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GEOGRAPHIC VARIATION OF INTER-SPECIFIC DIFFERENTIATION BETWEEN *QUERCUS ROBUR* L. AND *QUERCUS PETRAEA* (MATT.) LIEBL.

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SUMMARY

Genetic variation was estimated among 14 populations of two species of white oaks, *Quercus robur* and *Quercus petraea*, distributed over a wide range in Europe, and clustered in seven geographic pairs. Eleven allozymes coding for 13 loci were extracted from radicles and analysed using starch gel electrophoresis. Ten loci were polymorphic in *Q. petraea* and nine in *Q. robur*. The two species shared the same alleles, and exhibited only small differences in allelic frequencies. They showed similar levels of diversity at the species and population level (the expected heterozygosity within populations was 0.245 for *Q. petraea* and 0.252 for *Q. robur*). Variation among populations within species was low for both species (G_{ST} = 0.024 for *Q. robur* and 0.032 for *Q. petraea*). Despite the low level of population differentiation, *Q. petraea* appeared more differentiated than *Q. robur* in 10 of the 11 polymorphic loci. The total gene differentiation between populations was 0.064, which could be separated equally into between populations differentiation (0.031) and between species differentiation (0.033). Comparison of local inter-specific genetic distances among the seven different regions indicated that there was no clear geographic pattern of species differentiation in this data set although differentiation between *Q. petraea* and *Q. robur* was higher in the Balkans than in the other regions.

Key words: *Quercus petraea*, *Quercus robur*, allozymes, genetic differentiation

INTRODUCTION

Quercus petraea (MATT.) LIEBL. (sessile oak) and *Q. robur* L. (pedunculate oak) have the widest geographic distribution among European white oaks. They cover most of the continent except northern Scandinavia (Fig.1). In the Mediterranean regions scattered stands are reported (GIL SANCHEZ *et al.* 1994), whereas in the central part of their natural ranges their distribution is continuous. Their natural ranges largely overlap, except at their eastern limit. *Q. robur* extends to the Ural mountains and *Q. petraea* attains only the Polish-Belorussian border. Within their common geographic distribution, the two species can coexist in mixed stands (RUSHTON 1979, GRANDJEAN & SIGAUD 1987), although they occupy different ecological niches. As shown by those authors, *Q. robur* preferentially grows on rich, humid soils whereas *Q. petraea* occurs on drier, more acidic soils.

Extensive inter-specific gene flow has been reported in mixed stands (BACILIERI *et al.* 1993, 1994). *Quercus petraea* pollinates preferentially *Q. robur* whereas the reciprocal crosses seldom occur. These results, obtained by studying the mating system in a mixed stand, have been confirmed by artificial inter-specific cross pollinations (STEINHOFF 1993). If no other evolutionary force is interfering in the equilibrium between the two species, inter-specific gene flow should considerably decrease the genetic differentiation between the two species and maintain equal levels of gene diversity in *Q. petraea* and *Q. robur*. The purpose of the present investigation was to compare the levels of diversity and population differentiation in these two inter-fertile species and to assess their genetic differentiation in different geographic regions.

Previous studies based on allozymes have shown that both species exhibit similar levels of within population diversity, whereas *Q. petraea* shows slightly higher population differentiation (MÜLLER-STARCK & ZIEHE 1991; KREMER *et al.* 1991; MÜLLER-STARCK *et al.* 1993; KREMER & PETIT 1993). Due to different population sampling strategies, the geographic variation of inter-specific differentiation could not be addressed these studies. The present allowed a comparaison of the local species differentiation from one geographic region to another. The proposed sampling strategy involved the collection of populations in pairs (corresponding to each species) from seven geographic regions throughout the sympatric distribution of both species (except for the Scandinavian pair). Thus, it will be possible to estimate inter-specific differentiation separately in each region.

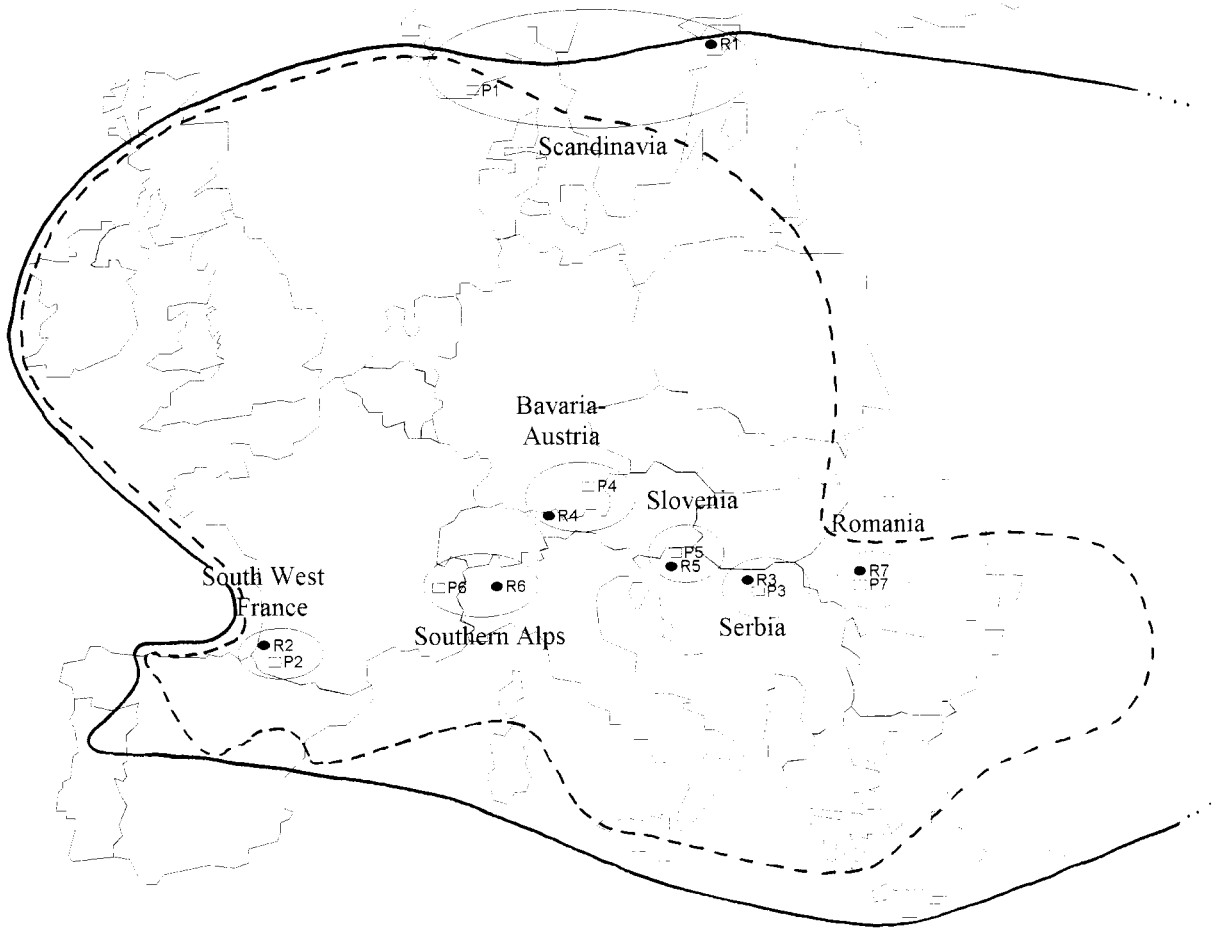

Figure 1 Locations of *Quercus robur* and *Quercus petraea* populations: ●– *Q. petraea*, ▣– *Q. robur*; – – – – natural limit of *Q. petraea*, ———— natural limit of *Q. robur* (the eastern limit, the Urals mountains, not shown)

MATERIAL AND METHODS

Material

Seven indigenous populations in pure stands of *Quercus petraea* and *Quercus robur* respectively were selected in geographic pairs, from a larger sample of populations originating from across the natural range (Fig. 1 and Table 1). The populations were clustered in pairs according to geographic proximity. Seeds were collected in each population either as a bulk collection or as open pollinated progenies, in stands where at least 50% of the trees were fruiting. In the case of bulk collections, seeds were harvested at the ground from 50 collecting points distributed over an area of 15 to 20 ha. At each point, 100 – 200 seeds were collected and bulked into a single lot. A random sample of 120 seeds was used for electrophoresis. In the case of open pollinated progenies, collections were made harvesting 20 seeds from at least 10 (to 30) trees. Subsequentely, a random subset of four to twelve seeds per family was used for electrophoresis. Except for one population, the total sample size used was 120.

Electrophoresis

Acorns were soaked in water for 24 h and germinated on vermiculite in an incubator (20°C/15°C). Enzymes were extracted from young radicles (2 to 3 cm long) and separated from crude homogenates by standard horizontal starch-gel electrophoresis (ZANETTO *et al.* 1993). Eleven enzyme systems were analysed: acid phosphatase (ACP, EC 3.1.3.2), alanine amino-peptidase (AAP, EC 3.4.11.1), diaphorase (DIA, EC - 1.6.4.3), glutamate oxaloacetate transaminase (GOT, -

Table 1 Sampled populations of *Quercus robur* and *Quercus petraea*

Region	Sample			Species	Latitude N	Longitude	Altitude (m)
	No	Population	Size				
Scandinavia	R1	Stockholm	120	*Q. robur*	59°17'	18°00'E	40
	P1	Tjore	120	*Q. petraea*	58°19'	8°31'E	40
Southwest France	R2	Mont de Marsan	120	*Q. robur*	43°42'	1°06'W	10
	P2	Oloron	120	*Q. petraea*	43°11'	0°40'W	120
Serbia	R3	Sremska	121	*Q. robur*	44°59'	19°23'E	80
	P3	Iriski Venac	115	*Q. petraea*	45°20'	19°50'E	450
Bavaria-Austria	R4	Stamser	76	*Q. robur*	43°30'	11°00'E	660
	P4	Riedenburg	120	*Q. petraea*	48°20'	13°16'E	450
Slovenia	R5	Babjilozic	124	*Q. robur*	46°25'	16°10'E	175
	P5	Kobilje	122	*Q. petraea*	46°36'	16°20'E	220
Southern Alps	R6	La Fagiana	120	*Q. robur*	45°27'	8°47'E	50
	P6	Nt.Dame du Cruet	142	*Q. petraea*	45°25'	6°18'E	700
Romania	R7	Dumbrava	127	*Q. robur*	45°50'	24°10'E	300
	P7	Resinari	116	*Q. petraea*	45°50'	24°10'E	400

EC 2.6.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1-.42), leucine aminopeptidase (LAP, EC 3.4.11.1), menadione reductase (MR, EC 1.6.99.2), malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucoisomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44). Buffer formulations for starch gel electrophoresis and staining of enzymes are given elsewhere (ZANETTO *et al.* 1993).

These enzymes were encoded by 13 loci (*Acp-C, Aap-A, Dia-A, Got-B, Idh-A, Lap-A, Mr-A, Mdh-B, Mdh-C, Pgi-B, Pgm-A, 6Pgd-A, 6Pgd-B*). The alleles were labelled with numbers from the cathode to the anode.

Gene diversity statistics

The number of alleles, gene diversity and effective number of alleles were first calculated at the species level (A_s, H_{es}, A_{es}). Within population gene diversity statistics were estimated for each locus (BROWN & WEIR 1983). These included the number of alleles per locus (A_p), the observed heterozygosity (Hop), the expected heterozygosity ($H_{ep} = 1 - \Sigma p_i^2$, where p_i is the frequency of allele i) and the effective number of alleles [$A_{ep} = 1 / (1 - H_{ep})$]. Confidence intervals for each parameter were calculated empirically by a hierarchical bootstrapping across populations and individuals (EFRON 1979). One thousand bootstrap samples were generated within each species, and gene diversity statistics calculated for each sample. Limits of the confidence intervals were determined by truncating both extremities of the distribution of diversity statistics at 2.5% .

Population differentiation

The total gene diversity (H_t) can be divided into a within population component (H_s) and an among populations component (D_{st}; $H_t = H_s + D_{st}$). Within each species, parameters of gene differentiation between populations ($G_{st} = D_{st} / H_t$) were calculated using Nei's methods (NEI 1973, 1977). Confidence intervals of G_{st} were calculated by using the bootstrap method (1000 samples).

Distribution of gene diversity in the *Q. robur – Q. petraea* complex

The structure of gene diversity in the complex formed by the two inter-fertile oak species was analysed by subdividing the total diversity in the complex (H_t) into three components ($H_t = H_s + D_{sg} + D_{gt}$; NEI 1973, 1977). H_s is the within population diversity within species, D_{sg} is the component of diversity due to

subdivision into population within species, and D_{gt} is the component of diversity due to species subdivision. These components were further calculated as ratios of the total diversity ($G_s = H_s / H_t$; $G_s = 1 - G_{st}$; $G_{st} = G_{sg} + G_{gt}$; $G_{sg} = D_{sg} / H_t$; $G_{gt} = D_{gt} / H_t$).

Genetic distances

Mean Nei's genetic distances were calculated for all possible pairwise comparison within and between populations and species. Genetic distances between populations were computed across all loci (NEI 1976). These distances are average distances across loci and not multilocus measures *per se*. Since several loci have been shown to form a linkage group (ZANETTO *et al.* in preparation) and in order to take into account desequilibrium that may have occurred between loci, Mahalanobis distances were computed as follows:

$$d_{ij} = (\mathbf{p}_i - \mathbf{p}_j)' \mathbf{W}^{-1} (\mathbf{p}_i - \mathbf{p}_j)$$

where $\mathbf{p}_i$ is the vector of the allelic frequencies in population i, $\mathbf{p}_j$ is the vector of the allelic frequencies in population j, d_{ij} is the Mahalanobis distance between the population i and the population j, $\mathbf{W}^{-1}$ is the inverted matrix of variance-covariance within populations, averaged over populations i and j. Computations of $\mathbf{W}^{-1}$ were made after transformations of the genotypic data according to the scoring procedure of SMOUSE *et al.* (1982). Mahalanobis distances were computed using only the 11 polymorphic loci.

RESULTS

Species and population gene diversity

At the species level, the mean number of alleles per population (A_s) was 3.692 for *Quercus petraea* and 3.538 for *Q. robur*. Additionally, values for the other gene diversity statistics were similar for the two species: gene diversity (H_{es}) and effective number of alleles (A_{es}) were respectively 0.262 and 1.355 for *Q. petraea* and 0.260 and 1.351 for *Q. robur*.

Within each species, levels of gene diversity at the population level differed markedly among loci. These variations were mostly due to differences in frequency profiles (Table 2). Four different classes could be identified. Class 1 comprised monomorphic loci (*Mdh-B, Mdh-C, 6Pgd-A* and *6Pgd-B* in *Q. robur*). Class 2 was characterized by one predominant allele (frequency > 0.9) and other rare alleles (*Got-B, Pgi-A, Pgm-A* in *Q. petraea*). In class 3, the frequency of the predominant allele varied between 0.75 and 0.90 (*Mr-A, Idh-A*) and finally class 4 gathered loci that exhibited several frequent alleles (*Aap-A, Acp-C, Dia-A* and *Lap-A*). Interestingly this classification encompassed the metabolic classification of enzymes: enzymes of class 1 and 2 were predominantly involved in the primary metabolism (Group I, BERGMANN 1991), whereas class 3 and 4 comprised enzymes involved in the secondary metabolism (Group II, BERGMANN 1991). In general, enzymes of group I exhibited higher heterozygosities than enzymes of group II, whereas differences in number of alleles were less pronounced between the two groups.

Levels of within population gene diversity were remarkably similar in both species (Tables 3 and 4), when comparisons were made on average values across loci. Among the 13 loci, ten were polymorphic in *Quercus petraea* (82%) and nine in *Q. robur* (73%). Other gene diversity parameters were of the same amount in both species (A_p = 2.703 and 2.725; A_{ep} = 1.488 and 1.472; H_{ep} = 0.252 and 0.245, respectively for *Q. robur* and *Q. petraea*) except H_o, which was higher in *Q. petraea* (0.222) than in *Q. robur* (0.184). Both species shared the same alleles, including rare alleles (Table 2), except for *Pgm-A-3* for *Q. robur* and *Aap-A-1, 6Pgd-B-1, 6Pgd-B-6* and *6Pgd-B-8* for *Q. petraea*. Here extremely rare alleles were present in one species and absent in the other. However, these latter variations may have been due to chance, since extremely rare alleles may be absent in the sample only because the size was not large enough. Differences between the two species appeared at specific loci. For example, expected heterozygosities for *Pgm-A* was 0.502 for *Q. robur* and 0.150 for *Q. petraea*, whereas corresponding values for *Idh-B* were 0.251 and 0.429, and for *Pgi-B* 0.104 and 0.238. These differences between the two species were significant, since there was no overlap between the confidence intervals. Within population, inter-specific differences in the levels of diversity appeared to be more pronounced for enzymes of group I than enzymes of group II.

Population differentiation within each species

Most of the diversity was intrapopulational for both species (Table 5), although allelic frequency differences between populations were significant in each species. Low levels of differentiation were reflected by the maintenance of the same frequency profiles between the different populations. For most of the loci, the most frequent allele was constant across the populations, except for *Acp-C* and *Lap-A* (in *Q. petraea*). G_{st} values varied among loci, and consider-

Table 2 Allelic frequencies in *Quercus robur* and *Quercus petraea*

Regions	Species	*Acp-C*						*Dia-A*					
		1	2	3	4	5	χ^2	2	3	4	6	7	χ^2
Scandinavia	*Q. robur*	0.000	0.667	0.008	0.325	0.000		0.014	0.743	0.207	0.036	0.000	
	Q. petraea	0.000	0.571	0.000	0.429	0.000	ns	0.000	0.629	0.300	0.071	0.000	*
SW France	*Q. robur*	0.000	0.733	0.004	0.263	0.000		0.009	0.551	0.432	0.009	0.000	
	Q. petraea	0.000	0.529	0.017	0.454	0.000	***	0.000	0.440	0.371	0.190	0.000	***
Serbia	*Q. robur*	0.000	0.752	0.021	0.227	0.000		0.042	0.706	0.202	0.050	0.000	
	Q. petraea	0.000	0.197	0.000	0.741	0.061	***	0.005	0.410	0.446	0.140	0.000	***
Bavaria. &	*Q. robur*	0.000	0.947	0.000	0.053	0.000		0.000	0.713	0.187	0.100	0.000	
Austria	*Q. petraea*	0.000	0.554	0.000	0.446	0.000	***	0.000	0.517	0.389	0.094	0.000	***
Slovenia	*Q. robur*	0.000	0.847	0.000	0.153	0.000		0.053	0.676	0.139	0.127	0.004	
	Q. petraea	0.000	0.402	0.021	0.560	0.017	***	0.008	0.471	0.404	0.117	0.000	***
South. Alps	*Q. robur*	0.004	0.752	0.000	0.244	0.000		0.018	0.548	0.364	0.070	0.000	
	Q. petraea	0.000	0.592	0.004	0.405	0.000	***	0.004	0.451	0.278	0.246	0.021	***
Romania	*Q. robur*	0.000	0.873	0.004	0.119	0.004		0.000	0.586	0.351	0.063	0.000	
	Q. petraea	0.004	0.565	0.000	0.409	0.022	***	0.000	0.575	0.320	0.105	0.000	ns

Regions	Species	*Aap-A*						*Pgm-A*					
		1	2	3	4	5	χ^2	2	3	4	5	6	χ^2
Scandinavia	*Q. robur*	0.000	0.432	0.093	0.475	0.000		0.317	0.000	0.500	0.134	0.049	
	Q. petraea	0.000	0.386	0.157	0.436	0.021	ns	0.127	0.000	0.853	0.020	0.000	***
SW France	*Q. robur*	0.000	0.408	0.160	0.420	0.013		0.378	0.000	0.617	0.005	0.000	
	Q. petraea	0.000	0.592	0.100	0.292	0.017	***	0.042	0.000	0.907	0.051	0.000	***
Serbia	*Q. robur*	0.000	0.317	0.300	0.379	0.004		0.358	0.000	0.600	0.025	0.017	
	Q. petraea	0.005	0.473	0.136	0.373	0.014	***	0.027	0.000	0.969	0.004	0.000	***
Bavaria &	*Q. robur*	0.000	0.441	0.086	0.467	0.007		0.270	0.000	0.730	0.000	0.000	
Austria	*Q. petraea*	0.000	0.538	0.071	0.358	0.033	ns	0.012	0.000	0.921	0.067	0.000	***
Slovenia	*Q. robur*	0.000	0.385	0.234	0.365	0.016		0.407	0.015	0.500	0.024	0.054	
	Q. petraea	0.000	0.708	0.076	0.195	0.021	***	0.000	0.000	1.000	0.000	0.000	***
South. Alps	*Q. robur*	0.000	0.352	0.178	0.452	0.017		0.350	0.000	0.622	0.000	0.028	
	Q. petraea	0.000	0.588	0.063	0.338	0.011	***	0.102	0.000	0.891	0.007	0.000	***
Romania	*Q. robur*	0.000	0.545	0.101	0.354	0.000		0.184	0.010	0.760	0.005	0.041	
	Q. petraea	0.000	0.471	0.175	0.354	0.000	ns	0.100	0.000	0.870	0.005	0.025	*

Regions	Species	*Idh-A*						*6Pgd-A*	*6Pgd-B*				
		1	2	3	4	5	χ^2	2	1	3	6	8	χ^2
Scandinavia	*Q. robur*	0.000	0.000	0.389	0.607	0.004		1.000	0.000	1.000	0.000	0.000	
	Q. petraea	0.162	0.000	0.046	0.792	0.000	***	1.000	0.000	1.000	0.000	0.000	nc
SW France	*Q. robur*	0.000	0.029	0.271	0.700	0.000		1.000	0.000	1.000	0.000	0.000	
	Q. petraea	0.071	0.000	0.079	0.850	0.000	***	1.000	0.000	1.000	0.000	0.000	nc
Serbia	*Q. robur*	0.000	0.000	0.358	0.638	0.004		1.000	0.000	1.000	0.000	0.000	
	Q. petraea	0.009	0.000	0.144	0.842	0.005	***	1.000	0.000	1.000	0.000	0.000	nc
Bavaria &	*Q. robur*	0.000	0.000	0.211	0.790	0.000		1.000	0.000	1.000	0.000	0.000	
Austria	*Q. petraea*	0.033	0.000	0.004	0.942	0.021	***	1.000	0.000	1.000	0.000	0.000	nc
Slovenia	*Q. robur*	0.033	0.000	0.293	0.670	0.004		1.000	0.000	1.000	0.000	0.000	
	Q. petraea	0.131	0.000	0.029	0.824	0.016	***	1.000	0.000	1.000	0.000	0.000	nc
South. Alps	*Q. robur*	0.009	0.004	0.313	0.674	0.000		1.000	0.000	1.000	0.000	0.000	
	Q. petraea	0.063	0.000	0.011	0.922	0.004	*	1.000	0.004	0.894	0.004	0.098	***
Romania	*Q. robur*	0.013	0.000	0.197	0.790	0.000		1.000	0.000	1.000	0.000	0.000	
	Q. petraea	0.009	0.000	0.202	0.785	0.004	ns	1.000	0.000	1.000	0.000	0.000	nc

Table 2 (continued)

Regions	Species	*Lap-A*					*Mr-A*						
		2	3	4	5	χ^2	1	2	3	4	5	7	χ^2
Scandinavia	*Q. robur*	0.462	0.017	0.521	0.000		0.008	0.021	0.904	0.000	0.025	0.042	
	Q. petraea	0.609	0.000	0.319	0.071	***	0.000	0.000	0.912	0.038	0.004	0.046	ns
SW France	*Q. robur*	0.588	0.008	0.404	0.000		0.000	0.004	0.938	0.025	0.013	0.021	
	Q. petraea	0.313	0.000	0.617	0.071	***	0.000	0.013	0.811	0.000	0.017	0.160	***
Serbia	*Q. robur*	0.546	0.000	0.342	0.113		0.000	0.045	0.893	0.017	0.000	0.045	
	Q. petraea	0.474	0.017	0.435	0.074	ns	0.000	0.039	0.783	0.078	0.000	0.100	***
Bavaria &	*Q. robur*	0.553	0.000	0.434	0.013		0.000	0.000	0.875	0.026	0.000	0.099	
Austria	*Q. petraea*	0.388	0.000	0.558	0.054	**	0.000	0.000	0.853	0.055	0.000	0.092	ns
Slovenia	*Q. robur*	0.728	0.000	0.252	0.020		0.000	0.081	0.786	0.012	0.020	0.101	
	Q. petraea	0.329	0.000	0.632	0.038	***	0.000	0.030	0.791	0.068	0.004	0.107	***
South. Alps	*Q. robur*	0.654	0.000	0.320	0.026		0.000	0.013	0.904	0.026	0.009	0.048	
	Q. petraea	0.362	0.000	0.580	0.058	***	0.000	0.004	0.754	0.028	0.011	0.204	***
Romania	*Q. robur*	0.449	0.000	0.505	0.046		0.008	0.008	0.819	0.079	0.031	0.055	
	Q. petraea	0.563	0.000	0.379	0.058	*	0.004	0.004	0.822	0.087	0.043	0.039	ns

Regions	Species	*Got-B*				*Pgi-B*						*Mdh-B*	*Mdh-C*
		1	2	4	χ^2	1	2	3	5	6	χ^2	3	3
Scandinavia	*Q. robur*	0.025	0.971	0.004		0.000	0.033	0.000	0.967	0.000		1.000	1.000
	Q. petraea	0.088	0.892	0.021	*	0.004	0.067	0.000	0.763	0.167	***	1.000	1.000
SW France	*Q. robur*	0.017	0.921	0.063		0.004	0.038	0.000	0.921	0.038		1.000	1.000
	Q. petraea	0.017	0.953	0.030	ns	0.000	0.042	0.000	0.908	0.050	ns	1.000	1.000
Serbia	*Q. robur*	0.004	0.988	0.008		0.004	0.046	0.000	0.909	0.041		1.000	1.000
	Q. petraea	0.022	0.978	0.000	nc	0.000	0.052	0.000	0.930	0.018	ns	1.000	1.000
Bavaria &	*Q. robur*	0.034	0.966	0.000		0.000	0.007	0.000	0.993	0.000		1.000	1.000
Austria	*Q. petraea*	0.076	0.924	0.000	ns	0.000	0.158	0.004	0.771	0.067	***	1.000	1.000
Slovenia	*Q. robur*	0.023	0.977	0.000		0.000	0.025	0.004	0.938	0.033		1.000	1.000
	Q. petraea	0.065	0.930	0.004	ns	0.000	0.099	0.004	0.754	0.143	***	1.000	1.000
South. Alps	*Q. robur*	0.063	0.911	0.027		0.000	0.030	0.026	0.918	0.026		1.000	1.000
	Q. petraea	0.102	0.891	0.007	ns	0.000	0.021	0.000	0.933	0.046	ns	1.000	1.000
Romania	*Q. robur*	0.034	0.956	0.010		0.004	0.004	0.004	0.987	0.000		1.000	1.000
	Q. petraea	0.026	0.961	0.013	ns	0.013	0.022	0.000	0.957	0.009	nc	1.000	1.000

χ^2 tests, * p 0.05, ** p 0.01, *** p 0.001, ns – non significant, nc – not calculated.

able amount of variation existed for the coefficient of population differentiation. However, at this level, no systematic trend of variation due to group I or II could be seen for G_{st}. *6Pgd-B* was polymorphic in only one population of *Q. petraea* and showed an unusually high value of G_{st} (8.2%). Similarly, high levels of population differentiation for *Acp-C* in both species (4.4% in *Q. robur* and 5.5% in *Q. petraea*)appeared due to unusual allelic frequencies in one population (Serbia) compared to the others (*Acp-C* in *Q. petraea*, Table 2). Levels of differentiation at other loci were due to minor variations in allelic frequencies spread over several populations.

Among the ten polymorphic loci, nine showed higher levels of population differentiation in *Q. petraea* than in *Q. robur*, *Dia-A* being the only exception (Table 5). As a result, multilocus G_{st} values, calculated on the basis of average values of H_s and H_t, were higher in *Q. petraea* (3.3%) than in *Q. robur* (2.4%) although their confidence intervals overlap (Table 5).

Distribution of gene diversity in the *Q. robur* - *Q. petraea* complex

The total gene diversity in the *Q. petraea* - *Q. robur* complex was partitioned into a within-population (G_s), between species (G_{gt}) and across populations (within-species) (G_{sg}) components (Table 6). The total differ-

Table 3 Gene diversity statistics for *Quercus robur* at the population level

Enzyme group	Locus	A_p	A_{ep}
I	*Idh-A*	3.143 (2.465–3.434)	1.751 (1.611–1.867)
I	*Got-B*	2.714 (2.156–2.867)	1.093 (1.048–1.148)
I	*Mdh-B*	1.000 –	1.000 –
I	*Mdh-C*	1.000 –	1.000 –
I	*Pgi-B*	3.429 (2.342–3.769)	1.117 (1.062–1.176)
I	*Pgm-A*	3.714 (2.819–4.167)	2.008 (1.763–2.268)
I	*6Pgd-A*	1.000 –	1.000 –
I	*6Pgd-B*	1.000 –	1.000 –
II	*Aap-A*	3.714 (3.143–3.883)	2.644 (2.402–2.819)
II	*Acp-C*	2.857 (2.263–3.064)	1.477 (1.313–1.651)
II	*Dia-A*	3.857 (3.296–4.164)	1.960 (1.787–2.113)
II	*Lap-A*	3.000 (2.695–2.981)	1.995 (1.844–2.162)
II	*Mr-A*	4.714 (3.874–5.136)	1.296 (1.198–1.614)
Mean values over all loci		2.703 (2.518–2.888)	1.488 (1.456–1.520)
Enzyme group	Locus	H_{ep}	H_{op}
I	*Idh-A*	0.429 (0.379–0.464)	0.415 (0.354–0.472)
I	*Got-B*	0.085 (0.045–0.129)	0.071 (0.038–0.112)
I	*Mdh-B*	0.000 –	0.000 –
I	*Mdh-C*	0.000 –	0.000 –
I	*Pgi-B*	0.104 (0.059–0.149)	0.108 (0.061–0.154)
I	*Pgm-A*	0.502 (0.432–0.558)	0.233 (0.164–0.291)
I	*6Pgd-A*	0.000 –	0.000 –
I	*6Pgd-B*	0.000 –	0.000 –
II	*Aap-A*	0.622 (0.587–0.645)	0.281 (0.218–0.352)
II	*Acp-C*	0.323 (0.239–0.394)	0.322 (0.238–0.428)
II	*Dia-A*	0.490 (0.439–0.526)	0.453 (0.393–0.510)
II	*Lap-A*	0.499 (0.458–0.537)	0.294 (0.235–0.347)
II	*Mr-A*	0.228 (0.164–0.293)	0.212 (0.151–0.278)
Mean values over all loci		0.252 (0.238–0.266)	0.184 (0.166–0.202)

A_p: mean number of alleles per population
A_{ep}: mean effective number of alleles per population
H_{op}: observed heterozygosity
H_{ep}: expected heterozygosity
(...) : confidence interval.

entiation across populations, over the two species, amounted to 6.4% ($G_{gt} + G_{sg}$). The total differentiation may be subdivided equally as a species component (3.3%) and a population (within-species) component (3.1%). In other words, intra-specific variation within a given species is about as important as inter-specific variation.

However there were important differences among loci. *Pgm-A, Acp-C* and *Idh-B* showed important species differentiations (respectively 11.1%, 8.9% and 5.5%). These were the only loci for which species differentiation was higher than population differentiation. Although average values of G_{gt} and G_{sg} were similar, comparison of levels of species and population differentiation might be misleading due to the important variation between loci.

Genetic distances between populations

Nei's genetic distance

Mean Nei's genetic distance over 13 loci between *Quercus petraea* and *Q. robur* in all pairwise combinations was 0.027 (±0.013) and 0.039 (±0.017), if combinations within species were excluded (Table 7). When considering all combinations separately, there

Table 4 Gene diversity statistics for *Quercus petraea* at the population level

Enzyme group	Locus	A_p	A_{ep}
I	*Idh-A*	3.713 (3.004–3.866)	1.335 (1.222–1.465)
I	*Got-B*	2.714 (2.275–2.978)	1.145 (1.086–1.212)
I	*Mdh-B*	1.000 –	1.000 –
I	*Mdh-C*	1.000 –	1.000 –
I	*Pgi-B*	3.571 (2.999–3.725)	1.313 (1.168–1.504)
I	*Pgm-A*	2.857 (2.034–3.259)	1.176 (1.088–1.273)
I	*6Pgd-A*	1.000 –	1.000 –
I	*6-Pgd-B*	1.429 (0.000–2.071)	1.029 (1.005–1.097)
II	*Aap-A*	4.000 (3.555–4.293)	2.319 (2.070–2.573)
II	*Acp-C*	3.000 (2.291–3.457)	1.961 (1.839–2.056)
II	*Dia-A*	3.571 (3.023–4.028)	2.461 (2.233–2.681)
II	*Lap-A*	3.143 (3.000–3.441)	2.126 (2.015–2.227)
II	*Mr-A*	4.429 (3.564–4.737)	1.270 (1.332–1.576)
Mean values over all loci		2.725 (2.597–2.853)	1.472 (1.452–1.492)
Enzyme group	Locus	H_{ep}	H_{op}
I	*Idh-A*	0.251 (0.182–0.319)	0.415 (0.354–0.472)
I	*Got-B*	0.127 (0.079–0.175)	0.071 (0.038–0.112)
I	*Mdh-B*	0.000 –	0.000 –
I	*Mdh-C*	0.000 –	0.000 –
I	*Pgi-B*	0.238 (0.142–0.336)	0.108 (0.108–0.154)
I	*Pgm-A*	0.150 (0.080–0.214)	0.233 (0.233–0.164)
I	*6Pgd-A*	0.000 –	0.000 –
I	*6-Pgd-B*	0.028 (0.000–0.088)	0.028 (0.000–0.089)
II	*Aap-A*	0.569 (0.516–0.611)	0.369 (0.290–0.436)
II	*Acp-C*	0.490 (0.457–0.514)	0.459 (0.396–0.519)
II	*Dia-A*	0.594 (0.552–0.627)	0.563 (0.478–0.649)
II	*Lap-A*	0.530 (0.504–0.551)	0.430 (0.368–0.494)
II	*Mr-A*	0.312 (0.250–0.366)	0.309 (0.244–0.368)
Mean values over all loci		0.245 (0.236–0.254)	0.222 (0.200–0.243)

was a clear overlap between intra- and inter-specific distances. For example, the lowest inter-specific genetic distance was 0.012 whereas the highest within *Q. petraea* distance was 0.026 (0.021 for *Q. robur*). Genetic distances between *Q. petraea* populations were generally higher than for *Q. robur* (Table 7 and Figure 2), showing lower population differentiation in *Q. robur* than in *Q. petraea*.

There was no clear geographic pattern for inter-specific differentiation (Table 7). In Romania, the genetic distance between both species was extremely low (0.012). The greatest values were found in Serbia and Slovenia, whereas inter-specific genetic distances in other regions (Scandinavia, SouthWest France, Southern Alps and Bavaria-Austria) were of average amount. In general, the local (within a given region) inter-specific differentiation was lower than the between region inter-specific differentiation. However this was not the case in Serbia and Slovenia, where the genetic distances between *Q. robur* and *Q. petraea* 2were much higher, as compared to inter-regional inter-specific genetic distances. Important local differentiation for these regions were due to major allelic frequency differences for a few loci (*Acp-C* and *Aap-A* for Serbia, and *Lap-A* and *Pgm-A* for Slovenia). Differentiation between the two species may have either been due to divergence of one given species from the other (being stable), or from common divergence. In order to identify the source of differentiation, the mean genetic distance between *Q. robur* of the designate region and the *Q. petraea* of all other regions (D_1) and the mean genetic distance between *Q. petraea* of the designate region and the *Q. robur* of all other regions (D_2) were calculated (Table 8). The data shown in Table 8 support the second hypothesis since D_1 and D_2 values for Serbia and Slovenia are of the same amount, *e.g.* common divergence was most likely the source of differentiation.

Table 5 Components of the total diversity in the two species

Locus	*Quercus robur*		
	H_t	H_s	G_{st}
Aap-A	0.631 (0.595–0.657)	0.623 (0.580–0.648)	0.014 (0.003–0.036)
Acp-C	0.328 (0.234–0.404)	0.313 (0.221–0.397)	0.044 (0.007–0.088)
Dia-A	0.506 (0.450–0.541)	0.490 (0.441–0.528)	0.032 (0.010–0.053)
Lap-A	0.519 (0.480–0.551)	0.504 (0.464–0.539)	0.030 (0.007–0.062)
Mr-A	0.231 (0.168–0.293)	0.228 (0.167–0.289)	0.016 (0.003–0.031)
Idh-A	0.433 (0.381–0.469)	0.425 (0.372–0.466)	0.017 (0.001–0.040)
Got-B	0.086 (0.046–0.131)	0.085 (0.046–0.128)	0.012 (0.000–0.030)
Pgi-B	0.101 (0.055–0.148)	0.100 (0.054–0.147)	0.013 (0.003–0.028)
Pgm-A	0.512 (0.443–0.571)	0.499 (0.431–0.557)	0.025 (0.004–0.056)
6Pgd-B	0.000	0.000	0.000
Mean values	0.335 (0.310–0.350)	0.327 (0.301–0.344)	0.024* (0.012–0.029)
Locus	*Quercus petraea*		
	H_t	H_s	G_{st}
Aap-A	0.588 (0.529–0.625)	0.575 (0.519–0.617)	0.023 (0.003–0.053)
Acp-C	0.521 (0.483–0.531)	0.492 (0.433–0.516)	0.055 (0.000–0.114)
Dia-A	0.604 (0.562–0.636)	0.595 (0.554–0.627)	0.016 (0.004–0.033)
Lap-A	0.556 (0.522–0.571)	0.533 (0.506–0.555)	0.041 (0.005–0.073)
Mr-A	0.317 (0.256–0.370)	0.312 (0.251–0.366)	0.017 (0.005–0.035)
Idh-A	0.266 (0.192–0.329)	0.256 (0.186–0.319)	0.038 (0.014–0.062)
Got-B	0.127 (0.080–0.175)	0.125 (0.079–0.174)	0.013 (0.003–0.030)
Pgi-B	0.252 (0.149–0.349)	0.240 (0.142–0.339)	0.046 (0.013–0.068)
Pgm-A	0.158 (0.084–0.222)	0.153 (0.082–0.218)	0.028 (0.007–0.061)
6Pgd-B	0.030 (0.000–0.092)	0.027 (0.000–0.087)	0.082 (0.000–0.109)
Mean values	0.342 (0.326–0.352)	0.331 (0.317–0.342)	0.032* (0.019–0.044)

H_t: total gene diversity,
H_s: gene diversity within populations,
G_{st}: coefficient of differentiation between populations,
*G_{st} calculated as $(1 - \overline{H_s} / \overline{H_t})$, where $\overline{H_s}$ and $\overline{H_t}$ are the mean values over all loci,
(...) confidence interval.

diversity at the species level was 0.260 for *Q. robur* and 0.262 for *Q. petraea*. Although absolute values of gene diversity statistics were quite similar between the two species, in the three studies published, *Q. petraea* showed slightly higher values. This trend has been confirmed in a recent study where comparisons between the two species were based on nuclear DNA fragments (MOREAU *et al.* 1994).

Levels of diversity in the two European white oaks were similar to those found for North American species of the same section *Lepidobalanus*. The mean value of H_{es} obtained from 8 different species was, in that case, 0.257 (GUTTMAN and WEIGHT, 1989). Other North American examples showed lower levels of variation (H_{es} = 0.206 and 0.215 for *Q. macrocarpa* and *Q. gambelii*, in the section *Erythrobalanus*, SCHNABEL and HAMRICK, 1990). Levels of diversity in the white oaks were systematically higher to those found in the red oaks (H_{es} = 0.178 for the 10 species studied by GUTTMAN and WEIGHT 1989; 0.184 for *Q. laevis*, BERG & HAMRICK 1993). The values found in European and North American white oaks were amongst the highest in woody plants, when compared with data of the reviews made by HAMRICK and GODT (1990) and HAMRICK *et al.* (1992). Life history traits specific to oaks contributed to maintenance of high genetic diversities. The two species are almost strictly allogamous (BACILIERI *et al.* 1933), they usually grow in wide pure stands and their geographic distribution is extremely large. Allogamy, large population sizes and vast geographic distribution have been shown to be statistically related to levels of diversity (HAMRICK-

Table 6 Components of the total diversity in the *Quercus robur – Quercus petraea* complex

Locus	H_t	G_s	G_{sg}	G_{gt}
Aap-A	0.616	0.973	0.017	0.011
Acp-C	0.470	0.858	0.053	0.089
Dia-A	0.564	0.962	0.023	0.015
Lap-A	0.545	0.952	0.033	0.016
Mr-A	0.276	0.978	0.016	0.006
Idh-A	0.368	0.926	0.019	0.055
Got-B	0.107	0.985	0.011	0.004
Pgi-B	0.180	0.948	0.037	0.015
Pgm-A	0.374	0.871	0.018	0.111
6Pgd-B	0.015	0.913	0.080	0.007
Mean values	0.351	0.936	0.031	0.033

H_t: total gene diversity
G_s: coefficient of gene differentiation among individuals (within populations)
G_{sg}: coefficient of gene differentiation among populations (within species)
G_{gt}: coefficient of gene differentiation between species
Values are based on polymorphic loci only.

Table 7 Mean inter- and intra-specific genetic distances

	Nei's distance		Mahalanobis distance	
	Quercus petraea	*Quercus robur*	*Quercus petraea*	*Quercus robur*
Quercus petraea	0.015 (0.004–0.026)		2.950 (1.202–5.334)	
Quercus robur	0.039 (0.012–0.082)	0.011 (0.002–0.021)	5.122 (0.821–8.791)	1.854 (0.441–3.809)

Values within brackets indicate minimum and maximum genetic distances.

DISCUSSION

Level of genetic diversity

Levels of diversity in *Quercus petraea* and *Q. robur* were of a similar amount in the two species, whether calculations were made at the species or the population level. The values obtained were similary to those previously published. In a study comprising five German populations of each species MÜLLER-STARCK *et al.* (1993) found gene diversities of 0.272 and 0.288 at the species level for *Q. robur* and *Q. petraea* respectively (KREMER & PETIT 1993 from data of MÜLLER-STARCK *et al.* 1993). KREMER *et al.* (1991) found 0.264 for *Q. robur* and 0.266 for *Q. petraea* on a reduced sample of French populations. In the present study, covering a large part of the natural distribution, the diversity at the species level was 0.260 for *Q. robur* and 0.262 for *Q. petraea*. Although absolute values of gene diversity statistics were quite similar between the two species, in the three studies published, *Q. petraea* showed slightly higher values. This trend has been confirmed in a recent study where comparisons between the two species were based on nuclear DNA fragments (MOREAU *et al.* 1994).

Levels of diversity in the two European white oaks were similar to those found for North American species of the same section *Lepidobalanus*. The mean value of H_{es} obtained from 8 different species was, in that case, 0.257 (GUTTMAN and WEIGHT, 1989). Other North American examples showed lower levels of variation (H_{es} = 0.206 and 0.215 for *Q. macrocarpa* and *Q. gambelii*, in the section *Erythrobalanus*, SCHNABEL and HAMRICK, 1990). Levels of diversity in the white oaks were systematically higher to those found in the red oaks (H_{es} = 0.178 for the 10 species studied by GUTTMAN and WEIGHT 1989; 0.184 for *Q. laevis*, BERG & HAMRICK 1993). The values found in European and North American white oaks were amongst the highest in woody plants, when compared with data of the reviews made by HAMRICK and GODT (1990) and HAMRICK *et al.* (1992). Life history traits specific to oaks contributed to maintenance of high genetic diversities. The two species are almost strictly

Table 8 Interspecific Nei's genetic distances

Regions	Local distance	Inter–regional distance	
		D_1	D_2
Scandinavia	0.030	0.042 (0.018–0.032)	0.024 (0.021–0.058)
Southwest France	0.032	0.033 (0.016–0.057)	0.036 (0.014–0.050)
Serbia	0.061	0.040 (0.051–0.079)	0.059 (0.019–0.065)
Bavaria–Austria	0.036	0.041 (0.022–0.058)	0.039 (0.020–0.070)
Slovenia	0.082	0.051 (0.039–0.082)	0.055 (0.027–0.082)
Southern Alps	0.037	0.032 (0.019–0.052)	0.037 (0.014–0.058)
Romania	0.012	0.029 (0.012–0.027)	0.019 (0.012–0.039)

Table 9 Interspecific Mahalanobis genetic distances

Regions	Local distance	Inter–regional distance	
		D_1	D_2
Scandinavia	6.626	4.607 (3.635–6.626)	5.996 (2.881–8.709)
Southwest France	3.622	4.262 (2.918–6.318)	4.344 (1.682–6.861)
Serbia	5.342	5.633 (4.216–8.520)	5.143 (1.710–7.513)
Bavaria–Austria	5.153	5.102 (4.499–7.858)	5.069 (1.793–7.533)
Slovenia	8.791	6.802 (5.503–8.791)	6.725 (3.053–8.791)
Southern Alps	6.386	7.230 (5.587–8.709)	3.968 (1.305–6.386)
Romania	0.821	2.071 (0.821–3.053)	4.461 (0.821–5.856)

D_1: mean genetic distance between *Q. robur* of the designated region and *Q. petraea* of all other regions
D_2: mean genetic distance between *Q. petraea* of the designated region and *Q. robur* of all other regions
Values within brackets indicate minimum and maximum genetic distances.

allogamous (BACILIERI *et al.* 1933), they usually grow in wide pure stands and their geographic distribution is extremely large. Allogamy, large population sizes and vast geographic distribution have been shown to be statistically related to levels of diversity (HAMRICK- *et al.* 1992).

Population differentiation within species

Population differentiation within the two species was extremely low, even though populations originated from a wide geographic range. G_{st} was 3.2% in *Q. petraea* and 2.4% in *Q. robur* (Table 5). A slightly larger differentiation could be observed in *Q. petraea* for all loci except *Dia-A*. These results were also apparent from the genetic distances (Nei's or Mahalanobis (Table 7), where intra-specific distances were higher in *Q. petraea* than in *Q. robur*. Similar results were found in a study based on German populations (KREMER & PETIT 1993 from data of MÜLLER-STARCK *et al.* 1993). In a set of 32 French populations, the differentiation amounted to 1.7% (KREMER *et al.* 1991). Our results differed markedly with previous data obtained in other oak species (G_{st} = 12% in *Q. gambelii*, 8% in *Q. macrocarpa*, SCHNABEL & HAMRICK 1990) whereas in *Q. laevis* differentiation was of similar amount to that in our species (G_{st} = 3.2%, BERG & HAMRICK 1993).

Important gene flow was generally advocated for reduced population differentiation (HAMRICK *et al.* 1992); extended gene flow appeared facilitated in oaks forming a continuous distribution. Although the original distribution has been fragmented by farmers since three to five generations, numerous scattered stands still exist between larger forests, so that gene flow is still favoured. Whereas *Q. petraea* usually forms large stands, *Q. robur* is widespread throughout the natural range with numerous small stands regularly distributed. These differences might also have contributed to maintenance of greater gene flow in *Q. robur* than in *Q. petraea*.

Species differentiation

Genetic differentiation between the two species was extremely low, as shown by several results obtained in this study: (i) both species shared all alleles including rare alleles (Table 2), (ii) population differentiation within a species was of the same amount than species differentiation (Table 6), (iii) the range of

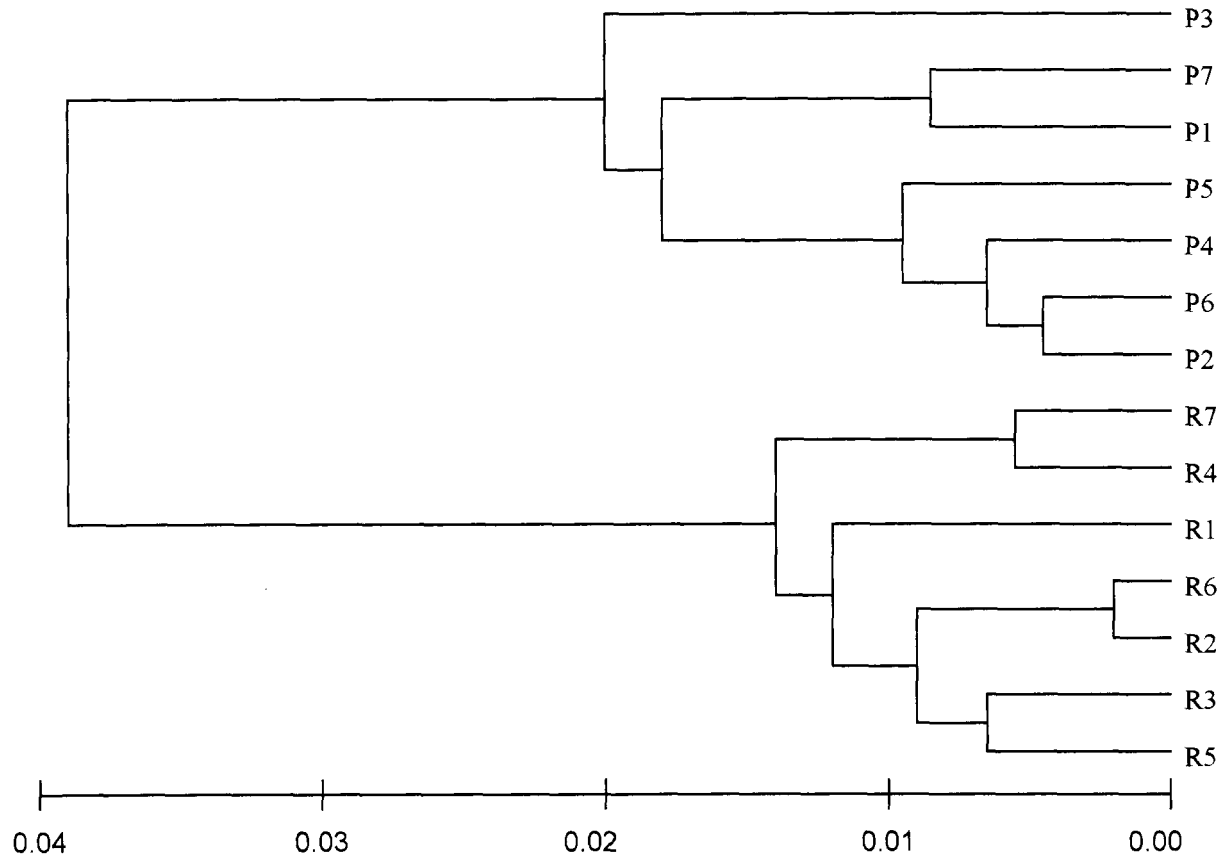

Figure 2 Diagram of Nei's genetic distances (for identification of populations, see table 1)

intra-specific genetic distances encompassed the range of inter-specific distances, although mean inter-specific values were superior to mean intra-specific values (Table 7). Major differences between the two species were due to their allelic frequency profiles, which were generally maintained from one region to another (Table 2). These differences appear to be the smallest encountered in oak complexes. In other examples (KREMER & PETIT 1993), the proportion of loci having the same common alleles, in all species forming a complex, varied from 44% to 94%. In our case, all loci shared alleles in the two species.

The comparison of local inter-specific distances did not indicate any geographic pattern of differentiation between the two species. In general, inter-regional inter-specific distances were of the same amount than local inter-specific distances, which indicates that the differentiation was maintained in a similar way in different geographic regions with the exception of the Balkans regions (Slovenia and Serbia). These results could be interpreted in two different ways. On the one hand, one might advocated an equilibrium between intra-specific gene flow (between different populations of the same species) and the local inter-specific gene flow. This hypothesis would result in similar values for intra-specific and inter-specific differentiation for all loci (Table 6). On the other hand, intra-specific and local inter-specific gene flow might have been counterbalanced by selective pressures within each species, leading to similar allelic frequency profiles in all populations of a given species. The discrepancy of G_{gt} and G_{sg} values between different loci (Table 6) could be considered to support of the second hypothesis.

As the number of populations studied in this paper was no very high and the populations not spreaded regularly in all the natural range of the species, these results have to be confirmed by a larger sampling. This is a programme running at present in the laboratory.

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