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# Mitochondrial DNA Heritage of Cres Islanders – Example of Croatian Genetic Outliers

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## ABSTRACT

*Diversity of mitochondrial DNA (mtDNA) lineages of the Island of Cres was determined by high-resolution phylogenetic analysis on a sample of 119 adult unrelated individuals from eight settlements. The composition of mtDNA pool of this Island population is in contrast with other Croatian and European populations. The analysis revealed the highest frequency of haplogroup U (29.4%) with the predominance of one single lineage of subhaplogroup U2e (20.2%). Haplogroup H is the second most prevalent one with only 27.7%. Other very interesting features of contemporary Island population are extremely low frequency of haplogroup J (only 0.84%), and much higher frequency of haplogroup W (12.6%) comparing to other Croatian and European populations. Especially interesting finding is a strikingly higher frequency of haplogroup N1a (9.24%) presented with African/south Asian branch almost absent in Europeans, while its European sister-branch, proved to be highly prevalent among Neolithic farmers, is present in contemporary Europeans with only 0.2%. Haplotype analysis revealed that only five mtDNA lineages account for almost 50% of maternal genetic heritage of this island and they present supposed founder lineages. All presented findings confirm that genetic drift, especially founder effect, has played significant role in shaping genetic composition of the isolated population of the Island of Cres. Due to presented data contemporary population of Cres Island can be considered as genetic »outlier« among Croatian populations.*

**Key words:** population isolate, mitochondrial DNA, Island of Cres, founder effect

## Introduction

The Island of Cres is the largest Croatian Island, located in the northern Adriatic Sea (Figure 1). In spite of its geographic proximity to mainland, its population is considered as relatively isolated and suffers from the constant population decline and low level of immigration.

Analyses of mtDNA in isolated populations showed to be very informative in microevolutionary studies, especially in observing effects of genetic drift/founder effect<sup>1</sup>, due to mtDNAs four times smaller effective population size.

The oldest archaeological evidence imply that Eastern Adriatic was inhabited from prehistoric times by hunter-gatherers, which were in some extent mixed with early farmers around 6000 B.C.<sup>2</sup>. Complex ethnohistorical pro-

cesses shaped its population. Sequential migratory episodes in the early B.C. period brought Illyrians, Greeks and Romans. The first considerable immigration of Croats onto the Adriatic islands occurred between 6th and 8th century. The second large immigration wave resulted from migrations from the mainland of the Balkan Peninsula during the expansion of the Ottoman Empire with the greatest influx of the immigrants in the 17th century.

Genetic diversity of mitochondrial DNA (mtDNA) in Croatian population was previously studied in Croatian Adriatic islands of Krk, Brač, Hvar, Korčula, as well as in continental and coastal part of Croatia<sup>3–6</sup> on the basic level of haplogroup resolution. In this study we pursue in analyzing maternal genetic heritage on the Island of

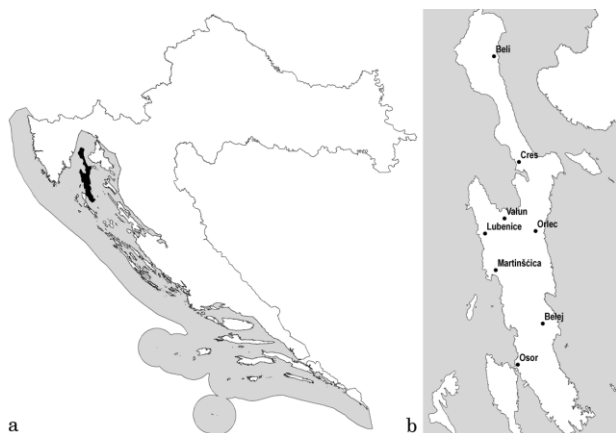


Fig. 1. Geographic position of the Island of Cres within Croatia (a) and location of sampled villages (b).

Cres. The aim was to resolve phylogenetic affiliation of mtDNA lineages in Cres Islanders, using high-resolution phylogenetic analysis, and to assess level of mtDNA diversity of this isolated Island population; as well as to make comparison with other island populations in order to determine its genetic position between them.

## Sample and Methods

### Sample

Blood samples were taken from randomly chosen 119 autochthonous unrelated adult individuals from 8 different settlements of the island of Cres (the small town of Cres and 7 villages: Beli, Belej, Orlec, Lubenice, Valun, Martinićica, Osor), after giving the informed consent. Genomic DNA was extracted from whole blood samples using the NucleoSpin Blood kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

### DNA analysis

The hypervariable segment I (HVS-I) of the control region of mtDNA was PCR amplified, purified and sequenced on Applied Biosystems 3730xl DNA Analyzer using the Big Dye Terminator kit (Applied Biosystems, CA, USA). To confirm the exact haplogroup affiliation of mtDNA subhaplogroups, SNP polymorphisms diagnostic for main Eurasian (sub)haplogroups<sup>7</sup> from coding and HVSII/III region, were typed by RFLP or sequencing.

### Data analysis

Sequences were aligned and analyzed according to CRS<sup>8</sup> using ChromasPro software. Phylogenetic networks of mtDNA haplotypes were constructed using the program Network 4.502 (Fluxus Engineering Web site). Both reduced median and median joining algorithms were applied<sup>9</sup>. Different weights were assigned to substitutions<sup>10</sup>. Gene diversity was calculated according to standard formula:  $H = \frac{(1 - \sum x_i^2) \cdot N}{N - 1}$ . In order to place the

analysed population between other island populations PCA analysis was performed in statistical package Primer 6.1.6. Only those haplogroups that had a noticeable impact on the scatterplot were used for the analysis (the sum of the absolute values for both coordinates of each allele was at least 0.2).

## Results and Discussion

Altogether, with high-resolution phylogenetic analysis of mitochondrial lineages we determined at least 27 subhaplogroups and 47 haplotypes in Cres Island population (Table 1), whose phylogenetic relationships are seen from Figure 2. In contrast to majority of European<sup>11</sup> and Croatian<sup>3-5</sup> (Pavao Rudan, Personal communication, 2009) populations, where haplogroup H is almost with no exception the most predominant one, mtDNA haplogroup analysis in Cres Island population revealed the highest frequency of haplogroup U (29.4%) with the predominance of one single lineage of subhaplogroup U2e (20.2%). Expectedly, second most prevalent is haplogroup H (27.7%) presented with lower frequency than in other Croatian populations.

The results show some other very interesting findings among Cres islanders. In contrast to other Croatian and European populations, haplogroup J is almost totally absent and found in only 1 individual, while it is notable a much higher frequency of haplogroup W (12.6%).

The most interesting and unexpected finding is a strikingly higher frequency of haplogroup N1a (9.24%). In modern Europeans this haplogroup is presented with extremely low frequency (0.2%)<sup>12</sup>. Moreover, it should be emphasized that almost all European findings of this haplogroup fall into its European/central Asian branch designated »N1a1«, characterized by the 16147A variant (together with transitions 16320 and 3336)<sup>13</sup>, which is sparse but widespread in Europe and adjacent parts of Asia and North Africa. Although very rare in contemporary Europeans, it has been proved that haplogroup N1a was formerly prevalent among Neolithic farmers in Central Europe reaching about 25% of mtDNA gene pool<sup>12</sup>, what is about 150-times higher frequency comparing to modern Europeans. All Neolithic types also fall into the European N1a sub-branch.

Conversely, N1a presented in Cres population (HVS-I haplotype 147G-172-223-355) belongs to its ancient eastern African/southern Asian sister branch characterized by the 16147G variant, which is almost absent from European population. This is to our knowledge the northernmost till now reported finding of this branch in Europe, the nearest previously reported one was from Thessaloniki, Greece but with different HVS-I haplotype (147G-172-223-224-248-355-357)<sup>12</sup>. In contemporary Croats, except in the Island of Cres, 147G variant is found only in 1 individual from the island of Pag (147G-172-223-248-295-355) and 1 individual from the town of Delnice (147G-172-223-248-355) in mountainous region of Croatia<sup>4</sup> (Pavao Rudan, Personal communication, 2009). However, this haplogroup has found the way to the Cres

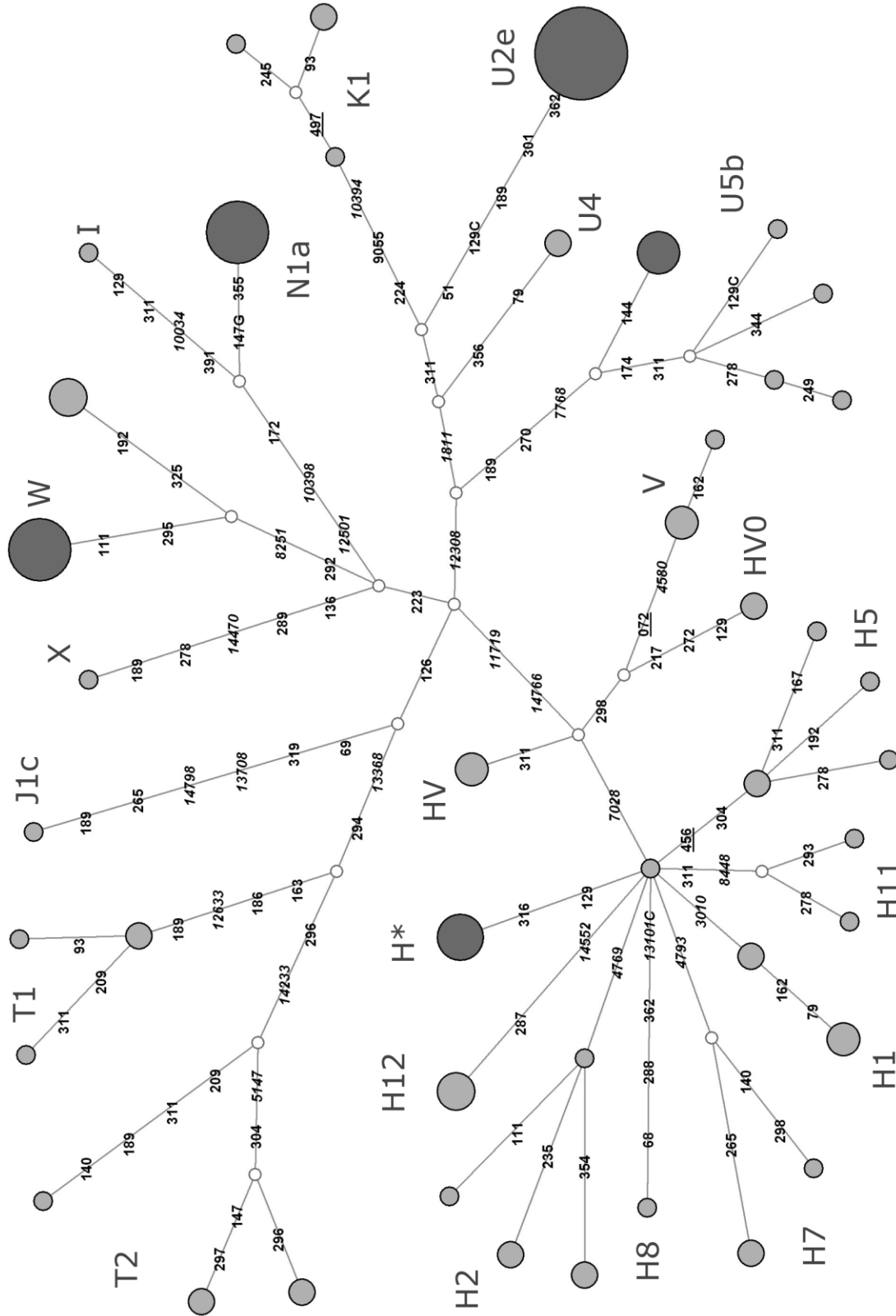


Fig. 2. Reduced-Median-Joining phylogenetic network of mtDNA haplotypes gathered by high-resolution analysis in the Island of Cres population. The size of the node is proportional to the number of individuals. Supposed founder lineages are coloured dark grey. White nodes present median vectors. Coding region mutations are in italic; all other mutations are from HVS-I region (+16000), except underlined numbers that present mutations from HVSII/III region.

**TABLE 1**  
DISTRIBUTION OF (SUB)HAPLOGROUPS IN CRES ISLANDERS

(Sub)haplogroup	%	HVS-I sequence	n
H1	4.20	CRS	2
H1a		79–162	3
H11	1.68	278–311	1
H11a		293–311	1
H12	3.36	287	4
H2a	5.04	111	1
		235	2
		CRS	1
H2a1		354	2
H5	4.20	304	2
		167–304–311	1
		192–304	1
		278–304	1
H7	2.52	265	2
		140–298	1
H8	0.84	68–288–362	1
H*	5.04	129–316 <sup>#</sup>	6
H*	0.84	CRS	1
HV	2.52	311	3
HVO	1.68	129–217–272–298	2
I	0.84	129–172–223–311–391	1
J1c4	0.84	69–126–189–265–319	1
K1*(xK1a/b/c)	0.84	224–311	1
K1a	2.52	224–245–311	1
		93–224–311	2
N1a	9.24	147G–172–223–355 <sup>#</sup>	11
T1a	3.36	126–163–186–189–209–294–311	1
		126–163–186–189–294	2
		93–126–163–186–189–294	1
T2	4.20	126–140–189–209–294–296–311	1
T2b		126–294–304	2
T2b3		126–147–294–296–297–304	2
U2e	20.17	51–129C–189–301–311–362 <sup>#</sup>	24
U4	1.68	79–356	2
U5b1	7.56	129C–174–189–270–311	1
		144–189–270 <sup>#</sup>	5
		174–189–249–270–278–311	1
		174–189–270–278–311	1
		174–189–270–311–344	1
V	3.36	298	3
		162–298	1
W1	9.24	111–223–292–295 <sup>#</sup>	11
W*(xW1,W3,W5)	3.36	192–223–292–325	4
X	0.84	136–189–223–278–289	1

<sup>#</sup>supposed founder lineages

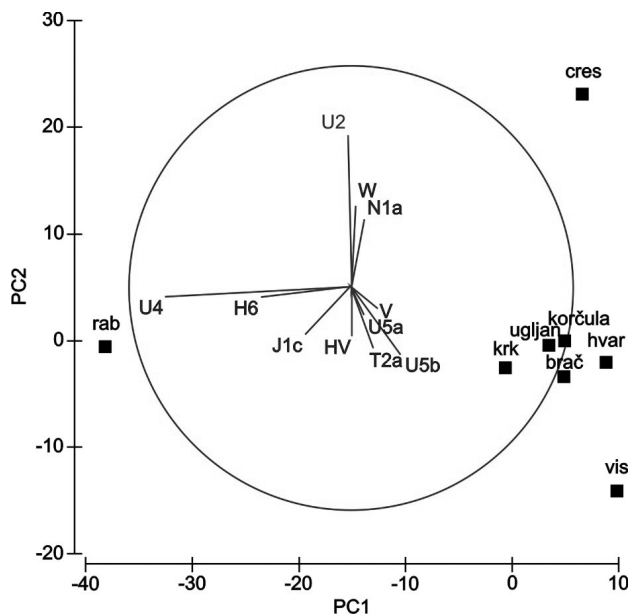


Fig. 3. PCA plot based on frequencies of subhaplogroups in analyzed island populations. The first 2 principal components describe 73.4% of variation.

Island and has successfully spread throughout the Island population, where it is found in 6 different villages.

According to current data about this haplogroup, it is hard to predict how this lineage reached northern Adriatic region: did it come to Croatian territory by migrations in Palaeolithic times starting from southwestern Asia, where the source of lineage diversification within haplogroup N1 is considered<sup>13</sup>. It is also possible that founder came much later; the unique HVS-I haplotype in all N1a samples from the island of Cres indicate a relatively recent founder effect. Further complete sequencing of samples with 147G variant from Island of Cres together with other Eurasian (as well as African) findings of this branch should enable calculation of the coalescence time of this branch in Eurasia and of founder of this haplogroup in the Island of Cres; as well as elucidation of phylogeography of this haplogroup.

Furthermore, by haplotype analysis we noticed that there are 5 most frequent lineages in Cres Island mtDNA gene pool that account for almost 50% (precisely 47.89%) of it, as follows: U2e (51-129C-189-301-311-362) – 20.17%; N1a (147G-172-223-355) – 9.24%; W1 (111-223-292-295) – 9.24%; H\*129-316 – 5%; U5b1 (144-189-270) – 4.2%. We

further checked the distribution of these lineages throughout the island and found that they are present in 7 (U2e), 6 (N1a), 3 (W1), 2 (H\*129-316) and 4 (U5b1) out of 8 sampled settlements. These lineages are presented with  $\geq 5\%$ , except U5b1(144-189-270), but this lineage is found in 4 different settlements indicating not so recent arrival to the island. These mtDNA types can therefore be considered as founder lineages for the Island of Cres.

Moreover, the Island of Cres has the lowest observed gene diversity index – only 0.937, what is even lower than in geographically more remote islands such as Korčula and Vis (Pavao Rudan, Personal communication, 2009).

Altogether, relatively low level of diversity is found in this population. Having in mind relatively small geographic distance from the mainland and the proximity of the city of Rijeka, Island of Cres population is surprisingly isolated.

To place the Cres Island population in the context of some other Croatian Island populations (islands of: Krk, Rab, Ugljan, Brač, Hvar, Korčula and Vis) we performed interpopulation comparison by using subhaplogroup frequencies through the PCA analysis (Figure 3). The outlying position of this Island population is notable and is due to U2e, W1 and N1a haplogroups. Only the Island of Rab seems to be even greater outlier.

Relatively low level of mtDNA diversity is found in the population of the Island of Cres. Only five (supposed founder) lineages account for almost 50% of maternal genetic heritage of this island.

All presented findings confirm that the effects of evolutionary forces, especially founder effect, have had significant part in designing the genetic composition of mtDNA pool of contemporary Cres islanders and that they may be considered as genetic »outliers« among Croatian and other European populations.

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## MAJČINSKO NASLJEĐE STANOVNIŠTVA OTOKA CRESA – PRIMJER GENETIČKE IZNIMKE U HRVATSKOJ POPULACIJI

### SAŽETAK

Raznolikost linija mitohondrijske DNK (mtDNK) određena je pomoću analize visoke filogenetske rezolucije na uzorku od 119 odraslih autohtonih stanovnika otoka Cresa iz osam naselja. Ustanovljeno je da se sastav haplogrupa ove otočne populacije znatno razlikuje od ostalih hrvatskih i europskih populacija. Najviši udio ima haplogrupa U (29,4%) i to s prevagom jedne linije podhaplogrupe U2e (20,2%). Haplogrupa H je slijedeća po udjelu sa samo 27,7%. Ostale posebnosti današnjeg otočnog stanovništva su ekstremno niska učestalost haplogrupe J (samo 0,84%) te znatno povećani udio haplogrupe W (12,6%) u usporedbi s ostalim hrvatskim i europskim populacijama. Nadalje, posebno je zanimljiv nalaz znatno povećanog udjela haplogrupe N1a (9,24%), koja pripada afričkoj/južno azijskoj grani gotovo odsutnoj u stanovništvu Europe, dok je njezina sestrinska europska grana, za koju je dokazano da je bila visoko zastupljena kod neolitičkih zemljoradnika, prisutna sa samo 0,2% u današnjoj europskoj populaciji. Analiza haplotipova pokazala je da na samo 5 linija mtDNK otpada 50% majčinskog naslijeđa ove otočne populacije te se one smatraju osnivačkim linijama. Dobiveni rezultati potvrđuju da je genetički drift, poglavito učinak utemeljitelja, imalo važnu ulogu u oblikovanju genetičke zalihe mtDNK izolirane populacije otoka Cresa. Na temelju dobivenih rezultata može se zaključiti da današnja populacija otoka Cresa predstavlja genetičku »iznimku« u hrvatskoj populaciji.