

VARIATION PATTERNS OF MITOCHONDRIAL DNA  
OF *ABIES ALBA* MILL. IN SUTURE ZONES  
OF POSTGLACIAL MIGRATION IN EUROPE

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ABSTRACT

Thirty silver fir populations originating from the putative suture zones of the postglacial recolonization (Slovenia, Bosnia and Hercegovina, Ukraine) were studied using a mitochondrial *nad5-4* gene marker. The geographical distribution of mtDNA haplotypes in the Ukrainian Carpathians and their northern foothills indicates a very recent meeting of migration streams arriving from the Romanian Carpathians and Central Europe. In the western part of the Balkan Peninsula, two counterparallel migration streams are the most plausible explanation of the pattern observed. The haplotype typical for the Balkan Peninsula predominates along the Adrian coast, whereas the Central-European haplotype is more represented in the inland.

KEY WORDS: *Abies alba*, mitochondrial DNA, postglacial migration, Balkans.

INTRODUCTION

Silver fir (*Abies alba* Mill.) is the most productive native European tree species (Korpel' et al. 1982). In Central Europe (Carpathians, Alps, Hercynic range), its distribution range is relatively continuous, except for marginal regions. On the other hand, disrupted and insular occurrences are typical especially of the Pyrenees, Massif Central, Corsica, Apennines and the southern and southwestern parts of the Balkan Peninsula. Some of these occurrences are extremely small today. For example, the net area of silver fir in Calabria is only 11 000 ha and it is fragmented into several small insulas (Menguzzato 1988).

Periodical outbursts of the dieback of silver fir are known since the 15th century (Larsen 1986). Whereas at the beginning, the decline was limited to the northern marginal populations in Saxony and Thuringia, since the last century it has been a phenomenon endangering the persistence of local small populations and strongly reducing population sizes in areas with a more continuous occurrence of silver fir over the whole central part of the range. During the last decade, a recovery has been reported from Germany, the Czech Republic, Slovakia and Slovenia, but very probably a new phase of decline will appear again. Conversion of indigenous forests to spruce and pine plantations and the use of silvicultural systems requiring arti-

cial reforestation also contributed to a gradual withdrawal of fir from the Central European forests. Gene conservation is thus a relevant issue in silver fir.

Among the factors, shaping the genetic variation in forest trees, the processes associated with the survival of tree population in refugial populations during the Pleistocene glacial periods and the postglacial recolonization of the European continent belong to the most important ones. In addition to paleobotanical investigations, based on the occurrence of fossil pollen and macrofossils (needles, wood, charcoal), genetic evidence can very efficiently be used in the identification of refugia and migration routes. Recent variation patterns in cytoplasmic genes with maternal inheritance, which are thus transferred exclusively by seeds, allow to outline genetically homogeneous areas and make inferences about the migration (Taberlet et al. 1998). In the Pinaceae family, it is the mitochondrial DNA, which generally exhibits maternal inheritance, whereas the chloroplast genes are transferred by the father (Mogensen 1996). Mitochondrial markers have widely been used for reconstructing phylogenies in many conifer species (Gugerli et al. 2001; Johansen and Latta 2003; Mitton et al. 2000; Sinclair et al. 1999; Sperisen et al. 2001).

In an extensive rangewide study of *Abies alba* Mill., Liepelt et al. (2002) investigated the geographical distribution of haplotypes at an mtDNA locus with two highly conserved alleles, which can be assigned to at least two distinct refugial areas: the Balkans and Central Europe. Since these areas are separated by the treeless Great Hungarian Plain, there are two rather narrow suture zones between them, the eastern one in the Carpathians and the western one in the Dinarian range. However, the suture zones are either underrepresented or even completely unrepresented in the collection. The aim of this study is completing the gaps in these areas of interest and making inferences on the postglacial migration process of silver fir.

## MATERIAL AND METHODS

Samples were collected from 388 trees representing 6 populations from Slovenia, 13 populations from Bosnia and Herzegovina and 11 populations from the Ukrainian Carpathians and their northeastern foothills (Table 1). Only indigenous populations were chosen for sampling. From each population, 10 to 14 adult trees (aged over 80 years) were studied. Dry needles of the 2nd year-class were used for the analyses. To complete the geographic distribution patterns of mtDNA haplotypes in the studied regions, the results presented by Liepelt et al. (2002) for Croatian populations and/or populations from northern Romania, eastern Slovakia and eastern Poland are included in the presentation (Figs 1 and 2).

Total genomic DNA was extracted from 5 to 8 needles per tree, using a modified CTAB protocol following Dumlouin et al. (1995). DNA concentration was measured spectrophotometrically.

The assessment of the mtDNA variation followed Liepelt et al. (2002) with slight modifications. The PCR-amplified fragment is located within the fourth intron of the mitochondrial NAD dehydrogenase subunit 5 gene (*nad5-4*). The PCR mixture contained 1×PCR buffer (Invitrogen, Frankfurt a.M., Germany), 1.75 mM MgCl<sub>2</sub>, 0.2 μM forward and re-

TABLE 1. List of the investigated *Abies alba* Mill. Populations.

Name	Longitude	Latitude	Country
Veretskij pereval	23°10'	48°48'	Ukraine
Korostiv	23°28'	49°00'	Ukraine
Sojmy	23°30'	48°32'	Ukraine
Myslivka	23°42'	48°45'	Ukraine
Yasinya	24°20'	48°14'	Ukraine
Yasin'skij pereval	24°28'	48°19'	Ukraine
Nadvirna	24°35'	48°36'	Ukraine
Majdan	24°36'	49°00'	Ukraine
Knyazhdvir	24°55'	48°34'	Ukraine
Berehomet	25°26'	48°12'	Ukraine
Storozhyniets	25°41'	48°08'	Ukraine
Šator	16°24'	44°12'	Bosnia and Herzegovina
Oštrej	16°24'	44°28'	Bosnia and Herzegovina
Grmeč	16°39'	44°35'	Bosnia and Herzegovina
Busije	16°50'	44°01'	Bosnia and Herzegovina
Prusačka Rijeka	17°17'	44°04'	Bosnia and Herzegovina
Vlašič	17°41'	44°20'	Bosnia and Herzegovina
Kamenica	18°09'	44°22'	Bosnia and Herzegovina
Igman	18°16'	43°47'	Bosnia and Herzegovina
Zvijezda	18°28'	44°13'	Bosnia and Herzegovina
Jahorina	18°36'	43°44'	Bosnia and Herzegovina
Ravna Romanija	18°42'	43°56'	Bosnia and Herzegovina
Petroviči-Klis	18°44'	44°10'	Bosnia and Herzegovina
Sjemeč	19°10'	43°49'	Bosnia and Herzegovina
Trnovski gozd	13°51'	45°58'	Slovenia
Hotedršica	14°08'	45°56'	Slovenia
Kočevlje	14°58'	45°38'	Slovenia
Rajhenavski Rog	15°01'	45°39'	Slovenia
Slovenske Konjice	15°23'	46°20'	Slovenia
Zgornja Velka	15°46'	46°41'	Slovenia

verse primer (for primer sequences, see Liepelt et al. 2002), 0.2 μM each dNTP, 0.2 unit *Taq* DNA polymerase and 25 ng of template DNA. The cycle profile consisted of an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 52.5°C for 1 min, and extension at 72°C for 1 min 20 sec, and a final extension step at 72°C for 8 min. The amplified fragments were separated by electrophoresis in a 1.2% agarose gel for 2 hours at 4.5 V/cm (8 μl of each PCR product).

## RESULTS AND DISCUSSION

Liepelt et al. (2002) identified two variants of the amplified fragment, differing by an 80 bp insertion/deletion, whereby the type occurring over the major part of the range (central and western Europe) represents the longer variant (allele 1) and the type occurring in the southeastern Europe represents the shorter one (allele 2).

The distribution of mtDNA haplotypes in the Ukrainian Carpathians and their northern foothills is shown in Fig. 1. The boundary between the ranges of both types is unexpectedly sharp in this region. No mixed population samples were found West and Northwest of the line Rakhov–Yasinya–Chernivtsi, all of them contained allele 1. On the other side of this line, two population samples were fixed for allele 2 and two samples contained a mixture of alleles, but with a strong predominance of allele 2.

The situation in the suture zone West of the Great Hungarian Plain is slightly different (Fig. 2). The Western-Bosnian populations belong contain predominantly the allele 1. However, to the East of the 16°40' meridian, all popula-

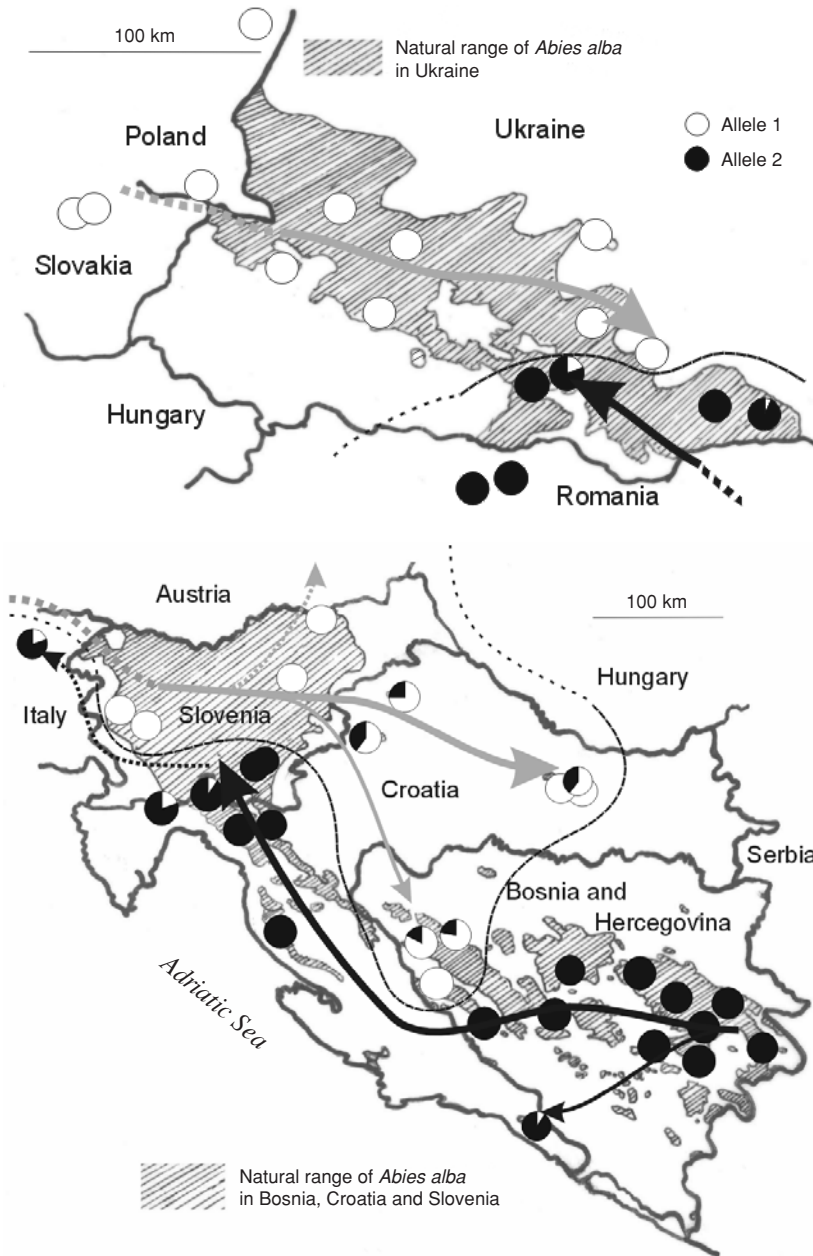


Fig. 1. Frequencies of the *nad5-4* haplotypes in the Ukrainian Carpathians including the proposed migration routes (black arrow – migration route of fir originating from the putative North-Apennine refugium, grey arrow – migration route of fir originating from the putative Balkan refugium, dashed line – proposed border between migration streams). Polish, Slovak and Romanian samples taken from Ziegenhagen (unpubl.) and Liepelt et al. (2002).

Fig. 2. Frequencies of the *nad5-4* haplotypes in the western Balkans including the proposed migration routes (symbols as in Fig. 1). Croatian populations taken from Liepelt et al. (2002).

tion samples contained purely the allele 2. In Slovenia, the two North-Dinaric populations were found to be fixed for allele 2, whereas in the populations from the foothills of Alps both in the West and the East of the country, allele 1 predominated strongly. This pattern corresponds to the findings of Liepelt et al., who found the predominance of allele 2 in Dalmatia and a prevalent occurrence of allele 1 in Slavonia and central Croatia.

In both suture zones, the revealed trends correspond completely to those observed in previous studies. In the Eastern and Southern Carpathians in Romania, all the fir populations are fixed for allele 2, whereas Slovak and Polish populations in the Western Carpathians and adjacent hilly regions North of the Carpathian arc exhibit fixation for allele 1 (Liepelt et al. 2002; Ziegenhagen unpubl.). In the western part of the Balkan Peninsula, the pattern is more complicated. Practically all populations are mixed and two parallel clines can be observed: allele 2 is fixed in the southern Balkans populations (Bulgaria), it predominates along the Adriatic coast (from Dalmatia, over Velebit and Kapela Mts., Julian Alps, reaching even northeastern Italy), whereas in

the inland (eastern Slovenia, Slavonian hills), allele 1 has generally higher frequencies (Liepelt et al. 2002).

The existence of only two haplotypes in the investigated region of the mitochondrial DNA does not mean that only two glacial refugia existed. In fact, a quite strong differentiation between the southern Balkans (Bulgaria, Macedonia) and the Romanian Carpathians, as well as differences in the representation of regionally specific alleles, indicate rather two (or more) refugial populations in the Balkans, both sharing the same haplotype (Longauer 1996). The same may be true for the central and western Europe.

For the Ukrainian Carpathians, the immigration of fir from the Southeast along the Carpathian arc is generally suggested (Krippel 1986; Środoń 1983). The junction of migration streams from the Balkans and the western refugia was supposed at the eastern limit of the Hercynic range (Krippel 1986; Samek 1967). Based on isozyme studies, Konnert and Bergmann (1995) proposed a very broad introgression zone between these migration streams, covering the whole Western Carpathians and Bohemian Quadrangle, and Llamas Gómez and Braun (1995) suppose the influence of the Car-

pathian silver fir even more westwards (Saxony). The presented results prove that the Carpathian migration stream stopped in fact much more eastern, very close to the present-day state border of Romania and Ukraine. This corresponds well with the results of Longauer (1996), who observed, despite a generally clinal trend of differentiation and allele frequency distribution at isozyme genes, a clear discontinuity between Romanian and Ukrainian Carpathians. Of course, this does not mean that introgression has not taken place, but it is driven more by pollen flow than by seed dispersal, and the center of the introgression zone is located more in the East than originally assumed.

In the western Balkans, Horvat-Marolt and Kramer (1982) proposed the migration of fir from the refugia in Macedonia along the Dinarian range to northern Bosnia and Croatia. Further migration should have been stopped by the presence and strong competition of beech. Konnert and Bergmann (1995) supported this view and suggested a second introgression zone between the populations of Balkan and Central-European origins, covering northwestern Croatia, Slovenia, and southeastern Austria. On the other hand, Kral (1980) admitted the spread of fir originating from the Northern-Appennine refugium from Slovenia towards Slavonia and Dalmatia.

The distribution patterns in the suture zones indicate that the concepts of the glacial and postglacial history of *Abies alba*, as they have been presented both in paleobotanical and genetical studies, must be slightly revised. The presented results, completed by those of Liepelt et al. (2002) allow to conclude about two counterparallel migration streams in this region. In eastern Bosnia and Bulgaria, populations are fixed for the allele 1, so that probably they were settled from the Balkan refugium. Along the Adriatic coast, fir migrated to the Northwest. In the opposite direction, fir originating from the western refugia occupied the hills between the Sava and Drava rivers, as well as the easternmost part of Bosnia. The introgression zone in the western Balkans is much broader than the Carpathian one, the predominance of populations containing both mitochondrial haplotypes indicates that not only pollen flow, but also seed migration contributed to the introgression. The absence of a sharp border between both haplotypes allows to conclude that the contact of migration streams in the western Balkans must have happened much earlier than in the Carpathians. However, genetic data cannot provide the information on temporal scales of migration.

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