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Chloroplast DNA polymorphisms provide evidence for postglacial re-colonisation of oaks (*Quercus* spp.) across the Swiss Alps

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Abstract The spatial pattern of chloroplast DNA (cpDNA) variation in *Quercus robur*, *Quercus petraea* and *Quercus pubescens* was studied in 1036 trees from 181 locations throughout the Swiss Alps and adjacent regions in order to gain a deeper insight into the postglacial history of these species. A total of ten different cpDNA types (haplotypes) were identified, six of which are described for the first time. The genetic variation was mainly found between collection sites (G_{ST}=0.881). Spatial autocorrelation indicated that the two dominant haplotypes had a structured, non-random distribution. The spatial pattern of these two haplotypes, which are associated with different ice-age refugia, reveals: (1) that oaks did not immigrate into regions of the Swiss Alps together, rather they immigrated separately in space and/or time, and (2) that the spatial mixing of haplotypes as a consequence of seed dispersal was low. Furthermore, the geographic distribution of the haplotypes suggests that the Alps only partially blocked the re-colonisation of oaks: *Quercus* species, which originated after range expansion from a refugium in Italy, have most likely crossed the Swiss Alps. Previously proposed postglacial migration pathways are reviewed and possible re-colonisation routes are discussed.

Keywords Postglacial migration · Contact zone · *Quercus* spp. · cpDNA · Maternal gene marker

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Introduction

The present range of plant and animal species in Europe is largely the result of migration from sheltered refugia where the species survived during the last ice age (115000–10000 BP). Many of these refugia were located in mountainous regions of Southern Europe (Bennett et al. 1991; Hewitt 1999), and it is becoming apparent that northward expansion from these refugia was strongly influenced by the Alps and/or the Pyrenees (Taberlet et al. 1998; Hewitt 1999). Depending on the effect of these mountains as impediments to postglacial migration, distinct refugia have contributed differently to the colonisation of Central and Northern Europe. Because populations in different refugia probably diverged genetically during the last ice age, the mode and routes of colonisation may have largely determined the present patterns of genetic variation. Detailed information on postglacial migration routes would therefore greatly contribute to our understanding of the current genetic structure of populations.

The postglacial history of plant species has been traditionally inferred from fossil pollen records. Recently, further insight into species postglacial history has come from the analysis of chloroplast DNA variation (e.g. Hewitt 1999; Petit 1999; Tremblay and Schoen 1999). CpDNA is inherited maternally in most angiosperms (Corriveau and Coleman 1988; Reboud and Zeyl 1994; Dumolin et al. 1995) and thus provides a seed-specific marker. The routes of seed dispersal therefore can be inferred from the geographical distribution of cpDNA variation. The relatively slow rate of sequence evolution (Wolfe et al. 1987; Frascaria et al. 1993) and the absence of sexual recombination make cpDNA an ideal marker for molecular phylogeographic studies (Petit et al. 1993; Newton et al. 1999).

Deciduous oaks (*Quercus* spp.) of Europe comprise several species, some of which undergo interspecific hybridisation (Aas 1998; Müller 1999). Data from fossil pollen distributions and cpDNA variation show that oaks survived the last ice age in refugia in Iberia, Italy, the

Balkans, and possibly the Caucasus (Huntley and Birks 1983; Frenzel et al. 1992; Gliemeroth 1995; Dumolin-Lapègue et al. 1997). Northward expansion from these refugia started about 13000 BP, and Central Europe was mainly colonised from refugia in Iberia and the Balkans. The Italian refugium has also contributed to the colonisation of regions north of the Alps but the routes of migration are unclear. Oaks from the Italian refugium may have colonised Central Europe via bypassing the Alps or may have crossed the Alps through narrow corridors (Kral 1979; Beug 1982; Küster 1996; Dumolin-Lapègue et al. 1997; Burga and Perret 1998).

In this study, we have explored the postglacial recolonisation routes of oaks in the region of the Swiss Alps. We examined whether oaks from the Italian refugium crossed the Alps or if the oaks on the southern border of the Swiss Alps remained isolated. Additionally, we examined whether or not different migration routes met in this region, and evaluated the extent of their mixing. To address these questions, we assessed variation in the chloroplast *trn*L intron and in four intergenic spacers of chloroplast genes. The *trn*L intron contains a single base change (a thymine to cytosine transition) which reveals an east/west division of oaks in Central Europe running through Switzerland; this polymorphism occurs in a highly conserved part of the *trn*L intron and to-date has not been detected in any photosynthetic organisms (Ferris et al. 1993, 1997). The polymorphism may therefore represent a single mutation event and appears highly informative for phylogeographic studies of oaks. The additional four cpDNA fragments were selected to determine the origin of oaks in the Swiss Alps region.

Materials and methods

Species

Each of the closely related species *Quercus robur* L., *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. were studied (Camus 1938). Sharing of the same haplotypes between these oak species (Dumolin-Lapègue et al. 1999) showed that all three taxa can be used to detect footprints of the postglacial northward expansion. Six individuals of *Quercus cerris* were investigated from the southern border of the Alps, with the aim of serving as an outgroup in the phylogenetic analysis.

Study sites and sampling methods

A total of 1036 oak trees were sampled from 1996 to 1998 representing 181 locations from Switzerland, Northern Italy and Southeastern France. Collection sites were primarily selected based on published information on the presence of oaks (Mátyás 1999). Material from 24 collection sites throughout the studied region was kindly provided by Beat Müller (ETH, Zürich, Switzerland). We expected the geographic pattern of the cpDNA variation in Switzerland to be more complex than in Northern Italy due to the differing topography and previous results of cpDNA analyses (Ferris et al. 1993, 1997, 1998; Dumolin-Lapègue et al. 1997; Fineschi et al. 1997). For this reason, Switzerland was more extensively sampled than the surrounding areas (see Fig. 1). From the majority of the collection sites (158) five oak trees were sampled. This allowed the detection of the dominant haplotype (>0.5) in the collection sites with a probability of >96.9% (Spiegel 1976; Crossa 1989). From 19 collection sites only 1–4 trees were analysed (for full details of collection sites see Mátyás 1999). Additionally, 50 trees were sampled from each of four populations (Fig. 1). This extensive sampling regime was used to examine whether or not five individuals were representative for the cpDNA variation in these populations. The four additional populations were chosen to represent all three *Quercus* species investigated as well as different postglacial re-colonisation routes. A further criterion used was the occurrence of different haplotypes in two of the four populations. In all collection sites, the sampled trees were randomly chosen, separated by at least 30 m. Whenever possible, material (leaves and/or buds) was collected from all three oak species studied. A portion of leaves and/or buds was used for DNA extraction, the remaining portion was dried (herbarium located at the WSL, Birmensdorf, Switzerland).

DNA extraction and PCR-RFLP analysis of cpDNA

Total DNA was extracted using 20–200 mg of fresh or frozen leaves or buds, or dried leaves, following a modification of the protocol described by Ziegenhagen et al. (1993). Samples were ground to a fine powder under liquid nitrogen in a shaking mill (Retsch type MM2000, Haan, Germany). The powder was dispersed in 1100 µl of extraction buffer [100 mM sodium acetate, 50 mM (Na_2)EDTA, 500 mM NaCl, 0.2% (w/v) sodium hydroxide, 2% (w/v) polyvinylpyrrolidone (MW approximately 10000), 1.4% (w/v) SDS, pH 5.5, supplemented with 1% (w/v) sodiumbisulphite and 65 µl of RNase A (20 mg/ml)] and incubated for 30–60 min at 65°C. After the addition of 385 µl of 3 M potassium acetate pH 5.2, the mixture was incubated for 15–30 min on ice and centrifuged at 20000 g for 10 min. The supernatant (1100 µl) was added to 800 µl of dichloromethane and thoroughly mixed. After centrifugation at 6000 g for 10 min, 400 µl of AP3 buffer (Qiagen, Hilden, Germany) and 800 µl of ethanol (>96%) were added to 800 µl of supernatant. DNA was then purified by the QIAamp DNA blood kit (Qiagen) as recommended in the tissue protocol of the handbook supplemented with the kit (steps 1–4 were omitted). DNA was eluted with 200 µl of pre-heated (70°C) TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 9.0). The concentration of DNA extracts was estimated by fluorometric measuring (DyNA Quant 200, Hoefer Pharmacia Biotech, San Francisco, USA).

PCR was used to amplify the *trn*L intron as well as four intergenic spacers (IGS) between the *trn*D-*trn*T (DT), *trn*C-*trn*D (CD), *psa*A-*trn*S (AS) and *trn*T-*trn*F (TF) chloroplast genes. The primer sequences are given in Taberlet et al. (1991) and Demesure et al. (1995). The 25 µl of PCR mix was as follows: 20 ng of total DNA, 1×PCR buffer (Sigma or Gibco), 1.6 or 2.1 mM $MgCl_2$ (Sigma or Gibco), 0.1 mM of each dATP, dCTP, dGTP, dTTP (Promega), 0.2 µM of each primer (Intron, Kaltbrunn, Switzerland) and 2.5 units of *Taq* DNA polymerase (Sigma or Gibco). The cycling profile for the *trn*L intron was as follows: one cycle of 3 min at 94°C, 1 min at 57°C, 2 min 72°C, 40 cycles of 1 min at 94°C, 1 min at 57°C, 2 min 72°C, followed by 8 min at 72°C. The cycling profile for the remaining four cpDNA fragments (DT, CD, AS and TF) was performed as detailed in Demesure et al. (1995). All thermal cycles were run on a PTC-100 thermocycler (MJ Research, Watertown, USA).

The *trn*L amplification products were digested with the restriction enzyme *Cfo*I (Promega) or its isoschizomer *Hha*I (Promega) to reveal the T>C transition described by Ferris et al. (1993). The resulting restriction fragments were visualised on 1.0–1.5% agarose gels as given in Ferris et al. (1993). The *trn*D–*trn*T and *trn*C-*trn*D amplification products were digested with *Taq*I (Promega), the *psa*A-*trn*S products with *Hinf*I (Promega), and the *trn*T-*trn*F products with *Alu*I (Promega). All digestions were performed according to the manufacturer's instructions. The resulting restriction digests were separated on 8% (w/v) Long Ranger (FMC, Rockland, USA) polyacrylamide gels run in 1×TBE (89 mM Tris-Borate, 2 mM EDTA) buffer for 3.5 h at 500 V (C.B.S. Scientific DSG-250 gel apparatus, Del Mar, USA). The gels were stained

using SYBR Gold (Molecular Probes, Eugene, Oregon USA; 0.1 µl/ml) and visualised under ultraviolet (254 nm) light. The digestion profiles of all four fragments were compared simultaneously with the digestion fragments of control DNAs of known haplotypes (kindly provided by Rémy Petit, INRA, Pierroton, France, and Ulrike Csaikl, ARCS, Seibersdorf, Austria) to identify haplotypes as described by Dumolin-Lapègue et al. (1997).

Data analysis

For the observed relative haplotype frequencies (P), upper and lower confidence limits (p) were calculated using the method as described by Spiegel (1976):

$$p = \frac{P + \frac{z_k^2}{2N} \pm z_k \sqrt{\frac{P(1-P)}{N} + \frac{z_k^2}{4N^2}}}{1 + \frac{z_k^2}{N}}, \quad (1)$$

where N is the sample size and z_k is chosen according to the desired level of confidence (z_k is 1.96 for a level of 95% of the z-distribution, Rohlf and Sokal 1995). This equation allows an estimate of confidence limits from small sample sizes (Agresti and Coull 1998; Newcombe 1998).

Genetic differentiation among collection sites (G_{ST}) was estimated using procedures appropriate for haploid loci as described by Pons and Petit (1995). Only collection sites having at least two individuals were used for the calculation of G_{ST} (1029 trees from 174 sites). The significance of G_{ST} was tested by applying an exact test for population differentiation as described by Raymond and Rousset (1995) with 50000 replications (1000 steps of de-memorisation) using the software R×C (Miller 1997).

The distribution of different haplotypes, as well as their frequencies in the collection sites, was visualised with the GIS program ArcView Version 3.0a (Environmental Systems Research Institute, ESRI). In addition, the spatial structure of haplotypes was investigated by means of spatial autocorrelation (Sokal and Oden 1978; Slatkin and Arter 1991) under the null hypothesis that individuals are distributed at random with regard to their haplotype. Only the collection sites having at least five individuals were taken into account (990 trees from 162 collection sites). Geographical distances in kilometres were calculated between all pairs of these 162 collection sites by using trigonometric equations, and distances were assigned to distance classes with class intervals of 10 km and 50 km, respectively. For each class and each of the two most frequent haplotypes, a coefficient of correlation I between paired frequencies was computed by the method of Moran (1950) using the program Rook's Case (Sawada 1998). The values of Moran's I were then plotted as a function of the distance. The significance testing of I values was conducted by the Monte-Carlo procedure with 1000 permutations (Upton and Fingleton 1985) using the software Rook's Case (Sawada 1998).

To infer a phylogenetic relationship among the different haplotypes observed, a distance matrix was calculated based on the presence and absence of restriction fragments of haplotypes with the algorithm described by Nei and Li (1979) using the software package RAPDistance (Armstrong et al. 1994). Based on this matrix, the phylogenetic tree was constructed with the neighbor-joining algorithm (Saitou and Nei 1987) using the program Neighbor of the software package Phylip (Felsenstein 1993). The resulting tree file was drawn using the program Treeview (Page 1996). The robustness of the phylogenetic tree was tested by bootstrap analysis with 2000 replications (Felsenstein 1985; Hedges 1992) with the program Treecon (Van de Peer and De Wachter 1993).

Results

Restriction fragment analysis of the *trn*L intron of *Q. robur*, *Q. petraea*, and *Q. pubescens* revealed both (eastern and western) haplotypes described by Ferris et al. (1993). In 649 samples (63%), the *trn*L intron was cut by the restriction enzyme *Cfo*I or its isoschizomer *Hha*I and thus contains the single base change (T>C, eastern haplotype). The *trn*L intron of the remaining 387 (37%) samples remained uncut and thus contains the original T base (western haplotype; Ferris et al. 1993). Both the western and eastern haplotypes were shared by all three *Quercus* species studied. The six individuals of the outgroup *Q. cerris* had exclusively the western haplotype (Table 1). Sixty collection sites (322 trees) showed only the western haplotype and 101 collection sites (526 individuals) only the eastern haplotype. In 20 sites (188 individuals) both haplotypes occurred. The spatial distribution of the western and eastern haplotypes is given in Fig. 1 and shows: (1) that collection sites containing both haplotypes are mainly found in transition zones

Table 1 Haplotypes (1–10) identified in *Q. petraea* (Pet), *Q. robur* (Rob), *Q. pubescens* (Pub) and *Q. cerris* (QC) based on the combination of 39 polymorphic PCR-RFLP fragments. The haplotypes are given according to Dumolin-Lapègue et al. (1997). Each column represents one restriction fragment. The numbers denote the relative size of the fragments (1=longest fragment, 9=shortest fragment). Apostrophes (') and letters (a to d) denote a variant of fragments and haplotypes described by Dumolin-Lapègue et al. (1997). In the column *trn*L/*Cfo*1 the single base change (T>C) in the *trn*L intron is given: 1=T (western haplotype), 9=C (eastern haplotype). Abbreviations: DT=*trn*D–*trn*T, CD=*trn*C-*trn*D, AS=*psa*A-*trn*S, TF=*trn*T-*trn*F; *trn*L=*trn*L intron; restriction enzyme: *Taq*=*Taq*I, *Alu*=*Alu*I, *Hinf*=*Hinf*I, *Cfo*=*Cfo*I or *Hha*I

Haplotype	*trn*L *Cfo*1	DT *Taq*1	DT *Taq*2	DT *Taq*3	CD *Taq*1	CD *Taq*2	CD *Taq*3	CD *Taq*4	AS *Hinf*1	AS *Hinf*2	AS *Hinf*4	AS *Hinf*5	TF *Alu*2	Number of individuals			
														Pet	Rob	Pub	Total
1	1	9	2'	2	1	2	3	2'	2	2	2	3	2	172	79	121	372
1a	1	9	2'	2	1	1''	3	2'	2	2	2	3	2	0	1	0	1
1b	1	9	2'	2	2	2	3	2'	2	2	2	2	2	0	0	1	1
1c	1	9	2'	2	1	2	2'''	2'	2	2	2	2	2	0	0	10	10
1d	1	9	2'	2	5	2	3	2'	2	2	2	2	2	0	0	1	1
5	9	2'	1	2	1	1	2''	2	1	3	2	3	3	0	1	0	1
7	9	2	1	5	2	1	2'	2	1	3	2	4	3	227	171	243	641
7a	9	2	1	5	2	1	2''	2	1	3	2	4	3	0	1	0	1
7b	9	2	1	5	3'	1	2'	2	1	3	2	4	3	4	0	2	6
10	1	2'	2	3	1	1	2'''	2	1	2	2	2	3	0	2	0	2
QC	1	1	1	1'	4	1'	4	2	2	3	2'	1	3	–	–	–	6

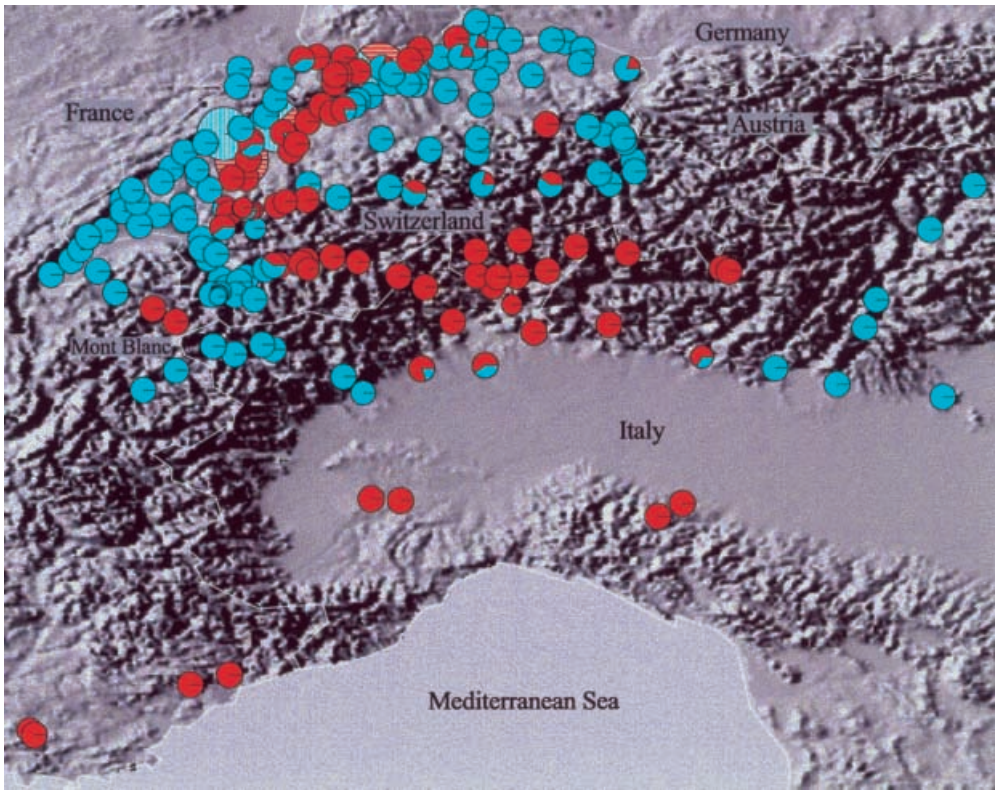

Fig. 1 Geographic distribution of a single base change (T>C) in the chloroplast *trn*L intron of oaks across the Swiss Alps and adjacent regions. *Blue coloured symbols* indicate C (eastern haplotype) as revealed by the presence of a *Cfo*I restriction site. *Red coloured symbols* indicates T (western haplotype) as revealed by the absence of the *Cfo*I restriction site. For polymorphic locations, *shading* is proportional to haplotype frequencies. *Symbol size* represents relative sample size. From four plots (*hatched*) 50 trees were sampled

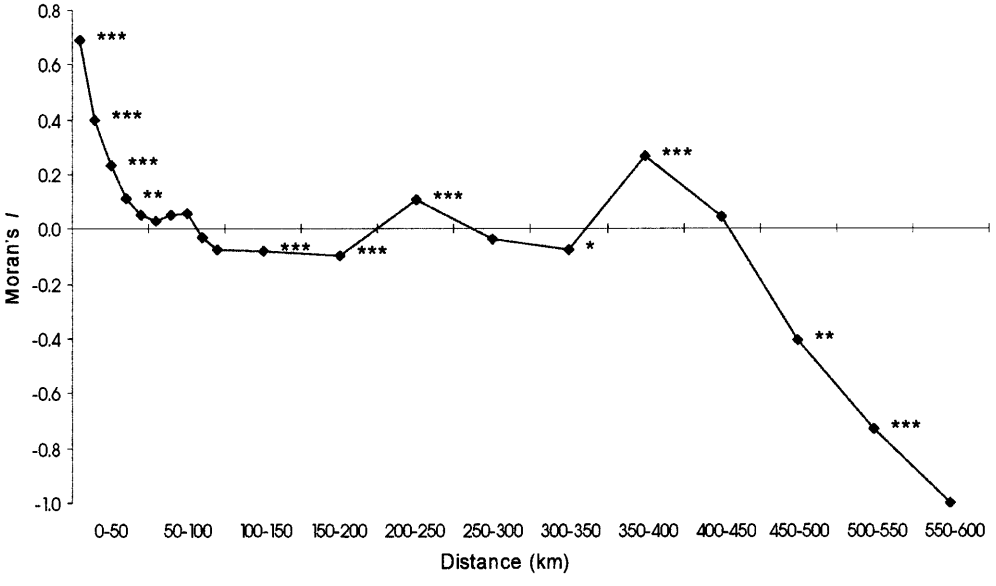

Fig. 2 Correlogram of the Moran's *I* spatial autocorrelation indices of a single base change (T>C) in the *trn*L intron. The correlogram of the two haplotypes (western and eastern) is identical due to their complementary frequencies. The value of *I* is given for 10-km distance classes for distances <100 km and for 50-km classes for distances >100 km. *Asterisks* (*) indicate whether the values of *I* significantly differ from 0 and E(*I*), respectively, where E(*I*) is the expected value of *I* under a randomisation hypothesis (Sokal and Oden 1978) (*=P<0.05; **=P<0.01; ***=P<0.001)

where the western and the eastern haplotypes meet, and (2) that the western haplotype occurs from the Mediterranean Sea across the Swiss Alps to Northern Switzerland in a corridor between regions showing the eastern haplotype. Autocorrelation analysis indicates that both haplotypes are not randomly distributed in space since Moran's *I* significantly differs from 0 and *E* (*I*) (expected value of *I* under a randomisation hypothesis), respectively, in several distance classes (Fig. 2). Moran's *I* values of distance classes up to 40 km were all positive and highly significant. The genetic differentiation among collection sites calculated from frequencies of the eastern and the western haplotypes was very high (G_{ST}= 0.881, P<0.001), as expected from the spatial structure.

Further polymorphisms were identified within the fragments DT, CD, AS and TF. A total of 39 polymorphic restriction fragments were detected. These fragments allowed us to distinguish 11 different haplotypes (Table 1, nomenclature of haplotyes as described by Dumolin-Lapègue et al. 1997). Seven haplotypes, in-

Table 2 Relative frequencies of haplotypes in the four extensively sampled populations CH145–CH148. Asterisks (*) denote relative frequencies obtained from five individuals which significantly differ from frequencies obtained from 50 individuals at $P<0.05$. Lower and upper confidence limits (calculated according to Equation 1) are given in parentheses

Population	Sample size N=50						Sample size N=5			
	Haplotype 1		Haplotype 7		Haplotype 10		Haplotype 1		Haplotype 7	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
CH145	50	1.00 (0.93–1.00)	0	0.00 (0.00–0.07)	0	0.00 (0.00–0.07)	5	1.00	0	0.00
CH146	0	0.00 (0.00–0.07)	50	1.00 (0.93–1.00)	0	0.00 (0.00–0.07)	0	0.00	5	1.00
CH147	5	0.10 (0.04–0.21)	44	0.88 (0.76–0.94)	1	0.02 (0.00–0.10)	0	0.00*	5	1.00*
CH148	19	0.38 (0.26–0.52)	31	0.62 (0.48–0.74)	0	0.00 (0.00–0.07)	2	0.40	3	0.60

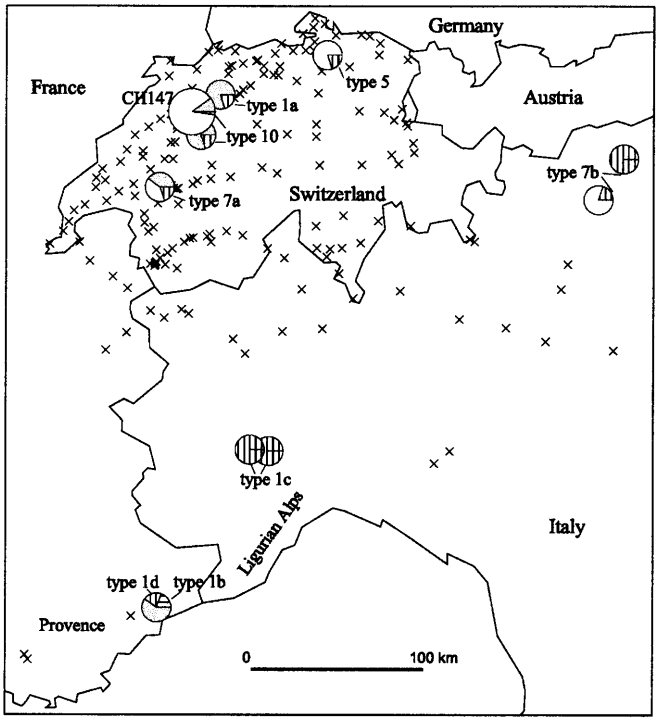

Fig. 3 Geographic distribution of eight rare haplotypes (*hatched*) as revealed by the DT, CD, AS and TF restriction fragments (cf. Table 1). *Symbol size and shading* is proportional to sample size and haplotype frequencies, respectively. Location of collection sites, where only haplotype 1 (*grey*) and/or haplotype 7 (*white*) occur, are denoted (×). In these locations, haplotype 1 correponds to the western haplotype and haployte 7 to the eastern haplotype as given in Fig. 1

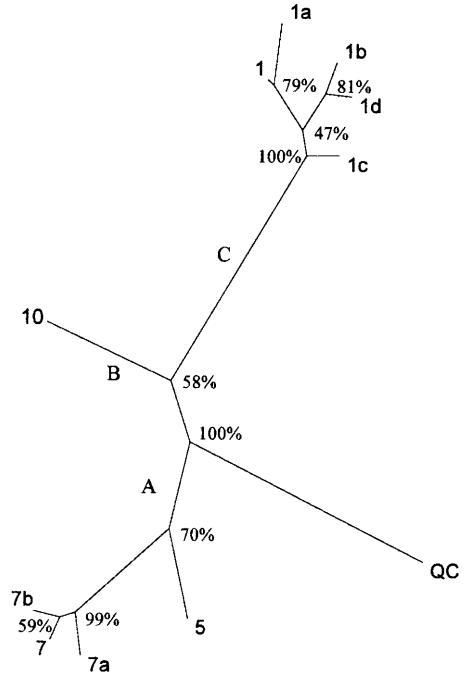

Fig. 4 Unrooted neighbor-joining tree of cpDNA haplotypes in the four *Quercus* species studied. The chloroplast lineages *A*, *B*, and *C* are designated according to Dumolin-Lapègue et al. (1997). *QC* indicates the lineage of *Q. cerris*. Bootstrap values are given

cluding that of *Q. cerris*, contained the original T base in the *trn*L intron, of which haplotype 1 was the most frequent. The remaining four haplotypes showed the T>C transition with haplotype 7 being the most frequent. Rare haplotypes were found in a total of ten collection sites (Fig. 3), although it cannot be ruled out that further non-detected haplotypes occur in the collection sites at a maximal frequency of 43% at $P<0.05$ (Equation 1; cf. Hanley and Lippman-Hand 1983). By applying phylogenetic analysis, the identified haplotypes (Table 1) were assigned to four chloroplast lineages (Fig. 4).

Two of the extensively sampled four populations were in a region where haplotype 1 (CH145, *Q. petraea*) or haplotype 7 (CH146, *Q. pubescens*) dominated, and two were located in a contact zone of these haplotypes (CH147, *Q. robur* and CH148, *Q. petraea*). Restriction-fragment analysis of 50 trees from each of these populations showed that all trees from populations CH145 and CH146 had only haplotypes 1 or 7, respectively (Table 2). In populations CH147 and CH148, both haplotypes oc-

curred. These results correspond to the spatial pattern as obtained from the five individuals per collection site (Figs. 1 and 3). The frequencies of haplotypes 1 and 7 calculated from five (first five trees in the populations) and 50 trees of populations CH145, CH146 and CH148 did not significantly differ from each other (Table 2). In population CH147, three different haplotypes occurred (types 1, 7 and 10; cf. Fig. 3).

Discussion

Our study was based on PCR-RFLP analysis of five cpDNA fragments which have been shown to be informative for the analysis of the postglacial migration history of oaks in Europe. A total of ten different haplotypes were identified in *Q. petraea*, *Q. robur* and *Q. pubescens*, six of which are described for the first time. The two most-frequent haplotypes comprised 36% (haplotype 1) and 62% (haplotype 7). The remaining eight haplotypes were rare (2%). The spatial distribution of the two most-frequent haplotypes allowed us to draw conclusions on the origin of oaks in the region of the Swiss Alps, the detection of routes of re-colonisation, and the identification of contact zones between different migration routes.

Origin of oaks in the Swiss Alps

Phylogenetic analysis of the identified haplotypes revealed four cpDNA lineages, three of which correspond to the lineages A, B and C described by Dumolin-Lapègue et al. (1997). An additional lineage (QC) is represented by the haplotype of *Q. cerris* (Fig. 4). The three lineages (A, B, and C) are associated with the three putative ice-age refugia of oaks located in the Balkans, Iberia and Italy, respectively (Dumolin-Lapègue et al. 1997). Accordingly, the haplotypes of lineage C (1, 1a, 1b, 1c, 1d) detected in our study are associated with the refugium in Italy. Haplotype 1 presumably has its origin in Southern Italy (Dumolin-Lapègue et al. 1997; Fineschi et al. 1998). Haplotypes 1b, 1c and 1d were all found in North-western Italy (Ligurian Alps) and in South-eastern France (Provence; Fig. 3). This relatively high number of variants of haplotype 1 supports the hypothesis that oaks survived the last glaciation also in these regions. Such a scenario is consistent with the observation that populations located in refugial areas typically show high levels of haplotype diversity (Hewitt 1999). Haplotype 7 of lineage A presumably has its origin in the Balkans and haplotype 5 in Italy, and the rare haplotype 10 of lineage B is associated with the Iberian refugium (Dumolin-Lapègue et al. 1997). In view of this, most of the oaks of the Swiss Alps have immigrated from refugia located in the Balkans, Italy, and possibly South-eastern France. The region of the Swiss Alps therefore appears to be part of a transition zone where oaks from different refugia met and consequently may have exchanged genes.

The two most-frequent haplotypes were shared among *Q. petraea*, *Q. robur* and *Q. pubescens* (Table 1). This indicates that all three species were present in the same refugium and that these two haplotypes were transferred among the three species (Ferris et al. 1993; Dumolin-Lapègue et al. 1997). It remains to be determined, however, whether all three species immigrated from all three putative refugia into the Swiss Alps since interspecific exchange of genetic information may have occurred at least locally among the three oak species during and after re-colonisation (Petit et al. 1997; Dumolin-Lapègue et al. 1999). All trees identified as *Q. cerris* revealed a haplotype distinct from the haplotypes of the remaining *Quercus* species (Table 1 and Fig. 4). This result could be expected since *Q. cerris* belongs to a different section of the subgenus *Euquercus* (Camus 1938).

Postglacial re-colonisation of the Swiss Alps

The spatial distribution of haplotypes is highly structured, and the genetic differentiation among collection sites (G_{ST}=0.881) is very high (cf. G_{ST} values reviewed in Petit 1999). These results indicate that the different lineages immigrated into the region of the Swiss Alps separately in space and/or time. Simultaneous re-colonisation of the different lineages on common migration routes would have resulted in a random distribution of the haplotypes (Nichols and Hewitt 1994; Le Corre et al. 1997). In case of the simultaneous dispersal of different haplotypes, selection and/or human activities must have been directed such that a single haplotype would have been fixed in a region. However, such a scenario appears rather unlikely because regions with the same haplotypes are rather large and, in the case of haplotype 1, are also strongly oriented (south-north; cf. Fig. 5). It can be assumed that the detected spatial pattern of cpDNA variation is the result of postglacial re-colonisation events.

Fossil pollen records show that oaks colonised the region of the Swiss Alps from about 11000 BP to 8000 BP and that oaks first arrived at the southern border of these mountains (Burga and Perret 1998). From the spatial distribution of the detected haplotypes (Figs. 1 and 3) it appears reasonable to suppose that the first oaks in the Swiss Alps had the Italian haplotype 1. Oaks with this haplotype colonised various regions at the southern border of the Alps (Val Poschiavo, Bregaglia, Ticino) and may have crossed the Swiss Alps at the Simplon pass [2006 m above sea level (a.s.l.); Fig. 5]. From the upper region of Valais (a major east-west oriented valley of the Swiss Alps), oaks might have migrated northwards into the Swiss central lowland across the Gemmi pass (2322 m a.s.l.) and/or the Rawil pass (2429 m a.s.l.), and/or even the Col du Sanetsch pass (2242 m a.s.l.; Fig. 5). In the case of the eastern haplotype, the results presented here do not rule out the possibility that oaks also crossed the Alps further westward at the Grand St. Bernard pass (2469 m a.s.l.) as well as the Petit St. Bernard pass

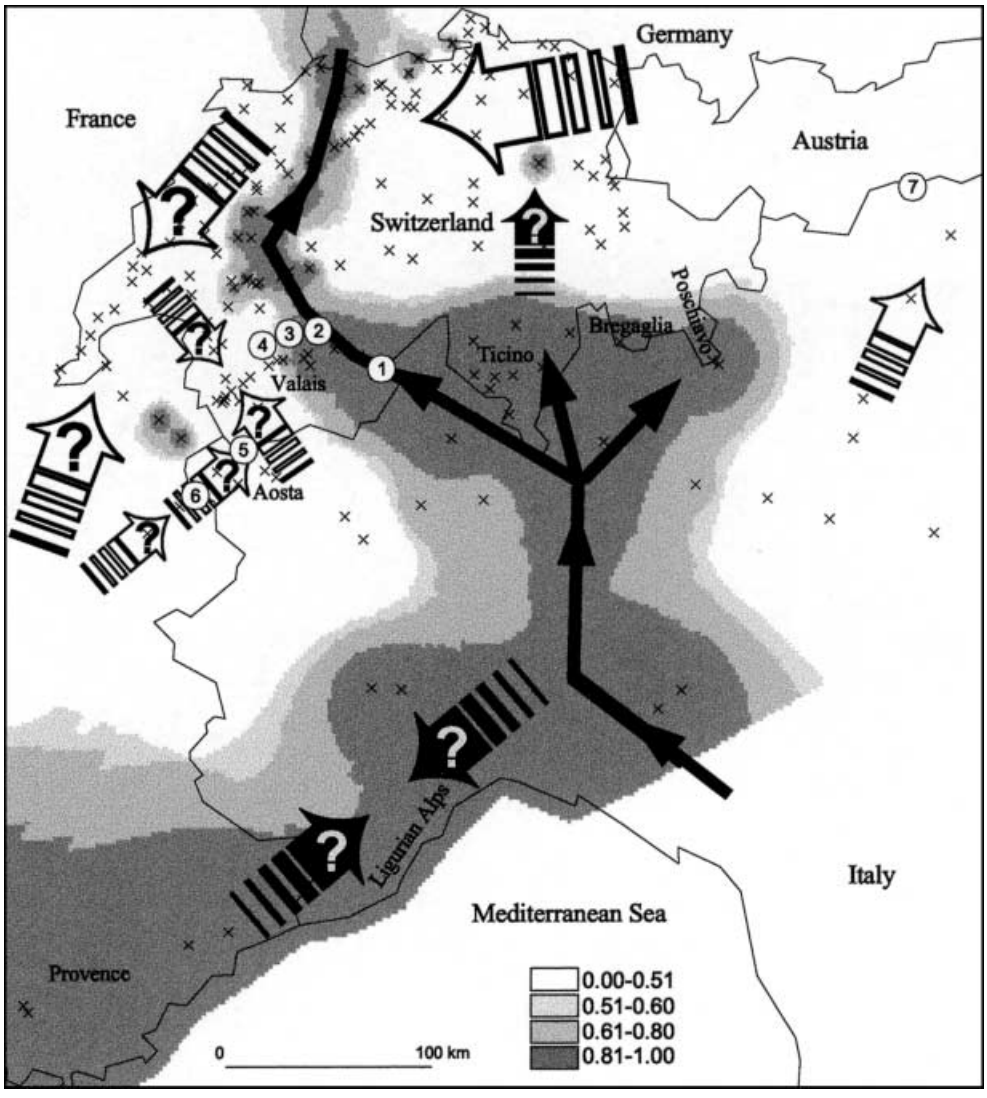

Fig. 5 Schematic representation of possible postglacial recolonisation routes of haplotype 1 and its variants (*black arrows*), as well as haplotype 7 and its variants (*white arrows*), as inferred from their spatial distribution (cf. Figs. 1 and 3). Inverse distance-weighted interpolated frequencies of haplotype 1 and its variants are presented (calculated as described by Mátyás 1999). The *different levels of grey* correspond to intervals covering haplotype frequencies from 0.51 to 1.00. Locations of the collection sites are denoted (×) and the passes mentioned in the text are *numbered*: 1: Simplon pass, 2: Gemmi pass, 3: Rawil pass, 4: Col du Sanetsch pass, 5: Grand St. Bernard pass, 6: Petit St. Bernard pass, 7: Brenner pass

(2188 m a.s.l.) since the same haplotype 7 occurs on both sides of these passes (Figs. 1 and 5). Similarly, the distribution of haplotype 1 in the central part of the Swiss Alps (Figs. 1 and 3) indicates that oaks may have crossed the Alps also in this area but did not expand further north (cf. Fig. 5). However, the different frequencies of haplotypes 1 and 7 in the region around Mont Blanc (4807 m a.s.l.) suggest that the Alps have formed a significant barrier to the migration of oaks in this region (Fig. 1). These findings correspond to the results of several previous studies of European biota showing that the Alps acted as impediments to postglacial migration (reviewed in Taberlet et al. 1998; Hewitt 1999).

How could oaks cross such high passes? Fossil pollen records show that at the maximum of the postglacial warm period (8500–5500 BP), the upper limits of the forests were higher than today: pollen deposits of *Quercus* have been found in mires of the Alps at 2000–2100 m a.s.l. (Keller 1932). Although these findings can be influenced by long-distance dispersal of pollen grains (Jochimsen 1986), oak migration cannot be ruled out since oaks may have occurred at a density too low to be detected by fossil pollen counts (Bennet et al. 1991). Additionally, the crossing of passes could be explained by transfer of seeds by the European jay (*Garrulus glandarius*) as a consequence of the behaviour of this bird. In several studies, the burying of viable acorns has been observed at a distance of 8 (–16) km from oak trees (Schuster 1950; Bossema 1979; Stimm and Böswald 1994; Kollmann and Schill 1996). Moreover, occasional long-distance transport of acorns by the Spotted Nutcracker (*Nucifraga caryocatactes*), *Corvus* species, and the Common Wood-Pigeon (*Columba palumbus*) may have played an important role in oak dispersal (Ritchards 1958; Webb 1986).

Comparison of our results with the cpDNA data described by Ferris et al. (1993) and Dumolin-Lapègue et al. (1997, 1998) reveals that the continuous area of haplotype 1 in the Swiss Alps is the unique detectable footprint of the postglacial northward expansion of oaks from the Italian refugium (Fig. 5). The spatial pattern of haplotype 7 (probably with a Balkan origin) supports the

hypothesis based on fossil pollen records (Kral 1979) that *Quercus* species also crossed the Alps in the area of the Brenner pass (1371 m a.s.l.; cf. Figs. 1 and 5). Our data also support the hypothesis of Burga and Perret (1998) that oaks immigrated into Northern Switzerland from the west as well as from the east, and it seems reasonable to assume that these oaks had haplotype 7 (Figs. 1 and 3). This suggestion contradicts the proposal of Dumolin-Lapègue et al. (1997) that haplotype 7 colonised the region north of the Alps only from the east (Balkans). The following reasoning supports an immigration of haplotype 7 also from the west. Oaks, probably with haplotype 1, occurred already about 11000 BP in the region of present-day South-eastern France and Northern Italy (Huntley and Birks 1983; Burga and Perret 1998). Their expansion into Western Switzerland, as well as into the Aosta valley, would have therefore been possible before the arrival of haplotype 7 from the Balkan refugium, if not then oaks with haplotype 7 would have already occurred in this region and thus would have formed an ecological barrier (i.e. the dispersal of seeds and pollen is usually negatively correlated with the density of populations; Handel 1983). However, some other forest tree species (e.g., *Abies alba*, *Picea abies*) could have also formed a significant barrier to the migration of oaks with haplotype 1 into Western Switzerland and the Aosta valley (cf. Konnert and Bergmann 1995). Further studies of chloroplast and/or mitochondrial DNA (possibly using dated fossil material) are necessary to confirm whether oaks were also present in South-eastern France and/or North-western Italy at the end of the last glacial period.

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