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# Distribution of haplotypes of common oak (*Quercus robur*) in Croatia

**Abstract**

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The investigation was based on geographic distribution of haplotypes of oak chloroplast DNA (ct DNA) on species from *Quercus sensu stricto* group with particular stress on common oak (*Q. robur* L.) in Croatia. Study of the Croatian populations was a part of wide European investigation in frame of FAIR OAK project. In Croatia, five haplotypes (2, 5, 6, 7, 17) of ct DNA were found. Our results showed that all oak species from *Quercus sensu stricto* group shared the same haplotypes which could be a consequence of the hybridisation events that have occured more frequent in the past than today.

## Introduction

Climate is constantly changing through long periods of tens and hundred of millenniums. In Earths history cold glacial periods alternated with warm interglacials. During each transition dramatical changes occurred. In dry glacial periods ice covered the most of Europe, while, with warming on the beginning of interglacial, ice melted, see level increased and climate was more humid. These changes were followed by migration of plant and animal world, which was repetitively retreated to glacial refugia on the extreme south of Europe during cold periods and was migrating back to the north with more favourable conditions. Last glacial lasts around 100000 years, and its last maximum was before 18000 – 15000 years. Spreading of many plant species can be traced by fossil polen (Brewer & Cheddadi 2001), but the data says almost nothing about routes of colonisation and refugia of certain genotypes. In the frame of EU Fair Oak project, distribution of ct haplotypes of oaks from *Quercus sensu stricto* group (*Q. robur* L., *Q. petraea* (Matt.) Liebl, *Q. pubescens* Wild., *Q. virgiliana* Ten., *Q. frainetto* Ten.), and their migration routes after last maximum of glaciation of each haplotype are reconstructed. Many populations in the entire Europe were investigated (Petit & al. 2001), except the south part of Balkan peninsula. This work focusses on distribution of haplotypes in Croatia with particular stress on common oak (*Quercus robur* L.). Investigation was based on geographic distribution of haplotypes of chloroplast DNA (ct DNA). Chloroplasts and mitochondria are inherited exclusively from mother-trees in oaks. Because of that, their genetic material do not spread by pollen in gametophyte phase and unique migrating phase is acorn. Consequently, ct

DNA remained relatively conserved inside populations from the time of colonisation. Thus, colonisation left footstep on today's distribution of haplotypes of oaks.

## Materials and methods

Investigation of Croatian populations was part of wide European investigations in frame of FAIR OAK project, and it was realised thanks to bilateral cooperation with INRA, France. In the analysis of 39 populations (Fig. 3) the same methods were used. Total DNA from buds or leaves was isolated using c-tab protocol (Dumolin-Lapegue & al. 1995). Four segments of ct DNA were amplified by PCR, marked with AS, DT, CD and TF. (Fig. 1 and Table 1). Each segment was digested with restriction enzymes Hinf1 or Taq1 and obtained fragments were separated by vertical electrophoresis on poliacrilamyde gel (Fig. 2). Haplotypes were detected on the basis of their different sizes of fragments and appearance of new restriction sites. There are marked with number and colour/simbols, standardized within the project.

Table 1. Pares of primers used for amplification of ct DNA fragments.

| Fragment | 1st primer | | 2nd primer | |
|---|---|---|---|---|
| | name | sequence | name | sequence |
| AS | Psa A [PSI (P700 apo-protein A1)] | 5'-ACTTCTGGT TCC GGCGAACGAA-3' | Trn Sr [tRNA-Ser(GGA)] | 5'-AACCACTC GGC CATCTCTCCTA-3' |
| DT | Trn D [tRNA-Asp(GUC)] | 5'-ACCAATTGA ACTACAATCC-3' | Trn Tr [tRNA-Thr(GGU)] | 5'-CTACCACTG AGTTAAAAGGG |
| CD | Trn C [tRNA-Cys(GCA)] | 5'-CCAGGTCA AATCTGGGTGTC-3' | Trn Dr [tRNA-Asp(GUC)] | 5'-GGGATTGT AGT TCAATTGGT-3' |
| TF | Trn T [tRNA-Thr(UGU)] | 5'-CATTACAAA TGCGATGCTCT-3' | Trn Fr [tRNA-Phe(GAA)] | 5'-ATTGAACTG GTG ACACGAG-3' |

## Results and discussion

Haplotypes were detected on the basis of the position of bands on all four gels (for each fragment, Fig. 2). Individuals with the same bands on all four gels were identified as the same haplotype.

In Croatia five haplotypes of ct DNA were found. Their distribution is shown on Figure 3. On the basis of distribution of haplotypes in Europe, as well as from their genetic distance we could conclude that closely related haplotypes 5 and 6 originated from Balkan refugia. They were found in Eastern Croatia, so we could suppose that Eastern Croatia was colonised from south-east and east. To the west there is transition zone in which we still found haplotypes 5 and 6, but haplotypes 7, 2 and 17, found in Western and South-western Croatia were found as well. This transition zone extends from Novska and Sisak in Central Posavina to Repaš and Kloštar Podravski in Podravina region. In Central Croatia

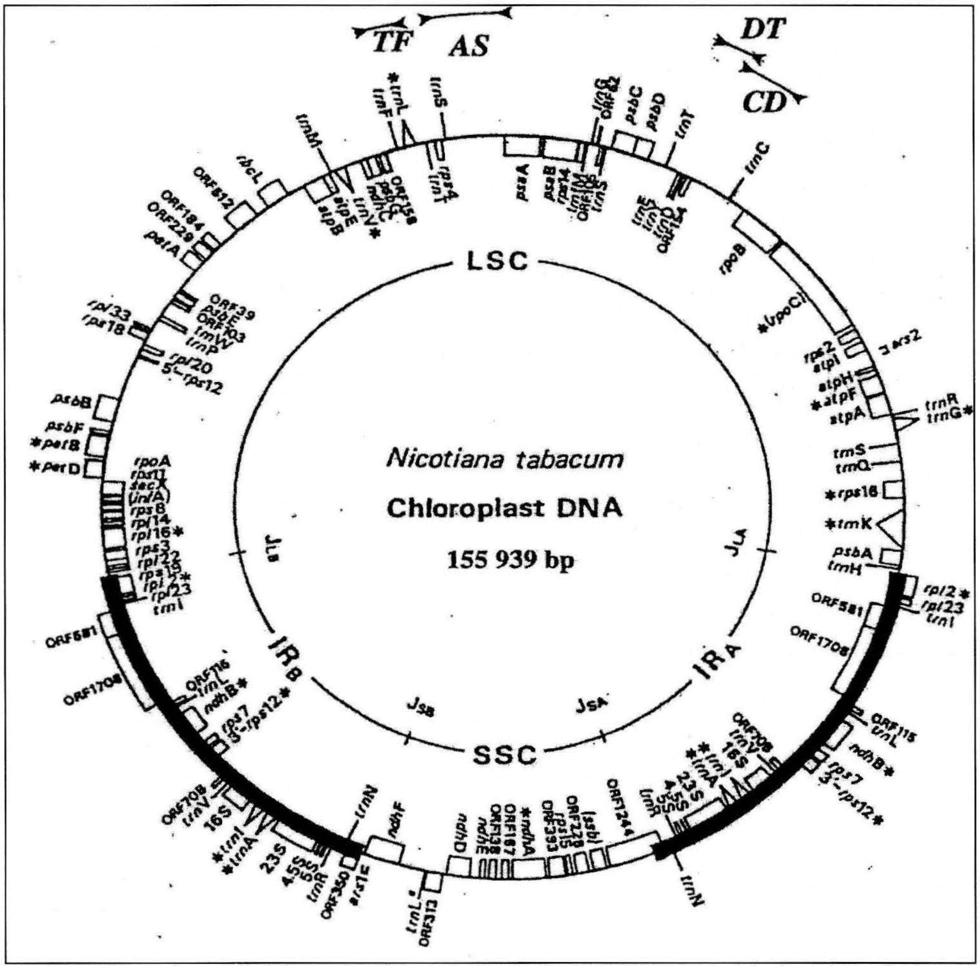

Fig. 1. Position of amplified fragments (TF, AC, DT, CD) on ct DNA on model of ct DNA of tobacco.

haplotype 7 of Eastern Pyrenean origin is prevalent, but influence from south is also pronounced that is reflected in presence of haplotypes 2 and 17 from Apennine glacial refugia. On southwest haplotype 2 is dominant, while on second place is haplotype 7. It is consequence of nearby Apennine refugium and shallow Northern Adriatic that was mainland at the end of last glaciation. In Southern Croatia (Dalmatia) one population of hungarian oak (*Quercus frainetto* Ten.) and pubescent oak (*Quercus pubescens* (Matt.) Liebl.) was analysed. Both populations are of haplotype 5. Although number of investigated populations is to small to bring to general conclusion about the region, it is interesting that here, as on the south of Apennine peninsula, haplotype 6 was not found. It is found in Eastern Croatia, Eastern Hungary and Western Rumania, which can lead to conclusion about their origin from the cost of Black see, instead from Balkan. Based on all this, Croatia is divided on zones shown on Figure 3.

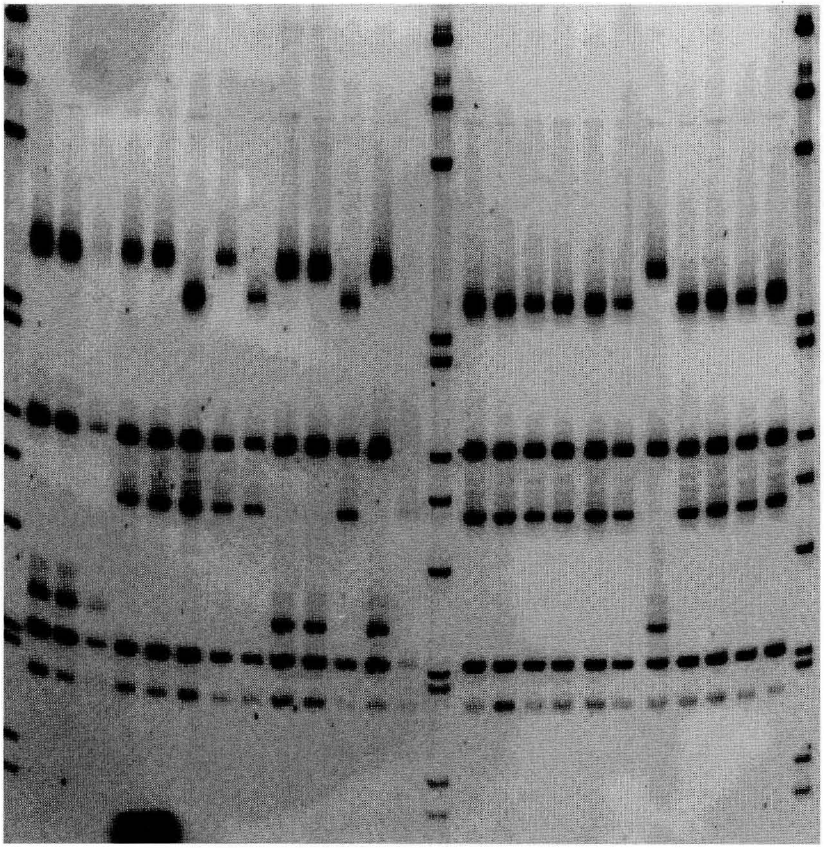

Fig. 2. Digest of fragment DT of 24 individuals on polyacril-amide gel upon electrophoresys. Notice that different haplotypes have different positions of bands. On the edge of the gel and in the middle 1 kb marker is visible.

From all the results it is clear that all species of oaks from *Quercus sensu stricto* group share the same haplotypes. It is consequence of their hybridisation, which was more frequent in the past than today. During colonisation oaks from *Quercus sensu stricto* group met different and changing conditions, on which they were pushed to adapt. Todays "species" of oaks resulted from the process of repetitive hybridisation and selective pressure. Thus, different species of this group can be considered as different ecotypes rather than as different species. At the same time oaks from other groups have different haplotypes. For instance haplotypes of *Quercus suber* L. and *Quercus cerris* L. have similar haplotypes, but their haplotypes differ from any haplotype of *Q. sensu stricto* group (Dumolin-Lapegue & al. 1997).

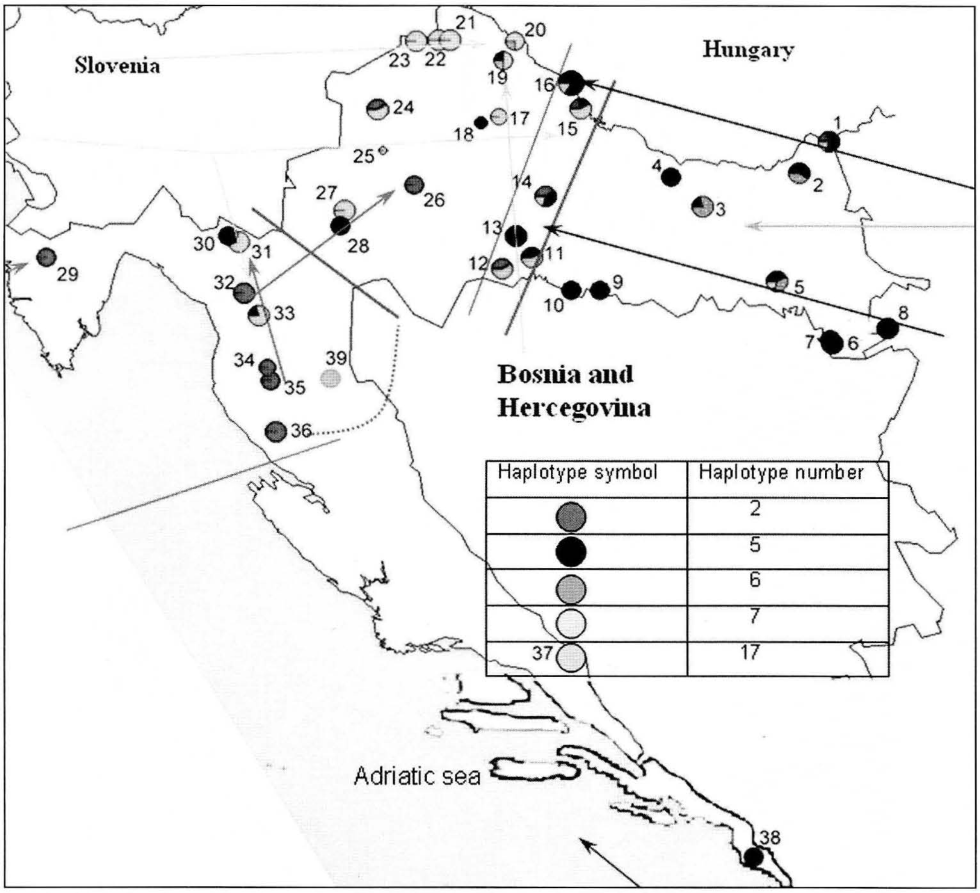

Fig. 3. Distribution of haplotypes of oak in Croatia. Each symbol repesents haplotype indicated on table overlayd upon the map.

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