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Chloroplast DNA variation of white oaks in Italy

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Abstract

Polymorphism in non-coding regions of the chloroplast genome was studied in four white oak species (*Q. robur* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd. s.l., and *Q. frainetto* Ten.) in Italy (mainland and associated islands, including Corsica). A total of 924 trees (194 populations) was analysed. This data set also includes results previously obtained on 20 Italian and Corsican populations [Genetics 146 (1997) 1475]. Most of the sampled individuals were classified as *Q. pubescens* (73.5%). Thirty-four populations out of 194 (17.5%) were polymorphic. The level of population sub-division was very high, as expressed by the value of the coefficient $G_{ST} = 0.870$ ($h_S = 0.100$, $h_T = 0.776$). The highest value of total genetic diversity was calculated in Sardinia and in central Italy; the lowest in southern Italy. The highest values of differentiation among populations occurred in Sardinia ($G_{ST} = 1$), and in Corsica (0.927). We found evidence that the Italian oak populations of today mainly originated from Sicilian and Balkan refugia. Their origin and migration routes are more easily seen by considering data separately for the four sampling regions: the north, the centre, the south, and the three main islands. The C (=blue) lineage was common in the southern part of Italy. This may be a trace of a migration from the Balkans via the Adriatic bridge during quaternary cold periods. The northern part of the country had a set of haplotypes similar to the other Alpine regions. Most Sicilian populations are fixed for one of the two haplotypes that probably originated in the island: one spread over the whole Italian peninsula, whereas the other one did not reach the Alps. Results showed that the Appennines may have played a role in the distribution of the haplotypes western and eastern of the mountains chain.

Keywords: Chloroplast DNA; *Quercus petraea*; *Q. robur*; *Q. pubescens*; *Q. frainetto*; Phylogeography

1. Introduction

Present-day tree vegetation in Europe is believed to result from the migration of tree populations that survived in southern refugia during the last ice-age. Bennet et al. (1991), Hewitt (1996), and Taberlet et al. (1998) identified the three Mediterranean peninsulas as the major refugial areas for most temperate tree species. Fossil pollen data strongly support this model of post-glacial recolonisation (Huntley and Birks, 1983; Brewer et al., 2001).

During the last decade there have been several studies based on chloroplast DNA (cpDNA) variation in a wide range of plants, including trees. Organellar DNA markers are very informative for studying the postglacial history of many species (Soltis et al., 1992; Schaal et al., 1998; Petit, 1999). The uniparental nature of inheritance of organelle genomes does not

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involve recombination, and this makes them particularly suited for phylogenetic and phylogeographic studies. Chloroplasts are maternally inherited in most angiosperms, including oaks (Dumolin et al., 1995); for this reason cpDNA based markers are more suited than nuclear ones for the study of seed dispersal and the geographic structure of genetic diversity in plants (Petit et al., 1997).

Results based on cpDNA polymorphisms obtained in *Fagus sylvatica* L. (Demesure et al., 1996), *Alnus glutinosa* (L.) Gaertn. (King and Ferris, 1998), and the white oaks complex (Dumolin-Lapègue et al., 1997) have been used to study the most likely pathways from southern refugia into central and northern Europe. The first extensive study of the white oak complex at the European level (Dumolin-Lapègue et al., 1997) identified three major cpDNA lineages along with their possible migration routes from the Iberian, Italian and Balkan regions. Species surviving in the three southern European peninsulas would have encountered mountain chains such as the Pyrenees, Alps and Carpathian which may have acted as natural barriers for their northern migrations (Hewitt, 1996).

The role played by these mountain chains for the northward migration has been discussed (Taberlet et al., 1998). The mountain chains acted as barriers to the migration of species such as beech from southern Italy (Demesure et al., 1996) and silver fir from Calabria (Konnert and Bergman, 1995) but failed to prevent the migration of black alder (King and Ferris, 1998) and, at least for some cpDNA haplotypes, of oak (Dumolin-Lapègue et al., 1997). As the Italian peninsula lies in the central part of the Mediterranean area, it could act as both an origin of migration, as well as a meeting point of different migration routes. This situation has been recently discussed for *Castanea sativa* Mill., a species which has experienced considerable long term human interference during the last thousands of years; cpDNA polymorphism revealed the absence of a clear geographic structure of the genetic diversity. The presence of several different haplotypes in Italy might indicate that present day chestnut populations originated from both eastern and western refugia (Fineschi et al., 2000).

Four white oak species are present in Italy: *Q. robur* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd. s.l., and *Q. frainetto* Ten., but intermediate forms between *Q. robur* and *Q. petraea*, and between *Q. petraea* and *Q. pubescens* are common in mixed stands (Pignatti, 1982; Schwarz, 1993, Bernetti, 1995; Gellini and Grossoni, 1997). However, the different ecology and the history of these oak species in relation to human activities are expected to keep them separate in many environments. The sporadic occurrence of pedunculate oak is probably due to its preference for wet lowlands; a type of environment which has become rare in Italy today. Sessile oak is more drought resistant, but despite being able to tolerate drier conditions, also has a sporadic distribution due to changes in land use from forestry to agriculture. In spite of their sporadic occurrence, both pedunculate and sessile oaks can be found over the whole Italian mainland. Both these species are completely absent in Sardinia; their occurrence on the other main islands is, however, confined to a few natural populations. In Sicily, these consist of a single *Q. petraea* population growing on the mountain chain of Madonie (Pignatti, 1982; Bernetti, 1995) and in Corsica of two known *Q. robur* populations, and probably not more than two *Q. petraea* populations.

The most common white oak species in Italy is *Q. pubescens*, which is well adapted to dry, warm environments. Its distribution in Italy covers the entire territory, including Sicily and Sardinia, where this species is particularly common. Under the definition of *Q. pubescens*, we also include other forms, whose taxonomic status is particularly difficult to assess and which have been elevated to the species level. We opted for a broad use of the species name *Q. pubescens* (*Q. pubescens sensu lato*). *Q. pubescens* grows in and around forests, mostly in the form of coppice in less fertile environments. Its economic value is low, particularly when compared with *Q. petraea* and *Q. robur*, and for this reason the human impact on this species can be considered limited.

The final white oak species, *Q. frainetto*, occurs naturally although sporadically throughout the central and southern part of the country, with the exclusion of the three main islands. Its ecology is similar to that of *Q. robur*, and the two species often grow in the same conditions.

Initial studies on cpDNA polymorphism in British and continental white oaks, *Q. robur* and *Q. petraea*, identified an east/west divide across the continent (Ferris et al., 1993, 1995). A more extensive study on 345 European white oak populations by Dumolin-Lapègue et al. (1997) traced the most likely role

played by the Italian oak populations in colonising the European continent. However, the following questions remain unanswered: how did the migration occur from the southern Italian refugia towards Europe? Did the Alpine chain represent a barrier to the northward migration of all identified Italian cpDNA haplotypes or did these mountains act as a barrier only to some of them? Did populations from other Mediterranean refugia, particularly from the Balkans, migrate into Italy? If so, did the Apennine chain prevent an east–west population movement? Finally, did human activity modify the natural distribution of cpDNA diversity in Italy?

To examine these questions, we analysed oak populations collected in the whole Italian territory utilising the previously described cpDNA mutations (Dumolin-Lapègue et al., 1997).

2. Materials and methods

A total of 924 trees from 194 populations was sampled from throughout Italy (Table 1). The results of the 20 populations analysed in the study of Dumolin-Lapègue et al. (1997) were included in the data set presented here. In the majority of populations five trees were sampled, but in a few rare cases samples were only taken from three or four trees. The area was divided into 50 km squares with the aim of collecting from at least one population per square. Ultimately, however, no samples were taken from some squares in northern Italy and in contrast, some squares in southern and central Italy were more intensively sampled. Samples were allocated to the following four species on the basis of leaf characters: *Q. robur* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd. s.l., and *Q. frainetto* Ten., even though hybrid individuals between these species may have formed part of the sample. The majority of the trees sampled were classified as *Q. pubescens* (73.5%) though all four species were represented (Table 1).

Fifteen stands in our collection were mixed populations (Table 1). Total DNA was extracted from bud material using the CTAB method of Doyle and Doyle (1990) modified by Dumolin et al. (1995).

Four universal cpDNA primer pairs were chosen to detect mutations and to identify the different haplotypes described by Dumolin-Lapègue et al. (1997). Chosen primers were designed by Taberlet et al. (1991) and Demesure et al. (1995) (Table 2). Reaction mixes (25 μl) contained 2.1 mM of $MgCl_2$, 100 μM of each dNTP, 0.2 μM of each primer and 2.5 U of Taq polymerase with the respective 1 × PCR buffer (Taq polymerase and 10 × buffer purchased from Gibco BRL—Life Technologies) and about 20 ng of template DNA. DNA amplification was performed in a DNA thermal cycler *Genius* Techne (Cambridge, UK). An initial 4 min denaturation at 94 °C was followed by 30 cycles of 94 °C for 45 s, annealing at different temperatures according to the primers for 45 s, and extension at 72 °C for 2–4 min according to the length of the fragments to be amplified. Amplification cycles were followed by a final 10 min extension at 72 °C. Details on the amplification for each fragments are given in Table 2.

The amplification product of 10 μl was digested with 5 U of each restriction enzyme for at least 3 h and at most overnight.

Restriction fragments were separated by electrophoresis in 8% polyacrilamide gel using Tris Borate EDTA buffer (1×) at a constant e.m.f. of 300 V for 120–150 min. After electrophoresis, gels were stained

Table 1
Number of populations and individuals sampled per species

	Populations sampled per species		Individuals sampled per species	
	Total number	Relative frequency	Total number	Relative frequency
Q. pubescens	152	0.784	679	0.735
Q. petraea	7	0.036	32	0.035
Q. robur	15	0.077	64	0.069
Q. frainetto	5	0.026	26	0.028
Mixed	15	0.077	123	0.133
Total	194		924	

Table 2
Details on primers, restriction enzymes, and amplification conditions

Gene	Primer pairs and sequence	Code	Annealing temperature (°C)	Extension time (′)	Endo-nuclease	Reference
*trn*C *trn*D	CCA GTT CAA ATC TGG GTG TC GGG ATT GTA GTT CAA TTG GT	CD	58	4	*Taq* I	Demesure et al. (1995)
*trn*D *trn*T	ACC AAT TGA ACT ACA ATC CC CTA CCA CTG AGT TAA AAG GG	DT	54.5	2	*Taq* I	Demesure et al. (1995)
*psa*A *trn*S	ACT TCT GGT TCC GGC GAA CGA A AAC CAC TCG GCC ATC TCT CCT A	AS	57.5	4	*Hinf* I	Demesure et al. (1995)
trnT *trn*F	CAT TAC AAA TGC GAT GCT CT ATT TGA ACT GGT GAC ACG AG	TF	57.5	2	*Hinf* I	Taberlet et al. (1991)

with ethidium bromide and photographed under UV light with Polaroid 667 film using an MP4 Polaroid Land Camera.

Gibco BRL (Life Technologies) ladder of 100 bp was used as a molecular weight marker.

The classification of the haplotypes was performed according to the presence or absence of mutation (insertion/deletion or site mutation) as previously described by Dumolin-Lapègue et al. (1997) and Petit et al. (2002a).

However, one primer/enzyme combination (TF Alu I) was not scored because the analysis of the three other combinations could unambiguously identify the most common haplotypes found in southern Europe.

All measures of diversity, including their standard errors were computed according to Pons and Petit (1995). The level of population sub-division was examined by computing G_{ST} (Pons and Petit, 1995). The level of differentiation was also calculated within regions, after sub-division of the area into the following regions: north (46–44.50°N), centre (44.50–42°N), south (42–39°N) and the three major islands, Corsica, Sardinia and Sicily.

The frequency of haplotypes for each population was plotted from geo-coded data (MapInfo Professional Version 3.5, MapInfo).

3. Results

The description of the electrophoretic profiles of the haplotypes is indicated in Annexes 1 and 2 of Petit et al. (2002a). The distribution of the cpDNA haplotypes found in Italy is shown in Fig. 1. The relative frequency of each haplotype is reported in Table 3. The majority of the sample consists of haplotypes 1, 2, 5 and 17. Haplotype 7 occurs less than 5% of the sample and is restricted to the Alpine region (with the exception of one individual in central Italy and one in southern Italy). Haplotypes 3, 4 and 19 were each detected in two samples and haplotype 6 was identified only once. The composition of all lineages (A–F) is indicated in Figs. 1 and 2 of Petit et al. (2002a). Two of the three main lineages identified by Dumolin-Lapègue et al. (1997) are represented in Italy: lineage C with haplotypes 1 (23.5%) and 2 (18.4%), and lineage A consisting mostly of haplotypes 5, which is the most frequent one (31.8%) but also haplotype 7 (4.1%). Lineage E is represented in Italy by three haplotypes: 17, 19 and 20. Haplotype 17 occurs at a frequency of 17.2% and haplotypes 19 and 20 are both present at a frequency lower than 5%.

Haplotype 5, which is the most common one in Italy, predominates in the central and southern part of the peninsula (43 and 59%, respectively), it is present in Corsica (9%), but with the exception of one individual, is absent in the other main islands. Haplotype 5 is absent in the northern region, where haplotype 7, which is confined to this part of Italy, dominates (50.7%). Lineage E (haplotypes 17, 19 and 20) dominates in Sardinia and Corsica but is completely absent in northern Italy at latitudes greater than 43°N. In the centre and south, where haplotype 5 dominates, the frequency of haplotype 1 is low (19 and 6.8%, respectively). The frequency of haplotype 1 is very high in the north and in the islands. Haplotype 2 occurs at a frequency of 55.4% in Sicily but is less common in the centre (20.3%) and completely absent in all other areas. The occurrence of haplotype 2 in two

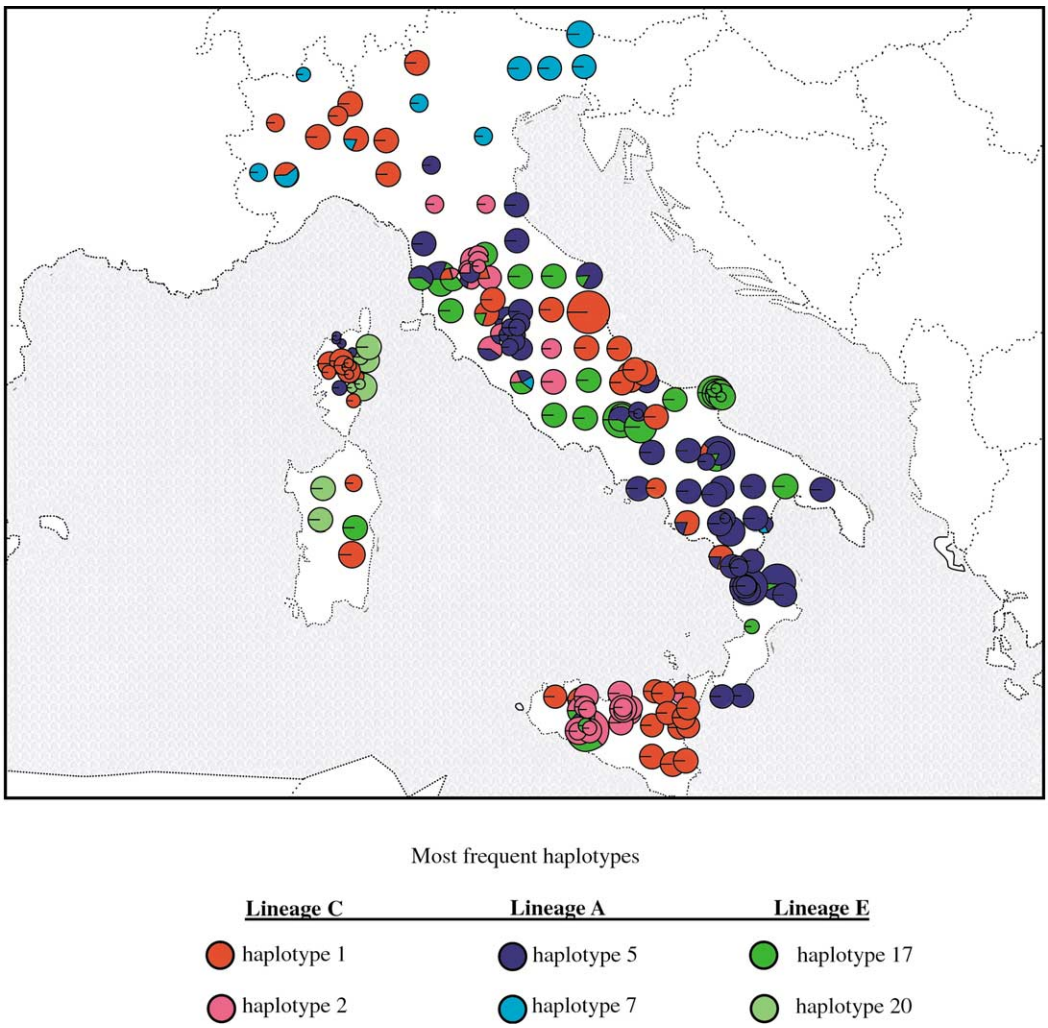

Fig. 1. Map of cpDNA haplotypes in Italian and Corsican oak forests. Size of pies indicates the number of individuals sampled from each population (from 1 to 5).

populations at 44°N latitude marks the northern limits of this haplotype in Italy. Despite the wide distribution of lineage C in central and northern Europe, haplotype 2 in Italy seems to have not reached the Alps. The dominance of haplotype 5 in southern Italy, particularly in the southern part of Calabria, contrasts sharply with its almost complete absence in Sicily (one individual only).

Separate consideration must be given to the three main islands, Corsica, Sardinia and Sicily. In Corsica, lineage E (haplotype 20) and lineage C (haplotype 1) occur at high frequency, but lineage A (haplotype 5), which is completely absent in Sardinia, is also present. In Sardinia only haplotypes 1, 17 and 20 have been identified. The Sardinian population *Orgosolo* is to date the most westerly location detected for haplotype 17 in Europe. This haplotype was not detected in the neighbouring island of Corsica.

In Sicily, haplotypes 1 and 2 are the most common (remarkably, almost half of the Sicilian populations are fixed for haplotype 2, which is completely absent in the whole southern Italian region) and haplotype 17 was also detected in four mixed haplotype populations.

Table 3
Relative frequency of the haplotypes detected in Italy and Corsica

Region	Haplotype number										Number of individuals	Number of populations	Polymorphic populations
	1	2	3	4	5	6	7	17	19	20			
North	35	0	0	0	0	0	36	0	0	0	71	16	2
	0.493	0.000	0.000	0.000	0.000	0.000	0.507	0.000	0.000	0.000			0.125
Centre	58	62	0	2	132	0	1	49	1	0	305	61	16
	0.190	0.203	0.000	0.007	0.433	0.000	0.003	0.161	0.003	0.000			0.262
South	18	0	0	0	155	1	1	87	1	0	263	52	8
	0.068	0.000	0.000	0.000	0.589	0.004	0.004	0.331	0.004	0.000			0.154
Corsica	31	0	0	0	6	0	0	0	0	29	66	22	1
	0.470	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.439			0.045
Sardinia	9	0	0	0	0	0	0	5	0	10	24	5	0
	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.208	0.000	0.417			0.000
Sicily	66	108	2	0	1	0	0	18	0	0	195	38	7
	0.338	0.554	0.010	0.000	0.005	0.000	0.000	0.092	0.000	0.000			0.184
Italy	217	170	2	2	294	1	38	159	2	39	924	194	34
	0.235	0.184	0.002	0.002	0.318	0.001	0.041	0.172	0.002	0.042			0.175

Table 4
Distribution of haplotypes and lineages in different oak species in different Italian regions and major islands (including Corsica)

	Species																										Total
	Q. robur					*Q. petraea*		*Q. pubescens*								*Q. frainetto*			Mixed populations							
	Lineage																										
	C		A			C		C			A			E		A	E		C		A		E			
	Haplotype																										
	1	2	5	6	7	1	2	1	2	3	4	5	7	17	20	5	17	19	1	2	5	7	17	19	20	
North																											
Number of individuals	15	0	0	0	14	10	0	10	16	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	71
Total sample size in northern Italy (%)	21.1	0	0	0	19.7	14.1	0	14.1	22.5	0	0	0	0	0	0	0	0	0	0	0	0	8.5	0	0	0	
Centre																											
Number of individuals	10	7	0	10	0	0	0	40	53	0	2	117	1	37	0	0	0	0	8	2	5	0	12	1	0	305
Total sample size in central Italy (%)	3	2	0	3	0	0	0	13	17	0	1	38	0.3	12	0	0	0	0	3	1	2	0	4	0.3	0	
South																											
Number of individuals	0	0	7	1	0	0	0	17	0	0	0	97	0	32	0	24	1	1	1	0	27	1	54	0	0	263
Total sample size in southern Italy (%)	0	0	2.7	0.4	0	0	0	6.5	0	0	0	36.9	0	12.2	0	9.1	0.4	0.4	0	0	10	0	21	0	0	
Corsica																											
Number of individuals	0	0	0	0	0	3	0	28	0	0	0	6	0	0	23	0	0	0	0	0	0	0	0	0	6	66
Total sample size in Corsica (%)	0	0	0	0	0	4.5	0	42	0	0	0	9.1	0	0	35	0	0	0	0	0	0	0	0	0	9.1	
Sardinia																											
Number of individuals	0	0	0	0	0	0	0	9	0	0	0	0	0	5	10	0	0	0	0	0	0	0	0	0	0	24
Total sample size in Sardinia (%)	0	0	0	0	0	0	0	38	0	0	0	0	0	21	42	0	0	0	0	0	0	0	0	0	0	
Sicily																											
Number of individuals	0	0	0	0	0	0	19	66	89	2	0	1	0	18	0	0	0	0	0	0	0	0	0	0	0	195
Total sample size in Sicily (%)	0	0	0	0	0	0	9.7	33.8	45.6	1.0	0	0.5	0	9.2	0	0	0	0	0	0	0	0	0	0	0	
Total per haplotype and species	25	7	7	11	14	13	19	170	158	2	2	221	1	92	33	24	1	1	9	2	32	7	66	1	6	924
Total sample size per haplotype and per species (%)	0.4	0.1	0.1	0.2	0.2	0.4	0.6	0.3	0.2	0.003	0.003	0.3	0.001	0.1	0.05	0.9	0.04	0.04	0.1	0.02	0.3	0.1	0.5	0.01	0.05	

Table 5
Levels of diversity and differentiation by region

	Number of populations ≥3 individuals	Harmonic mean number of individuals per population	Number of haplotypes	h_S[a]	h_T[a]	G_{ST}[a]
North	15	4.39	2	0.067 (0.046)	0.531 (0.010)	0.874 (0.089)
Centre	60	4.71	7	0.143 (0.032)	0.696 (0.036)	0.794 (0.046)
South	45	5.08	6	0.080 (0.027)	0.521 (0.059)	0.847 (0.052)
Corsica	10	4.67	2	0.040 (0.040)	0.551 (0.013)	0.927 (0.073)
Sardinia	5	4.55	3	0.000 (0.000)	0.800 (0.080)	1000 (NC)
Sicily	36	4.83	5	0.100 (0.033)	0.572 (0.041)	0.826 (0.053)
Italy	171	4.79	10	0.100 (0.016)	0.776 (0.013)	0.870 (0.020)

[a] Values in the parenthesis indicate the standard errors.

The partitioning of forests by species, by regions, and by haplotype is reported in Tables 1 and 3.

Thirty-four populations out of 194 (17.5%), most of which were *Q. pubescens* consisted of more than one haplotype. Of the 61 populations sampled in the central region 16 were mixed haplotype, so that this region has both the highest concentration of polymorphic populations (26.2%) as well as the largest number of scored haplotypes in Italy (7). In the north only two polymorphic populations out of 16 (12.5%), both pure *Q. robur* stands, were identified. In the south, eight populations out of 52 (15.4%) were polymorphic. A similar proportion of mixed haplotype populations (18.4%) was present in Sicily. Mixed haplotype woods were absent in Sardinia; in Corsica one population out of 22 (4.5%) was polymorphic.

Table 4 reports the distribution of haplotypes and lineages in different oak species in the Italian regions and main islands. There is no species-specific haplotype; the haplotype distribution per each species reflects the geographic distribution.

The level of population sub-division was very high, as expressed by the value of the coefficient $G_{ST} = 0.870$ ($h_S = 0.100$, $h_T = 0.776$). When the six major geographic regions were analysed separately (Table 5) total genetic diversity was higher in Sardinia and in central Italy. The highest values of differentiation among populations were estimated for the populations from Sardinia ($G_{ST} = 1$), and Corsica ($G_{ST} = 0.927$). Populations in central Italy were the most polymorphic: in this area the highest number of haplotypes and the highest level of differentiation within populations ($h_S = 0.143$) was detected.

4. Discussion

In general this population analysis confirms part of the results already discussed in a previous study (Dumolin-Lapègue et al., 1997). However, in this survey a larger part of the Italian territory was examined, on the basis of a 50 km grid system; in addition some geographic regions, not previously sampled, were also investigated, such as the Alpine region and the island of Sardinia.

This study demonstrated the presence of some additional haplotypes which have not previously been found to occur in Italy (Dumolin-Lapègue et al., 1997). These include haplotype 7, found in the Alpine region (see also Csaikl et al., 2002a), and haplotype 20 from trees growing in Sardinia and Corsica. The study also described in more detail the distribution of the different haplotypes throughout Italy.

In Italy a lower number of haplotypes (10) were scored than in the other refugial areas studied, the Iberian peninsula (12 haplotypes) and the northern Balkans (11 haplotypes) (Petit et al., 2002a). The value of total genetic diversity was higher in those areas ($h_T = 0.799$ in the Iberian peninsula and $h_T = 0.794$ in the northern Balkans) than in Italy ($h_T = 0.775$) (Petit et al., 2002a).

The coefficient of genetic differentiation is high for the European oaks($G_{ST} = 0.828$); the highest values are reported in the Iberian peninsula ($G_{ST} = 0.889$) and in Italy ($G_{ST} = 0.879$) indicating that a great proportion of the genetic diversity resides among populations (Petit et al., 2002a). When taking into account the phylogenetic relationships, the level of

population sub-division is significantly higher for the Italian populations ($N_{ST} = 0.890 > G_{ST} = 0.879$) (Petit et al., 2002a).

The coefficient of genetic differentiation for Italian oaks does not change if we remove either one of the less common species. The highest differentiation between populations occurs in Sardinia and in Corsica, where most populations are fixed for one haplotype, while the lowest values are in the central part of the peninsula. Central Italy is the area where the highest haplotypic diversity, the lowest genetic differentiation among populations and the highest proportion of polymorphic populations were sampled.

The distribution of the haplotypes demonstrated a clear divide between the south-central and the northern part of the peninsula. The three major islands need to be considered separately.

Three of the six European cpDNA lineages (A, C and E) described by Petit et al. (2002a) have been found in Italy and no new lineages have been identified. Lineage B, which colonised most of the Atlantic regions (Olalde et al., 2002; Petit et al., 2002b) is absent from Italy. In this sense no colonisation of the peninsula seems to have occurred from west to east. Instead, colonisation probably followed two pathways, one from south to north and the other from east (Balkan peninsula) to west. The existence of the Adriatic bridge during the quaternary cold periods (Taberlet et al., 1998) would have made very early contact between Balkan and Italian populations possible. During the Quaternary cold periods lineage C (haplotypes 1, 2, and 3) was probably restricted to the Italian refugium (Petit et al., 2002b). Haplotypes 1 and 2 seem to have migrated from Sicily northward following different pathways. Haplotype 1 colonised first the western side of the Apennines along the Tyrrhenian coast of Calabria, then it passed through the mountain chain and colonised the central part of the peninsula along the Adriatic coast. The westward movement from the eastern Apennines to the western part of the Alps (south-eastern France and north-eastern Spain) probably proceeded through the Po plain, without crossing the Alpine chain (Petit et al., 2002b).

In contrast, the northward migration of haplotype 2 apparently followed the opposite pathway: from the western part of the Apennines it moved to eastern Europe. Haplotype 2 could avoid the Alpine barrier because of the land bridge which existed in the northern and central part of the present Adriatic sea between the Italian and the Balkan peninsulas. The disjunction which characterises the presence of haplotypes 2 in Italy, with its high frequency in Sicily but complete absence in all southern Italian regions, could be explained by extinction episodes caused by late-glacial cold period, as discussed by Petit et al. (2002b).

The presence of lineage A (haplotypes 5, 6, and 7) in both the Italian and the Balkan peninsulas supports the hypothesis of exchange between refugial areas, which might have taken place in earlier interglacial periods. The Adriatic connection between Italian and Balkan flora is particularly evident in Apulia, the extreme south-eastern Italian region, where the highest number of native oak species in Italy as well as southern Europe occur. The only western European stands of *Quercus macrolepis* Kotschy and *Q. trojana* Webb occur in Apulia (Schirone and Spada, 1995): these oaks belong to the tree flora which is likely to have survived in scattered refugia in southern Italy even during the dominance of a steppe-like vegetation between 13,000 and 9000 years BP (Huntley and Birks, 1983; Schirone and Spada, 1995).

The possible exchange between the two peninsulas explains the high frequency of haplotype 5 in the southern Italian regions, however, there are still no data on southern Balkan countries. In this sense, we can only speculate on the Balkan or the Italian origin of haplotype 5. In both cases such migration, which may have preceded the last interglacial, from one side to the other one of the Adriatic sea, would have been made easier by the land bridge which was present at this time. The south–north migration of haplotype 5 in Italy stopped in the northern Apennines (44°N) and proceeded eastward to reach Slovenia and Croatia (Petit et al., 2002b).

Petit et al. (2002b) also suggest the hypothesis of the existence of two refugia for this haplotype, one in Italy and one in the Balkans, but this needs further investigation.

It could also be hypothesised that a first colonisation of haplotypes 5 in Italy started from the refugial area of Laghi di Monticchio located north from Calabria (40.93°N, 15.61°E) and moved in two directions, northward and southward, where it colonised the whole Calabrian region. The area of Laghi di Monticchio has been described as one of the *primary refugia*, i.e.

refugia located in areas able to sustain the species even during the glacial maximum (18 ka BP) and identified for *Quercus* only in the south of the European continent (Brewer et al., 2001).

From the distribution of haplotypes 5 in southern Italy, we can postulate that the Apennine chain crossing Calabria did not act as a barrier on an east–west gradient. In contrast, no migration took place from Calabria to Sicily, at least for this haplotype (if we ignore the only one individual with this haplotype detected in Sicily). This pattern lends support to the idea that Calabria was the meeting point between Balkan and Mediterranean flora: gene flow between Sicily and Calabria might have been prevented by the natural barrier represented by the depth of the Ionic sea, which never experienced any glacial event. Haplotype 7 belongs to the same lineage as haplotype 5 (lineage A). In Italy it is restricted to the pre-Alpine and Alpine region, where it is present mostly in fixed populations or mixed with haplotype 1. The trees in northern Italy (at latitudes greater than 44.50°N) are exclusively haplotype 1 or 7. However, haplotype 1 is more frequent at the western Italian Alpine border, which supports the idea of a migration pathway (from the eastern Apennines through the Po plain to western Alps) described previously and by Petit et al. (2002b). The distribution of haplotype 7 is common to the whole Alpine region (Csaikl et al., 2002a); its occurrence in northern Italy could be the result of a post-glacial northern Balkan migration.

Haplotypes 5 and 7 occur together in Romania, Poland, Hungary and eastern Austria (Bordács et al., 2002; Csaikl et al., 2002b), but in Italy they occur separately: haplotype 5 is found in the south and the centre, and haplotype 7 in the north.

The third lineage present in Italy is the newly described lineage E (Petit et al., 2002a) represented by the haplotypes 17, 19, and 20. As commented by Petit et al. (2002b), haplotype 17 is a complex group which includes different molecular types. This, in addition to their wide distribution, lends support the hypothesis that exchanges between refugial areas older than the last post-glacial occurred. In Italy only one form of haplotype 17 is missing: 17c, which is present in Rumania, Georgia and Russia. The other forms have different patterns of distribution over the Italian territory. Haplotype 17e is present in the western part of Sicily, haplotype 17b is limited to the small peninsula of Gargano in Apulia, haplotype 17a is the most common one and is more or less continuously distributed along the Apennine chain. Haplotype 17d, detected in Sardinia, is related to haplotype 20, whose occurrence is limited to the islands of Sardinia and Corsica. This endemic haplotype is particularly interesting because its presence suggests the possibility of a refugium in these islands, which were connected to each other during the glacial period (Petit et al., 2002b). However, the Sardinian/Corsican haplotype 20 was unable to colonise other territories as it was not found outside of these islands.

It should be remembered that Corsica and Sardinia represent the westernmost occurrence of lineage E (haplotypes 17 and 20) and that this lineage is phylogenetically related to lineage D detected in the Iberian peninsula and described by Olalde et al. (2002) and Petit et al. (2002b).

The sampling in Sardinia was restricted to five populations and all of these were fixed for one haplotype. The collection was limited by the reduction of forested areas caused by frequent episodes of habitat destruction, mostly forest fires, that occurred in recent times in this island.

In Corsica, where more populations were analysed, haplotype 5, absent in our Sardinian sample, was also detected. This haplotype probably migrated into Corsica from the Tyrrhenian coast. The possibility of oak material transferred from the main land to the island cannot be excluded if we consider the recent history of this island, which belonged to the Marine Republic of Pisa (Tuscany) between the 11th and the 14th century and later to the Marine Republic of Genova until 1768. However, the haplotype distribution is quite structured in Corsica, and shared across species, suggesting the local origin of the analysed material. In this sense, if oak material was introduced by humans into Corsica, this introduction must have occurred in earlier times. In contrast, Sardinia was always more isolated from the main land than the other Mediterranean islands and the exchange of oak material would have been more difficult. The distinction between Corsica and Sardinia is not surprising considering the differences between the dendroflora of these islands, emphasised by the presence in Corsica of tree species like *F. sylvatica* L. and *Pinus nigra* Arn. subsp. *Laricio* Poir., which are completely absent from Sardinia (Pignatti, 1982; Gellini and Grossoni, 1997).

This supports the theory of different history experienced by the tree populations of the two islands, and therefore probably also by oaks.

The disjunction observed in the European distribution of haplotypes 2, 5, and 17, and therefore their migration from southern Italian regions northward and eastward can be explained by the existence of *secondary refugia* during the cold phase of the Younger Dryas, as commented by Petit et al. (2002b).

In summary, the different Italian geographical territories experienced completely different colonisation histories:

1. The three major islands were either refugial areas (Sicily for lineage C; Sardinia and Corsica for haplotype 20) or, in the case of Sardinia and Corsica were colonised from the Tyrrhenian side of the Italian mainland.
2. Southern Italy was probably one of the refugial areas for lineage A; on the other hand southern Italy also experienced exchanges with other refugial areas, like the Balkans and Sicily.
3. Central Italy was colonised from both Sicilian and southern Italian (or Balkan) populations. More precisely from the northern Apennines, haplotypes 2, 5, and 17 followed different migration pathways, either westward, or eastward, or both, but not northward. Only haplotype 1 migrated to the north, colonising the Italian Alpine region.
4. Northern Italy was colonised by the northward migration of the Italian haplotype 1 and by the westward migration of the Balkan haplotype 7, one of the few haplotypes which did not originate from southern Mediterranean refugia. The colonisation of northern Italy was not interest at all by the migration of haplotypes 2, 5, and 17.

The combined results of the European oaks cpDNA analysis and fossil pollen data (Petit et al., 2002a,b; Brewer et al., 2001) extend our understanding of the history of post-glacial recolonisation and present-day vegetation. The three Mediterranean peninsulas played an important role as refugial areas conserving populations and genetic diversity. The current distribution of haplotypic diversity of white oaks in Italy seems to be the result of a complex pattern of migration during the post-glacial period. The most plausible hypothesis assumes the presence of two main migration routes: the first one starting from the southern Italian and Balkan refugia and moving northward; the second one originating from the refugia located in the northern Balkans and migrating westwards. According to this hypothesis, central Italy represents the confluence of migration routes radiating from separate refugia.

The effect of human activities, like the transfer of material, can be detected with these markers. In our sampling, the origin of few populations, which contained more than one haplotype could be easily recognised as artificial. In general, however, we can exclude an intense human impact on the distribution of the diversity in white oaks in Italy.

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References

Bennet, K.D., Tzedakis, P.C., Willesk, K.J., 1991. Quaternary refugia of the North Europeans trees. J. Biogeogr. 18, 103–115.

Bernetti G., 1995. Selvicoltura Speciale. UTET, Torino.

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., and Data contributors, 2001. The spread of deciduous *Quercus* throughout Europe since the last glacial period. For. Ecol. Manage., this issue.

Csaikl, U.M., Burg, K., Fineschi, S., König, A.O., Mátyás, G., Petit, R.J., 2002a. Chloroplast DNA variation of white oaks in the Alpine region. For. Ecol. Manage. 156, 131–145.

Csaikl, U.M., Glaz, I., Baliuckas, V., Petit, R.J., Jensen, J.S., 2002b. Chloroplast DNA variation of white oaks in the Baltic countries and Poland. For. Ecol. Manage. 156, 211–222.

Demesure, B., Sodzi, N., Petit, J.R., 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.

Demesure, B., Comps, B., Petit, R.J., 1996. Chloroplast phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. Evolution 50, 2515–2520.

Doyle, J.J., Doyle, J.H., 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13–15.

Dumolin, S., Demesure, B., Petit, R.J., 1995. Inheritance of chloroplast and miochondrial genomes in pedunculate oak investigated with an efficient PCR method. Theor. Appl. Genet. 91, 1253–1256.

Dumolin-Lapègue, S., Demesure, B., Le Corre, V., 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 chloroplast reveal an ancient divide across Europe. Mol. Ecol. 2, 337–344.

Ferris, C., Oliver, R.P., Davy, A.J., Hewitt, G.M., 1995. Using chloroplast DNA to trace postglacial migration routes of oaks into Britain. Mol. Ecol. 4, 731–738.

Fineschi, S., Taurchini, D., Vendramin, G.G., Villani, F., 2000. Chloroplast DNA polymorphism reveals little geographic structuration in *Castanea sativa* Mill. throughout southern European countries. Mol. Ecol. 9, 1495–1503.

Gellini, R., Grossoni, P., 1997. Botanica Forestale, Vol. II: Angiosperme. CEDAM, Padova.

Hewitt, G.M., 1996. Some genetic consequences of ice ages and their role in divergence and speciation. Biol. J. Linn. Soc. 58, 247–276.

Huntley, B., Birks, H.J.B., 1983. An Atlas of Past and Present Pollen Maps of Europe, 0–13,000 Years Ago. Cambridge University Press, Cambridge.

King, R.A., Ferris, C., 1998. Chloroplast DNA phylogeography of *Alnus glutinosa* (L.). Gaertn. Mol. Ecol. 7, 1151–1161.

Konnert, M., Bergman, F., 1995. The geographical distribution of genetic variation of silver fir (*Abies alba*, Pinaceae) in relation to its migration history. Plant Syst. Evol. 196, 19–30.

Olalde, M., Herrán, A., Espinel, S., Goicoechea, P.G., 2002. White oaks phylogeography in the Iberian peninsula. For Ecol. Manage. 156, 89–102.

Petit, R.J., 1999. Diversité Génétique et Histoire des Populations d'Arbres Forestiers. Dossier d'habilitation à diriger des recherches. Universté de Paris-Sud, UFR Scientifique d'Orsay.

Petit, R.J., Pineau, E., Demesure, B., Bacilieri, R., Ducousso, A., 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 2600 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, 49–74.

Pignatti, S., 1982. Flora d'Italia. Edagricole, Bologna.

Pons, O., Petit, R.J., 1995. Estimation, variance and optimal sampling of gene diversity. 1. Haploid locus. Theor. Appl. Genet. 90, 462–470.

Schaal, B., Hayworth, D.A., Olsen, K.K., Rauscher, J.T., Smith, W.A., 1998. Phylogeographic studies in plants: problems and prospects. Mol. Ecol. 7, 465–474.

Schirone, B., Spada, F., 1995. Anomalies in reproductive phenology and vegetation history: the case of SE Italy. Colloques Phytosociologiques 16, 847–857.

Schwarz, O., 1993. *Quercus* L. In: Tutin, T.G., Burges, N.A., Chater, A.O., Edmodnson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea. Cambridge University Press, Cambridge.

Soltis, D.E., Soltis, P.S., Milligan, B.G., 1992. Intraspecific chloroplast DNA variation: systematics and phylogenetic implications. In: Soltis, P.S., Soltis, D.E., Doyle, J.J. (Eds.), Molecular Plant Systematics. Chapman & Hall, New York, pp. 117–150.

Taberlet, P., Gielly, L., Patou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7, 453–464.