MtDNA Haplogroups in the Populations of Croatian Adriatic Islands

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A B S T R A C T

The number of previous anthropological studies pointed to very complex ethnohistorical processes that shaped the current genetic structure of Croatian island isolates. The scope of this study was limited to the general insight into their founding populations and the overall level of genetic diversity based on the study mtDNA variation. A total of 444 randomly chosen adult individuals from 32 rural communities of the islands of Krk, Brač, Hvar and Korčula were sampled. MtDNA HVS-I region together with RFLP sites diagnostic for main Eurasian and African mtDNA haplogroups were analysed in order to determine the haplogroup structure. The most frequent haplogroups were »H« (27.8–60.2%), »U« (10.2–24.1%), »J« (6.1–9.0%) and »T« (5.1–13.9%), which is similar to the other European and Near Eastern populations. The genetic drift could have been important aspect in history, as there were examples of excess frequencies of certain haplogroups (11.3% of »I« and 7.5% of »W« in Krk, 10.5% of »HV« in Brač, 13.9% of »J« in Hvar and 60.2% of »H« in Korčula). As the settlements on the islands were formed trough several immigratory episodes of genetically distant populations, this analysis (performed at the level of entire islands) showed greater genetic diversity (0.940–0.972) than expected at the level of particular settlements.

Introduction

The four islands in eastern Adriatic, Croatia (*Krk*, *Brač*, *Hvar* and *Korčula*) are among the best characterised human isolate resources in the world. As a result of the continuous anthropological research during the past quarter of century, more

than 100 publications refering to these populations have been recorded in the international biomedical and anthropological databases to date. The strategy of the research was set by Rudan[1–3], who reconstructed the ethnohistory and demography and analysed the linguistic pecularities of these populations with Sujoldžić et al.[5–7]. In addition, the detailed anthropometrical measurements were carried out by Rudan et al.[8], physiological measurements were performed by Smolej--Narančić et al.[9,10], dermatoglyphics studied by Rudan[11] and Miličić et al.[12,13], and the migrational analyses carried out by Jovanović et al.[14,15] and Špoljar-Vržina et al.[16–18]. Using the obtained data, the population structure in the studied region was assessed through the holistic approach relying on the model-bound methods[19,20], the model-free methods[21–24] and original experimental designs [25].

The insight into population structure of these islands has prompted further *population genetic* research. The initial results were obtained through isonymy studies[26,27] and the analyses of assortative mate choice[28]. These simple approaches were followed by blood sampling and the analysis of the distribution of allelic frequencies of some basic serogenetic polymorphysms in collaboration with the laboratory in Newcastle upon Tyne, UK. The most important findings were published by Janićijević et al.[29] and Roberts et al.[30]. Following the emergence of molecular genetic technology, the research has been extended to include the analysis of STR and VNTR DNA polymorphysms in the papers by Martinović et al.[31–34].

Recently, the collaboration was established between the Institute of Anthropological Research in Zagreb, Croatia, and the Institute of Molecular and Cell Biology in Tartu, Estonia to study the characteristics of mtDNA in Croatian island populations. This type of study has become very valuable in the emerging field of molecular anthropology due to the three important properties of mtDNA: high mutation rate[35], strictly maternal inheritance[36] and the absence of recombination. Due to maternal inheritance, mitochondrial genome as a single locus has four times smaller effective population size than nuclear autosomal loci. This increases the sensitivity of mtDNA diversity to fluctuations in population size and the effect of genetic drift in small isolated populations. Most studies conducted so far using mtDNA can be considered as macro-geographical in their approach (out--of-Africa, peopling of Americas, Europe etc.) while the micro-geographic studies are still quite exceptional and only starting to emerge[37, 38]. According to numerous previous studies, human mtDNA variation is highly region-specific, giving a good background for more detailed studies on isolated populations.

This paper presents the very first mtDNA study on Croatian population in order to describe the mtDNA structure in the context of European haplogroups providing an additional explanation to previous research conducted on the populations of the Adriatic islands. The aim of this study is to determine population genetic structure in isolated rural populations of four Adriatic islands (Krk, Brač, Hvar, and Korčula) using mtDNA variation, and to find out is there any »non--Caucasoid« mtDNA types as well as to study the diversity levels.

Brief genetic history of the studied populations

In terms of the genetic history of these four island populations, the most important characteristic that needs to be emphasised is that the current populations were formed through several immigratory episodes of genetically quite distant populations.

The archeological evidence recently reviewed by Forenbaher[39] implies that the first settlers of the eastern Adriatic islands were Mediterranean non-Indo--European people who probably came from the south of today's Italy and were later replaced by Indo-European Illyrians. The oldest archaeological evidence of human settlements on the Dalmatian islands date back to Neolithic, approximately 5,000 years before present (YBP). From about the 10th century BC onward the Illyrians ruled the entire region of eastern Adriatic coast.

Subsequent migratory episodes in the early B.C. period brought Greeks (4th century B.C.) and then Romans (2nd century B.C. onward). Romans organised their settlements at the coastline of the islands, as they were used as harbours on the trade routes from the western Adriatic coast to Greece, Middle East and Northern Africa. The extent of the Roman admixture with preexisting Illyrians is difficult to estimate.

The first great influx of Croats onto the Adriatic islands occurred between 6th and 8th century. The Croats gradually Slavicised the islands, but the extent of their genetic admixture with Romans, Greeks and Illyrians is unknown.

The second large immigration wave resulted from migrations from the mainland of the Balkans peninsula during the expansion of the Ottoman empire. The greatest influx of the immigrants occurred in the 17th century during the Candian and Morean wars. The Croatian immigrants brought their own customs and gene pool, but the settlements they founded remained isolated from those of the preexisting settlers throughout the centuries due the various political, cultural and legal barriers to intermarriage imposed by the governors[19].

The detailed ethnohistory of these populations and the historic events that were influencing the shaping of the current genetic structure was recently reviewed by Rudan et al.[40]. It is important to note that *the founder unit of the population is a settlement (village) rather than each island itself*, and that any study analysing the diversity at the level of entire island populations, such as the present one, is expected to reveal far more diversity than it would be expected within the single villages.

Sample and methods

A) Sample

As the scope of this research was limited to performing the general overview of the haplogroups in the populations of interest, and bearing in mind that the previous analyses of the other world populations were based on very small sample sizes, we decided to include 15–20 adult persons from each village found on each of these four islands. The examinees were then randomly chosen from parish registries in those villages. The final sample size was 105 in Brač island, 108 in Hvar island, 98 in Korčula island and 133 in Krk island, i.e. the total of 444 individuals from 32 rural communities (Figure 1). It is important to emphasise that the sampling was not based on the likely founder populations (villages) but merely on geography (islands) and that the selected individuals therefore constitute a very heterogenous group with respect to possible founders. The blood samples were collected during the field research by the staff of the Institute for Anthropological Research in Zagreb, Croatia in the late 1980's and early 1990's following the appropriate approval of the ethical comitee on this institution. All the subsequent molecular analysis of the mtDNA was performed in the Estonian Biocentre and Department of Evolutionary Biology at the Institute of Molecular and Cell Biology, University of Tartu, Estonia.

Fig. 1. Location of the study regions in Croatia

B) MtDNA analysis.

The whole blood from investigated individuals was obtained by venipuncture and collected into EDTA tubes, which were transferred for storage at –40 °C. The amount of 10 ml of whole blood was used for DNA extraction using the macromethod of Ponz et al.[41]. Hypervariable segment I (HVS-I) of the control region of mitochondrial DNA (mtDNA) was amplified with primers A15909 (5´-ACACCAGTCT-TGTAAACCGG-3´) and B16498 (5´-CCT-GAAGTAGGAACCAGATG-3´) followed by sequencing of the segment between nps16024 and 16383 with primers H15975 (5´-CTCCACCAT-TAGCACCCAAAG-3´) and F16420 (5´-TGATTTCACGGAGGAT-GGTGG-3') by use of DYEnamic™ ET terminator cycle sequencing premix kit (Amersham Pharmacia Biotech). The fluorescently labelled extension products we-

re analysed on an Applied Biosystems Model on a 377 DNA sequencer (Perkin--Elmer). A selection of RFLP sites (73 *Alw*44I, 663 *Hae*III, 1715 *Dde*I, 3592 *Hpa*I, 4577 *Nla*III, 7025 *Alu*I, 8249 *Ava*II, 8994 *Hae*III, 9052 *Hae*II, 10028 *Alu*I, 10237 *Hph*I, 10032 *Alu*I, 10394 *Dde*I, 10397 *Alu*I, 11465 *Tru*I, 12308 *Hin*fI, 12406 *Hinc*II, 12704 *Mbo*I, 13366 *Bam*HI, 13704 *Bst*OI, 14465 *Acc*I, 14766 *Tru*I, 15606 *Alu*I, 15904 *Tru*I), diagnostic for main Eurasian and African mtDNA haplogroups[42–47], was screened. The numbering of mtDNA is based on comparison with the Cambridge Reference Sequence – CRS[48,49].

C) Data analysis

The alignment of the sequences was carried out by the Wisconsin Package (GCG). The haplogroup classification was

carried out using the system based on both D-loop and RLFP markers[42–47,50–52]. Based on these studies, all Eurasian mtDNAs are descendants of the Sub-Saharan haplogroup L3. According to the Neanderthal sequences[53,54] the root of the mitochondrial DNA lies in the African-specific haplogroup L1. More than 90 per cent of extant European mtDNA lineages can be classified as belonging among 9 mtDNA haplogroups (H, I, J, K, T, U, V, W and X)[44, 46]. Eastern Asian populations are described as belonging to haplogroups A, B, F, M, and Y and there is no extensive sharing between these two groups of populations except for regional contact zones like Central Asia[55]. Chi-square test (with Yates correction) was used in order to determinate statistically significant difference between the frequencies of haplogroups.

The phylogenetic analysis was carried out applying the median network method[56] using both D-loop and RFLP data. The median network approach is useful in a case of high mutation rate, which causes many parallel mutations in phylogenetically distant branches. The method permits describing of numerous equally parsimonious phylogenetic trees at the same time.

Genetic diversity index[57], the distribution of pairwise differences, mean number of pairwise differences[58,59] using HVS-I (bps 16024–16383) data were calculated by software package Arlequin 2.0[60]. The shape of the distribution of pairwise differences (pairwise mismatch distribution, PMD) is proposed to give the idea about the expansion events in population histories. The bell-shaped distribution demonstrates the population expansion events in the past; whereas the »bumpy« distributions indicate constant population size or genetic bottleneck effects. The coalescence time calculations were based on mutation rate of 20,180 years calibrated for the HVS-I[61].

Results

A) The frequency distribution of mtDNA haplogroups

The most frequent mtDNA haplogroups found in the four islands were »H« (27.8–60.2%), »U« (10.2–24.1%), »J« (6.1––9.0%) and »T« (5.1–13.9%). This frequency pattern is generally similar to the other European and Near Eastern populations (Table 1). However, some single cases of the Asian-derived haplogroups »A« and »F« and a Sub-Saharan haplogroup »L« were also identified. However, it is recognised that »Non-European« haplogroups (i.e. »A«, »B«, »L«, »M«, »R*«, »U6« and some ambiguous groups) can usually be found in the very low frequency in the European and Near Eastern populations, with the approximate share of 1.65 % (68 of 4,108)[52]. In Europe, these can typically be found in the populations inhabiting the areas bordering other large geographical regions (e.g. Northern Africa, Near East, Caucasus and Urals), through which the transfer of African or Eastern Eurasian lineages could have happened.

Table 1 presents in detail the frequencies of the haplogroups found in the four Adriatic islands, as well as the frequencies of selected European and Asian populations that are presented for comparison. It should be noted that, although a general frequency pattern reasonably corresponds to other European populations, on each of the four islands there are statistically significant departures from frequencies noticed in the populations of East Medditerranean and south-east Europe for certain haplogroups or their subclusters. This supports the ethnohistorical evidence on the long-term isolation of these populations, as in such condition the effects of inbreeding, genetic drift and founder effect can theoretically cause very significant departures from the fre-

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TABLE 1 – HAPLOGROUP FREQUENCIES

	n	A	B	F	H	HV	I	J**	J1	J2	L	M	N1a	N1b	N1c	pre-HV	T**	T1
Egypt and Nubia[62]	147	0	0	0	12.9	1.4	2	4.8	1.4	0.7	39.5	9.5	0.7	2	0	6.1	12.2	6.8
Middle East[43,63]	520	0.4	0.4	0	25.2	6.2	1	13.3	5.4	0.8	7.3	1.5	0	1.2	0.6	5.8	8.5	2.9
Anatolia and S. Caucasus[52,64,65]	510	0.2	0.4	0.4	27.6	6.3	2.2	8.8	4.3	2	1.6	2.4	0.6	1.6	1	0.8	11.8	5.1
North Caucasus[43,52]	199	0	0	0	26.6	4	2	7.5	2	0.5	0	10.1	0.5	0.5	0	0	10.1	1.5
Medditerranean East[52,66]	149	0	0	0	35.6	3.4	4.7	14.1	5.4	0.7	0.7	1.3	0.7	0.7	0	1.3	6.7	2
Southeastern Europe[52,65]	233	0	0.4	0	38.2	2.6	1.7	8.2	2.6	0	0.4	1.3	0	0.4	0	1.7	11.2	7.3
Central Mediterranean[47,52,63,67]	248	0	0	0	29.8	4.4	2.4	9.3	1.6	3.2	2	0.8	0	1.2	0	0.4	12.1	2.4
Alps[50,68,69]	218	0	0	0	46.3	0.5	1.8	11.5	5	0	0.5	0.9	0	0	0	0.5	8.3	1.8
North Central Europe[50,52,70]	314	0	0	0	47.8	1.3	2.2	9.9	3.5	0.3	1	0.3	0.3	0.3	0	0	10.8	3.5
Mediterranen West[71,72]	217	0	0	0	48.8	0.5	0.9	7.4	1.8	0.9	3.7	0.5	0	0	0	1.4	6	1.8
Basque[52,71,73]	147	0	0	0	62.6	0	0	2.7	0.7	0	0.7	0	0	0	0	0	5.4	0
North West Europe[50,52,74]	456	0	0	0	48.5	1.3	3.1	13.8	2.6	0.2	0.7	0.4	0	0	0	0.2	7.2	2
Scandinavia[50,52,75–77]	316	0.3	0	0	51.6	0.3	1.3	8.5	2.2	0.6	0.6	0.6	0.3	0.6	0.3	0	8.2	1.3
North East Europe[50,52,76,77]	398	0.8	0	0	42.7	0.3	2.8	8	1.5	0.5	0.5	1.3	0.5	0	0	0	7.8	1.5
Krk (Current study)	133	0.8	0	0	33.8	4.5	11.3	9	0	0	0	0	0	0	0	0	5.3	0
Brač (Current study)	105	0	0	0	35.2	10.5	1	8.6	0	0	0	0	1.9	2.9	0	0	5.7	0
Hvar (Current study)	108	0	0	8.3	27.8	4.6	1.9	9.3	1.8	0	0	0	0	0	0	0	13.9	1
Korčula (Current study)	98	0	0	0	60.2	4.1	1	6.1	0	0	1	0	0	0	0	1	5.1	0

	n	U**	K	U1	U2	U3	U4	U5	U5a	U5b	U6	U7	V	W	X	N*	R*
Egypt and Nubia[62]	147	6.8	2	0.7	0	2	0	1.4	0.7	0	0.7	0	0	0	1.4	0.7	0
Middle East[43,63]	520	19	6	2.5	0.8	1.9	1.5	1.5	1.2	0.2	1.3	1.5	1.9	1.5	3.8	1.2	1.3
Anatolia and S. Caucasus[52,64,65]	510	26.3	6.3	3.9	1.4	5.3	2.9	3.3	2.4	0.8	0	1	0.8	2.7	2.5	0.4	1.8
North Caucasus[43,52]	199	23.1	4	5.5	1.5	2	2	7.5	7.5	0	0	0	2	2.5	5.5	1	3.5
Medditerranean East[52,66]	149	22.1	4	2	0.7	1.3	2.7	10.1	6.7	2.7	0	0	2	1.3	4.7	0.7	0
Southeastern Europe[52,65]	233	25.3	5.2	0.4	0.4	3	4.3	10.3	5.2	4.7	0	1.3	3.9	3	1.3	0	0.4
Central Mediterranean[47,52,63,67]	248	27	8.5	3.2	0.8	1.2	1.2	9.7	5.2	3.2	0.4	1.2	4	1.6	4	0	0.8
Alps[50,68,69]	218	23.4	5.5	0	0.9	1.4	4.6	8.3	5.5	2.3	0	0	3.7	1.8	0.9	0	0
North Central Europe[50,52,70]	314	16.9	3.5	0.3	0.6	1	2.9	8	5.4	2.2	0	0	5.1	1.6	2.2	0.3	0
Mediterranen West[71,72]	217	21.2	5.5	0	0.9	0.5	2.8	3.7	1.8	1.4	2.3	0	5.5	2.8	1.4	0	0
Basque[52,71,73]	147	13.6	4.8	0	0	0	0	6.8	5.4	0.7	0	0.7	11.6	0	1.4	0	2
North West Europe[50,52,74]	456	17.5	7.7	0	0.4	0	2.2	7	4.2	1.3	0	0	5	1.1	0.9	0.2	0
Scandinavia[50,52,75–77]	316	22.2	4.7	0	0	1.9	3.2	12.3	6.3	3.8	0	0	3.8	0.9	0.3	0	0
North East Europe[50,52,76,77]	398	25.4	2.5	0.3	1.5	0.3	5.5	13.3	7.5	5	0	0	4.5	4.5	0.8	0	0.3
Krk (Current study)	133	22.6	6.8	0	1.5	0	3	11.3	4.5	6.8	0	0	5.3	7.5	0	0	0
Brač (Current study)	105	23.8	9.5	1	0	2.9	1	8.6	7.6	1	0	0	7.6	2.9	0	0	0
Hvar (Current study)	108	24.1	3.7	0	4.6	0	0.9	14.8	5.5	9.3	0	0	6.5	1.9	1.9	0	0
Korčula (Current study)	98	10.2	4.1	2	1	0	1	2	1	1	0	0	8.2	1.9	0	0	0

** J, T and U summarise the frequencies of subclusters J, J1, J2; T, T1; and K, U1-U7 respectively

quencies that would be expected. In this study, the most prominent examples are:

(i) the increased frequency (f) of haplogroup »H« in Korčula island in comparison to the other islands (f(»H«)=60.2%, p=0.001); When this frequency is compared to the geographically close populations of south-east Europe[52,65] and East Mediterranean[52,66], the significant difference can also be found (p=0.001). The only Europeans with the recorded frequency of haplogroup H greater than the one in Korčula are the Basques (62.6%)[52,71,73].

(ii) the increased frequency of the subcluster »U2« in Hvar island, where it comprises 4.6% of island's mtDNA diversity. It is the highest frequency of subcluster U2 recorded in Europe;

(iii) the increased frequency of the haplogroup »J« in Hvar island (f(»J«)= =13.9%), which is significantly greater than the frequency recorded in the other investigated islands (p=0.006). The comparison with other eastern Mediterranean population[52,66], however, did not reveal statistically significant difference;

(iv) the increased overall frequency of the haplogroup »V« in eastern Adriatic islands (f(»V«)=6.8%) in comparison to other eastern Mediterranean populations[52,66] (f(»V«)=2%, p=0.048), although there was no significant difference when that frequency was compared to the overall one in the south-eastern Europe[52,65] (f(»V«)=3.9%).

(v) the increased frequency of haplogroup »HV« in Brač Island (f(»HV«)= =10.5%), which is significantly greater than the frequency recorded in the other investigated islands (p=0.038). The haplogroup »HV« represents merely the ancestral node shared by haplogroups H and V, and its frequency on Brač Island is also significantly greater from the reports on the east Mediterranean[52,66] (f(»HV«)= =3.4%; p=0.042) and south-eastern Europe[52,65] (f(»HV«)=2.6%; p=0.005).

(vi) the increased frequency of haplogroup »I« in Krk Island (f(»I«)=11.3%), which is significantly different from the other three islands (p=0.001). The increased frequency of this haplogroup can be found in the populations of eastern Mediterranean[52, 66] whereas the comparison ith the frequency reported for the southweastern Europe[52,65] (f(»I«)=1.7%) reveals significant difference (p=0.005).

(vii) the increased frequency of haplogroup »W« in Krk island (f(»W«)=7.5%). This haplogroup is the most frequent in the north-eastern Europe[50,76,77] (f(»W«)= =4.5%), whereas the frequencies in Mediterranean range form 1.3% (east)[52,66] to 2.8% (west)[71,72]. The frequency in Krk Island is significantly greater (p=0.017) than in other islands, as well as in the other populations of eastern Mediterranean[52,66] (p=0.023).

The occurrence of the minor haplogroups in European gene pool was detected as well. Most of them are derivatives of supergroup »N1«: haplogroup »I« was detected in all islands, several individualds belonging to haplogroups »N1a« and »N1b« are present in Brač, and two persons from Hvar belong to haplogroup »X«. The single case of haplogroup »pre-HV«, relatively frequent in north-eastern Africa (6.1%)[62], and Middle East (5.8%)[43,52,63], is present in Korčula.

Similarly, some of the other »non-Caucasoid« haplogroups were exclusive for some islands. Haplogroup »F«, characteristic for south-eastern Asian populations, was found only on Hvar island (8.3%). However, the rare occurence of unusual haplotypes due to individual migratory experiences in ancient history can be expected in any population in the world, especially those susceptible to the impact of the founder effects such as island isolates. Therefore, the report on this finding and the discussion of its implications will be discussed in a separate paper.

Haplogroup »A« (Asian-specific haplogroup) is extremely rare in Europe (with frequencies significantly less then 1% reported in north-eastern Europe and Scandinavia[50,52,75–77]). This haplogroup was detected in 1 examinee from Krk Island. A single case of Sub-Saharan haplogroup »L2a« is noticed in Korčula.

Overall phylogenetic relationship of mtDNA data of the investigated populations are shown in Figure 2. MtDNA types are grouped to haplogroups according to RLFP and D-loop data.

B) Insights into population origin and genetic diversity

To assess the genetic relationship at the mtDNA level among populations from the four studied eastern Adriatic islands, the 444 obtained samples were sequenced and 147 different haplotypes were obtained. The addition of the RLFP data allowed the identification of 151 lineages.

The values of genetic diversity index ranged from 0.944 in Korčula island to 0.976 in Hvar Island (Table 2). Although those estimates indicate greater diversity than in some other isolated populations such as Sardinians (0.936)[63], Saami (0.815)[76], and Basque (0.936)[73], but lower than in diverse population like Turks – 0.995[64,65], it should again be emphasised that this relatively high level of diversity was actually expected because the sample came from all the villages founded by genetically very distant founders in the various historic periods.

The pairwise mismatch distibution of the whole dataset was carried out for each island separately (Figure 3) in order to assess the historical changes in population size. The typical human population in expansion would reveal unimodal Poisson distribution, while the populations which have undergone severe bottleneck effects would reveal »bumpy« distribu-

TABLE 2

GENETIC DIVERSITY, MEAN NUMBER OF PAIRWISE DIFFERENCES AND THEIR COALESCENCE TIMES ACCORDING TO HVS-I DATA (BPS 16024-16383).

Island	Sample size	Gene Diversity	Mean number of pairwise differences (95% CI)	Coalescence time in years (95%CI)
Krk	133	0.960	4.18 (3.00–5.97)	42,200 (30,300–60,300)
Brač	105	0.968	4.29 (3.14–5.23)	43,300 (31,700–52,800)
Hvar	108	0.976	5.55 (3.50–7.50)	56,000 (35,400–75,700)
Korčula	98	0.944	3.31 (1.93–6.79)	33,500 (19,500–68,500)

Fig. 2. Median-joining network showing the phylogenetic relationship of mtDNA haplogroups detected in Krk (n=133, green circles), Brač (n=105, yellow circles), Hvar (n=108, blue circles), and Korčula (n=98, red circles). Bold lines denote diagnostic RLFP or HVS-I (transversions are shown with letters) sites according to CRS. Each hairline link denotes one mutation in HVS-I region (bps 16024-16383). Small white circles indicate putative predecessors not yet sampled in islands. Number inside the circle Đ indicates the number of individuals sharing the same mtDNA haplotype. Large black-lined circles are indicating haplogroups typical for western Eurasia; red-lined circle contains sub-Saharan haplogroup L2a, and blue-lined circle eastern Eurasian haplo-groups A and F.
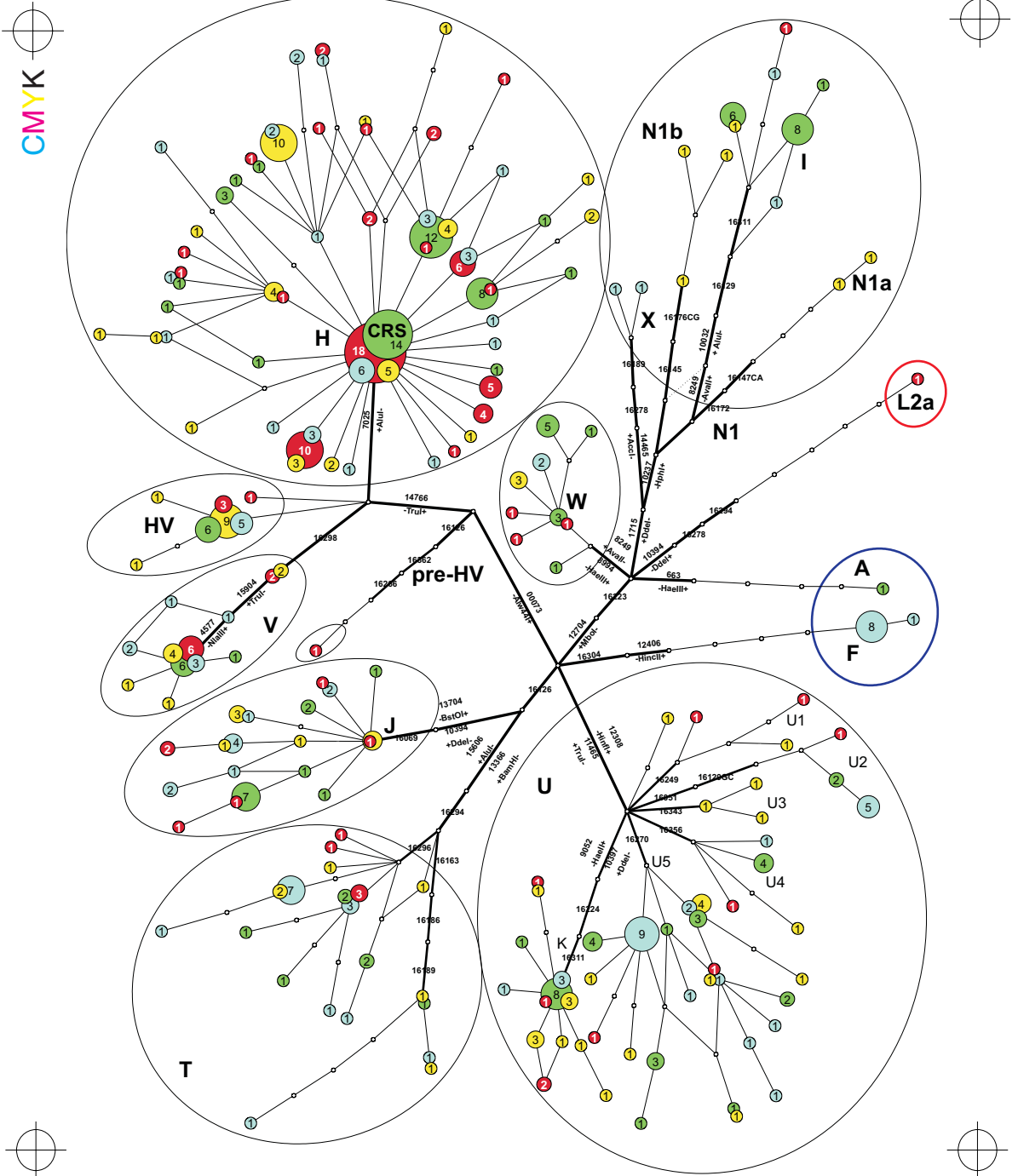
tion. The obtained distributions are rather typical for European populations, although the mismatch distribution of the population of Korčula island seems to be shaped more strongly by a founder effect. The Hvar Island's population reveals two peaks, indicating two expansion phases and/or the reduction in population size. However, it is possible that the bell--shaped graph could have been changed due to the significant proportion of the sequences from haplogroup F.
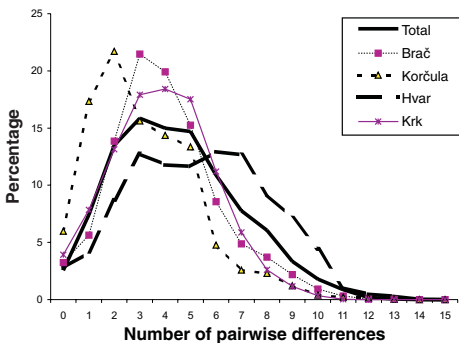

Fig. 3. Pairwise mismatch distribution of the HVS-I region (bps 16,024-16,383) of the four populations from the islands of Krk, Brač, Hvar, Korčula, and for the total sample size pooled together.

Using the mean number of pairwise difference and the mutation rate described by Forster et al.[6] the coalescence times were estimated. The results (Table 2) indicate that the coalescence times of mtDNA types of the populations of Krk and Brač are quite similar, between 40,000 and 50,000 years. The coalescence time of the population of the island of Hvar has is also quite similar, 56,000 years. Only Korčula has lower coalescence estimate approximately 33,500 years, which could indicate the different genetic history, possible genetic drift in recent past. But the 95% confidence interval of coalescence times of all investigated pop-

ulations is similar, having about the same range. None of these estimates reflect of course the settlement of investigated islands. What they reveal, however, is that the initial expansion of the mtDNA lineages found in the four islands, is a representative in this sense subset of the maternal lineage pool that started to expand around 40,000–50,000 YBP, possibly during the early phases of the settlement of Europe in early Upper Palaeolithic.

Discussion

As the number of previous anthropological studies pointed to the very complex ethnohistorical processes that shaped the current genetic structure of Croatian island isolates, the scope of this study was limited to the very rough insight into the founding populations of these islands and into the overall level of genetic diversity. It is expected that the sampling of considerable number of the examinees at the level of specific villages could point to the settlements which remained isolated and inbred for centuries due to specific political or cultural barriers. This could result in considerable reduction in genetic diversity, which could qualify such populations for the subsequent studies of quantitative traits or complex diseases in humans. However, the design of the present study, where the examinees were randomly drafted from all the villages regardless of their founder characteristics or the tendency towards isolation and inbreeding could only result in the greater diversity than it would be expected in an isolate, as the current populations were formed through a number of immigratory episodes pursued by genetically very distinct populations at the various points in time.

At least two historical events could have shaped the present distribution of mtDNA haplogroup frequencies regardless the founder effects. Due the plague

epidemics 4–5 centuries ago and the »wine crisis« at the beginning of the 20th century there were significant reductions in population size. Those two »bottleneck« effects could have caused a substantial non-random loss of certain haplogroups from the original population. However, the results of this study reveals that the mtDNA diversity is still relatively high. A large part of this genetic diversity could have been recreated due to several migration waves from the mainland areas during the Turkish wars[19].

The overall pattern of the frequency distribution of the mtDNA haplogroups was similar to the other European and Near Eastern populations. About 98% of the identified haplogroups are characteristic for contemporary western Eurasian populations, while the remaining 2% comprise of the sporadic cases of the haplogroups »L«, »A« and »F«. Those rare non-European haplogroups seem to have been brought to Europe relatively recently. The estimates of diversity, regardless of the sampling considerations, still show the lower levels of diversity than the populations of the Near East[63–65]. The fact that the overall diversity in Korčula Island (as a whole) is only slightly greater than in some well-known isolates including Basques, Saamis, and Sardinians[63,71,73,76] implies that the diversity in some villages of these islands could be extremely reduced. In addition, there are at least three more island populations that should have the levels of genetic diversity even lower than Korčula Island (i.e. Vis, Lastovo and Mljet), so the similar research should be extended to those islands in the future.

We conclude that the populations of the four eastern Adriatic islands (Krk, Brač, Hvar and Korčula) exhibit the mtDNA haplogroups characteristics similar to the other European populations, and the frequency pattern of various haplogroups also corresponds to the neighboring populations. There is evidence that founder effect could have played significant role in shaping the genetic structure of the contemporary islanders, as there were many examples of significantly increased frequencies of certain rare haplogroups in some islands. The level of genetic diversity was not as low as in some other established isolates, but it was not expected either because the sampling design of this study did not take in the account the likely founding population of each specific village. There is a good chance that the additional studies in the specific sub-populations of these islands could reveal extremely decreased genetic diversity in some villages, prompting future linkage disequilibrium studies of the genetic determinants of quantitative traits and complex diseases in humans.

Acknowledgements

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MtDNA HAPLOGRUPE POPULACIJA HRVATSKIH JADRANSKIH OTOKA

S A Ž E T A K

Brojna antropološka istraživanja provedena do danas na otocima Jadrana ukazala su na vrlo kompleksne etnopovijesne procese što su formirali genetičku strukturu suvremenih hrvatskih otočnih izolata. Predmet je ovog proučavanja ograničen na opći uvid u populacije utemeljitelja i na stupanj genetičke različitosti današnjeg stanovništva temeljem proučavanja varijacija mtDNA. Ukupno su proučene 444 slučajno izabrane odrasle osobe iz 32 seoske zajednice otoka Krka, Brača, Hvara i Korčule. MtDNA HVS-I regija zajedno s RFLP položajem utvrđene su za glavne Euroazijske i Afričke mtDNA haplogrupe i analizirane u cilju utvrđivanja strukture haplogrupa. Najuče-

stalije haplogrupe su »H« (27,8–60,2%), »U« (10,2–24,1%), »J« (6,1–9,0%) i »T« (5,1––13,9%), koje su slične drugim populacijama Evrope i Bliskog istoka. Genetički drift mogao je biti značajan aspekt u povijesti hrvatskih otočnih izolata, budući da postoje odstupanja u frekvenciji nekih haplogrupa (11,3% »I« i 7,5% »W« na Krku, 10,5% »HV« na Braču, 13,9% »J« na Hvaru i 60,2% »H« na otoku Korčuli). Kako su naselja na otocima oformljena tijekom nekoliko imigracijskih epizoda genetički udaljenih populacija, ova analiza (provedena na razini otoka) pokazala je veću genetičku različitost (0,940––0,972) od one što ju očekujemo na razini pojedinih naselja.

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