Isozyme Variation in Natural Populations of Cork-Oak (*Quercus suber* L.)

Population Structure, Diversity, Differentiation and Gene Flow

By J. A. ELENA-ROSSELLÓ and E. CABRERA

Departamento de Biologia Vegetal, Facultad de Biologia, Universidad de Salamanca, 37008 Salamanca (Spain)

(Received 26th July 1996)

Summary

Genetic variation of cork-oak (*Quercus suber* L.) was investigated in 7 Mediterranean populations in Spain using 13 loci from 7 enzyme systems. *Quercus suber* was found to possess higher values of heterozygosity (H = 0.288 ± 0.069), percent of polymorphic loci at the 99% criterion (P = 76.9%) and average number of alleles per locus (A = 2.46), than any other oak species. Interpopulation diversity was about 16.9% of the total diversity. The average estimated of Nm based on 10 loci is 2.57, and shows current or recent gene flow among populations. Genetic identities among populations were moderate, ranging from I = 0.829 to I = 0.999, and a cluster analysis revealed no patterns related to geography.

Our results revealed that the *Q. suber* populations studied share a common gene pool. The number of alleles and genotypes and the levels of genetic variability in the study populations, indicate that the Spanish populations are genetically rich. These high levels of genetic variability, coupled with the ecological and economic importance of the species make *Q. suber* a strong candidate for a gene conservation program.

Key words: Quercus suber, isozymes, population structure, gene diversity, gene flow, genetic differentiation, genetic identities.

FDC: 165.3; 165.5; 176.1 *Quercus suber;* (460).

Introduction

Quercus suber L. is a diploid (2n = 24) species. It is predominatly allogamous, anemophilous, and monoecious, with a life span to 500 years or more, and grows in climax communities of the Mediterranean area (COUNTINHO, 1889). Within its geographic range, cork-oak shows high levels of morphological and phenological variability; most of this diversity is considered to be the result of past introgressive hybridization (AMARAL FRANCO, 1990; RUSHTON, 1991). This polytypic species is thought to contain over 40 varieties (SCHWARTZ: *In:* TUTIN et al., 1964; VICIOSO, 1950; AMARAL FRANCO, 1990; VALSECHI, 1966). The species is widely distributed in the Mediterranean Basin, in both Europe and North Africa, where it occupies a total of 2.35 M Ha, of which 300,000 Ha to 500,000 Ha are in the Iberian Peninsula. *Quercus suber* is ecologically plastic and grows in warm humid conditions from sea level to 2,000 m. It occurs preferentially on siliceous soils, but it can also grow in poor and extremely acid soils of abrupt topography, where cork-oak is the only tree that can survive.

Cork-oak's economic importance is associated with harvesting of cork and acorns. Trees are first stripped of cork at about 14 years and subsequently at 9-year intervals and can survive this "surgery" from 100 to 500 years without apparent effect on the trees. In addition, the cork protects the trees against fire; therefore they have high capacity to regenerate after forest fire. Acorns are eaten by birds and they are highly valued as fattening feed for domestic Iberian pigs. Due to the economic value and also because cork-oak woodlands are renowned reservoirs of biodiversity, and home to a variety of threatened and endangered species (PEINADO-LORCA and MARTINEZ-PARRAS, 1987; HUNTSINGER et al., 1991) *Quercus suber* populations represent valuable material for genetic studies as well as gene conservation programs.

A description of the genetic structure of populations and of the distribution of genetic variation within and among populations in this species is necessary to permit informed decisions to be made about germplasm collections, tree breeding and the conservation of plant genetic resources (BROWN et al., 1990; ADAMS et al., 1992). Until recently, researchers analyzed only phenotypic (morphological, anatomical and physiological) traits of cork-oak populations (VALSECHI, 1966). However these traits are influenced by the interplay between environment and multiple genes. In order to find taxonomic criteria for the study of relationships among *Quercus* ssp., ever more sophisticated approaches are being used, including biochemical markers such as isozymes, cpDNA or rDNA (MANOS and FAIRBROTHERS, 1987; AFZAL-RAFII, 1988; GUTTMAN and WEIGT, 1989; BELLAROSA et al., 1990; MICHAUD, 1993; BORDACS and KORANYI, 1993).

Isozyme variation in the genus *Quercus* shows that genetic variability in oak species is high, and similar to that found in conifers (HAMRICK et al., 1992; HAMRICK and GODT, 1989; LEDIG and CONKLE, 1983; GONCHARENKO et al., 1994; WHITTEMORE and SCHAAL, 1991; KREMER et al., 1991; KREMER and PETIT, 1993). Analyses of the population structure and gene flow with WRIGHT's F-statistics show generally high levels of genetic variation within oak populations but moderate amount of geographic differentiation among them; gene flow is very high both within and among populations (DUCOUSSO et al., 1993).

Here we present analyses of the genetic structure, levels of variation and differentiation among Spanish populations in cork-oak using isozyme markers. Indirect methods for estimating levels and patterns of gene flow (WRIGHT's index) and distance and identity measures (NEI's coefficients) among populations were used. This is the first population-wide study of genetic variation in this Mediterranean oak species.

Table 1. – Codes, geographic locations and elevations of 7 *Quercus suber* populations sampled in the Iberian Peninsula.

Population designations	Geographic location	Elevation(m)
1. AQ	Cedillo (CC)	400
2. BQ	Rincón de Ballesteros (CC)	425
3. CQ	Sierra Madrona (J)	1000
4. DQ	Almoraima (CA)	200
5. EQ	Castellón (CS)	500-1000
6. GQ	Gabriel y Galán (CC)	520
7. HQ	Valdelosa (SA)	780

Materials and Methods

Materials

Collections of *Quercus suber* were taken from seven locations that were ecologically and geographically representative of all populations within Spain (*Figure 1* and *Table 1*).

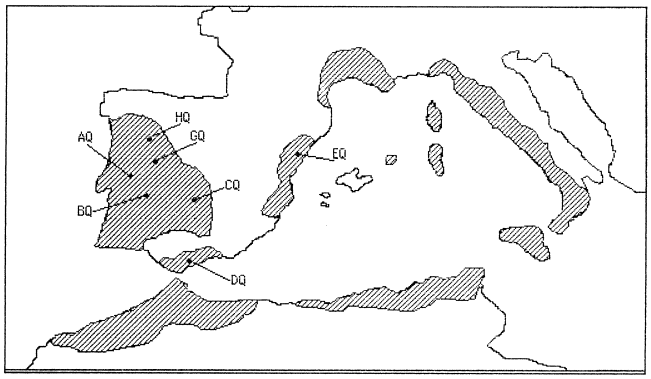

Figure 1. – Natural distribution of *Quercus suber* (hatched area) and locations of sampled populations (see *Table 1* for population codes).

Samples of 2 of the populations studied were collected in naturally generated forest (pop. GQ and HQ). The trees from the other 5 locations grew for 5 years in a nursery at "La Almoraima" (S.A. ICONA, Cadiz). These plants germinated from acorns collected in natural populations, grew in the greenhouse during their first year in "super-leach" tubes, and when 20 cm tall plants were placed into acid and sandy soil plots. Each plant was protected with a prismatic plastic ("tubex" or "finntubo") and watered only in summer.

At least 25 individuals were sampled per site, and at least 10 leaves per individual were collected to be used for electrophoresis. The leaves removed from the trees were placed with moist paper toweling into plastic bags, and the bags were kept refrigerated until processing.

Analyses of enzyme polymorphisms

Protein extraction was carried out using 350 mg to 700 mg leaves crushed with liquid N_2 in 2 ml Tris-HCl buffer (pH 7.6) and 30 mg of insoluble PVP. The homogenate was centrifuged for 15 min. at 20,000 rpm (40,000 x g) and the supernatant stored at −80 °C before analysis (ELENA-ROSSELLÓ, 1979). Horizontal starch electrophoresis (SELANDER et al., 1971) was employed using 12.5% Sigma starch (Sigma Chemical Co, St. Louis) and 3 buffer systems (*Table 2*). Staining protocols for 7 enzyme systems were those of SCANDALIOS, 1969 (ADH, EST, LAP, ACP), SHAW and PRASAD, 1970 (PER, SKD), and SHAW and KOEN, 1968 (SOD) with minor modifications in pH and concentrations of ingredients.

Table 2. – Enzymes and buffers systems used in electrophoresis analysis of cork-oaks.

Enzyme (Abbr.)	EC No	Loci	Gel&Tray Buffer	Alleles
Alcohol dehydrogenase (ADH)	1.1.1.1.2	Adh-1	A	2
		Adh-2		2
Leucine aminopeptidase (LAP)	3.4.1.1.1.	Lap-1	A	3
Peroxidase (PER)	1.11.1.7.	Per-1	A	4
		Per-2		4
		Per-3		3
		Per-4		1
		Per-5		1
Acid phosphatase (ACP)	3.1.3.2.	Acp-1	B	2
Alpha-esterase (α-EST)	3.1.1.1.	α-Est-1	A	4
Superoxide dismutase (SOD)	1.15.1.1.	Sod-1	B	1
		Sod-2		2
Shikimate dehydrogenase (SKD)	1.1.1.25.	Skd-1	C	2

A = Tris citrate, pH 8.3 & lithium borate, pH 8.3 (SCANDALIOS, 1969)
B = Tris citrate, pH 8.8 & sodium borate, pH 8.0 (FOWLER and MORRIS, 1977)
C = Tris citrate, pH 6.2 (NICHOLS and RUDDLE, 1973)

For the interpretation of banding patterns of gels, identification of monomeric vs. dimeric proteins, and homozygous vs. heterozygous individuals we followed methods discussed by PASTEUR et al. (1987), as well as similar studies in related species. Zones of activity varying independently of other zones were considered to be encoded by single loci. The loci are considered putative because no formal analyses of inheritance were done with this species. However, the patterns observed were consistent with those expected in species for which formal analyses have been carried out (e.g. YACINE and LUMARET, 1989; MICHAUD, 1993; BACILIERI, et al., 1994) The fastest anodally migrating zone of activity was designated 1, the next 2, and so on. When allelic variation occurred, alleles were numbered sequentially, with the fastest anodally migrating allele being number 1.

Computations of genotypic and allelic frequencies, statistics for HARDY-WEINBERG expectations and genetic diversity within populations, genetic identities and distances among populations and indices of population structure (F-statistics) were generated using the BIOSYS-1 (SWOFFORD and SELANDER, 1981) and GeneStrud computer programs (CONSTANTINE et al., 1994).

We obtained for each population the mean and variance of the number of alleles per locus (A), the percentage of polymorphic loci (Pl) at 99% and 95% criteria (the frequency of the most common allele was not greater than 0.99 or 0.95), the mean and variance of the observed (Ho) and expected (He) heterozygosity or total heterozygosity and WRIGHT's fixation

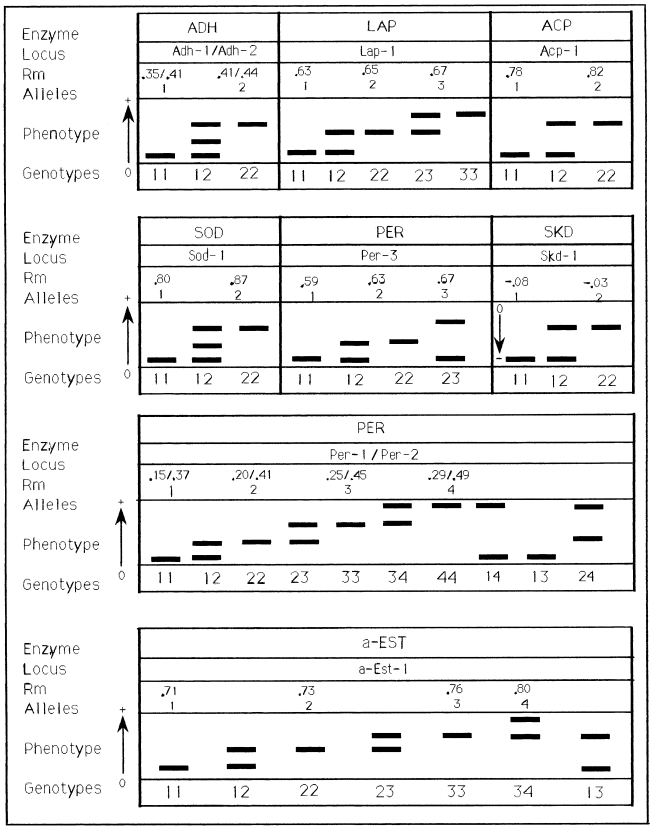

Figure 2. – Schematic representation of all electrophoretic variants revealed in *Quercus suber* populations. Individual alleles and their migration distances (Rm) are indicated.

Table 3. – Allelic frequencies for every locus in 7 populations of *Quercus suber*. Heterozygosity per locus: Ho (direct-count estimate), He (unbiased estimate) and WRIGHT's fixation index.

		Populations						
		AQ	BQ	CQ	DQ	EQ	GQ	HQ
	No. individuals	24	23	24	23	24	25	24
Adh-1	Allelic frequencies:							
	1	.125	.364	.354	.271	.229	.020	.146
	2	.875	.636	.646	.729	.771	.980	.854
	Ho	.167	.364	.292	.292	.208	.040	.125
	He	.223	.474	.467	.403	.361	.040	.254
	Variance	.176	.250	.250	.242	.233	.039	.143
	Fixation	.254	.232	.376	.277	.423	.000	.509
Adh-2	Allelic frequencies:							
	1	.625	.848	.667	.625	.688	.540	.583
	2	.375	.152	.333	.375	.313	.460	.417
	Ho	.583	.130	.417	.250	.458	.920	.667
	He	.479	.264	.454	.479	.439	.507	.496
	Variance	.250	.197	.249	.250	.248	.250	.250
	Fixation	-.219	.505	.082	.478	-.044	-.815	-.343
Lap-1	Allelic frequencies :							
	1	.271	.609	.479	.500	.500	.040	.208
	2	.729	.391	.521	.500	.500	.320	.771
	3	.000	.000	.000	.000	.000	.640	.021
	Ho	.375	.261	.375	.333	.333	.080	.458
	He	.403	.487	.510	.511	.511	.496	.370
	Variance	.242	.250	.250	.250	.250	.252	.236
	Fixation	.070	.464	.264	.347	.347	.839	-.240
Per-1	Allelic frequencies :							
	1	.271	1.000	.583	.667	.792	.700	.042
	2	.563	.000	.333	.271	.146	.240	.500
	3	.042	.000	.063	.063	.063	.060	.417
	4	.125	.000	.021	.000	.000	.000	.042
	Ho	.542	.000	.625	.500	.167	.600	.833
	He	.605	.000	.556	.488	.355	.458	.585
	Variance	.244	.000	.250	.253	.233	.252	.246
	Fixation	.086	.000	-.124	-.024	.531	-.310	-.424
Per-2	Allelic frequencies :							
	1	.022	.043	.000	.021	.000	.000	.021
	2	.044	.043	.021	.000	.063	.000	.042
	3	.511	.370	.417	.875	.896	.400	.500
	4	.422	.543	.563	.104	.042	.600	.438
	Ho	.533	.391	.458	.125	.125	.560	.417
	He	.571	.577	.520	.125	.125	.560	.565
	Variance	.248	.248	.251	.179	.160	.250	.248
	Fixation	.065	.322	.119	.451	.362	-.143	.267
Per-3	Allelic frequencies :							
	1	.087	.043	.563	.146	.188	.740	.750
	2	.913	.957	.417	.854	.813	.260	.250
	3	.000	.000	.021	.000	.000	.000	.000
	Ho	.087	.087	.542	.125	.208	.200	.167
	He	.162	.085	.520	.254	.311	.393	.383
	Variance	.138	.079	.251	.192	.217	.240	.238
	Fixation	.464	-.023	-.041	.509	.330	.491	.565
Acp-1	Allelic frequencies :							
	1	.000	.000	.000	.000	.000	.000	.188
	2	1.000	1.000	1.000	1.000	1.000	1.000	.813
	Ho	.000	.000	.000	.000	.000	.000	.125
	He	.000	.000	.000	.000	.000	.000	.311
	Variance	.000	.000	.000	.000	.000	.000	.217
	Fixation	.000	.000	.000	.000	.000	.000	.598
Est-1	Allelic frequencies :							
	1	.239	.022	.563	.188	.250	.120	.292
	2	.696	.978	.438	.813	.750	.140	.396
	3	.065	.000	.000	.000	.000	.720	.271
	4	.000	.000	.000	.000	.000	.020	.042
	Ho	.522	.043	.625	.375	.500	.320	.667
	He	.465	.043	.503	.311	.383	.456	.698
	Variance	.252	.042	.250	.217	.238	.253	.217
	Fixation	-.123	.000	-.243	-.205	-.306	.299	.044
Sod-2	Allelic frequencies :							
	1	1.000	1.000	1.000	.750	.979	.920	1.000
	2	.000	.000	.000	.250	.021	.080	.000
	Ho	.000	.000	.000	.417	.042	.000	.000
	He	.000	.000	.000	.383	.042	.150	.000
	Variance	.000	.000	.000	.238	.041	.130	.000
	Fixation	.000	.000	.000	-.088	.000	1.000	.000
Skd-1	Allelic frequencies :							
	1	.000	.000	.000	.000	.250	.000	.000
	2	1.000	1.000	1.000	1.000	.750	1.000	1.000
	Ho	.000	.000	.000	.000	.417	.000	.000
	He	.000	.000	.000	.000	.383	.000	.000
	Variance	.000	.000	.000	.000	.238	.000	.000
	Fixation	.000	.000	.000	.000	-.088	.000	.000

index (F) estimated as 1- Ho/He. Finally, we obtained distance (D) and identity (I) measures between all pairs of populations (NEI, 1978; ROGERS, 1972). Distance and Identity measures are based only on those loci polymorphic in all populations. Measures of gene diversity include the estimates, for each locus, of (Ho) total observed heterozygosity, the allelic diversity within-population (Hs) and the total (Ht) allelic diversity. WRIGHT's fixation index, Fis, Fst and Fit, were calculated for each locus separetly, and then averaged over all loci. The basic formula used was 1-Fit = (1-Fis) (1-Fst). The deviation of Fis

and Fst from 0 was tested by the contingency Chi-square. We estimated Nm, the number of migrants omong populations per generation, using a numerical method (WRIGHT, 1951) where Fst = 1/(1 + 4Nm).

The genetic relationship among populations were summarized with an UPGMA dendrogram using NEI's genetic identity coefficient.

Results and Discussion

Enzyme phenotypes

All the electrophoretic allelic variants detected in our study are shown schematically in *figure 2*. LAP, PER, ACR, α-EST and SKD were monomeric, ADH and SOD were dimeric.

Allelic diversity

A total of 30 alleles at 13 putative loci could be identified in the 168 individual cork-oak trees analyzed. Allelic frequencies for all the alleles at 10 polymorphic loci, observed and expected heterozygosity and WRIGHT's fixation index values are listed in *table 3*. From the table it can be seen that of 30 alleles, 8 appeared to be rare, with frequencies under 10%. Three alleles distributed among three loci appear to be private to specific populations and two others belonging to two different loci were found in only two populations.

Six loci (60%) were polymorphic in all the populations (Adh-1, Adh-2, Lap-1, Per-2, Per-3 and Est-1) and they were the most variable loci. Their expected heterozygosities (He) ranged between 30% to 50% in almost all populations, although we observed marked interpopulation differences in these values. For example expected heterozygosity in Est-1 ranges from 4.3% in population BQ to 69.8% in HQ. Acp-1, Sod-2 and Skd-1 appear to be the least variables with heterozygosity less than 9.5%. Per-4, Per-5 and Sod-1 were monomorphic over all the populations studied.

WRIGHT's fixation index (*Table 3*) indicates a deficiency of heterozygotes at four loci (Adh-1, Lap-1, Per-2 and Per-3) in many populations. In contrast, excesses of heterozygotes at loci Adh-2, Per-1 and Est-1 were found in almost all populations. At locus Sod-2 and Skd-1, F-values were zero in almost all populations.

Table 4. – Estimates of genetic diversity for *Q. suber* populations. Mean and variance of the number of alleles per locus (A), percentage of polymorphic loci at 99% and 95% criteria (Pl), the mean and variance of observed (Ho) and expected (He) heterozygosity or total diversity. Mean average values across the 7 populations (Av). Q* (combined population, N = 168) values at species level.

	Ho	A	Pl freq<.99	Pl freq.<.95	He
AQ	.214±.050	1.92±1.244	53.8	53.8	.222± .068
BQ	.098±.010	1.61± .756	46.1	30.7	.148± .061
CQ	.251±.068	2.07± .744	76.9	53.8	.289± .069
DQ	.186±.037	1.77± .526	61.5	61.5	.235± .058
EQ	.189±.039	1.85± .474	69.2	61.5	.229± .054
GQ	.209±.047	1.92± .910	61.5	53.8	.230± .065
HQ	.266±.077	2.15±1.474	61.5	61.5	.282± .072
Av.	.202±.054	1.90± .875	65.8	62.8	.233± .064
-Q*	.202±.044	2.46±1.103	76.9	61.5	.288± .069

Variables summarizing genetic variation are presented in *table 4*. The mean for the average number of alleles per locus (A) was 2.46. This value varies from 1.61 in population BQ to 2.15 in population HQ. Percent of polymorphic loci (Pl) at the 99% criteria varies from 0.77 in population CQ to 0.46 in population BQ; 0.77 and 0.61 were the means for the species at 99% and 95% criteria, respectively. The mean values for He and A were 0.29 and 2.46, respectively. Population BQ had the lowest level of genetic variation, with He = 0.148 and A = 1.61. Populations CQ and HQ were the most variable, with He = 0.289 and 0.282, and A = 2.07 and 2.15, respectively.

Genetic structure

Estimates of population structure and gene flow were determined using all seven populations (*Table 5*). The Fis values calculated over all the populations ranged from –0.094 at Adh-2 to 0.530 at Acp-1, where only 3 individuals in population HQ were heterozygotes. Of the 10 polymorphic loci, 6 (60.0%) had positive and significant F-value. The value of observed heterozygosity (Ho = 26.2%) was lower than expected (Hs = 30.4%) heterozygosity and total diversity (Ht = 37.3%) within populations. The average Fis value was positive (0.173) showing a slight deficit of heterozygotes within populations. At the same time, the Fit value was 0.273 indicating a heterozygote deficiency in Spain cork-oak as a whole.

Table 5. – NEI's gene diversity calculated over all populations, for each locus. Variables are as follows: Total observed heterozygosity (Ho), within populations expected heterozygosity (He), gene diverity (Ht), WRIGHT's fixation index within (Fis) among populations (Fst) and total genetic differentiation (Fit). Number of migrants per generation (Nm).

Locus	Ho	Hs	Ht	Fis	X^2	Fst	X^2	Fit	Nm
Adh-1	.208	.315	.331	.339	19.2**	.050	16.6*	.372	4.75
Adh-2	.486	.444	.453	-.094	1.5	.021	7.0	-.071	11.6
Lap-1	.319	.473	.571	.326	17.7**	.171	115 **	.441	1.21
Per-1	.474	.440	.575	-.077	1.0	.235	235 **	.176	0.81
Per-2	.366	.458	.535	.201	6.7**	.143	143 **	.315	1.50
Per-3	.199	.300	.469	.337	19.0**	.360	240 **	.576	0.44
Acp-1	.024	.051	.058	.530	47.0**	.120	40.1**	.587	1.83
α-Est-1	.424	.405	.561	-.047	0.4	.277	278 **	.243	0.65
Sod-2	.065	.095	.107	.310	16.1*	.110	36.8**	.386	2.02
Skd-1	.060	.055	.069	-.090	1.3	.209	69.7**	.138	0.95
Av.	.262	.304	.373	.173	130**	.169	1181**	.316	2.57

*) level of significance < 0.05
**) level of significance < 0.01

We observed high levels of gene diversity within populations; conversely, inter-population variability, Fst average value (*Table 5*) indicated that most of the total genetic diversity in the species is found within rather than among populations. More than 83% of the total diversity in this species is within populations. The level of genetic diversity among populations was estimated at 16.7%. The lack of population divergence, and the presence of high within-population variation have been found for several conifers (MITTON et al., 1977; LINHART et al., 1981; HATTEMER et al., 1991), and can be interpreted in relation to the main characteristics of forest trees, i.e., long life – span, allogamy, wind-pollination, monoecy and continuous geographical distribution (HAMRICK et al., 1981) and perhaps in terms of the age of the populations and the extent of gene flow (DUCOUSSO et al., 1993).

When these results are compared to those obtained with other oak species, cork-oak seems like other oak species showing a deficit of heterozygotes within populations. The values obtained in most of the oak species studied, *Q. macro-*

carpa and *Q. gambelii* (SCHNABEL and HAMRICK, 1990), *Q. rubra* (SORK et al., 1993) and *Q. agrifolia, Q. lobata* and *Q. douglasii* (MICHAUD, 1993), indicated a slight deficit of heterozygotes too. Only in *Q. ilex* (YACINE and LUMARET, 1989; MICHAUD, 1993) and *Q. rubra* (SCHWARZMANN, 1991) fixation indices were negative, but near zero, indicating a situation close to panmixia.

The estimate of Nm based on Fst values was 2.57. This means that gene flow among *Quercus suber* populations is slightly greater than 1 migrant per generation. This value is one of the lowest values reported for any other species of oak (YACINE and LUMARET, 1988; MANOS and FAIRBROTHERS, 1987; SCHNABEL and HAMRICK, 1990; KREMER et al., 1991; SCHWARZMANN, 1991; MICHAUD, 1993; SORK et al., 1993); only the Nm among populations of *Q. rubra* (KREMER et al., 1991) and the interspecific gene flow among members of the *Q. alba* "complex" (WHITTEMORE and SCHAAL, 1991) showed lower values than that found in *Q. suber*.

Three loci (Per-3, Est-1 and Per-1) differ sufficiently from the others in this values of Fst that it is reasonable to conclude, according to SLATKIN (1987), that these loci or other loci closely linked to them are subject to strong natural selection favoring different alleles in different populations.

Our results about genetic structure of cork-oak populations (i.e. slight deficit of heterozygotes within populations, and low Nm value) show that *Q. suber* is rather different from other European oak species, which are actively exchanging genes (DUCCOUSSO et al., 1993), but has a gene flow among populations sufficiently high to prevent local differentiation due to genetic drift (WRIGHT, 1951).

Genetic distance

Cluster analysis based on unbiased genetic distance (NEI, 1978) and the UPGMA dendrogram (SNEATH and SOKAL, 1973) reveal low levels of genetic distance among populations. As seen in *table 6* and *figure 3*, most of the populations (AQ, CQ, DQ and EQ) have high levels of similarity to each other with mean identities values ranging between 0.94 and 0.93.

Table 6. – Matrix of NEI's genetic identity (above diagonal) and ROGERS' genetic distance (below), between all populations of *Quercus suber*. I* values: average of paired identities between each population and other population.

	AQ	BQ	CQ	DQ	EQ	GQ	HQ	I*
AQ	*****	.932	.953	.972	.963	.869	.942	.938
BQ	.143	*****	.938	.955	.959	.829	.842	.909
CQ	.138	.156	*****	.946	.941	.913	.959	.943
DQ	.116	.133	.135	*****	.999	.851	.901	.937
EQ	.132	.134	.144	.068	*****	.856	.900	.937
GQ	.203	.243	.157	.222	.237	*****	.914	.872
HQ	.121	.246	.135	.211	.219	.161	*****	.909

Major differencies in genetic constitution occurred between GQ (from Gabriel y Galán) and BQ (from Rincon de Ballesteros); the genetic identity between them is 0.829. The forest in Rincon de Ballesteros, which has low levels of isozyme variability, represents a plant community where the mesomediterranean cork-oaks reach their optimum state (acid soils, mean temperatures over 25 °C and 602 mm/year precipitation); meanwhile the Valdelosa (HQ) population is located in an ecologically marginal site for the species. At this site cork-oak reaches its Northern limits, grows on basic soils exposed to extreme temperatures (ranging between 38 °C to −10 °C) and some of the lowest precipitation (472 mm/year) found in the species' range. In this population, the heterozygosity atains one

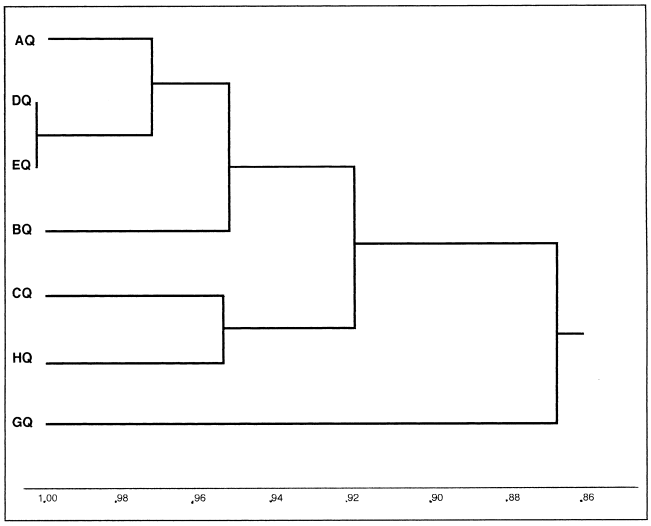

Figure 3. – Dendrogram of populations, relationships using NEI (1978) unbiased genetic identity.

of the highest values of all populations studied. This results is in contrast to the hypothesis of low genetic variability in marginal populations (MAYR, 1963). In this case the adaptative strategy of the species in marginal populations appears to be associated with a high genetic variability as already was reported by PRUS-GLOWACKI and STEPHAN (1994) for coniferous species.

The most differentiated (I = 0.872) population was from Gabriel y Galán (GQ); it shows significant differences from the others, caused by divergent genotype frequencies. This divergence from the other populations could be partialy explained as a result of introgressive hybridization between the 2 sympatric oak species (cork-oak and holm-oak (*Quercus ilex*)) present in this location. Previous studies in this area (ELENA-ROSSELLÓ et al., 1992) suggested that the interspecific hybridization may occur. The results obtained from this study provide further support for this hypothesis.

From the dendrogram (*Figure 3*) it can be seen that clustering did not depend on geographical proximity of distance among locations.

Conclusions

Cork-oak is a highly variable species, with levels of genetic variability comparable to those in *Q. ilex* (LUMARET and MICHAUD, 1991; MICHAUD, 1993) and *Q. douglasii* (MICHAUD, 1993), but containing more variation than those reported by MANOS and FAIRBROTHERS (1987), in 6 American red oaks (*Quercus* sp.), KREMER and PETIT (1993) in 21 oak species, or HAMRICK et al., (1992) in 322 long-lived tree species.

The high genetic diversity found at the isozyme level in *Q. suber* populations is congruent with the high variability observed among cork-oak individuals within populations in relation to quality and cork production, tree architecture, phenology and bitter vs. sweet acorn production. It is interesting that these morphological and physiological characters also show greater levels of within than among population variation (VALDECANTOS, pers. com.; VICIOSO, 1950; VALSECHI, 1966). One of the main causes of the high polymorphism found in cork-oak as well as its relative the holm-oak may be attributable to the physiological plasticity of the species, which enables them to adapt to variable and unpredictable climatic conditions, characteristic of the mediterranean climate. For example, cork-oak in particular, shows 2 well-differentiated

reproductive strategies (ELENA-ROSSELLÓ et al., 1993), "annual", when fruit ripening occurs within the same season as flowering, vs. "biennal" which requires two full years to ripen their fruits. This difference may reflect an adaptative response to subhumid or to harsh climatic conditions.

The two species -holm and cork-oak- also show variation both in flowering time and in investment to reproductive and or vegetative activities (LUMARET et al., 1991; MICHAUD et al., 1992; ELENA-ROSSELLÓ et al., 1993). Investment in reproductive structures and in vegetative functions was found to be extremely variable among individuals within cork-oak populations and for individuals amoung years.

Most of the isozyme variability in *Quercus suber* is found within populations showing a significant heterogeneity of allele frequencies among populations. This result suggests that, within the range of cork-oak distribution that we studied, a common gene pool is shared.

Gene flow among populations is estimated as more than one migrant per generation and is theoretically sufficiently strong to prevent genetic drift from causing local genetic differentiation and therefore population divergence (SLATKIN, 1987). Gene flow can occur owing to transfer of pollen and seeds; in any case our data show that *Q. suber* populations exchange genetic material at present, or exchanged it not long ago, quite intensively, and as a result they have similar gene pools, regardless of the fact that most of them are isolated today.

In fact, the principal human activities in the cork-oak woodlands called "dehesa", including vegetation clearing, forestry management and the cork-oak reafforestation, appear to be one of the most important causes opperating to reduce genetic variability among populations. Reafforestation is usually done with selected acorns belonging to the natural populations (MONTOYA-OLIVER, 1988) and may have contributed significantly to fashion the present genetic makeup of the cork-oak populations because these acorns are moved about quite liberally over distances of several km. The moderate differentiation among populations seems to be therefore a consequence of partial domestication of the original species.

Some cork-oak populations show some differentiation perhaps associated with local conditions. For example, our results suggest that the genetically richest populations (in number of polymorphic loci, alleles and genotypes) seem to be associated with marginal sites of the species; in contrast populations in which cork-oak grow under optimum conditions appear to be less variable. The cluster analysis does not show close relationships among populations from each geographic area (latitude, longitude, altitude). This pattern has also been observed in most of the studied populations of forest trees, including oak species (CHECHOWITZ and CHAPPEL, 1990).

The comparisons between morphological and isozymic variation on cork-oak individuals studied confirm the absence of a clearly-identifiable relationship between morphology and allozymes, as was indicated in previous studies carried out by CLAVERO (1988). The disparity among morphological and electrophoretic data suggests that natural selection is operating differently on the morphological and isozymic characteres. This has been reported for other trees including oaks and appear to be common observed in many species (CHECHOWITZ and CAPPEL, 1990; LINHART, 1988).

On the whole our results with the Spanish populations of *Quercus suber* show a genetically variable species, which may help to explain its great ecological plasticity. The sexual reproductive characteristics of the cork-oak, and the occurrence of inter-specific hybridization, associated with its long life span and allogamy, have permitted the existence and maintainance of the great genetic diversity within the species. These high levels of genetic variability, coupled with the ecological and economic importance of the species make *Q. suber* a strong candidate for a gene conservation program.

Acknowledgements

We would like to thank Prof. Y. B. LINHART for review and comments on an earlier draft of this paper and for linguistic help, Profs. J. B. MITTON, Y. A. EL-KASSABY and an anonymous referee for valuable comments and suggestions. We also thank Drs. A. PREMOLI and J. L. G. VALDECANTOS for their assistance. Support was provided by a grant from INIA (Proy. n. 9547) Spain.

Literature

ADAMS, W. T., STRAUSS, S. H., COPES, D. L. and GRIFFIN, A. R.: Population genetics of forest trees. Kluwer Academic, Dordrecht and Boston (1992). — AFZAL-RAFII, Z.: Caracteristiques taxonomiques, morphologiques et isoenzymatiques du complexe "chene vert". Bull. Soc. Bot. Fr. **135**: 343–352 (1988). — AMARAL FRANCO, J. DO,: Flora Iberica. II. Real Jardin Botanico. C.S.I.C., Madrid (1990). — BACILIERI, R., LABBE, T. and KREMER, A.: Intraspecific genetics structure in a mixed populations of *Quercus petraea* (MATT.) LEIBL and *Q. robur* L. Heredity **73**: 130–140 (1994). — BELLAROSA, R., DELRE, V., SCHIRONE, B. and MAGGINI, F.: Ribosomal RNA genes in *Quercus* ssp. (Fagaceae). Pl. Syst. Evol. **172**: 127–139 (1990). — BORDAS, S. and KORANYI, P.: Electrophoretic differentiation possibilities within the genus *Quercus* by means of protein monomers. Silvae Genetica **42**(6): 285–288 (1993). — BROWN, A. H. D., CLEGG, M. T., KAHLER, A. L. and WEIR, B. S.: Plant population genetics, breeding, and genetic resources. Sinauer Associates, Sunderland, Massachussetts (1990). — CHECHOWITZ, M. and CHAPPEL, D. M.: Morphological, electrophoretic and ecological analysis of *Quercus macrocarpa* populations in the Black Hills of South Dakota and Wyoming. Can J. Bot. **68**: 2185–2194 (1990). — CLAVERO, L.: Aproximacion al estudio del polimorfismo enzimatico en *Quercus suber* L. Memoria de Licenciatura. Departamento de Biologia Vegetal, Universidad de Salamanca (1988). — CONSTANTINE, C. C., HOBBS, R. P. and LYMBERRY, A. J.: Fortran Programs for Analyzing Population structure from Multilocus Genotypic Data. The Journal of Heredity **85**(4): 336–337 (1994). — COUTINHO, A. X. P.: Os *Quercus* do Portugal. Boletin da Soc. Broteriana **6**: 47–116. Coimbra (1889). — DUCOUSSO, A., MICHAUD, H. and LUMARET, R.: Reproduction and gene flow in the genus *Quercus* L. Ann. Sc. For. **50**(1): 91–106 (1993). — ELENA-ROSSELLO, J. A.: Identification of a species-specific enzyme marker (Locus Esterase-E) in *Thymus* sp. J. of Heredity **70**: 147–149 (1979). — ELENA-ROSSELLO, J. A., LUMARET, R., CABRERA, E. and MICHAUD, H.: Evidence for hybridization between sympatric holm-oak and cork-oak in Spain based on enzyme markers. Vegetatio **99–100**: 115–118 (1992). — ELENA-ROSSELLO, J. A., DEL RIO, J. M., G. VALDECANTOS, J. L. and G. SANTAMARIA, I.: Ecological aspects of the floral phenology of the cork-oak (*Quercus suber* L.); why do annual and biennial biotypes appear? Ann. Sc. For. (1993). — FOWLER, D. P. and MORRIS, R. W.: Genetic diversity in red pine: evidence for low genetic hetrozygosity. Can. J. Forest Res. **7**: 343–347 (1977). — GONCHARENKO, G. G., SILIN, A. E. and PADUTOV, V. E.: Allozyme variation in natural populations of eurasian pines. III. Population structure, Diversity, Differentiation and gene flow in Central and Isolated populations of *Pinus sylvestris* in Eastern Europe and Siberia. Silvae Genetica **43**, 2–3 (1994). — GUTTMAN, S. I. and WEIGT, L. A.: Electrophoretic evidence of relationships among *Quercus* (oaks) of eastern North America. Can. J. Bot. **67**: 339–351 (1989). — HAMRICK, J. L. and GODT, M. J.: Allozyme diversity in plant species. *In:* A. H. D. BROWN, M. T. CLEGG, A. L. KAHLER and B. S. WEIR (eds.): Populations genetics and germplasm resources in crop improvement. 44–64. Sinauer, Suderland, M. A. (1986). — HAMRICK, J. L., GODT, M. J. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — HAMRICK, J. L., MITTON, J. B. and LINHART, Y. B.: Levels of genetic variation in trees: Influence of life history characteristics. In: CONCKLE, M. T. (ed.): Proceedings of the Symposium on Isozymes of North American Forest Trees and Forest Insects. Berkeley. Forest Service, General Technical Report, PSW **48**: 35–41 (1981). — HATTEMER, H. H., FINESCHI, S., CANNATA, F. and MALVOLTI, M. E. (eds.): Biochemical markers in the population genetics of forest trees. SPB Academic Publishing bv., The Hague (1991). — HUNTSINGER, L., BARTOLOME, J. W. and STARRS, P. F.: Comparison of management strategies in the oak woodlands of Spain and California. Gen. Tech. Rep. PSW-126 (1991). — KREMER, A. and PETIT, R. J.: Gene diversity in natural populations of oak species. Ann. Sci. For. **50**: 186–202 (1993). — KREMER, A., PETIT, R. J., ZANETTO, A., FOUGERE, V., DUCOUSSO, A., WAGNER, D. and CHAUVIN, C.: Nuclear and organelle gene diversity in *Quercus robur* and *Quercus petraea*. MÜLLER-STARCK, G. and ZIEHE, M.

(eds.). Genetic variation in European populations of forest trees. J. D. Sauerländer's Verlag, Frankfurt am Main (1991). — LEDIG, F. T. and CONCKLE, M. T.: Gene diversity and genetic structure in a narrow endemic. Torrey pine (*Pinus torreyana* PARRYLEX CARR). Evolution **37**: 79–85 (1983). — LINHART, Y. B.; Ecological and evolutionary studies of Ponderosa pine in the Rocky Mountains. In: D. M. BAUMGARTNER and LOTAN, J. E. (eds.): Ponderosa Pine: The species and its management. Cooperative Extension. Washington State University, Pullman, WA. (1988). — LINHART, Y. B., MITTON, J. B., STURGEON, K. B. and DAVIS, M. L.: Genetic variation in space and time in a population of ponderosa pine. Heredity **46**: 407–426 (1981). — LUMARET, R. and MICHAUD, H.: Genetic variation in Holm oak populations. In: G. MÜLLER-STARCK and M. ZIEHE (eds.). Genetic variation in European population of Forest Trees. Sauerländer's Verlag, Frankfurt. 167–172 (1991). — LUMARET, R., YACINE, A., BERROD, A., ROMANE, F. and LI, T. X..: Mating system and genetic diversity in holm oak (*Quercus ilex* L. Fagaceae). FINESCHI, S., MAVOLTI, M. E., CANNATA, F. and HATTEMER, H. H. (eds.). Biochemical markers in the populations genetics of forest trees. SPB Academic Publishing bv, The Hague, The Netherlands (1991). — MANOS, P. S. and FAIRBROTHERS, D. E.: Allozyme variation in population of six Northeastern American red oaks (Fagaceae: *Quercus* subg. *Erytrobalanus*). Systematic Botany **12**(3): 365–373 (1987). — MAYR, E.: Animal species and Evolution. Harvard Univ. Press, Cambridge (1963). — MICHAUD, H.: Etude de la variabilite genetique du chene vert (*Quercus ilex* L.) a l'aide de marqueurs enzymatiques et moleculaires. These the doctorat, Univ. Droit, Econ. Sc. Aix-Marseille (1993). — MICHAUD, H., LUMARET, R. and ROMANE, F.: Variation in the genetic structure and reproductive biology of holm oak populations. Vegetatio **99–100**: 107–113 (1992). — MITTON, J. B., LINHART, Y. B., HAMRICK, J. L. and BECKMAN, J. S.: Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor. Appl. Genet. **57**: 5–13 (1977). — MONTOYA OLIVER, J. M.: Los Alcornocales. Ministerio de Agricultura pesca y Alimentacion. Series Manuales Tecnicos SEA, Madrid (1988). — NEI, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583–590 (1978). — NICHOLS, E. A. and RUDDLE, F. H.: A review of enzyme polymorphism, linkage and electrophoretic conditions for mouse and somatic cell hybrids in starch gels. J. Histochem. and Cytochem **21**: 1066–1081 (1973). — PASTEUR, N., PASTEUR, G., BONHOMME, F., CATALAN, J. and BRITTON-DAVIDIAN, J.: Manuel technique de genetique par electrophorese des proteines. Technique et Documentation (Lavoisier) (1987). — PEINADO LORCA, J. and MARTINEZ PARRAS, P.: La vegetacion de España. Serv. Publicaciones Universidad de Alcala, Madrid (1987). — PRUS-GLOWACKI, W. and STEPHAN, B. R.: Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. Silvae Genetica **43**(1): 7–14 (1994). — ROGERS, J. S.: Measures of genetic similarity and genetic distance. Studies in Genetics. Univ. Texas Publ. 7213: 145–153 (1972). — RUSHTON, B. S.: Natural hybridization within the genus *Quercus* L. IUFRO Meeting. Genetics of Oak species. Nogent sur Vernisson (1991). — SCANDALIOS, J. G.: Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Genet. **3**: 37–79 (1969). — SCHNABEL, A. and HAMRICK, J. L.: Comparative analysis of population genetic structure in *Quercus macrocarpa* and *Q. gambelii* (Fagaceae). Syst. Bot. **15**: 240–251 (1990). — SCHWARTZ, O.: *Quercus*. In: TUTIN, T. G. et al. (eds.): Flora Europaea. Vol. I. Cambridge University Press, London (1993). — SCHWARZMANN, J. F.: Genetic Structure and mating system of northern red oak (*Quercus rubra* L.) in Pensylvania. For. Sci. **37**: 1376–1389 (1991). — SELANDER, R. K., SMITH, M. M., YANG, S. Y., JOHNSON, W. E. and GENTRY, J. B.: Biochemical polimorphism in the genus Peromyscus. I. Variation of the old-field mouse. Stud. Genetics, Univ. Texas, 7103: 49–90 (1971). — SHAW, CH. R. and KOEN, A. L.: Starch gel zone electrophoresis of enzymes. In: SMITH, I. (ed.): Chromatographic and electrophoretic techniques. Volume II. Bath, England, Pitman Press: 325–364 (1968). — SHAW, CH. R. and PRASAD, R.: Starch gel electrophoresis of enzymes a compilation of recipes. Biochem. Genet. **4**: 297–320 (1970). — SLATKIN, M.: Gene flow and the geographic structure of natural populations. Science **236**: 787–792 (1987). — SNEATH and SOKAL: Numerical Taxonomy. Freeman, San Francisco (1973). — SORK, V. L., HUANG, S. and WIENER, E.: Macrogeographic and fine scale genetic structure in North American oak species, *Quercus rubra* L. Ann. Sci. For. **50**: 261–270 (1993). — SWOFFORD, D. L. and SELANDER, R. B.: BIOSYS-1: a FORTRAN programe for the comprehensive analysis of electrophoretic data in population genetics and systematics. The Journal of Heredity **72**: 281–283 (1981). — VALSECHI, F.: Contributo alla conescenza delle *Quercus suber* L. presenti in Sardegna. I, II and III. Staz. Sperim. Sughero. Temp. Pausania. Mem. **14**: 1–6 (1966). — VICIOSO, C.: Revision del genero *Quercus* en Espana. I.F.I.E., Madrid (1950). — WHITTEMORE, A. T. and SCHAAL, B. A.: Interspecific gene flow in sympatric oaks. Proc. Natl. Acad. Sci., USA **88**: 2540–2544 (1991). — WRIGHT, S.: The genetical structure of populations. Ann. Eugenics **15**: 323–354 (1951). — YACINE, A. and LUMARET, R.: Distribution spatial des genotypes dans une population de chene vert (*Quercus ilex* L.), flux genique et regime de la reproduction. Genet. Sel. Evol. **20**(2): 181–198 (1988). — YACINE, A. and LUMARET, R.: Genetic diversity in holm-oak (*Quercus ilex* L.): Insight, from several enzyme markers. Silvae Genetica **38**: 140–148 (1989).

Genetic Parameter Estimates for Resistance to Rust (*Cronartium quercuum*) Infection from Full-Sib Tests of Slash Pine (*Pinus elliottii*), Modelled as Functions of Rust Incidence[1])

By M. J. DIETERS[2]), G. R. HODGE[3]) and T. L. WHITE

Department of Forestry, University of Florida, Gainesville, FL 32611, USA

(Received 9th July 1996)

Abstract

Data from 171 slash pine progeny tests, incorporating over 700 different families from more than 2100 first-generation parents and approximately 170000 trees, were used to estimate variance and covariance components by Restricted Maximum Likelihood (REML) in both single-site and paired-site analyses. From these REML estimates, genetic parameters (heritabilities, proportion of dominance, type B genetic correlations, and age-age genetic correlations) were estimated for resistance to fusiform rust infection at 4 to 15 years of age. Predictive models were developed for biased (single-site) heritability, unbiased (paired-site) heritability and the type B genetic correlation. Biased heritability exhibited a maximum of 0.20 at an average rust infection of 72%. Unbiased heritability estimates from paired-site analyses increased linearly with increasing average rust infection in the tests; however, in very few test pairs did the average rust infection exceed 75%, and

[1]) This is Florida Agricultural Experiment Station Journal Series No. R-03963 of the Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611. We gratefully acknowledge members of the Cooperative Forest Genetics Research Program who supplied the data that made these analyses possible. Also the authors wish to thank Drs. D. L. ROCKWOOD, M. DELORENZO, R. C. LITTELL, R. BURDON and N. BORRALHO for their critical review of this paper.

[2]) Current address: Queensland Forestry Research Institute, MS 483, Fraser Rd., Gympie, Qld. 4570, Australia

[3]) Current address: CAMCORE, Box 7626, Grinnels Lab., North Carolina State University, Raleigh, NC 27695-7626, USA.