

quency of polygyny than polyandry, and demographic differences between the sexes (greater male than female mortality and greater variance in reproductive success of men than women). The mutation rate in mtDNA is about ten times higher than that of nuclear DNA and Y chromosome (14,15), which provides the abundance of polymorphisms and larger number of maternal than paternal haplogroups. A lower mutation rate in Y chromosome is compensated by the greater length of DNA on NRY than in the mtDNA. Despite differences in mutation rates and abundance of polymorphisms, the general structure of maternal and paternal genealogies is indicative of the common origin of all-non African contemporary populations from a small subset of Africans (6).

Special Features of mtDNA

Mitochondrion is a cytoplasmatic organelle that serves as the principle site for the process of oxidative phosphorylation. The number of mitochondria per cells vary substantially, from thousands of copies in cells that require high ATP production to only a few hundred copies in cells with low energy requirement. About 100,000 copies of mitochondria are contained in oocytes, whereas only 50 to 75 copies may be found in the sperm cell. Mitochondrion is the only organelle in human cell cytoplasm that possesses its own DNA. Human mitochondrial DNA (mtDNA) is a circular double-stranded molecule 16,569 bp in length, whose entire sequence is known (16,17). Functionally, mtDNA is divided into two regions, control and coding. It codes 37 genes, 22 transfer RNAs, 2 ribosomal RNAs, and 13 protein genes mostly involved in the process of oxidative phosphorylation. Mammalian mtDNA is maternally inherited (18) without recombination. Although the non-recombining mode of human mtDNA heritage was challenged from time to time (19-21), claims favoring the presence of recombination have received strong criticism (22-25). Another important characteristic that differentiates mitochondrial from nuclear genome is its mutation rate. Base substitution rate in mtDNA is about 10 to 100 times higher than the average rate in nuclear DNA (14,15) due to its higher turnover rate than nuclear genome, lack of histones and less effective repair system (26). The observed mutation rate in mitochondrial genome is higher in the control than in the coding region (27), but with sub-

stantial differences depending on the nature of nucleotide positions (28,29). Some nucleotide positions within the control region mutate more often than others, and they are known as mutational "hotspots" (5,22,30). There are numerous mutations, mainly substitutions within coding and control regions, which play a key role in revealing the origin of modern humans. Brown et al (31) found different mtDNA restriction fragment patterns in individuals of different geographic and ethnic origin. After publishing the full mitochondrial sequence (16) and the 1987 paper by Cann et al (32), it became clear that mtDNA could be successfully used as a genetic marker for reconstructing human evolution. Furthermore, combination of control and coding region data allows grouping of mtDNA variants into haplogroups defined by one or more mtDNA coding region polymorphisms and particular control region sequences. The majority of haplogroups show geographic specificity, thus making mtDNA suitable for studies of maternal genetic history. Molecular phylogeny of mtDNA haplogroups and their continental affiliations are shown in the phylogenetic tree in Figure 1A.

Special Features of Y Chromosome

Human Y chromosome is 60 million bp long. Through the action of sex determining region (SRY), it defines the male sex. It is the only haploid chromosome in the human genome and it is characterized by holandric transmission. Since it is haploid, most of its length (approximately 95%) does not recombine with X chromosome. This region is called the non-recombining region (NRY) of Y or male-specific region (MSY). The NRY is flanked on both sides by a pseudoautosomal region, where X-Y crossing-over occurs regularly. NRY splits into euchromatic and heterochromatic portions (33), completely sequenced and characterized by Skaletski et al (34). NRY polymorphisms are valuable in evolutionary studies because of their low rate of parallel and recurrent mutations that allow reconstruction of paternal lineages thousands and thousands years backwards. The interest for paternal genetic history has been present since mid 1980s, when the first polymorphisms were being reported (7-9). Significant acceleration in the number of discovered Y chromosomal single nucleotide polymorphisms (SNP) came with the implementation of denatur-

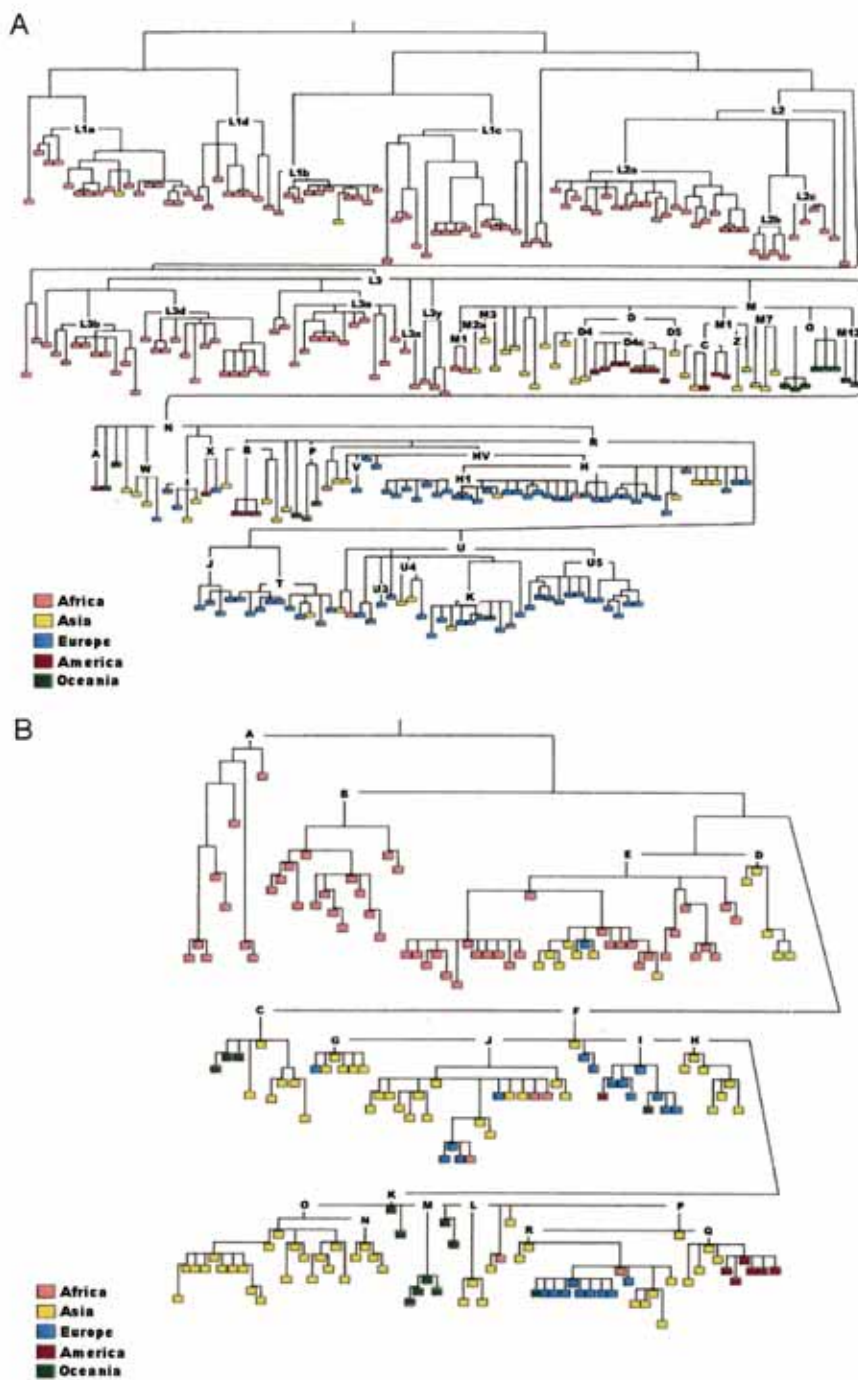


Figure 1. High-resolution molecular phylogeny of human mtDNA (A) and Y chromosome (B) haplogroups and their continental affiliation. From Cavalli-Sforza and Feldman (6), with the permission of the Nature Publishing Group.

ing high-performance liquid chromatography (38). Currently, there are more than 200 known SNPs and small indels (39). Combinations of slow-mutating and stable non-recurrent SNPs define Y-chromosomal haplogroups, whereas highly-mutating recurrent short tandem repeat loci (STRs) define haplotypes. STRs can be used to investigate demographic events that occurred in a more recent time-scale and, in combination with binary markers, they enable inferences to diversity (35) and thus to estimates of mutation rate and time to the most recent common ancestor (TMRCA) (36,37). Molecular phylogeny of Y chromosome haplogroups and their continental affiliations are shown in the phylogenetic tree in Figure 1b.

Data Sources

Paternal and maternal genetic heritage of Croatian populations was studied on DNA from individuals from continental part of Croatia and the Eastern Adriatic islands of Krk, Brač, Hvar, and Korčula (Fig. 2). There were 721 individuals analyzed for mitochondrial markers (40,41) and 451 individuals analyzed for Y chromosome markers (42-44). Detailed protocols for typing of Y-chromosomal polymorphisms and the selection of informative markers were reported by Barać et al (42) and Perićić et al (44). This review is based on the data obtained from the analysis of 16 SNP/indel markers plus 8 microsatellites analyzed in Barać et al (42), and 3 additional Y chromosome polymorphisms-M78, M172 typed in the entire sample and P37 typed in the samples from Adriatic islands for the purposes of this review. A detailed



Figure 2. Geographic location of the investigated islands.

procedure for mtDNA analysis and the exact affiliation of mtDNA HVS-I lineages with the set of 24 RFLP sites diagnostic for main Eurasian haplogroups were reported in Tolk et al (40).

Phylogeographic Distribution of mtDNA in Europe and Croatia

The most frequent mitochondrial haplogroup in the overall investigated Croatian sample (Table 1) and undoubtedly the most frequent in Europe, is haplogroup H (4,45,46). It has been suggested that haplogroup H originated in the Middle East approximately 30 to 25 kilo years ago (KYA), expanded into Europe contemporary to the diffusion of the Gravettian culture about 25 to 20 KYA, and was involved in numerous episodes of gene flow in western Eurasia but particularly during postglacial repopulation of Europe (3,4,46). In Europe, H displays wide distribution and high fre-

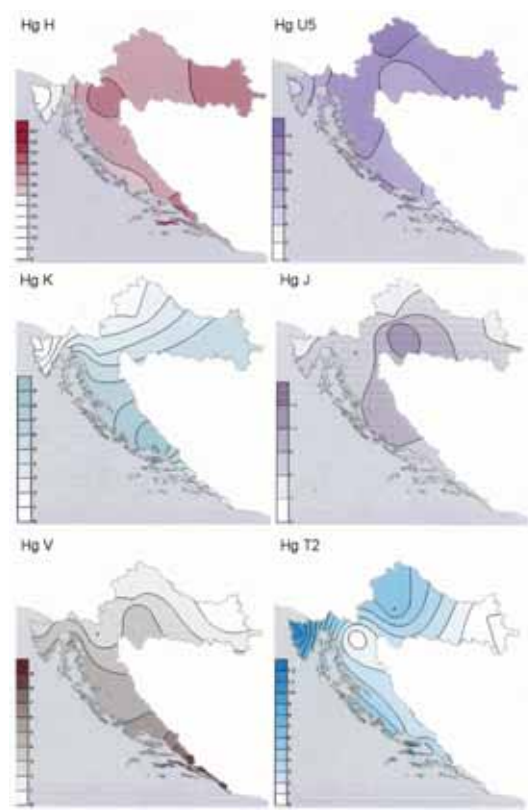


Figure 3. The spatial distribution of the most frequent mtDNA haplogroups in Croatia (Hg H, Hg U5, Hg K, Hg J, Hg V, and Hg T2). The haplogroup frequency surfaces were graphically reconstructed by use of the data reported in Table 1 and the computer Surfer System (Golden Software). The data on the subdivision of the mainland sample is not shown but is available on request.

Table 1. Frequencies of selected mtDNA haplogroups (%) in the investigated Croatian populations

mtDNA haplogroup	Haplogroup frequency (%) in Croatian population (reference source)					total (n=721)
	Mainland (26,27) (n=277)	Krk (26) (n=133)	Brač (26) (n=105)	Hvar (26) (n=108)	Korčula (26) (n=98)	
A		0.8				0.01
F	0.4			8.3		1.4
H	45.1	33.8	35.2	27.8	60.2	41.1
HV	0.4	4.5	10.5	4.6	4.1	3.7
I	1.4	11.3	1	1.9	1	3.2
J	11.9	9	8.6	9.3	6.1	9.7
K	3.6	6.8	9.5	3.7	4.1	5.1
M	0.4					0.1
N1a	0.7		1.9			0.06
N1b	0.4		2.9			0.06
preHV	0.7				1	0.04
preV			1.9	1.9		0.06
T*	0.7	0.8	1			0.06
T1	0.22	0.8	1.9	1.9		1.5
T2	5.8	3.8	2.9	12	5.1	5.8
U	0.7		1.9			0.06
U1	0.4				2	0.04
U2	1.4	1.5		4.6	1	1.7
U3	0.7		2.9			0.07
U4	2.2	3	1	0.9	1	1.8
U5	11.6	11.3	8.6	14.8	2	10.3
U6	0.4					0.01
U8	0.4					0.01
V	4	5.3	5.7	4.6	8.2	5.1
W	2.2	7.5	2.9	1.9	3.1	3.3
X	2.2			1.9		1.1
L2a3	0.4				1	0.03

*The haplogroup classification was carried out using system based on D-loop and RFLP markers (2,23).

quencies, e.g. 63% in Basques (4,47), 52% in Scandinavians (1,4,48-50), ~ 50% in northern and western Europe (1,4,51), 49% in western Mediterranean (47,52), and 41-47% in southeastern Europe (41). Haplogroup H retains high frequencies in Croatia with a frequency maximum on the island of Korčula (Fig. 3), probably as a result of the genetic drift (40). Since haplogroup H displays uniform and elevated frequency, a few groups of investigators are working on dissecting H into younger subhaplogroups in order to find yet undetectable spatial patterns (5,45,46,53).

Phylogenetically close clades of haplogroup H, Pre-HV, HV1, and HV2 are present in Europe at low frequencies. Pre-HV is very rare in Europe, where it occurs at 1.5% frequency in the east Mediterranean and south Europe (4,54,55). The overall frequency in Croatian population is 0.4% (Table 1). It is suggested that HV* (comprising HV1 and HV2) originated in the Near East (23) and today it is most frequent in southeastern Europe (41) and the middle Mediterranean (3,4,56), whereas it is present at very low frequencies in northern Europe (1,4). In the overall Croatian sample, HV* is present at 3.7% frequency, but with considerable differences among the investigated populations (Table 1). HV* frequency on the is-

land of Brač is higher than on other Croatian islands (Table 1) and the eastern Mediterranean (3,4,49). Other populations of southeastern Europe are also characterized by low (2.6%) HV* frequencies (4,54).

Haplogroup U is the most ancient haplogroup in Europe, embracing phylogeographically different subclades. Haplogroup U, in entirety, is the second most prevalent haplogroup in the investigated sample comprising 7 subhaplogroups U1-U6, U8, and K. Haplogroups U1, U2, U3, U6, and U8 occur at low frequencies in the investigated population (Table 1), as elsewhere in Europe (4). The oldest branch of haplogroup U is U5, the oldest European haplogroup whose age was estimated at 53 to 40 KYA (1,2,4). Phylogenetically more refined studies of U5 suggest that it underwent a postglacial expansion phase (57). U5 has a 10.3% frequency in Croatians, similarly to populations in the eastern Mediterranean and southeastern Europe (4,41,54,55). Its distribution in Croatia is uniform, with slightly decreasing frequency toward south (Fig. 3). The highest frequency of this haplogroup is found in northeastern Europe (1,4,49,50). The second most frequent U haplogroup is haplogroup U4. This haplogroup is dated at 25 to 16 KYA (4) and is most frequently found in

northeastern Europe (5.5%) (1,49,50,58). U4 frequency in Croatians (Table 1) is lower than in other southeastern European populations (~5%) (41), but is slightly higher than in populations of the middle Mediterranean, where its lowest frequency in Europe is noticed (~1%) (3,4,56). Phylogenetic studies showed that the first described haplogroup in European population, haplogroup K (59) also belongs to haplogroup U (60). Its frequency in Europe is less than 10% and it is more common in Western (8%) than in Eastern Europe (5%) (1,4). In Croatia, this haplogroup comprises 5.3% of the investigated sample (Table 1). Lower frequencies are noted in north Croatia and Istria, and the highest on the island of Brač (Fig. 3).

Another autochthonous European haplogroup is haplogroup V, which marks the postglacial population expansion from the Iberian Peninsula about 15 to 10 KYA (3). This haplogroup is present across Europe and shows a decreasing west to east frequency gradient. The highest frequency is noticed among Basques (42%) and Saami (12%) (57). In Croatia, V is found at 5.1% and shows a north-south increasing frequency gradient (Fig. 3) that can be explained by relatively recent founder events.

Haplogroups T and J are sister clades, present in European populations at similar frequencies. Haplogroup T comprises 8% of the maternal European gene pool variation (2), with the highest frequency among populations of the middle Mediterranean (12%) (2-4,56). In Croatian population, this haplogroup comprises 7.7% of the overall sample. The age of T is estimated at 46 KYA (4), but its subhaplogroups, T1 and T2, show considerable differences in age and migrational patterns, suggesting its multiple ways of arrival to Europe (4). Haplogroup T2 is found in all investigated Croatian populations, with frequency peaks in Istria, north Croatia, and the highest prevalence at the island of Hvar. A significantly higher frequency of T2 at the island of Hvar is another example of genetic drift and founder effect.

Haplogroup J comprises 11% of European maternal genetic variation, with the highest frequency of 14.1% in the east Mediterranean (4,54). The average frequency of haplogroup J in Croatians is 8.9% (Table 1) and a frequency peak may be noticed in central Croatia (Fig. 3). Richards et al (2) suggested that J originated before LGM and arrived to Europe at least 10 KYA.

Haplogroups that stem directly from the common node N (I, N1a, N1b, X, W) are relatively rare in Europe and do not usually exceed the level of 5%. The oldest among them is haplogroup I whose age is estimated at 34 KYA (2). I shows the highest frequency in Europe (at the level of 4.7%) in the eastern Mediterranean (4,54). The average frequency of 3.2% in Croatian population (Table 1) is similar to other population of southeastern Europe (41). Population of the island of Krk shows significantly higher frequency (11.3%) of I in comparison to other investigated Croatian populations and other populations from the eastern Mediterranean (40).

Another haplogroup from N node is haplogroup W, whose age is estimated at 18 KYA (2). W is most frequent in northeastern Europe (4.5%) (1,49,50). As in the majority of European populations where W is present at frequencies between 1 to 3% (4,41), its average frequency in Croatia is 3.4% (Table 1). Similar to the phenomenon observed in the case of haplogroup I, the island of Krk shows an elevated frequency of haplogroup W.

Haplogroup X, despite its minor contribution to mtDNA variation, has a surprisingly wide geographical distribution (61). In Europe, its highest frequency is noticed among the populations of the eastern and middle Mediterranean (4,56,61) and Republic of Macedonia (41). The average frequency of X in Croatians is 1% (Table 1).

The presence of haplogroup F on the island of Hvar (8.3%) and in one individual from Croatian mainland (Table 1) represents the first reported case of this southeast-Asian haplogroup in Europe (62). Based on worldwide phylogenetic analysis of haplogroup F, Tolk et al (62) suggested that this lineage could have reached Croatia (and Europe) during several documented historical episodes (communication along the ancient Silk Road, the 4th century invasions of Huns, the 6th century tribal alliance of Avars and Slavs, or 13th century raids of Mongols). Most likely, F became a significant component of mtDNA variety in the Hvar isolate because of a random genetic drift.

Phylogeographic Distribution of Y Chromosome in Europe and Croatia

The most frequent Y chromosome haplogroup in the overall Croatian population is I1b-P37

Table 2. The Y chromosome haplogroups frequencies (%) in the Croatian population samples*

Population (reference)	No.	E-M78	G-M201	J-M172	F	I(xP37)	I-P37	K(xP)	R1a-SRY1532	R1b	P*
Mainland (42-44)	108	5.6	0.9	1.9	1.9	4.6	32.4	1.9	34.3	15.7	1.9
Krk (42)	73	6.8		10.9		17.8	9.5		38.4	16.4	
Brač (42)	47	4.3	6.4		2.1	17	36.2	2.1	27.7	6.4	
Hvar (42)	91	4.4	1.1	3.3	1.1		65.9	1.1	8.8	6.4	14.3
Korčula (42)	132	3.8	10.6	2.3	1.5	0.7	52.3	1.5	20.5	1.1	6.1
Total	451	4.9	4.2	3.5	1.3	5.9	41.7	1.3	25	7.5	5.1

*Haplogroup nomenclature was based on Y Chromosome Consortium suggestions (39).

(Table 2). I is the only autochthonous European Y chromosome haplogroup which is almost exclusively confined to European continent (12,43), where it reaches approximately 50% frequencies in two regions – Scandinavia and southeastern Europe (12,42,63,64). Semino et al (12) proposed that M170, defining the haplogroup I; originated about 22 KYA in the northern Balkans in an Epi-Gravettian group. Based on the highest frequencies of M170 reported in Europe on three Adriatic islands, Brač, Hvar, and Korčula (54-66%), and high associated STR variance, Barać et al (42) indicated that the northern Adriatic could have been a Last Glacial Maximum (LGM) refugium and a likely source of the post-glacial expansion of the M170. Rootsi et al (43) sub-lineated haplogroup I and noticed that I1a-M253 shows the highest frequency in Northern Europe (among Norwegians, Swedes, and Saami), that I1c-M223 and unresolved I* lineages display low or moderate frequencies across Europe, whereas I1b* lineages peak in eastern Europe and in the Balkans. Furthermore, Rootsi et al (43) suggested that I originated in the Middle Upper Paleolithic and that different lineages diverged from I* in the Late Upper Paleolithic/Mesolithic, I1a at 15.9 ± 5.2 KYA, I1b at 10.7 ± 4.8 KYA, and I1c at 14.6 ± 3.8 KYA. I1a expansion to Scandinavia and I1b diffusion throughout the east Adriatic-North Pontic continuum signal the colonization from two distinct refugia, possibly Franco-Cantabrian and Adriatic, during two different prehistoric episodes (43). Our latest study indicated that I1b-P37 lineages reached maximum frequency in southeastern Europe, whereas associated variance peaked over a larger geographic region, encompassing both southeastern and central Europe (44). A Southeastern European I1b-P37 frequency peak is further supported by a recent study reporting massive frequencies of this lineage in Bosnia and Herzegovina (65). In Croatia, I1b-P37 lineages peak in the south and around the capital (Fig. 4). The estimated coalescent time of I1b-P37 in southeastern Europe

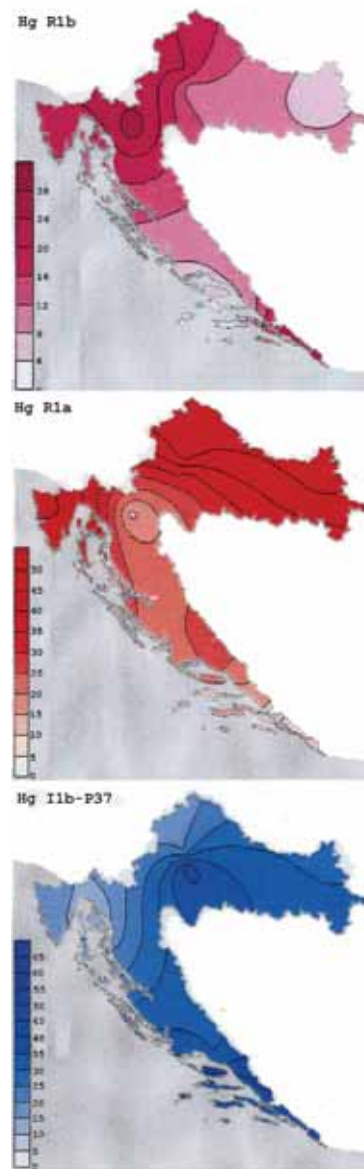


Figure 4. The spatial distribution of Y chromosome haplogroups I1b-P37, R1a and R1b in Croatia. The haplogroup frequency surfaces were graphically reconstructed by use of the data reported in Table 2 and the computer Surfer System (Golden Software). The data on the subdivision of the mainland sample is not shown but is available on request.

(11.1 ± 4.8 KYA) (44) is substantially older than the estimate reported by Rootsi et al (43). Based on current level of phylogenetic resolution of haplogroup I, observed phylogeographic pattern and estimated coalescence times, we suggest that I1b-P37 lineages might have expanded from the larger geographic region than the northern Adriatic plane, that is, from southeastern to central, eastern, and southern Europe, presumably not earlier than the Younger Dryas to Holocene transition and not later than the early Neolithic (44).

The second most prevailing haplogroup is R1a. The age of R1a has been approximated to 15 KYA (12,66). Kivisild et al (67) suggested that southern and western Asia might be the source of R1 and R1a differentiation. Semino et al (12) attributed the spread of R1a to the post-LGM recolonization of Europe from the refuge located in the territory of present-day Ukraine. An alternative possibility has been suggested by Rosser et al (11), who linked the spread of R1a to the movement of the Kurgan people dated to approximately 7 KYA. Currently observed R1a distribution in Europe shows an increasing west-east frequency and variance gradients with peaks among Finno-Ugric and Slavic speakers (11,12). Similarly to I1b*, R1a frequency gradient decreases slowly to the south of Europe and abruptly in the west (11,12). R1a frequency in Croatia shows a north-south frequency gradient (Fig. 4), very possibly due to the fact that the Dinaric Alps could have served as a natural mountain barrier to migrations from northeastern Europe to southern Croatia. At present, R1a diffusion throughout Croatia and southeastern Europe could be attributed to the early post-LGM recolonizations, expanding from the refugium in Ukraine, migrations from northern Pontic steppe from 3000 to 1000 B.C., as well as possibly massive Slavic migrations from 5th to 7th century A.D. It is important to note that prehistoric gene flows of I1b-P37 and R1a between southeastern and eastern Europe (and *vice versa*) mainly contributed to paternal genetic pool of contemporary Slavic populations, who share up to two thirds of common paternal lineages (e.g. Croats, Poles, and Ukrainians) marked by mutations that are generally absent in the large part of Western Europe (44).

Even though haplogroup R1b is phylogenetically close to R1a, it shows opposite frequency distribution in Europe. R1b-M173 lineages are considered to trace an Upper Paleolithic mi-

gration from West Asia to European regions then occupied by the Aurignacian culture (12,13). This ancient Eurasiatic haplogroup reaches frequencies of $\geq 50\%$ in western Europe (12,66,68). R1b lineages are present at relatively low frequency (8.5%) in southeastern Europe (44) and Croatia (7.4%) (Table 2). R1b displays frequency maximums in north and south both of Croatia (Fig. 4) and southeastern Europe (44), possibly due to its dual origin from two different source populations during recolonization of Europe from Iberia and Asia Minor during the Late Upper Paleolithic and Holocene (69).

The remainder of Croatian paternal genetic pool consists of haplogroups E, J, and G that have been traditionally considered to represent male contribution of demic diffusion of farmers from the Near East to Europe (12,63,70) that was more pronounced along the Mediterranean coast than in continental Europe (12,71). Results of Peričić et al (44) showed that at least one subset of E haplogroup originated in south and southeastern Europe from where it spread to the rest of the continent via the Vardar-Morava-Danube rivers corridor, whereas the expansion of southeastern European variants of J lineages was associated with the post-Neolithic period, approximately at 2.8 ± 1.6 KYA. Haplogroups E, J, and G are present in the investigated Croatian populations at frequencies less than 5%, with two major exceptions (Table 2). Population of the island of Korčula shows the highest frequency of haplogroup G (10.4%) among investigated populations, probably as a result of drift or a maritime Neolithic route (42). Another exception is the elevated frequency of haplogroup J at the island of Krk (10.8%) that might be a consequence of drift and the fact that this island was very exposed to the immigrations from the mainland (42).

The presence of central-Asian haplogroup P* on the islands of Hvar (14.3%), Korčula (6.1%), and in two individuals from the mainland (Table 2) recapitulates the finding of mtDNA haplogroup F. All Croatian P* lineages have a C→T transition at M242, accompanied by a typical presence of the unusually long DYS392-15 allele and are classified as Q-M242 (our unpublished data). Interestingly, among 681 men from southeastern Europe, only one Herzegovinian male was typed as Q-M242 (44).

Conclusion

The phylogeography of mtDNA and Y chromosome variants of Croatians can be adequately explained within typical European maternal and paternal genetic landscape, with the exception of mtDNA haplogroup F and Y-chromosomal haplogroup P*, which indicate a connection to Central Asian populations. Similar to other European and Near Eastern populations, the most frequent mtDNA haplogroups in Croatians were H (41.1%), U5 (10.3%), and J (9.7%). The most frequent Y chromosomal haplogroups in Croatians, I-P37 (41.7%) and R1a-SRY1532 (25%), as well as the observed structuring of Y chromosomal variance reveal a clearly evident Slavic component in the paternal gene pool of contemporary Croatian men.

Present-day Croatia, as a part of a larger geographic region of southeastern Europe, might be considered as a reservoir and a starting point of I1b-P37 (not including M26) post-glacial dispersal. Even though western Balkans has not been unambiguously accepted as a glacial refugium by archeologists, our genetic data and data on reoccupation of northern Europe from the Balkans by other mammals such as brown bear (72) and European hedgehog (73) indirectly support its existence.

Both genetic systems show the predominance of mutations that can be classified as "Paleolithic." This finding is in concordance with the range of "Paleolithic genes" in the present-day European gene pool, estimated to be between 70 to 80% in most Y-chromosomal and mtDNA studies (1,11,12,74,75). A low percentage of "Neolithic mutations" in Croatia is in good agreement with neighboring populations in the case of the observed mitochondrial profile, whereas in the case of Y chromosome this finding is somewhat surprising due to the fact that a large part of Croatia belongs to the Mediterranean region, characterized by higher frequencies of "Neolithic haplogroups."

Increased incidence of mitochondrial and Y-chromosomal haplogroups on Eastern Adriatic islands is a consequence of the fact that these islands represent reproductive isolates of relatively small size, where genetic drift and founder effect have particularly significant role in shaping the observed genetic diversity.

Lastly, even though each population and groups of populations are well characterized by maternal and paternal haplogroup distribution, it is important to keep in mind that linking phylogeography of various haplogroups with known historic and prehistoric scenarios used for explaining the age or origin of any population cannot be used without caution. It is important to bear in mind that estimated coalescence times do not represent the timing of a migration that spread certain lineages, but the upper bound to the age of the migration, since coalescence estimate for a set of alleles is typically much older than the population in which they are found. Only in the case of severe bottleneck or founder effect, the coalescence estimate becomes similar to the age of the population split.

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