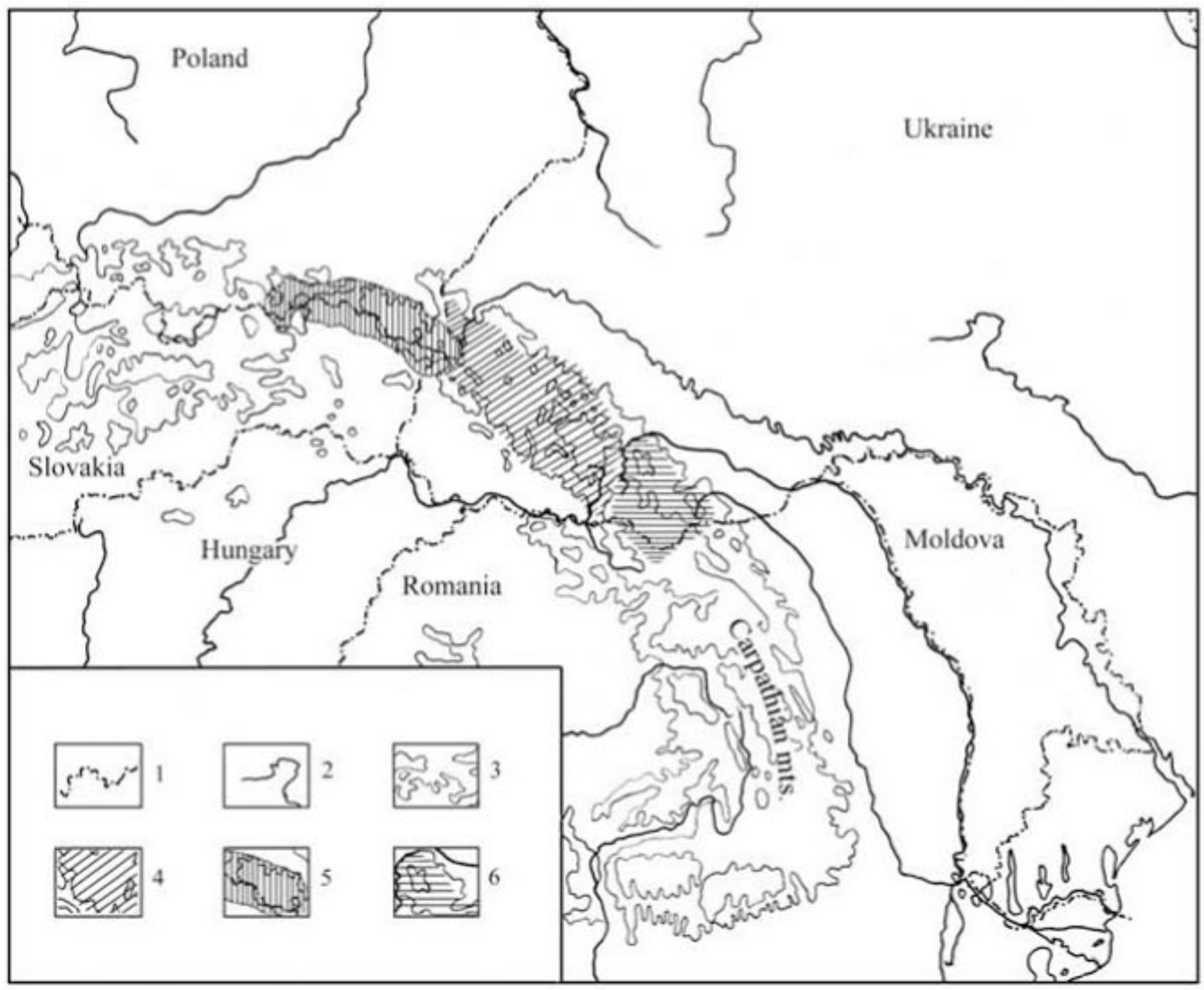

Figure 1. Central Europe with an outline of the Carpathian Mountains showing the approximate locations of the Boyko, Hutsul, and Lemko territories. (1) Country borders, (2) rivers, (3) mountains, (4) Boyko territory (Boykivschyna), (5) Lemko territory (Lemkivschyna) before 1944, (6) Hutsul territory (Hutsulschyna).

ethnic group, it also incorporates features characteristic of non-Slavic ethnicities, which are especially prominent in Hutsuls (Dyachenko 1987).

There is no consensus on the origins of Boykos, Hutsuls, and Lemkos, partly because of the scarceness of historical sources of information in the region. Documented information about Carpathian highlanders is virtually absent before the 14th century (Zakrevska 1997). Their ethnocultural identities are also not well established within the respective jurisdictions where these groups reside. Although in Slovakia Lemkos are considered a distinct ethnic minority, Lemkos, Boykos, and Hutsuls living in Ukraine are considered part of the Ukrainian nationality. In Poland, Lemkos only recently began to enjoy some ethnic recognition (Mihalasky 1997). Yet each group has its own linguistic dialect as well as distinctive cultural attributes, including a rich oral history, in which each group claims to have distinct ethnocultural roots (Chopovsky 2003; Domashevsky 2001).

The aim of this study was to analyze mtDNA haplogroup composition in these three highlander groups of the Carpathian Mountains with the goal of better

understanding their origins and connecting them genetically to contemporary and historical populations in the region.

Materials and Methods

Sample Collection and DNA Extraction. Buccal swab samples were collected throughout the Ivano-Frankivsk Province, Ukraine, in full accordance with applicable human subject research protocols. Samples were obtained from 111 anonymous, unrelated volunteers who were able to identify their maternal grandmother's ethnicity as Boyko (20 subjects), Hutsul (38 subjects), or Lemko (53 subjects). The volunteers themselves hailed from the respective ethnic territories. DNA was extracted from the swabs with a Qiagen QIAmp DNA Kit using buccal swab spin protocol. The DNA extractions were eluted in 150 µl of water.

Molecular DNA Analysis. Samples were assigned mtDNA haplogroup designations by using PCR-RFLP high-resolution analysis of diagnostic SNPs based on the hierarchical method proposed by Santos et al. (2004) for samples with a predominantly West Eurasian background; previously published PCR primers and PCR conditions were used (Santos et al. 2004). The presence (+) or absence (−) of enzymatic restriction sites was determined by PCR-RFLP analysis of the following diagnostic nucleotide positions:

H: 14766 *Mse*I (−), 7025 *Alu*I (−)
Pre-HV: 14766 *Mse*I (+), 7025 *Alu*I (+), 4577 *Nla*III (+)
HV, pre*-V: 14766 *Mse*I (−), 7025 *Alu*I (+), 4577 *Nla*III (+)
V: 14766 *Mse*I (−), 7025 *Alu*I (+), 4577 *Nla*III (−)
K: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (+), 9052 *Hae*II (−)
U: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (+), 9052 *Hae*II (+)
J: 14766 *Mse*I (+), 4216 *Nla*III (+), 10394 *Dde*I (+), 13704 *Bst*0I (−)
T: 14766 *Mse*I (+), 4216 *Nla*III (+), 10394 *Dde*I (−), 15606 *Alu*I (+)
I: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (+), 10032 *Alu*I (+), 1715 *Dde*I (−)
W: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (−)
X: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (+), 10032 *Alu*I (−), 14465 *Acc*I (+)
L*: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (+), 10032 *Alu*I (−), 14465 *Acc*I (−), 11719 *Sma*I (−), 10871 *Mnl*I (−), 3592 *Hpa*I (+)
M*: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (+), 10032 *Alu*I (−), 14465 *Acc*I (−), 11719 *Sma*I (−), 10871 *Mnl*I (−), 3592 *Hpa*I (−), 10397 *Alu*I (+)
N*: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (+), 10032 *Alu*I (−), 14465 *Acc*I (−), 11719 *Sma*I (−), 10871 *Mnl*I (+), 12705(T) (confirmed by sequencing)

R*: 14766 *Mse*I (+), 4216 *Nla*III (−), 12308 *Hin*fI (−), 8994 *Hae*III (+), 10032 *Alu*I (−), 14465 *Acc*I (−), 11719 *Sma*I (−), 10871 *Mnl*I (+), 12705(C) (confirmed by sequencing)

Samples typed to haplogroup H were further assigned H subhaplogroup designations. Based on the relative frequencies and putative origins in Europe, five H subhaplogroups were selected for screening—H1, H2, H3, H5a, and H10—by both PCR-RFLP analysis and DNA sequence analysis of the corresponding diagnostic positions. H1 (3010(A)) and H3 (6776(C)) are the most frequent H subhaplogroups in Europe; H2 (1438(A), 4769(G)) is a frequent eastern European H subhaplogroup, and H5a (4336(C)) appears at its highest frequency in central Europe (Pereira et al. 2005). H10 was chosen because its diagnostic polymorphism, 4216(C), lies within the PCR region amplified for the H5a diagnostic polymorphism screening.

PCR conditions were essentially those described by Pereira et al. (2005). PCR primers for H1, H2, and H3 have been previously published (Finnilä et al. 2001; Pereira et al. 2005). We used PCR primers designed for the detection of the 4216 *Nla*III polymorphism (Santos et al. 2004) for H5a and H10 diagnostic SNP screening. All H samples were first screened for H1 and H3 polymorphisms. The non-H1 non-H3 samples were then screened for the H2 diagnostic SNPs. The remaining samples were screened for H5a and H10. Samples not showing H1, H2, H3, H5a, and H10 diagnostic polymorphisms were designated as H*.

PCR sample cleanup for sequencing was accomplished using a Qiagen MinElute PCR Purification kit. Sequencing was done at the University of Michigan Sequencing Core. Sequences were aligned manually, and all polymorphism positions were visually checked on the chromatograms.

Statistical Analysis. To investigate the clustering of population samples, we performed a principal components analysis on the haplogroup frequency distribution using Statistica, version 6.1, with haplogroup frequencies from our samples and with data obtained from the literature. The principal components analysis can be used to visualize high-dimension data by projecting the populations on a graph by means of the most important factors explaining the variation among groups. Euclidean distances between populations were calculated with haplogroup frequency composition using the joining (tree clustering) algorithm with single linkage (nearest neighbor) (Statistica 6.1). In this method the distance between two clusters is determined by the distance between nearest neighbors in the different clusters. An unrooted hierarchical tree plot based on the cluster data set was also constructed.

Results

Table 1 lists mtDNA haplogroup frequencies of the Boyko, Hutsul, and Lemko populations compared with those previously published for other central and eastern European populations. All common European mtDNA haplogroups

Table 1. MtDNA Haplogroup Frequencies (%) for the Boyko, Hutsul, and Lemko Populations Along with Frequencies for Eight Central and Eastern European Populations from the Literature[a]

Population (N)	*H*	*HV, pre*-V*	*V*	*U*	*K*	*N**	*R**	*M**	*I*	*W*	*X*	*T*	*J*	*Reference*
Boyko (20)	20	5	5	15	5	5	5	0	0	5	0	30	5	Present study
Hutsul (38)	50	0	0	15.8	5.3	5.3	5.3	0	0	0	2.6	2.6	13.2	Present study
Lemko (53)	32.1	3.8	0	7.5	5.7	0	1.9	5.7	11.3	3.8	1.9	13.2	13.2	Present study
Hungarians (98)	46.9	8.2	1	17.4	2	0	0	1	0	4.1	0	2	12.5	Semino et al. (2000b)
Poles (436)	45.2	5.7	0	16.1	3.4	0.5	0.5	1.8	1.8	3.7	1.8	11.5	7.8	Malyarchuk et al. (2002b)
Romanians (105)[b]	41	5.7	1.9	10.5	7.6	1.9	1.9	0	1	1	4.8	9.5	13.3	Bosch et al. (2005)
Belorussians (92)	40.2	9.8	0	26.1	2.2	1.1	0	2.2	2.2	1.1	1.1	8.7	4.4	Belyaeva et al. (2003)
Croatian mainland (277)	45.1	0.4	4	17.8	3.6	1.1	0	0.4	1.4	2.2	2.2	6.7	11.9	Peričić et al. (2005)
Czechs (179)	44.1	3.9	0	14	3.9	1.7	0	1.7	2.8	0.6	1.7	12.3	11.7	Malyarchuk et al. (2006)
Russians (251)	41.4	8.4	0.8	16.7	2.8	0	0.4	1.2	2.4	2	3.6	12.4	7.6	Malyarchuk and Derenko (2001)
Ukrainians (54)	42.6	0	3.7	20.4	7.4	0	0	0	0	1.9	1.9	11.1	7.4	Malyarchuk and Derenko. (2001), Malyarchuk et al. (2002a)

a. Data for haplogroups L* and pre-HV, for which no representatives were detected in all three Carpathian populations under study, have been omitted.

b. The combined frequencies for Romanian populations (Constanta and Ploiesti) are presented.

Table 2. mtDNA Subhaplogroup H Frequencies (%) in the Boyko, Hutsul, and Lemko Populations and in Neighboring Populations and Representatives of Populations Close to the Franco-Cantabrian Region

Population (N)	*H (Total)*	*H1*	*H2*	*H3*	*H5a*	*H10*	*Reference*
Boyko (20)	20.0	0	0	0	0	0	Present study
Hutsul (38)	50.0	18.4	2.6	2.6	5.3	2.6	Present study
Lemko (53)	32.1	7.5	1.9	0	0	0	Present study
Croatians (84)	44.0	8.3	–	6.0	–	–	Achilli et al. (2004)
Hungarians (130)	42.3	12.3	–	6.2	–	–	Achilli et al. (2004)
Slovaks (119)	42.0	7.6	1.0	2.0	1.0	–	Loogväli et al. (2004)
Czechs (102)	41.2	10.8	–	2.0	–	–	Achilli et al. (2004)
Poles (86)	37.2	9.3	–	3.5	–	–	Achilli et al. (2004)
Ukrainians (191)	40.8	9.9	–	2.1	–	–	Achilli et al. (2004)
Russians (312)	40.1	13.5	–	1.6	–	–	Achilli et al. (2004)
Spaniards (132)	39.4	18.9	–	3.8	–	–	Achilli et al. (2004)
Spanish Galicians (266)	45.0	17.6	3.2	8.1	2.7	–	Quintáns et al. (2004)
French Basques (40)	40.0	17.5	–	5.0	–	–	Achilli et al. (2004)

with the exception of the pre-HV haplogroup were found in all three populations under study. No representatives of the L node were found in any of the three Carpathian populations. There were no ambiguous samples for which a haplogroup could not be assigned based on the PCR-RFLP analysis.

Compared to European mtDNA databases, Boykos were different from most of the European populations studied to date. The Boyko sample analysis showed atypically low haplogroup H frequency (20%) for a European population. Boyko samples showed no H subhaplogroup subdivision to the extent of the resolution capacity of the current study (Table 2). Boykos displayed the highest haplogroup T frequency (30%) in the region and the second lowest frequency of haplogroup J in central and eastern Europe after the Belorussian population (5% and 4.4%, respectively) (Table 1).

The Lemko sample analysis showed the second lowest haplogroup H frequency (32.1%) in the region after Boykos. Among the 17 haplogroup H samples in Lemko, 4 (23.5% of the haplogroup H samples, 7.5% overall) were H1 and 1 (5.9%, 1.9% overall) was H2 (Table 2). The Lemko sample also contained the highest frequency of haplogroup I (11.3%) in Europe, identical to that of the population of Krk Island (Croatia) in the Adriatic Sea (Peričić et al. 2005). Three samples (5.7%) in the Lemko group belonged to the M* clade, similar to the number of M* specimens uncovered in a subset of the Hungarian population (5.5%) in a recent study (Nádasi et al. 2007). These populations present the highest frequencies of haplogroup M* reported in the region.

The Hutsul samples had the highest frequency of haplogroup H (50%) in the region after the Hungarian Paló́c group of Carpathian highlanders (50.6%; Semino et al. 2000b), who reside in the Inner Western Carpathians and territorially

are proximal to Lemkivschyna and Hutsulschyna. The combined Hungarian-Palóc haplogroup H frequency (46.9%) was comparable to that of Hutsuls (Table 1). All five H subhaplogroups selected for screening in the current study were found in Hutsuls (Table 2). Of the 19 haplogroup H samples in Hutsuls, 7 (36.8%; 18.4% overall) were H1; H2, H3, and H10 had one representative each (5.3%; 2.6% overall); and 2 samples were H5a (10.5%; 5.3% overall) (Table 2). The overall H1 frequency in Hutsuls was higher than that of their neighbors, such as Russians (13.5%), Hungarians (12.3%), Czechs (10.8%), Ukrainians (9.9%), Poles (9.3%), and Slovaks (7.6%) (Table 2). In fact, the H1 frequency in Hutsuls appears to be the highest of all central and eastern European populations studied to date. It is almost the same as the Spanish H1 frequency (18.9%) and is higher than the frequencies in Spanish Galicians (17.6%) and French Basques (17.5%) (Table 2). The last two groups represent populations proximal to the Franco-Cantabrian glacial refuge, where the H1 frequency is the highest in Europe (Achilli et al. 2004). Hutsuls had the second lowest frequency (2.6%) of haplogroup T in the region after the Hungarian-Palóc population (2%). The Hutsul sample had a haplogroup J frequency similar to the frequency for the Hungarian-Palóc sample (13.2% and 12.5%, respectively) (Table 1).

The principal components analysis of haplogroup frequencies extracted two components that explained 95.74% of the variance in haplogroup frequencies. Factor 1 explained 89.6% of the variance, and factor 2 explained 6.14% of the variance. When the mtDNA haplogroup frequencies of the three Carpathian populations were plotted in the principal components space along with frequencies from other central and eastern European populations, the Czech, Polish, Russian, and Ukrainian data points formed a cluster, with the rest of the population data points scattered around it and the Boyko data point appearing on the plot's margin (Figure 2). The distribution of the Czech, Polish, Ukrainian, Russian, Romanian, and Croatian population data points on the principal components plot appears to resemble the northwest-southeast geographic distribution of the corresponding populations; however, the Belorussian and Hungarian-Palóc data points as well as the Boyko, Hutsul, and Lemko population data points did not fit into that relationship (Figure 2).

The analysis of the Euclidean distance matrix revealed that the largest distance of all central and eastern European mtDNA data sets used in the analysis was the distance between Boykos and Hutsuls (0.42) (Table 3). Boykos showed the greatest overall distance from all other groups. Overall, Hutsuls were closer to the neighboring populations than to Boykos or Lemkos. The shortest distance to Boykos was that for Lemkos (0.28), although for Lemkos the Boyko population was the farthest of all populations used in the analysis. Lemkos were closest to the Romanian and Czech populations (0.17), whereas Hutsuls were closest to the Croatian mainland population (0.11). For Hutsuls the Lemko population was the second farthest (0.27) after Boykos. Hutsuls were closer to the Ukrainian population (0.16) than either Boykos (0.32) or Lemkos (0.23). Thus, based on the Euclidean distance analysis of mtDNA haplogroup frequencies, the three Carpathian

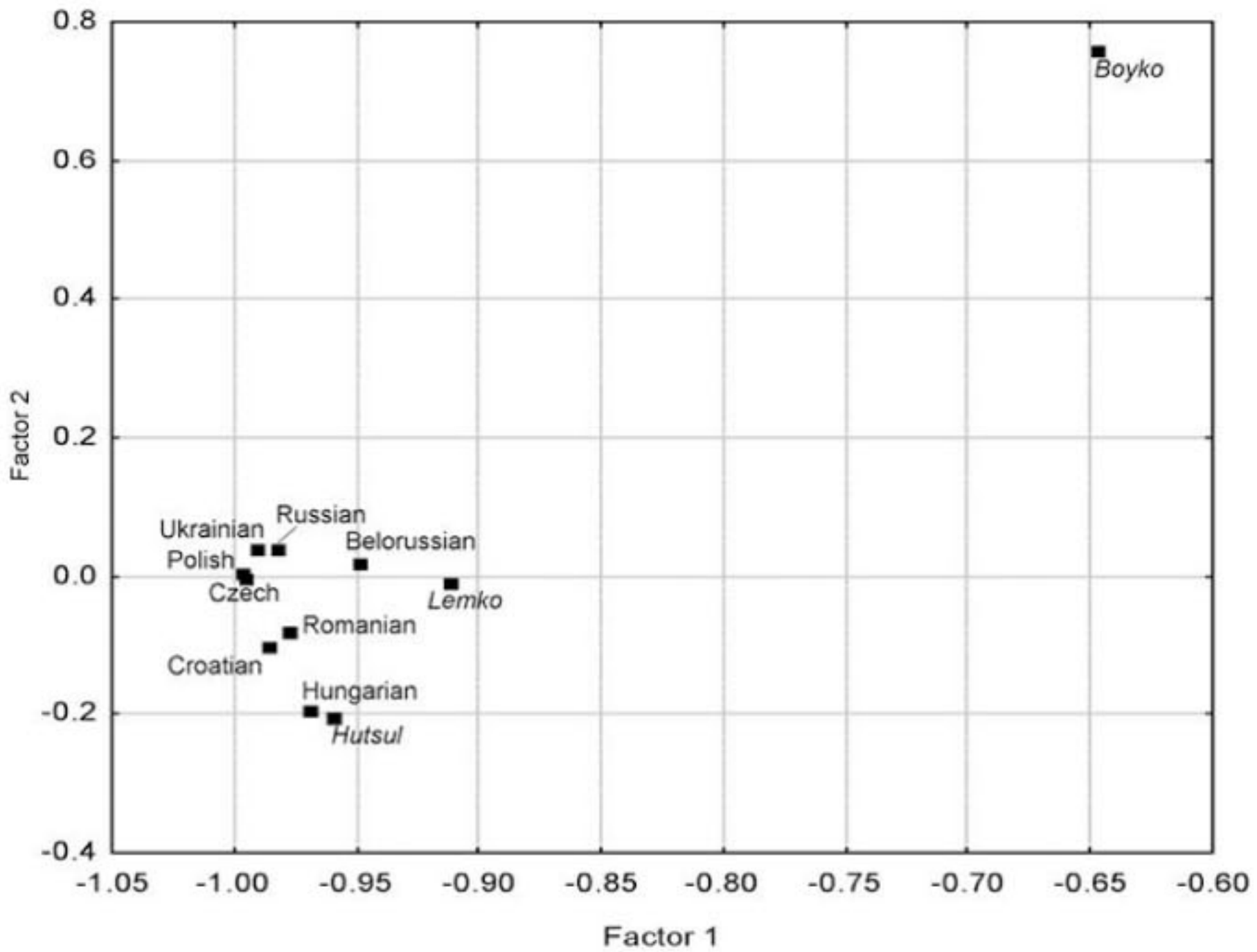

Figure 2. Distribution of mtDNA frequencies in the Boyko, Hutsul, and Lemko along with eight other central and eastern European populations in the principal components space. Factor 1 accounts for 89.6% of the total variability of frequency distribution, and factor 2 accounts for 6.14% of the total variability. Population data used were from the sources listed in Table 1.

highlander populations showed greater distances from each other than from other eastern and central European populations used in the analysis. Another noteworthy observation that can be made from analyzing the Euclidean distance distributions is the clustering of the Ukrainian and Croatian populations as well as the Romanian, Czech, Polish, and Russian populations, with the last two populations forming the lowest hierarchy cluster (Figure 3).

Discussion

In the present study we sought to determine the mtDNA haplogroup composition of three geographically isolated populations in the Carpathian Mountains. We obtained a good representation of the population mtDNA variation in all three groups. The studied populations contained representatives of major Eurasian mtDNA haplogroups such as H, U, T, and J as well several minor ones, such as I, N*, R*, pre*-V, and HV, which occur in Europe at low frequencies (Richards

Table 3. Euclidean Distances for 11 Central and Eastern European Populations, Including the Boyko, Hutsul, and Lemko, Based on Corresponding mtDNA Haplogroup Frequencies[a]

	Boyko	*Hutsul*	*Lemko*	*Ukrainians*	*Russians*	*Poles*	*Belorussians*	*Croatians*	*Romanians*	*Czechs*	*Hungarians*
Boyko	–	0.42	0.28	0.32	0.30	0.33	0.33	0.36	0.32	0.32	0.41
Hutsul	0.42	–	0.27	0.16	0.19	0.15	0.22	0.11	0.15	0.14	0.13
Lemko	0.28	0.27	–	0.23	0.19	0.20	0.26	0.22	0.17	0.17	0.25
Ukrainians	0.32	0.16	0.23	–	0.11	0.10	0.14	0.08	0.14	0.11	0.16
Russians	0.30	0.19	0.19	0.11	–	0.06	0.11	0.12	0.11	0.08	0.14
Poles	0.33	0.15	0.20	0.10	0.06	–	0.13	0.10	0.11	0.06	0.12
Belorussians	0.33	0.22	0.26	0.14	0.11	0.13	–	0.16	0.20	0.16	0.16
Croatians	0.36	0.11	0.22	0.08	0.12	0.10	0.16	–	0.12	0.09	0.11
Romanians	0.32	0.15	0.17	0.14	0.11	0.11	0.20	0.12	–	0.09	0.15
Czechs	0.32	0.14	0.17	0.110	0.08	0.06	0.16	0.09	0.09	–	0.13
Hungarians	0.41	0.13	0.25	0.16	0.14	0.12	0.16	0.11	0.15	0.13	–

a. Sources of the data are listed in Table 1.

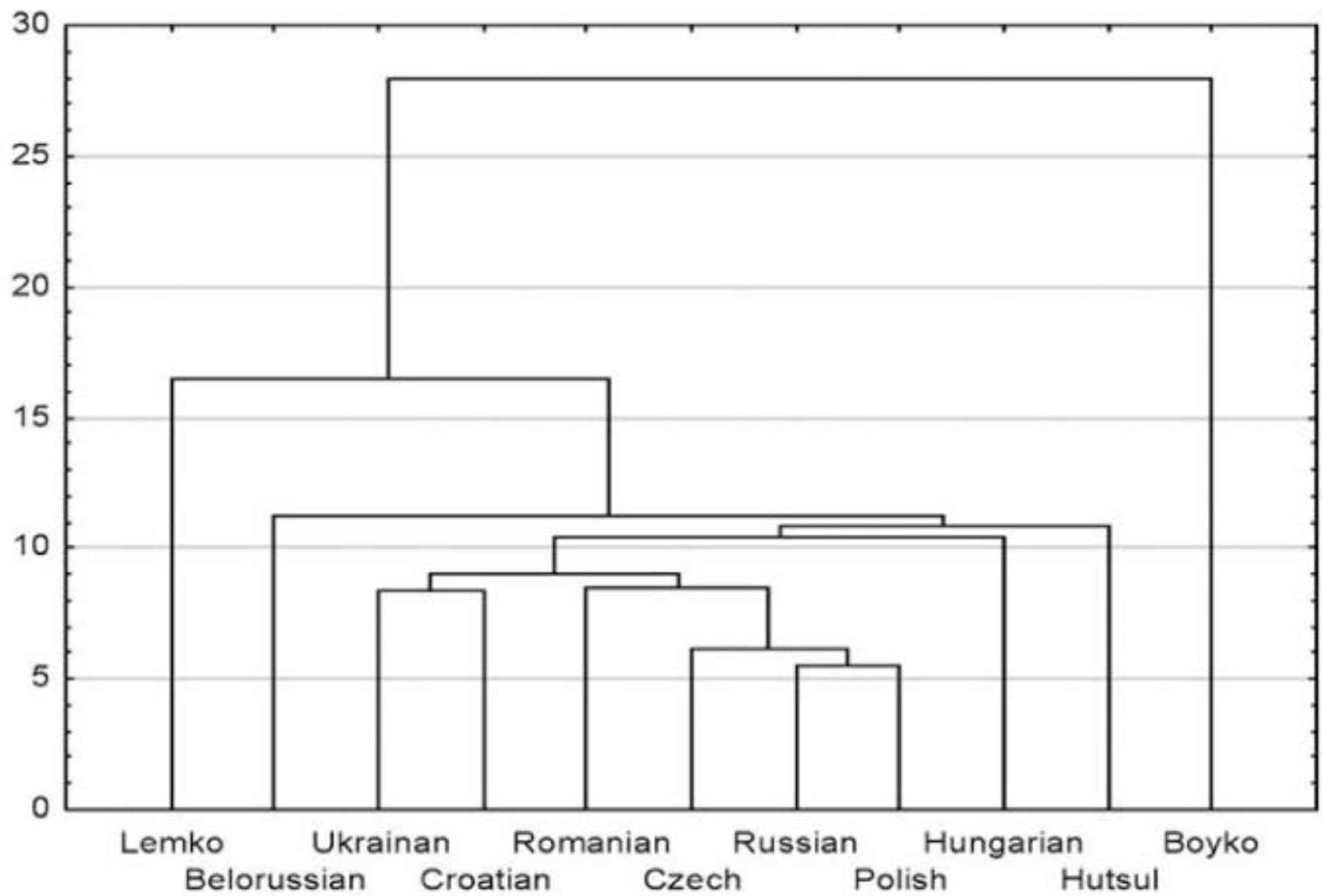

Figure 3. Single linkage clustering diagram for central and eastern European populations, based on the data from Table 3.

et al. 2002; Torroni et al. 2000, 2006). The overall haplogroup diversity in our population samples suggests that our sampling had appropriate resolution.

The Euclidean distance analysis of mtDNA haplogroup distribution revealed that the three Carpathian mountain groups in our study are not as closely related to each other as would be predicted based on their territorial proximity (Table 3, Figure 3). The results of the clustering analysis likely reflect the influence of different population factors throughout the history of each of the three Carpathian populations.

The low frequency of haplogroup H and the high frequency of haplogroup T in Boykos are atypical for a European population. The frequency of haplogroup J in Boykos appears to be in line with frequencies in the neighboring eastern European populations, which are overall lower than those of their central European counterparts (Table 2). A search of the pertinent literature revealed that at the H, T, and J frequency levels, Boykos appear closest to Udmurts, a population of Finno-Ugric speakers of the Uralic linguistic family currently residing along the eastern slopes of the Ural Mountains in Russia (21.8% for haplogroup H, 38.6% for haplogroup T, 2% for haplogroup J; Bermisheva et al. 2002). Although no archeological evidence exists to connect Boykos and Udmurts at the material culture level, the Carpathian anthropological type, with the exception of Hutsuls, shows characteristics of the Baltic anthropological type with pronounced Uralic

influences (Dyachenko 1987), likely a result of the expansion of Uralic tribes into the Carpathian region in the early Middle Ages (Tömöry et al. 2007 and references therein). The genetic roots of Boykos, however, may go even deeper into history.

The H, T, and J haplogroup frequencies in Boykos appear to be similar to those of the Neolithic Linear Pottery Culture (Linearband-keramik, or LBK) and its later counterpart, the eastern Hungarian Alföld Linear Pottery Culture (AVK) groups of the first European farmers, for which mtDNA haplogroup frequencies have recently become available (Haak et al. 2005). The frequency of haplogroup T in the LBK-AVK group was 20.8%, which would be the second highest T frequency in the region after Boykos. Haplogroup H frequencies in Boykos and in the LBK-AVK group are also comparably low (20% and 16.7%, respectively) (Haak et al. 2005). Also similar between Boykos and the LBK-AVK group are the low frequencies of haplogroup J (5% and 4.2%, respectively). Although archeological evidence points to LBK presence in Boykivschyna (I. T. Kochkin, unpublished data, 2007), the regional chronological successor to the LBK group, the Eneolithic agricultural civilization of Trypillia-Cucuteni, which likely shared its genetic pool with the LBK group (A. G. Nikitin et al., unpublished data, 2009), had a higher chance of leaving a genetic footprint in the modern inhabitants because of its more pronounced presence in the area (M. P. Sokhatsky, personal communication, 2008). We plan to expand the study of the genetic connection between the modern Carpathian inhabitants and these prehistoric European populations.

The Lemko group distinguishes itself by high frequencies of haplogroups M* and I. Haplogroup M* is an Asian-specific haplogroup, and its presence in populations of the Carpathian basin is attributed to Asian genetic contributions (Nádasi et al. 2007). Because we did not establish the specific subhaplogroups of the M* samples in our study, we cannot draw any conclusions about their phylogenetic age or origin in the Lemko population. Haplogroup I is the oldest subclade of the N* node, with an estimated age of 34,000 years (Richards et al. 1998). Haplogroup I, much like the other members of the N* node, is rare in Europe. To our knowledge, haplogroup I in the Lemko, along with a population from the Croatian Island of Krk (Peričić et al. 2005), has the highest frequency of all other European populations for which haplogroup frequency data are available. We can speculate that the higher than average European frequency for haplogroup I in Lemkos and Krk islanders may be a result of a high frequency of this haplogroup in the corresponding founding populations. A founder effect has been implicated as a major factor in the shaping of the mtDNA haplogroup frequencies of Croatian islander populations, including Krk islanders (Tolk et al. 2000).

The high frequency of H1 and the overall high diversity of haplogroup H in Hutsuls may be correlated with prehistoric migratory events in the region. Studies of distribution frequencies of haplogroup H in Eurasia suggest that haplogroup H entered southeastern Europe from the Near East at the peak of the Ice Age and subsequently dispersed to the European southwest, later expanding during the early Holocene out of the Franco-Cantabrian refuge back into the parts of Europe from which it originally entered (Pereira et al. 2005 and references therein; Richards et

al. 2000; Torroni et al. 1998). However, the postglacial population dynamics in Europe were probably more complex than what can be explained by the radiation out of the southwestern refuge and could have involved multiple reexpansion points (Simoni et al. 2000; Villems et al. 2002 and references therein). The haplogroup H frequency findings in the Hutsuls suggest a local early Holocene repopulation source.

The existence of a periglacial population refuge in eastern Europe has been suggested elsewhere (Semino et al. 2000a; Soffer 1990; Torroni et al. 2001 and references therein), and it is well supported by Y-chromosome data analysis (Lucotte et al. 2003; Malaspina et al. 2000; Semino et al. 2000a; Stefan et al. 2001). Data from Semino et al. (2000a) indicate that 50% of modern European Y chromosomes belong to just two related Y-chromosome haplogroups, each of which shows contrasting geographic distribution. Haplogroup Eu18 seems to dominate in the Iberian peninsula and decreases in frequency eastward. Haplogroup Eu19 shows the highest frequency in Hungary, Poland, and Ukraine and the highest corresponding microsatellite diversity in Ukraine and appears to decline westward. Thus the two Y-chromosome haplogroups might have expanded from two different geographic centers—a western, Iberian one and an eastern, Ukrainian one—at the end of the last glacial maximum (Semino et al. 2000a). Another study confirms these frequency observations for haplogroup Eu19 (haplotype XI, Hg3, or haplogroup R1a) and further shows that Ukraine, Russia, and Hungary have the highest frequencies of this Y-chromosome haplogroup in eastern Europe (Lucotte et al. 2003). Remarkably, Russians and Hungarians appear to have the highest frequency of mtDNA subhaplogroup H1 in the region (Achilli et al. 2004) after Hutsuls. Taken together, the frequency distribution data for haplogroup H in the Carpathian Hutsuls, along with the frequency distribution of mitochondrial H1 and Y-chromosomal Eu19 in eastern and central Europe, strengthen the possibility for a regional reexpansion at the end of the last glacial maximum from a local geographic center.

In conclusion, the current study further emphasizes the need for partitioning analysis of composite population groups of large nationalities to create a more comprehensive high-resolution picture of the distribution of lineage-specific genetic determinants at the regional, national, and continental levels. In such a component population analysis approach, the relationships between discrete ethnocultural groups and their putative ancestral genetic pools stand out from the homogenized genetic background of a more broadly defined nationality to which the smaller ethnic conglomerates are assigned.

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