CHLOROPLAST HAPLOTYPE DIVERSITY OF WHITE OAK SPECIES IN SLOVAKIA AND THE CZECH REPUBLIC: RESULTS FROM PCR-RFLP ANALYSIS AND PHYLOGEOGRAPHIC INTERPRETATIONS

Marcela Tutková-van Loo[1]* & Kornel Burg[2]

[1)] Department of Higher Plant Systematics and Evolution, Institute of Botany and Botanical Garden, University of Vienna, Rennweg 14, A-1030 Vienna, Austria
[2)] Division of Biogenetics and Natural Resources, Department of Bioresources, ARC Seibersdorf research GmbH, A-2444 Seibersdorf, Austria

Received January 31, 2003; accepted November 20, 2004

ABSTRACT

This paper presents a detailed study of white oak chloroplast DNA (cpDNA) variation in Slovakia and the Czech Republic. A total of 276 oak trees representing 41 populations of *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Quercus frainetto* Ten., *Quercus pubescens* Willd., and their several subspecies were screened for polymorphisms in up to four PCR-amplified cpDNA fragments. Using the banding pattern and haplotype nomenclature as described by PETIT *et al.* (2002a), eight chloroplast haplotypes were detected. Six of them can be regarded as autochthonous for the region studied. However, the separation system used in this study has a higher resolution compared to that used by PETIT *et al.* (2002a). Therefore, by scoring of bands using the refined banding pattern nomenclature, the number of haplotypes found increased from eight to ten belonging to three lineages. These lineages can be linked to glacial refugia in the Italian, and Balkan Peninsula. The haplotypes originating from the Balkans are particularly well represented in the region studied, but there is also a significant contribution from the other refugium, which explains the high overall level of cpDNA diversity. Despite the strong human impact in these countries during the past centuries resulting in clearance of many forests, followed by reforestation, sometimes involving seed transfers, geographic patterns of lineages and haplotypes could still be detected.

Key words: *Q. petraea*, *Q. robur*, *Q. pubescens*, *Q. frainetto*, PCR-RFLP, cpDNA, postglacial colonization, geographical variation.

INTRODUCTION

During the last glacial period (115,000–10,000 BP) many species, including oak, became restricted to the southern part of their present distribution, where climatic conditions allowed survival of the species (TALLIS 1991). Data from fossil pollen records indicate that the primary glacial refugia of *Quercus* were located mostly along the Mediterranean borderlands and around the Black Sea; on the Iberian Peninsula, the Apennine Peninsula, in the Balkans and possibly in the Caucasus (HUNTLEY & BIRKS 1983; BREWER *et al.* 2002). The northward expansion (the colonization process) of oaks took place in two steps. First, in the late-glacial interstadial (13–11 ka BP) when the climate became warmer/moister, *Quercus* reached the main central European mountains (Alps, Pyrenees and Carpathians). Second, with the stabilization of climate favorable to deciduous trees species in the Holocene (10 ka BP), oak spread into northern Europe (BREWER *et al.* 2002).

Recently, further insight into oak postglacial migration history has come from the analysis of cpDNA. The cpDNA is maternally inherited in most angiosperms (BIRKY 1995). DUMOLIN *et al.* (1995) demonstrated maternal inheritance in *Quercus* using controlled intraspecific crosses of *Q. robur*, where polymorphisms of cpDNA were passed from a single mother tree to its entire progeny. The relatively slow rate of mutations (CLEGG *et al.* 1994) and the lack of recombination make cpDNA an ideal marker for phylogenetic studies, even if homoplasy due to convergent or reverse mutations has to be considered. Furthermore, extensive cytoplasmic exchange due to hybridization and introgression between sympatric species of white oaks has been observed, both in the eastern United States (WHITTERMORE & SCHAAL 1991) and in Europe (FERRIS *et al.* 1993; PETIT *et al.* 1993). This results in similar polymorphisms of their cpDNA. The lack of differentiation of cpDNA markers among white oak species at a broad scale but also at a much finer geographical scale (PETIT *et al.* 1997) implies that interspecific exchanges were not restricted only to rare episodes during long periods of sympatry (for instance in refugia during the last ice age), but also took place after the last ice age, either during or after postglacial colonization (PETIT *et al.* 2003a,b). DUMOLIN- LAPÈQUE *et al.* (1997) have demonstrated that cpDNA variation is geographically structured in white oaks and that related haplotypes often have a similar distribution. In order to conduct a detailed European-wide survey of cpDNA variation in white oaks, PETIT *et al.* (2002a) have extended their previous study to about 1,890, mostly autochthonous oak populations sampled throughout Europe. Chloroplast haplotypes of white oaks were grouped according to their lineage and to their inferred Pleistocene refugial area (PETIT *et al.* 2002b). The present distribution of oak haplotypes and available palynological informations were then used to formulate possible postglacial migrations out of ice-age refugia.

In the Czech Republic, the forest covers approximately 2.63 million ha corresponding to 33 % of the national territory, of which 5 % consists of oak (KOBLÍŽEK 1990). In Slovakia, the forest covers 40.6 % of the national territory corresponding to 1.9 million ha of forestland resources. In 2001, broadleaved trees covered 58 % and coniferous trees 42 % of forestland, whereby oaks represented 14 % of the main tree species (GREEN REPORT 2001).

In both the countries, four European white oak species belonging to the subg. *Quercus* (= *Lepidobalanus* (Endl.) Örsted.): *Q. petraea, Q. robur, Q. pubescens*, and *Q. frainetto* occur naturally. The most abundant are *Q. petraea* and *Q. robur* that grow throughout the region and even further north in Poland and Germany. *Q. robur* (pedunculate or common oak), as a traditionally favored silviculture species, has been most affected by the action of man, including seed transfers, artificial regeneration, and selective cutting (BORDÁCS *et al.* 2002). Due to excellent growing properties, and first-rate wood, acorns of pedunculate oak of Slavonian origin (*Q. robur* ssp. *slavonica*) were imported into both countries (MAGIC 1974). Both *Q. frainetto* (Hungarian oak) and *Q. pubescens* (pubescent oak) prefer warm and dry sites, where Slovakia and the Czech Republic form the northern boundary of their distribution. Several other white oak taxa were found and described in natural forests of Slovakia and the Czech Republic, like *Q. polycarpa* (Schur.), *Q. dalechampii* (Ten.), *Q. pedunculiflora* (C. Koch), and *Q. virgiliana* (Ten.). Although several Slovak and Czech authors (KOBLÍŽEK 1990; MAGIC 1975; POŽGAJ 1997) treat them as species, their taxonomical position is still not clear, because of a great intraspecific morphological variation making them difficult to distinguish.

The objective of this study was to supplement the postglacial history of white oaks for the area of the Czech Republic and Slovakia. The identification of the original migration routes and the evaluation of the human influence on the cpDNA structure within and among populations were of special interest.

MATERIAL AND METHODS

Plant material

A summary of white oak populations screened for the cpDNA variation in this study is given in Table 1, where population code number, number of individuals, name of location, geographical code (longitude, latitude, and altitude), country of origin, and nature of the stand are indicated.

To estimate cpDNA polymorphisms for the Czech and Slovak Republics, a total of 41 white oak populations (with 276 individual oak trees) were sampled. For collecting and processing of all these samples, a protocol according to CSAIKL *et al.* (2002a) was used.

Species determination of samples was done by the providers (see in Acknowledgements) and additionally checked by us using the leaf morphological characters (MAGIC 1975) and knowledge gained during assessment of population samples of mixed species. Because of the still not clear taxonomical position of *Q. pedunculiflora, Q. dalechampii, Q. polycarpa*, and *Q. virgiliana*, they are treated as subspecies in this study. Thus, trees labeled as *Q. pedunculiflora* and Slavonian oak (*Q. robur* ssp. *slavonica*) were considered as *Q. robur, Q. dalechampii* and *Q. polycarpa* individuals were treated as *Q. petraea*, and those named *Q. virgiliana* were grouped with *Q. pubescens*. Voucher specimens are deposited at the ARC Seibersdorf research GmbH at Seibersdorf, Austria.

The map in Fig. 1 presents the distribution of populations studied according to country and species. In the sample set, 130 trees (47.10 %) turned out to be *Q. petraea* sensu lato (after this *Q. petraea*), 110 trees (39.86 %) *Q. robur* sensu lato (after this *Q. robur*), and 28 trees (10.14 %) *Q. pubescens* sensu lato (after this *Q. pubescens*). All of these species were found in both countries. Trees of species *Q. frainetto* were found only in Slovakia. This species was represented by 8 trees (2.9 %), which were collected in the Martinský les population located in south-western Slovakia.

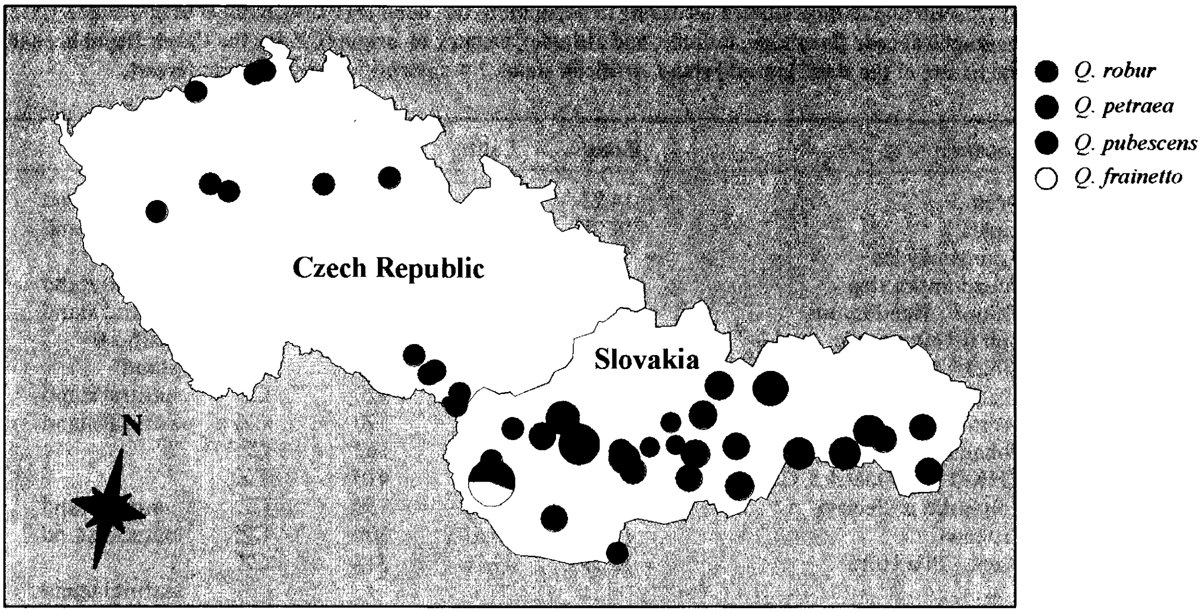

Figure 1. Distribution of all oak populations studied according to species. The size of circles is proportional to the population size investigated. Sectors are proportional to the species composition. The color codes correspond to the different species.

Analysis of cpDNA polymorphisms

The standard method used for analyses of cpDNA polymorphisms in oaks sampled throughout Europe (see PETIT *et al.* 2002a) was in this study replaced by a refined method with higher resolution developed at the ARC Seibersdorf research GmbH at Seibersdorf, Austria. The whole procedure is described in more detail in the following section.

DNA extraction and PCR-RFLP analysis of cpDNA

DNA extraction was done using the Dneasy Plant DNA Extraction Minikit (QIAGEN) according to the manufacturer's instructions. Total DNA was used as a template in PCR reactions involving amplification of cpDNA with primers *trnT/trnF* (TF) (TABERLET *et al.* 1991), *trnD/trnT* (DT), *psaA/trnS* (AS) and *trnC/trnD* (CD) (DEMESURE *et al.* 1995). 25 µl of PCR reaction mixture contained: 20–30 ng of template DNA, 12.5 µl of 2x buffer, 2 µM of each primer, 0.5 units of Expand Long Template PCR System (Boehringer Mannheim) and sterile water to volume upto 25 µl. To prepare 2x buffer (1 ml): 200 µl of 10x buffer 2 (provided with the kit, and containing 2.25 mM of $MgCl_2$), 0.2 mM dNTPs and sterile water upto 1 ml. Reaction mixtures were run on an MJ Research Thermal Cycler using the program: an initial denaturation for 4 min at 94 °C followed by 40 cycles of 45 s at 93 °C, 45 s at 58 °C (55 °C for DT, 62 °C for AS), 2 min (4 min for CD, and AS) at 72 °C, and final extension of 10 min at 72 °C. Amplification products (5 µl) were then digested with restriction enzymes: DT-fragment with TaqI, AS-fragment with HinfI, CD-fragment with TaqI, and TF-fragment with AluI, CfoI, and HinfI. After enzyme digestion (following the manufacturer's instructions), 10 µl of the TF/CfoI reaction mixture was separated on a 1 % agarose gel in 1x TBE buffer and stained by ethidium bromide to determine the restriction banding pattern. For the others (TF/AluI, AS/HinfI, CD/TaqI and DT/TagI), polyacrylamide gel electrophoresis (PAGE) with shark tooth combs was used. Samples were separated on a 0.5 mm thick non-denaturing 8 % PAGE gel in 1 % TBE at 500 V for 3 to 3 1/2 hours, and TF/AluI restricted samples for additionally 3 hours. Two gels were running simultaneously under the same conditions on 'Dual Vertical Slab Gel System' DSG-250 (CBS), along with a cooling unit (14°C). For visualization of cpDNA restriction fragments, PAGE gels were silver-stained (KRYSTUFEK 2001).

Scoring of gels and cpDNA haplotyping

The PCR-RFLP data were scored as multistate unordered characters: each polymorphic restriction band on the polyacrylamide gel was a character and the states were different mutants of this fragment. The combination of these characters within an individual corresponds to a characteristic restriction banding pattern of the restricted cpDNA fragment.

Table 1. Summary of *Quercus* populations studied according to population code number (PCN), number of individuals (NI), name of location, geographical code (longitude, latitude, and altitude), country of origin (CZ for the Czech Republic, and SK for Slovakia), and nature of the stand [natural stand, artificial stand, ? = unidentified origin, virgin forest].

PCN	NI	Location	Long.	Latit.	Altitude	Country	Origin
1	5	Děčín	14.23	50.79	266	CZ	natural stand
2	5	Lovoš	14.23	50.79	500	CZ	natural stand
3	5	Vranovický les	16.58	48.92	172	CZ	?
4	5	Pouzdřanská step	16.64	48.95	275	CZ	natural stand
5	1	Valtice – Rendezvous	16.80	48.75	185	CZ	natural stand
6	5	Luh u Třebechovic	16.00	50.18	248	CZ	artificial
7	5	Libický luh	15.17	50.11	183	CZ	stand?
8	5	Svatý jan pod Skalou	14.14	49.97	420	CZ	natural stand
9	5	Moravský Krumlov	16.40	49.04	370	CZ	natural stand
10	5	Pohansko u Břeclavi	16.91	48.73	163	CZ	?
11	5	CHKO Křivoklátsko, Červený kříž	13.93	50.00	410	CZ	?
12	5	Pohansko u Břeclavi	16.91	48.73	158	CZ	natural stand
13	5	Litvínov	13.61	50.61	300	CZ	natural stand
14	5	Plzeň – Bíla Hora	13.39	49.76	315	CZ	?
							natural stand
15	14	Cibajky	18.27	48.55	358	SK	artificial stand
16	10	Kulháň	18.07	48.72	910	SK	natural stand
17	5	Píla	17.35	48.38	245	SK	natural stand
18	5	Dobrá Voda	18.87	48.38	574	SK	natural stand
19	7	Piešťany	17.87	48.58	350	SK	natural stand
20	7	Palárikovo – Bažantnica	18.08	48.03	110	SK	natural stand
21	6	Revište	18.72	48.53	393	SK	artificial stand
22	5	Burda	18.77	47.83	395	SK	virgin forest
23	9	Kašivárová	18.77	48.48	602	SK	virgin forest
24	7	Sitno	19.87	48.40	1010	SK	virgin forest
25	8	Rohy	19.52	48.55	374	SK	natural stand
26	4	Hrochoť	19.30	48.60	636	SK	natural stand
27	7	Nedelište	19.47	48.38	570	SK	natural stand
28	8	Valaská	19.57	48.82	470	SK	natural stand
29	4	Príboj	19.23	48.75	430	SK	virgin forest
30	7	Hačava – Skálie	19.95	48.62	390	SK	?
31	4	Boky	19.03	48.57	561	SK	virgin forest
32	7	Veľké Kapušany	22.03	48.52	100	SK	natural stand
33	9	Kokošovská dubina	21.37	48.78	375	SK	virgin forest
34	7	Vinné	21.95	48.82	325	SK	natural stand
35	9	Dubky – Moldava nad Bodvou	21.12	48.62	220	SK	natural stand
36	7	Dargov	21.53	48.73	473	SK	natural stand?
37	8	Kurinecká dubina	20.02	48.35	242	SK	natural stand
38	9	Hrušovská lesostep	20.63	48.60	579	SK	natural stand
39	11	Kvetnica	20.28	49.03	700	SK	natural stand
40	18	Martinský les	17.38	48.23	122	SK	natural stand
41	8	Liptovský Hrádok	19.72	49.03	637	SK	natural stand

A combination of all restriction banding patterns in a tree allowed recognizing haplotypes. In order to compare and to supplement the postglacial history of white oak haplotypes found in this study with the so-called 'standard' haplotypes described by PETIT *et al.* (2002a), restriction banding patterns of (CD/TaqI, DT/TaqI, AS/HinfI, TF/HinfI, TF/CfoI) digests and haplotypes were labeled according to the nomenclature as developed in their study. Based on phylogenetic relationships between haplotypes, they were then grouped into lineages. The composition of the different lineages is indicated in Figs. 1 and 2 of PETIT *et al.* (2002a). The phylogenetic relationship of new haplotypes (found in this study) to 'standard' haplotypes was determined using the PAUP Software version 4.0b2a (SWOFFORD 1998). The geographical presentation of oak species and haplotypes was done with the aid of MapInfo Professional

Version 5.01 (MapInfo Corporation).

Nevertheless, the separation system used in this study has a higher resolution compared to that used by PETIT *et al.* (2002a) and allows recognizing more variable bands. Therefore, haplotypes were redefined (using the banding patterns of CD/TaqI, DT/TaqI, AS/HinfI, TF/HinfI, TF/CfoI, and additionally TF/AluI digests) and labeled according to the newly developed banding pattern nomenclature (for TF/Hinf, and TF/ AluI) and the refined banding pattern nomenclature (for CD/TaqI, and DT/TaqI) described by CSAIKL *et al.* (unpublished).

In more detail: for HinfI digests of the TF fragment following PETIT *et al.* (2002a), restriction bands TF1, TF3, TF5, TF10-12, and TF14 were considered; where TF14 exhibits the highest variation in its electrophoretic mobility. In the separation system used in this study the bands TF8-TF14 are not always clearly visible. Moreover, TF1 and TF7 showed more variability than in the resolution system used by PETIT *et al.* (2002a). Therefore, we developed a new banding pattern nomenclature for the TF/Hinfl restriction and consider only fragments TF1-TF3 and TF7. In addition, variations of the largest fragment in CD/TaqI, and DT/TaqI digests were labeled according to CSAIKL *et al.* (unpublished).

Genetic diversity analysis

In order to calculate the genetic diversity and differentiation measures, frequencies of haplotypes as well as the genetic distances between them were used following PONS & PETIT (1996), using the software HAPERMUT available at http://www.pierroton.inra.fr/genetics/labo/Software/. Following measures of diversity as well as their standard errors were computed: within population genetic diversity (h_S), the total diversity (h_T), the coefficient of genetic differentiation G_{ST} (where the genetic distance between haplotypes is ignored), and the coefficient of genetic differentiation N_{ST} (where the genetic distance between haplotypes is taken into account) (for more details see PETIT *et al.* 2002a). To evaluate the existence of phylogeographic structure of the data analysed, N_{ST} and G_{ST} were compared using permutation approach (BURBAN *et al.* 1999). A total of 1000 permutations of haplotypes identities were carried out. The 1000 permutated values of N_{ST} were ranked and the distribution compared to the observed value without permutation to infer significance levels. Levels of diversity and differentiation were computed separately for *Q. robur*, *Q. petraea*, and all species together from the whole data set using haplotypes labeled according to banding pattern and haplotype nomenclature as described by PETIT *et al.* (2002a), which make the results comparable with those of them. Only those populations represented by at least three trees were included in the analysis. The levels of diversity and differentiation were not separately computed for *Q. pubescens* (not enough populations with at least three species) and *Q. frainetto* (only one population). Finally the difference between *Q. robur*-, *Q. petraea*-, and all species sets was tested (pairwise tests) against zero by the *t*-test.

RESULTS

CpDNA polymorphisms

In Fig. 2, five restriction diagrams summarize the

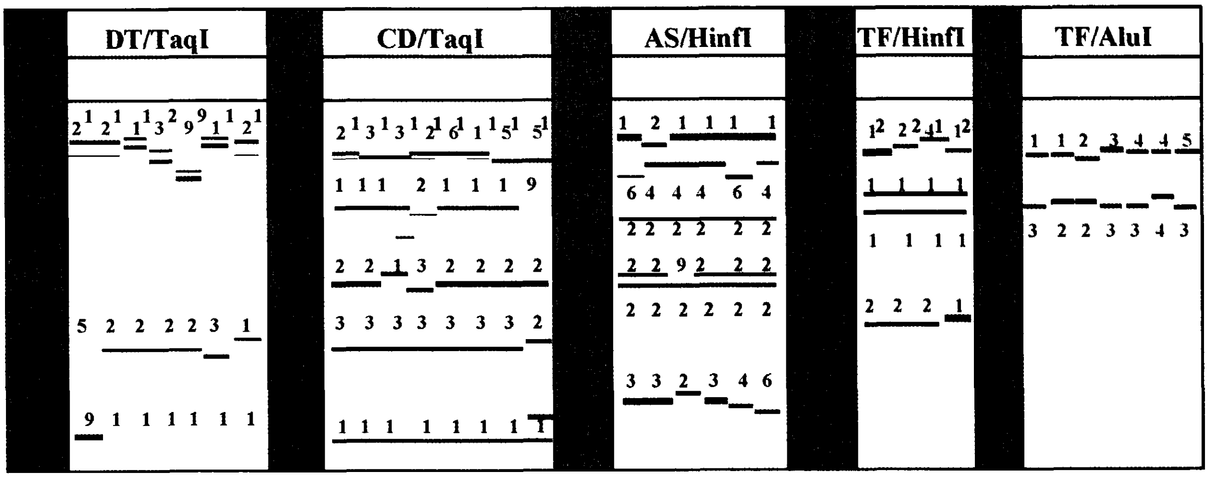

Figure 2. Restriction diagrams of polymorphisms observed in the sample set. Variation in the restriction patterns was scored according to PETIT *et al.* (2002a); (black numbered bands). Additionally, the bands have been numbered according to the refined banding pattern nomenclature developed: by CSAIKL *et al.* (unpublished) (see blue labeled bands), and in this study (see green labeling).

Table 2. White oak cpDNA haplotypes detected in the sample set according to banding pattern and haplotype nomenclature as described by PETIT *et al.* (2002a). In the 3rd row, the scored bands for the regions DT/TaqI (5), CD/TaqI (5), AS/HinfI (6), TF/HinfI (3) are shown. PCR-products of the TF-fragment digested with CfoI gave one or two distinct fragments depending on the absence (coded as 1) or presence (coded as 9) of the CfoI restriction site. A total of 8 white oak haplotypes were detected.

Haplotype	DT/Taq1					CD/Taq1					AS/HinfI						TF/HinfI			TF/CFoI
	DT1	DT2	DT3	DT3'	DT4	CD1	CD2	CD3	CD4	CD6	AS1	AS2	AS3	AS4	AS5	AS6	TF1	TF3	TF5	
1	9	1	2	1	1	1	2	3	3	1	2	4	2	2	2	3	2	0	2	1
2	9	1	2	1	1	1	9	2	2	1	1	4	2	9	2	2	2	0	2	1
4a	1	1	1	1	1	1	1	2	3	1	1	6	2	2	2	3	2	0	2	9
5	1	1	2	1	1	1	1	2	3	1	1	6	2	2	2	3	2	0	2	9
6	2	1	2	1	1	1	1	1	3	1	1	6	2	2	2	3	2	0	2	9
7	1	1	5	9	1	1	1	2	3	1	1	6	2	2	2	4	2	0	2	9
14b	1	1	2	1	1	1	1	2	3	1	1	4	2	2	2	6	1	0	2	1
17a	1	1	3	1	1	1	1	2	3	1	1	4	2	2	2	3	2	0	2	1

Table 3. White oak cpDNA haplotypes detected in the sample set using the more refined scoring. In the 2nd row, the scored bands for the regions DT/TaqI (5), CD/TaqI (5), AS/HinfI (6), TF/AluI (2), TF/HinfI(4), and TF/CfoI (1) are shown. PCR-products of TF-fragment digested with CfoI gave one or two distinct fragments depending on the absence (coded as 1) or presence (coded as 9) of the CfoI restriction site. Altogether, 10 haplotypes were detected.

	DT/TaqI					CD/TaqI					AS/HinfI						TF/ALuI		TF/HinfI				TF Cfo
	DT1	DT2	DT3	DT3	DT4	CD1	CD2	CD3	CD4	CD6	AS1	AS2	AS3	AS4	AS5	AS6	TF1	TF2	TF1	TF2	TF3	TF7	
1	9	1	2	1	1	2	2	3	3	1	2	4	2	2	2	3	2	2	2	1	1	2	1
2	9	1	2	1	1	5	9	2	2	1	1	4	2	2	2	2	1	2	2	1	1	2	1
4a	2	1	1	1	1	2	1	2	3	1	1	6	2	2	2	3	4	3	1	1	1	1	9
5A	2	1	2	1	1	6	1	2	3	1	1	6	2	2	2	3	5	3	1	1	1	1	9
5B	1	1	2	1	1	3	1	2	3	1	1	6	2	2	2	3	4	3	1	1	1	1	9
5C	2	1	2	1	1	2	1	2	3	1	1	6	2	2	2	3	4	3	1	1	1	1	9
6	3	1	2	1	1	3	1	1	3	1	1	6	2	2	2	3	4	3	1	1	1	1	9
7	2	1	5	9	1	3	1	2	3	1	1	6	2	2	2	4	3	3	1	1	1	1	9
14b	1	1	2	1	1	5	1	2	3	1	1	4	2	2	2	6	4	4	3	1	1	2	1
17a	1	1	3	1	1	1	1	2	3	1	1	4	2	2	2	3	1	3	1	1	1	2	1

polymorphisms of the 4 chloroplast fragments (loci) observed on 8 % non-denaturing polyacrylamide gels in the sample set.

The haplotypes were detected on the basis of the information provided by four (CD, DT, TF, and AS) fragments. According to the haplotype nomenclature as described by PETIT *et al.* (2002a), a total of 8 different white oak haplotypes (1, 2, 4a, 5, 6, 7, 14b, 17a) corresponding to three lineages (A, C, and E) were detected in this survey (see Table 2). One of these haplotypes was described for the first time. Among the 8 haplotypes, 20 fragment variants were detected.

The phylogenetic relationship of the new haplotype to the 'standard' haplotypes (PETIT *et al.* 2002a), which was examined using PAUP Software version 4.0b2a (SWOFFORD 1998) showed that the new haplotype named 14b is a new member of lineage E (Fig. 3). Haplotype 14b is similar to haplotype 14 (described by PETIT *et al.* 2002a), where it only differs in the characteristics found for the CD fragment.

When the banding patterns were numbered according to the refined scoring [CSAIKL *et al.* (unpublished), and scoring developed in this study] haplotype 5 was divided into 3 new haplotypes named by us 5A, 5B, and 5C (see Table 3). Thus the number of haplotypes found increased from eight to ten. Although PETIT *et al.* (2002a) have described in their phylogenetic trees haplotypes 5a, 5b, and 5c, these do not correspond to 'our' 5A, 5B, and 5C. Because Petit's 5a, 5b, and 5c haplotypes only differ in the small restriction fragment of the TF/HinfI combination, they were not clearly detected in all

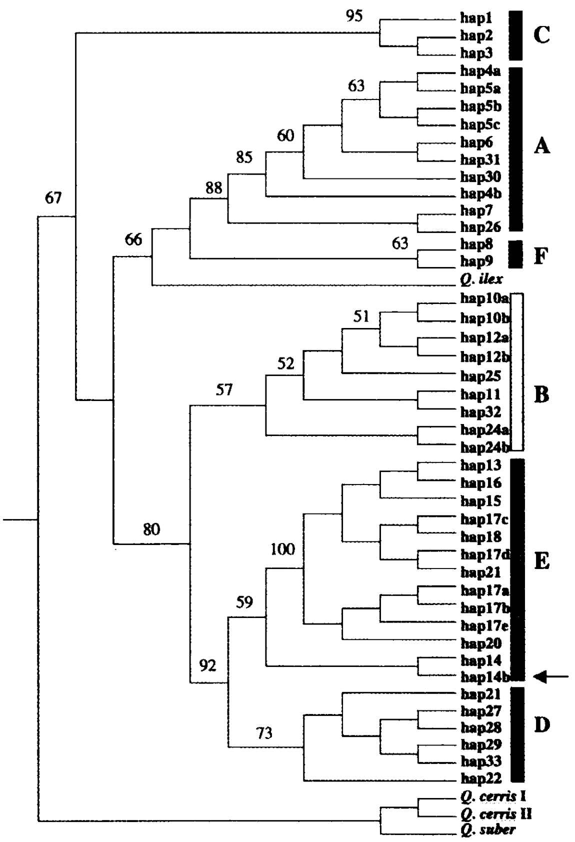

Figure 3. Phylogenetic tree (UPGMA) of 46 cpDNA 'standard' haplotypes (PETIT *et al.* 2002a) and the 'new' haplotype (see red arrow) obtained from white oaks (and *Q. ilex, Q. cerris1, Q. cerris2*), using the PAUP software version 4.0b2a (SWOFFORD 1998). The 'new' 14b haplotype is related to haplotype 14 and belongs to lineage E. Bootstrap values (percentages of 100 replicates) are shown above the branches. Only bootstrap values > 50 % are shown. Lineages have been assigned different colors according to PETIT *et al.* (2002a). *Q.lcerris* haplotypes were used as outgroup.

samples studied by them and thus subsumed under haplotype 5. The phylogenetic analysis using the PAUP software version 4.0b2a (SWOFFORD 1998) in which data from the Table 3 were used, grouped 5A, 5B and 5C together with haplotypes 4a, 6, and 7 into lineage A (data not show, more details in VAN LOO 2003). Although combination of banding patterns from all 4 fragments were used to characterize the haplotypes, the refined scoring of DT1 and DT3 bands (from the DT/TaqI restriction), and of CD1, CD2, and CD3 bands (from the CD/TaqI restriction) were sufficient to determine all 10 different haplotypes.

Genetic diversity and differentiation of cpDNA

Estimates of genetic diversity and differentiation are given in Table 4 for *Q. robur*, *Q. petraea* and all species together (inclusive *Q. frainetto* and *Q. pubescens*). *Q. petraea* has the highest level of total chloroplast diversity with 0.748, as measured by (h_T). The total chloroplast diversity analyzed in the all species set with 0.711 has the intermediate value, whereas *Q. robur* is characterized by the lowest diversity (h_T = 0.676). On the contrary, diversity within stands (h_S) is lower in *Q. petraea* than in *Q. robur.* All pairwise tests between species are significant for h_T and h_S (results not shown).

G_{ST} values vary from 0.698 in *Q. robur* to 0.863 in *Q. petraea*. The measures of differentiation that take into account the genetic distance between haplotypes (N_{ST}) show the same trend as those of G_{ST}. N_{ST} is lower for *Q. robur* (0.708) than for *Q. petraea* (0.886), whereas all N_{ST} values are slightly higher than the corresponding G_{ST} values. The difference between these two coefficients of differentiation (G_{ST} and N_{ST}) is significant only for the set, where all species were analysed.

Geographic distribution of haplotypes

The white oak group is represented by four different species, and 276 individuals in the sample set from the Czech and Slovak Republics. This, the 8 haplotypes found (when using the restriction pattern and haplotype nomenclature of PETIT *et al.* 2002a), and their lineages are shown in Tabs. 5 and 6. The two most frequent species, *Q. robur* and *Q. petraea,* share six of eight haplotypes (from lineages C, A, and E). Only the very rare haplotypes (1 and 14b) are restricted to one species.

In the white oak populations studied from the Czech and Slovak Republics, haplotypes 4a, 5, 6, and 7 of lineage A, originating predominantly from the Balkan refugial area are most common and occur in 88.77 % of the individuals (Table 5). The most frequent haplotype is haplotype 7, which was found in 46 % of the sample set in both countries. Six of 21 populations, which comprise this haplotype, are polytypic: mixed with individuals characterized by other white oak haplotypes. Haplotype 6 (4.7 % of the sample set) was only present in 3 populations from Slovakia. For this haplotype, one population (Dargov) detected in eastern Slovakia was monotypic. The remaining two (Liptovský Hrádok in northern Slovakia and Martinský les in south-western Slovakia) are polytypic. Haplotype 5 (26.4 %) has a more scattered distribution throughout both countries. It is the second most frequent haplotype in Slovakia (30 %) found in 15 populations, of which 3 were monotypic. In the Czech Republic, haplotype 5 was detected in 3 populations, all of them polytypic. The distribution of haplotype

Table 4. Levels of diversity and differentiation by species [number of populations with ≥3 individuals (A), harmonic mean number of individuals per population (B), number of haplotypes (C), within population genetic diversity (h_S), the total diversity (h_T), the coefficient of genetic differentiation G_{ST} (where the genetic distance between haplotypes is ignored), and the coefficient of genetic differentiation N_{ST} (where the genetic distance between haplotypes is taken into account)].

Species	A	B	C	h_s ± s.e.		h_T ± s.e.		G_{ST} ± s.e.		N_{ST} ± s.e.	
Q. robur	17	5.97	8	0.204	0.0671	0.676	0.0493	0.698	0.0938	0.708	0.1145
Q.petraea	26	4.75	6	0.102	0.0431	0.748	0.0532	0.863	0.0560	0.886	0.0250
All species	40	5.94	8	0.181	0.1170	0.71	0.0840	0.746	0.1679	0.794	0.1605

Table 5. Distribution of haplotypes and their lineages in Slovakia and the Czech Republic. White oak cpDNA haplotypes were detected in the sample set according to banding pattern and haplotype nomenclature as described by PETIT *et al.* (2002a).

Country	Lineage C		Lineage A				Lineage E		Total
	(C) 1	(C) 2	(A) 4a	(A) 5	(A) 6	(A) 7	(E) 17a	(E) 14b	
Slovakia	2	22	17	63	13	90	2	1	210
Czech Rep.	–	1	15	10	–	37	3	–	66
Total	2	23	32	73	13	127	5	1	276

ha plo ty- pe 2 wa s fo un d onl y i n 1

Table 6. Distribution of haplotypes among the species analyzed in the sample set from Slovakia and the Czech Republic. White oak cpDNA haplotypes were detected according to banding pattern and haplotype nomenclature as described by PETIT *et al.* (2002a).

Species	hap1	hap2	hap4a	hap5	hap6	hap7	hap14a	hap17a
Q. petraea	–	14	27	18	8	61	–	2
Q. robur	2	9	1	41	3	50	1	3
Q. pubescens	–	–	4	8	–	16	–	0
Q. frainetto	–	–	–	6	2	–	–	0
Total	2	23	32	73	13	127	1	5

4a appears clustered: one cluster was found in the northern part of the Czech Republic near to the German border, the other in middle southern Slovakia. In total, 11.6 % of the individuals belong to haplotype 4a. To lineage C (composed entirely of haplotypes of Italian origin) belong 9 % of the samples, with haplotype 1 found in only 2 individuals from eastern Slovakia, whereas haplotype 2 was found in both countries. In Slovakia, haplotype 2 comprises 7.97 % of the sample set, primarily from a group of 5 middle Slovak populations (Fig. 4). Additionally, two individuals of this haplotype were found in a haplotype-mixed population in eastern Slovakia indicating that these trees were probably introduced. Similarly, in the Czech Republic, individual from a haplotype-mixed population near the Austrian border.

The remaining samples (2.16 %) belong to lineage E of which refugia were most likely located in the Italian and the Balkan Peninsula. This lineage is mostly represented by haplotype 17a (1.8 %). In the Czech Republic, three samples containing this haplotype were found in a haplotype-mixed population. In Slovakia, only 2 trees with this type were found, also in a haplotype-mixed population. Additionally, haplotype 14b, a new member of this lineage was found in a polytypic population in eastern Slovakia near to the Hungarian and Ukrainian border. The *Q. robur*-individual with that haplotype was either introduced or exhibits a local

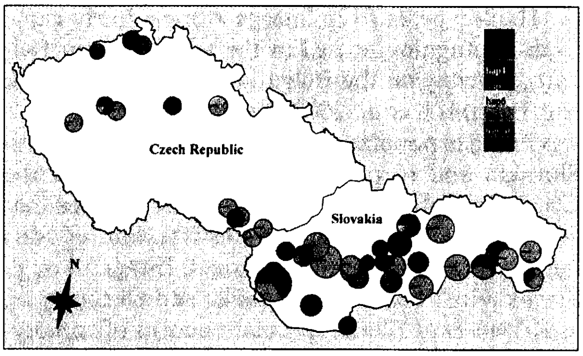

Figure 4. Haplotype distribution of white oaks in Slovakia, and the Czech Republic. Colored circles show relative frequencies of the eight different haplotypes found in populations. The color code corresponds to PETIT *et al.* (2002a). A circle with a uniform pattern shows that only one haplotype was found in the respective population, whereas a division of a circle into sectors of different colors indicates the detection of more than one haplotype. The size of the circles is proportional to the population size investigated, whereas the division of circles into sectors is proportional to the haplotype composition.

mutation. The distribution of individual haplotypes is shown in Fig. 4. No haplotypes of the lineages F, B, and D, wide-spread in W. and N. Europe, and predominantly originating from refugia on the Iberian Peninsula have been found in the Czech and Slovak Republics.

Splitting haplotype 5 (73 individuals) into the haplotypes 5A, 5B and 5C recognized by the more resolutive separation system used in this study, results in a more differentiated distribution pattern (Fig. 5). Haplotype 5A (represented by 30 individuals) occurs in 5 populations found in south-western Slovakia, in the south-eastern and north-western Czech Republic. In the Czech Republic, only 2 individuals of haplotype 5C were found in a mixed stand in the middle of this country. In contrast, haplotype 5C (with 38 individuals) is more frequent in Slovakia, where it was detected in one pure and 8 mixed stands throughout the country. Haplotype 5B (3 individuals) was found only in one haplotype-mixed population in northern Slovakia.

DISCUSSION

Despite centuries of anthropogenic forest exploitation (described in more detail in the next paragraph) and change in the Czech and Slovak Republics a phylogeographic structure of white oak plastid haplotypes still can be identified (N_{ST} was significantly higher than G_{ST} in the total sample set). For an interpretation several questions are relevant, e.g.: Which refugia have contributed to the colonization process? Which migration routes have been taken? How to define the autochthony and allochthony of the populations studied?

Results of similar investigations (especially those from neighboring countries) have to be taken into account, e.g., those of BORDÁCS *et al.* (2002), CSAIKL *et al.* (2002a,b), KÖNIG *et al.* (2002), PETIT *et al.* (2002b). In all these publications, haplotypes were determined and designated according to PETIT *et al.* (2002a). By using the same nomenclature, a total of 8 different haplotypes from three lineages could be detected in the Czech and Slovak Republics. The two dominant haplotypes (5 and 7) were found in more than half of the entire sample set. With the separation system used in this study, which has a more resolutive separation of restriction fragments compared to that of PETIT *et al.* (2002a), it was possible to increase the number of haplotypes from 8 to 10. The higher resolution can be attributed to improved electrophoresis for which thinner,

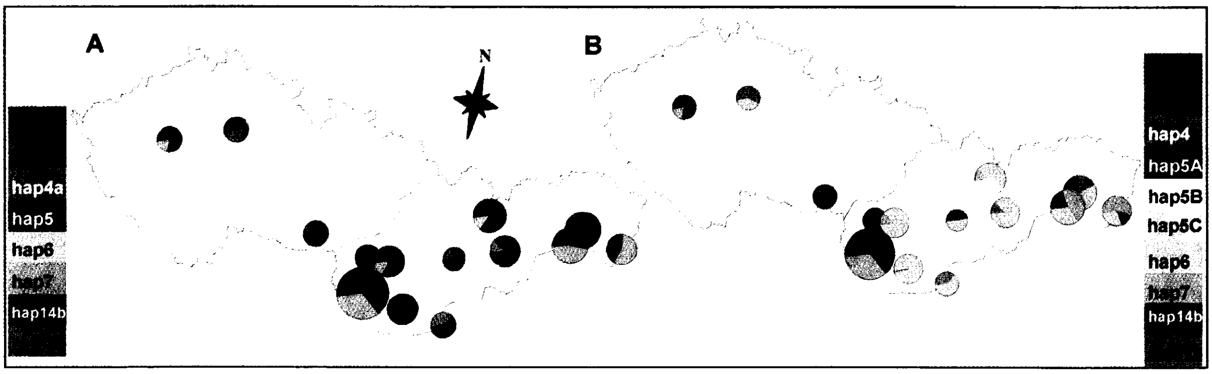

Figure 5. Distribution of haplotype 5 in the Slovak and Czech Republics. Populations containing haplotype 5 before (A) and after (B) splitting into the three haplotypes 5A, 5B, and 5C. The yellow color of haplotype 5B, and the orange color of haplotype 5C were chosen for better visibility and do not have any relation to the color of lineage B.

longer gels and shark toothcombs were used. Variants of DT1 and DT3 of the DT/TaqI restriction, and variants of CD1, CD2, and CD3 of the CD/TaqI restriction were sufficient to determine all 10 different haplotypes. With respect to the results by PETIT *et al.* (2002a), the new method would allow to increase the number of haplotypes and to decrease the analysis costs because not all fragments need to be analyzed.

Migration routes with respect to Slovak, and Czech samples

The analyzed populations from the Czech Republic do not cover the whole region, and therefore results of KÖNIG *et al.* (2002) that analyzed several oak populations in the Czech Republic are taken into account to reconstruct the migration routes of white oaks in this region more precisely. First, the historical information will be discussed, which can be obtained from lineage C originated most likely from Italy (DUMOLIN-LAPÈQUE *et al.* 1997; PETIT *et al.* 2002b). Its haplotype 1 has moved through Switzerland and Austria into southern and central Germany and from there on northwestward into Belgium and the Netherlands (KÖNIG *et al.* 2002), northward to southern Scandinavia, and northeastward into northern Poland, and up to south-western Finland (CSAIKL *et al.* 2002a; JENSEN *et al.* 2002). In the studied sample set, haplotype 1 was only found in one haplotype-mixed population (Dubky-Moldava nad Bodvou) in eastern Slovakia. However, in neighboring northern Hungary (BORDÁCS *et al.* 2002) and southern Poland (CSAIKL *et al.* 2002a) this haplotype was not detected. The great geographical distance to the nearest population with haplotype 1 in Austria and the 'mixed' character of this Slovak population studied suggest that it is most likely artificial (allochthonous).

In contrast to the more westerly route of haplotype 1, haplotype 2 also of lineage C migrated from an Italian refugium towards the north-east via Croatia, Austria, Hungary and Slovakia, with isolated populations in Poland and Lithuania (PETIT *et al.* 2002b). In the middle of Slovakia, a group of five populations containing this haplotype was detected. In addition, two other populations containing haplotype 2 were found. Both of them were haplotype-mixed. The first of these two is a Czech population (Slavonská prov. Vranovický les) containing *Q. robur* ssp. *slavonica* individuals and was artificially planted 90-100 years ago. The second is a Slovak population (Kokošovská dubina) sampled in eastern Slovakia. In this population, all *Q. robur* individuals were of haplotype 5 and the *Q. petraea* individuals a mixture of haplotypes 17a and 2.

Haplotype 4a from lineage A most likely came from a refugium located in the south-eastern Balkans, possibly on the Bulgarian coast of the Black sea (BORDÁCS *et al.* 2002; BREWER *et al.* 2002). It was found in populations of south-middle and south Slovakia and in populations in the north of the Czech Republic. There are 2 migration routes that could explain the current distribution of this haplotype in the countries studied: Either through Slovakia into the Czech Republic and Germany or, as KÖNIG *et al.* (2002) speculate, acorns of haplotype 4a in the Czech Republic are of 'German' origin and were shipped by man upstream along the river Elbe. In order to answer the disjunctive distribution of haplotype 4a, additional and more extensive sampling of oak populations located in western, and middle of the Czech Republic should be considered.

Haplotype 5 also of lineage A, does not have a clear origin. PETIT *et al.* (2002b) and BORDÁCS *et al.* (2002) proposed a hypothesis that it originated from two refugia, one in Italy and one in the Balkans. This haplotype is a composite haplotype. In the studied region, three 'variants' (haplotypes 5A, 5B, and 5C) of it were identified. Studying their distribution towards the south could reveal their glacial refugia more precisely.

Haplotype 7, which had a primary refugium located most likely in the Balkans, was the most frequent haplotype found: it comprised almost half of the sample set. Its extensive distribution in the whole studied region can be attributed to its occurrence in the secondary refugia. Based on palynological evidence and oak charcoal fossils found in the western Carpathians, KRIPPEL (1986) suggested that already at 9,000–10,000 BC oak existed in the south part of Slovak lowlands, in 'Juhoslovenská kotlina, Burda, and Cerová vrchovina'. This early presence of oak in Slovakia supports the hypothesis of secondary refugia, probably in the Mátra mountains (Hungary) (KRIPPEL 1986) or along the southern slopes of the higher mountains of Austria, Slovenia, and Croatia (BORDÁCS *et al.* 2002). After the cold and dry conditions of the Younger Dryas period, forests harboring oaks characterized by haplotype 7 expanded rapidly from such secondary refugia northward into large suitable areas.

Autochthonous versus allochthonous oak populations

The present study has shown that of the analyzed populations more than expected are polytypic (13 of 41 populations). However, we cannot conclude that all these populations are artificial, planted by humans. Natural processes can also bring about polytypic populations, for instance when the migration paths of two different haplotypes overlap. But when a population comprises haplotypes of different lineages or contains haplotypes which do not occur in the surrounding stands, strong evidence is provided that acorns have been introduced (at least partly: KÖNIG *et al.* 2002).

In other publications covering the same subject, often the notion of '*autochthony*' is used. For instance, PETIT *et al.* (2002a) mention that 'whenever possible, material of inferred *autochthonous* origin was sampled'. Furthermore, only variation in the cpDNA of *autochthonous* population was used to infer colonization routes of oak out of their glacial refugia.

Most dictionaries agree in their definition of autochthonous as 'native to a particular place', with the usually tacit assumption that this implies a longer existence at a place or site (SCHOPPA & GREGORIUS 2001). We define as an *autochthonous oak stand* one, which has regenerated naturally on its site over generations and is well adapted to it. The history of *autochthonous oak stands* begins in the studied region at about 9,000 BC (KRIPPEL 1986). It is difficult to prove which oak stands today really are autochthonous, especially when human influences are evidently strong. According to KRIPPEL (1986), 'The human intervention in forests on the Slovak territory was so intensive, that the original forests were completely destroyed' and 'Conscious cultivation and exploitation of forests changed these forests to such a degree, that one cannot speak about original forests anymore'. One has to conclude that today there are hardly any virgin and fully autochthonous oak populations. Even some nature reserves (Badín, Dobroč etc.) in Slovakia originally were forests, then cut and reforested by humans, and only secondarily left to nature a couple of centuries ago (KRIPPEL 1986).

In conclusion, our study has shown the following facts:

- In some cases monotypic populations (Cíbajky, Revište) contain haplotypes that are consistent with surrounding populations and migration routes; without knowing that they are artificial they would have been typed as autochthonous.
- Several 'autochthonous' populations from nature reserves turned out to be polytypic, comprising several haplotypes from different lineages (for instance Kokošovská dubina, Dubky – Moldava nad Bodvou).
- The Carpathian Basin is a meeting point of several colonization routes. Thus, the comparatively high level of cpDNA diversity may be a natural phenomenon (BORDÁCS *et al.* 2002).
- Despite the fact that forest exploitation (especially that of the oak) has a long history in the area studied, geographic patterns of individual haplotypes can still be identified.
- A higher level of within-population diversity (h_S) and lower (G_{ST}) in *Q. robur* compared to *Q. petraea* is a general trend which has been also found elsewhere in Europe (PETIT *et al.* 2002b; BORDÁCS *et al.* 2002). This supports the idea that acorns of *Q. robur* have been more frequently transferred by man and planted than those of *Q. petraea.*
- Where no written evidence exists on the history of populations, we conclude that even haplotype studies can not always give certain information on auto- or allochthony.

Therefore, it is proposed, to supplement the term autochthonous population by *autochthonous haplotype*. This is a haplotype that, based on generally accepted colonization routes, can be expected in the studied region. In summary, for the region studied the haplotypes originating from the Balkan refugia (mostly lineages A and E) and from the Italian refugia can be regarded as autochthonous.

ACKNOWLEDGEMENTS

This paper represents a part of the doctoral research of the first author, which was supported by an ARC Seibersdorf scholarship of ARC Seibersdorf research GmbH, Seibersdorf, Austria. Our special thanks go to U. Csaikl without her help the collecting and lab work would not have been possible. We are grateful to M. Pospíšková and J. Dostálek from the Silva Tarouca Research Institute for Landscape and Ornamental Gardening at Průhonice (Czech Republic), J. Schmidtová and L. Paule from the Faculty of Forestry, Technical University, Zvolen, (Slovakia) for providing the oak plant material. The collecting of the Czech plant material was financially supported by Ministry of Environment of the Czech Republic (grant Vav/640/4/00). We especially wish to thank M. Pospíšková and J. Schmidtová for help with the oak species taxonomy, and valuable discussions. Finally, we thank F. Ehrendorfer and two anonymous reviewers for constructive criticism on the previous version of the manuscript, and R. J. van Loo for the language corrections to the final manuscript.

REFERENCES

BIRKY, C. W. 1995: Uniparental inheritance of mitochondrial and chloroplast gens: mechanisms and evolution. *Proc. Natl. Acad. Sci. USA* **92**: 11331 -11338.

BORDÁCS, S., POPESCU, F., SLADE, D., CSAIKL, U. M., LESUR, I., BOROVICS, A., KÉZDY, P., KÖNIG, A. O., GÖMÖRY, D., BREWER, S., BURG, K. & PETIT, R. J. 2002: Chloroplast DNA variation of white oaks in northern Balkans and in the Carpathian Basin. *For.*

Ecol. Manage. **156**: 197–209.

BREWER, S., CHEDDADI, R., DE BEAULIEU, J. L., REILLE, M. & DATA CONTRIBUTORS. 2002: The spread of deciduous *Quercus* throughout Europe since the last glacial period. *For. Ecol. Manage.* **156**: 27–48.

BURBAN, C., PETIT, R. J., CARCREFF, E. & JACTEL, H. 1999: Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. *Mol. Ecol.* **8**: 1593–1602.

CLEGG, M. T., BRANDON, S. G., LEARN, G. H. & MORTON, B. 1994: Rates and patterns of chloroplast DNA evolution. *Proc. Natl. Acad. Sci. USA* **91**: 6795–6801.

CSAIKL, U. M., GLAZ, I., BALIUCKAS, V., PETIT, R. J. & JENSEN, J. S. 2002a: Chloroplast DNA variation of white oaks in the Baltic countries and Poland. *For. Ecol. Manage.* **156**: 211–222.

CSAIKL, U. M., BURG, K., FINESCHI, S., KÖNIG, A. O., MÁTYÁS, G. & PETIT, R. J. 2002b: Chloroplast DNA variation of white oaks in the Alpine region. *For. Ecol. Manage.* **156**: 131–145.

DEMESURE, B., SODZI, N. & PETIT, R. J. 1995: A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* **4**: 129–131.

DUMOLIN, S., DEMESURE, B & PETIT, R. J. 1995: Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theor. Appl. Genet.* **91**: 1253–1256.

DUMOLIN-LAPÈGUE, S., DEMESURE, B., LE CORRE, V., - FINESCHI, S. & PETIT, R. J. 1997: Phylogeographic structure of white oaks throughout the European continent. *Genetics* **146**: 1475–1487.

FERRIS, C., OLIVER, R. P., DAVY, A. J. & HEWITT, G. M. 1993: Native oak chloroplasts reveal an ancient divide across Europe. *Mol. Ecol.* **2**: 337–344.

GREEN REPORT, 2001: Report on Forestry in the Slovak Republic. Ministry of Agriculture of the Slovak Republic Bratislava–Forestry Section. CROCUS Ltd., Nové Zámky, 64 pp.

HUNTLEY, B. & BIRKS, H. J. B. 1983: An atlas of past and present pollen maps for Europe: 0–13000 years ago. Cambridge Univ. Press, Cambridge, 667 pp.

JENSEN, J. S., GILLIES, A., CSAIKL, U. M., MUNRO, R., MADSEN, S. F., ROULUND, H. & LOWE, A. 2002: Chloroplast DNA variation within the Nordic countries. *For. Ecol. Manage.* **156**: 167–180.

KÖNIG, A. O., ZIEGENHAGEN, B., VAN DAM, B. C., CSAIKL, U. M., COART, E., DEGEN, B., BURG, K., DE VRIES, S. M. G. & PETIT, R. J. 2002: Chloroplast DNA variation of oaks in western Central Europe and the genetic consequences of human influences. *For. Ecol. Manage.* **156**: 147–166.

KOBLÍŽEK, J. 1990: *Quercus* L., *In:* Květena České republiky 2. (ed. Hejný S., Slávik B). pp 21–35. Academia, Praha.

KRIPPEL, E. 1986: Postglaciálny vývoj Slovenska. Veda, Bratislava, 307 pp.

KRYSTUFEK, V. 2001: Population Genetic Analysis of Populus nigra in Austria Using Nuclear and Chloroplast DNA Markers, Ph.D. thesis, University of Vienna, Vienna, 150 pp.

MAGIC, D. 1974: Poznávame ďalšie druhy dubov v našich lesoch. *Les* **30**: 244–252.

MAGIC, D. 1975: Taxonomické poznámky z doterajšieho výskumu dubov v Západných Karpatoch. *Biológia* (Bratislava) **30**: 65–74.

PETIT, R. J., KREMER, A. & WAGNER, D. B. 1993: Geographic structure of chloroplast DNA polymorphisms in European oaks. *Theor. Appl. Genet.* **87**: 122–128.

PETIT, R. J., PINEAU, E., DEMESURE, B., BACILIERI, R., DUCOUSSO & KREMER, A. 1997: Chloroplast DNA footprints of postglacial recolonization by oaks. *Proc. Natl. Acad. Sci. USA* **94**: 9996–10001.

PETIT, R. J., CSAIKL, U. M., BORDÁCS, S., BURG, K., COART, E., COTTRELL, J., VAN DAM, B. C., DEANS, J. D., DUMOLIN-LAPÈGUE, S., FINESCHI, S., FINKELDAY, R., GILLIES, A., GLAZ, I., GOICOECHEA, P. G., JENSEN, J. S., KÖNIG, A., LOWE, A. J., MADSEN, S.F., MÁTYÁS, G., MUNRO, R. C., PEMONGE, M. H., POPESCU, F., SLADE, D., TABBENER, H., TAURCHINI, D., DE VRIES, S. M. G, ZIEGENHAGEN, B. & KREMER, A. 2002a: Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2,600 populations. *For. Ecol. Manage.* **156**: 5–26.

PETIT, R. J., BREWER, S., BORDÁCS, S., BURG, K., CHEDDADI, R., COART, E., COTTRELL, J., CSAIKL, U. M., VAN DAM, B. C., DEANS, J. D., FINESCHI, S., FINKELDEY, R., GLAZ, I., GOICOECHEA, P. G., JENSEN, J. S., KÖNIG, A. O., LOWE, A. J., MADSEN, S. F., MÁTYÁS, G., MUNRO, R. C., POPESCU, F., SLADE, D., TABBENER, H., DE VRIES, S. M. G., ZIEGENHAGEN, B., DE BEAULIEU, J. L. & KREMER, A. 2002b: Identification of refugia and postglacial colonization routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *For. Ecol. Manage.* **156**: 47–74.

PETIT, R. J., BODÉNÈS, C., DUCOUSSO, A., ROUSSEL, G. & KREMER, A. 2003a: Hybridization as a mechanism of invasion in oaks. *New Phytologist* **161**: 151–164.

PETIT, R. J., AGUINAGALDE, I., DE BEAULIEU, J.-L., BITTKAU, CH., BREWER, S., CHEDDADI, R., ENNOS, R., FINESCHI, S., GRIVET, D., LASCOUX, M., MOHANTY, A., MÜLLER-STARCK, G., DEMESURE-MUSCH, B., PALMÉ, A., MARTIN, J.P., RENDELL, S. & VENDRAMIN, G.G. 2003b: Glacial Refugia: Hotspots but not melting pots of genetic diversity. *Science* **300**: 1563 –1565.

PONS, O. & PETIT, R. J. 1996: Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics* **144**: 1237–1245.

POŽAJ, J. 1997: World areas and ecosystems of the native species of the *Quercus* L. genus in Slovakia. *Folia dendrologica* **1–2**: 21–38.

SCHOPPA, F. N. & GREGORIUS, H. -R. 2001: Is autochthony an operational concept? *In*: Genetic Response of Forest Systems to Changing Environmental Conditions. (ed. G. Müller-Starck and R. Schubert). pp. 173–185. Kluwer Academic Publishers, Dordrecht, The Netherlands.

SWOFFORD, D. L. 1998: PAUP Phylogenetic analysis using parsimony (and other methods): Version 4.

Sinauer Associates, Sunderland, Mass.
TABERLET, P., GIELLY, L., PAUTOU, G. & BOUVET J. 1991: Universal primers for the amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **17**: 1105–1109.
TALLIS J. H. 1991: Plant Community History. Chapman and Hall, London. 398 pp.
VAN LOO, M. 2003: Hybridization, cpDNA diversity, and phylogeography of Central European white oaks, Ph.D. thesis, University of Vienna, Vienna, 123 pp.
WHITTERMORE, A. T & SCHAAL, B. A. 1991: Interspecific gene flow in oaks. *Proc. Natl. Acad. Sci. USA* **84**: 2540–2544.