Genetic Variation in Central and Marginal Populations of *Quercus suber* L.

By P. JIMÉNEZ[1]), D. AGÚNDEZ[2]), R. ALÍA[2]) and L. GIL[1])[3])

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Summary

Eighteen spontaneous populations of cork oak (*Quercus suber*) from Spain (14), Portugal (1), Morocco (1) and Italy (2), were surveyed at 14 loci from 12 enzyme systems. Percentage of polymorphic loci (64%), mean number of alleles (2.07), and mean expected heterozygosity (0.158) values were within the ranges described for the genus. Populations from the central range of the species and from peripheral areas were evaluated, and differences between these two kinds were assessed. Significant lower diversity (number of alleles and expected heterozygosity) was found for the most isolated and small size populations in contrast to central forests, showing the existence of mechanisms maintaining the levels of diversity even in some isolated stands. Interpopulation diversity (F_{st}) is 3.3%, indicating extensive gene flows or recent postglacial expansion. A possible recent bottleneck is detected in two populations by comparing actual with expected heterozygosity from the number of detected alleles.

Key words: Quercus suber, isozymes, genetic variation, marginal populations, isolation.

Introduction

Cork oak (*Quercus suber* L.) forests are valuable Mediterranean ecosystems both economically and ecologically, although not as widely distributed as those of evergreen oak (*Q. ilex* L.). Cork oak is naturally distributed all around the western Mediterranean basin with the widest stands occurring in the Iberian Peninsula. Iberian cork oak forests cover more than 1.000.000 ha of which, 400.000 ha are located in Spain (MONTERO, 1987; MONTOYA, 1988). Specific ecological requirements of cork oak, e.g., siliceous soils, mean annual temperature around 15°C and 600 mm to 1,000 mm of annual rainfall, determine that the largest cork oak forests are found in Portugal and southwestern Spain, with a minor nucleus in the northeastern Spain (Catalonia). In the eastern, northern and interior regions of Spain, populations of cork oak become fragmented and scattered due to cold winter temperatures and/or calcareous soils.

Delineation of the genetic variation within cork oak is becoming an urgent task. Decline and mortality have been observed in cork oak forests over species' range (BRASIER, 1992; MONTOYA, 1992; FERNÁNDEZ and MONTERO, 1993; VARELA, 1993; VARELA and ERIKSSON, 1995) and have promoted several conservation programs. In addition, breeding schemes focused in cork production are being developed due to the species economic importance. However, the limited knowledge available on the genetic structure of the species has precluded the use of well-targeted approaches.

Several recent studies have addressed genetic variation in *Q. suber.* BELLAROSA *et al.* (1996) compared *Q. suber, Q. cerris* L. and a putative natural hybrid between them: *Q. crenata* LAM., by comparing seed storage proteins and rDNA genes. Hybridization between *Q. ilex* and *Q. suber* was studied by ELENA-ROSSELLÓ *et al.* (1992), and an introgression of *Q. ilex* and *Q. suber* have been described in mixed stands (TOUMI and LUMARET, 1998). In a range-wide isozyme study, TOUMI and LUMARET (1998) found high levels of heterozygosity and identified intraspecific differences in populations from two areas that are only partially geographically distinct. The first area contains most of the populations from the Iberian Peninsula and part of France (populations from Landes and Roussillon). The second area includes populations from Provence, Italy, Corsica, Sardinia, Sicily, north Africa and Galicia (northwestern Spain). TOUMI and LUMARET (1998) believed the first area corresponded to the species' centre of origin, and the second area represented a secondary range expansion. Allozyme variation restricted to seven Spanish populations (ELENA-ROSSELLÓ and CABRERA, 1996) confirmed the high levels of heterozygosity and inter-population differentiation in these populations, but did not ascribe differences to geographic area. The relevance of demographic and geographic factors to the maintenance of genetic variability of the species needs to be investigated. In this sense, the complex of fragmented cork oak forests found in Spain is of great interest for studying cork oak genetic variation.

The genetic importance of isolated (marginal) populations has been a topic of discussion. For example, CARSON (1959) and MAYR (1970) have stated that genetic variation should be reduced in marginal stands due to the smaller effective population size, a more limited gene flow and higher selective pressures. Isozyme studies, however, have not found a clear relationship between variation patterns and population characteristics. Lower heterozygosity and/or lower allelic richness were described for peripheral populations in *Pinus contorta* DOUGL. (YEH and LAYTON, 1979; CWYNAR and MACDONALD, 1987), *P. ponderosa* LAWS (HAMRICK *et al.,* 1989) and *Quercus ilex* L. (MICHAUD *et al.,* 1995). In contrast, LESICA and ALLENDORF (1995) listed a series of papers in which peripheral populations of other coniferous species did not displayed any loss of genetic variation (TIGERSTEDT, 1973; BETANCOURT *et al.,* 1991; YEH and O'MALLEY, 1980). Additionally, ELENA-ROSSELLÓ and CABRERA (1996) found the highest values of heterozygosity in a peripheral cork oak population. LESICA and ALLENDORF (1992) have proposed that small populations with moderate levels of stress may retain high levels of heterozygosity due to a heterozygous advantage. Therefore, assessment of genetic diversity in isolated stands and forests is of great importance for the conservation and improvement programs. In this study, the influence of isolation, population size and environmental stress on isozyme polymorphism was evaluated in cork oak populations from the Iberian peninsula, Morocco, Italy and Sicily.

Materials and Methods

Populations

Acorns were collected from 18 populations distributed throughout the natural range of the species: fourteen from Spain, and one each from Portugal, Morocco, Sicily and con-

[1]) Unidad de Anatomía, Fisiología y Genética Forestal, ETSI Montes, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain.

[2]) Dpto. Mejora y Brotecnología, CIFOR-INIA, Apdo 8111, 28080 Madrid, Spain.

[3]) Corresponding author.

Table 1. – Location and characteristics of the studied populations. * P = average annual rainfall; T = mean annual temperature. Type: C = central population; M = marginal population. (-: no data).

Pop (Province)	Code	Latitude	Longitude	Altitude (m)	P (mm)*	T (°C)*	Description	Cork production	Type
Liébana (Santander)	Sp-1	43° 09' N	4° 37' W	200-500	1.196	14.3	Isolated small forest	Medium	M
Valle del Sil (Orense-Lugo)	Sp-2	42° 29' N	7° 20' W	300-700	799	14.3	Several small stands	Low	M
Sestrica (Zaragoza)	Sp-3	41° 30' N	1° 36' W	800-1.200	396	12.8	Isolated small forest	Low	M
Ciudad Rodrigo (Salamanca)	Sp-4	40° 35' N	6° 26' W	700	547	13.3	Several small stands	Intense	M
El Pardo (Madrid)	Sp-5	40° 31' N	3° 45' W	700	438	13.9	Several small stands	Medium	M
Castillo de Bayuela (Toledo)	Sp-6	40° 07' N	4° 31' W	650-780	553	15.4	Several small stands	Intense	M
Cañaveral (Cáceres)	Sp-7	39° 50' N	6° 22' W	460-500	761	16.5	Central range	Intense	C
Cañamero (Cáceres)	Sp-8	39° 23' N	5° 23' W	600-800	783	14.6	Central range	Intense	C
Jerez de los Caballeros (Badajoz)	Sp-9	38° 13' N	6° 42' W	400-500	666	16.0	Central range	Intense	C
Sierra Madrona (Ciudad Real)	Sp-10	38° 29' N	4° 19' W	700-900	637	14.3	Central range	Intense	C
La Almoraima (Cádiz)	Sp-11	36° 16' N	5° 22' W	50-60	1.065	15.4	Central range	Intense	C
Haza del Lino (Granada)	Sp-12	36° 50' N	3° 18' W	1.300	742	13.0	Isolated forest	Intense	M
Espadán (Castellón)	Sp-13	39° 53' N	0° 22' W	600-800	637	16.2	Isolated forest	Intense	M
Sta. Coloma de Farnés (Girona)	Sp-14	41° 52' N	2° 35' E	200-500	795	15.3	Central range	Intense	C
Alportel (Portugal)	Po-1	37° 02' N	7° 54' W	460	874	15.9	Central range	Intense	C
Catania (Sicily-Italy)	It-1	37° 07' N	14° 30' E	250	448	17.7	Several small stands	—	M
Lazio (Italy)	It-2	42° 25' N	13° 02' E	160	936	16.4	Isolated forest	—	M
Aïn Rami (Morocco)	Mo-1	35° 07' N	5° 16' W	300	1482	19.0	Central range	Intense	C

tinental Italy. Acorns were collected from 23 to 33 morphologically typical cork oaks in each population. Geographical and climatic data and other information such as intensity of management and degree of isolation are provided in *table 1* and *figure 1.* Each population was classified as being central or marginal, depending on the degree of isolation. A stand was considered isolated if there is more than 50 km from another cork oak stand. Marginal populations, generally located in the periphery of the range, correspond to allopatric stands in contrast with the main sympatric range. Marginal populations can be subdivided into three different types: 1) a unique population that can be relatively large, e.g., Sp-12 or Sp-13; 2) a series of small and nearly sympatric population isolated in the same area, e.g., Sp-2, Sp-4; or 3) a unique and small stand, e.g., Sp-1, Sp-3.

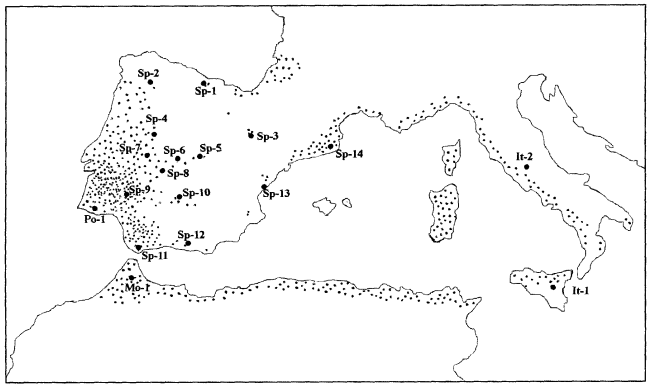

Figure 1. – Natural range of *Quercus suber* (dotted area) and location of populations included in the study (see *Table 1* for codes).

Starch gel electrophoresis

The acorns were kept at 5 °C in a hygroscopic material (peat) until analysis. Four to six acorns per mother tree were studied, in order to sample about 120 individuals per population. Isozyme analysis was conducted on cotyledons and leaf tissues. Initially, 100 mg of cotyledon were taken from each acorn, then the acorn was placed in a germination chamber (20 °C) until the first leaves appeared. A 100 mg sample of leaf material was used to complete the analysis. In both cases (cotyledon and leaf), extraction buffer was as described by AFZAL-RAFII (1988). Buffer systems, enzyme systems and tissue are indicated in *table 2.* Staining procedures were adapted from CONKLE *et al.* (1982), CHELIAK and PITEL (1984) and WENDEL and WEEDEN (1989). A total of 15 enzyme systems were analysed (AAT, ACPH, ADH, CAT, EST, IDH, LAP, MDH, MNR, 6PGD, PER, PGI, PGM, SKDH, SOD).

Allelic frequencies and the following parameters were calculated: *Na* (number of alleles), *Ne* (effective number of alleles), *Ho* (observed heterozygosity), *He* (expected heterozygosity), number and percentage of polymorphic loci at 99% and 95% criteria, and WRIGHT's F-statistics (F_{is}, F_{st}, F_{it}). NEI's genetic distances were computed (NEI, 1972). Mean values of parameters were obtained for central and marginal groups and compared by FISHER's least significant difference (LSD) procedure. Finally, the possible occurrence of a bottleneck was investigated by comparing the heterozygosity expected from the number of alleles and the observed gene diversity following CORNUET and LUIKART (1996).

Analyses were conducted using Popgene (YEH *et al.,* 1997), Bottleneck (PIRY *et al.,* 1997) and Statgraphics Plus for Windows (2.1) programs.

Results

Three enzyme systems were discarded: IDH (no interpretation achieved), EST (non-repeatable bands) and PER (too weak activity). Interpretation for PGI was in agreement with the segregation study reported by WUEHLISCH and NÓBREGA (1995). For the other systems, our interpretation (number of loci and enzyme structure) was congruent with the results of ZANETTO *et al.* (1996) and MÜLLER-STARK *et al.* (1996) in *Q. robur* L. and *Q. petraea* (MATT.) LIEBL. in which inheritance of enzymatic loci was verified by means of controlled crosses. The proposed pattern for ADH and SOD agreed with the interpretation of TOUMI and LUMARET (1998); finally, CAT was considered as a monomorphic locus since it showed an unique invariant band.

Fourteen loci were interpreted for the 12 enzymatic systems, 9 of them showing variation. Total number of alleles was 29, including the monomorphic loci. Only two private alleles, i.e., restricted to a single population, were found: Adh-3, in Sp-11

Table 2. – Enzyme systems included in the study. Tissue: C = cotyledon, L = leaf. (*): scored loci (loci are ordered from faster to lower); (**): references in which locus segregation has been verified for genus *Quercus;* 1 = WUEHLISCH and NÓBREGA, 1995; 2 = MÜLLER-STARCK *et al.,* 1996; 3 = ZANETTO *et al.,* 1996.

Enzyme system	Abbreviation	E.C. code	Tissue	Buffer system	Loci (*)	Refer (**)
Acid phosphatase	ACP	3.1.3.2	C		Acph-C	2, 3
Alcohol dehydrogenase	ADH	1.1.1.1	C	Tris-citrate –	Adh	–
Catalase	CAT	1.11.1.6	C	Litium borate	Cat	–
Aspartate amino transferase	AAT	2.6.1.1	C	PH 8.3	Aat-B,	2, 3
				(Scandalios,	Aat-C	
Leucine aminopeptidase	LAP	3.4.11.1	L	1969)	Lap-A	2, 3
Menadione reductase	MNR	1.6.99.2	L		Mnr	2, 3
Superoxide dismutase	SOD	1.15.1.1	C		Sod-B	–
Malate dehydrogenase	MDH	1.1.1.37	C	Morpholine-C	Mdh-A	2, 3
6-phosphogluconate dehydrogenase	6PGD	1.1.1.44	C	pH 7.0 (Clayton	6Pgd-A,	2, 3
				and Tretiak,	6Pgd-B	
				1972)		
Phosphoglucose isomerase	PGI	5.3.1.9	C	Morpholine-C	Pgi-B	1, 2, 3
Phosphoglucomutase	PGM	2.7.5.1	C	pH 6.1 (Clayton	Pgm	2, 3
Shikimate dehydrogenase	SKDH	1.1.1.25	C	and Tretiak,	Skdh-A	2, 3
				1972)		

and 6Pgd-B-1, in Mo-1. Both alleles appeared in very low frequencies (1% and 0.3%, respectively). Three other restricted alleles were found: Acph-C-1, appearing in populations from the northwest and centre of Spain; Pgi-B-3, absent from some northwestern Spanish populations and Italy; and Skdh-A-2, absent from several Spanish populations (Sp-3, Sp-4, Sp-7, Sp-10, Sp-12 and Sp-13) and an Italian population (It-2). Pgi-B-1 was the most frequent allele in all the populations except in the Italian and Sicilian populations. The frequency of this allele was higher than 70% in northern and western populations (Sp-1, Sp-8, Sp-7, Sp-3, Sp-2 and Po-1). Five loci (Acph-C, Got-B, 6Pgd-A, 6Pgd-B and Mdh-A) showed a very frequent allele (>90%) that becomes fixed in most of the marginal populations from eastern and northern Iberia (Sp-1, Sp-14, Sp-3, Sp-13, It-1, It-2).

Levels of expected heterozygosity within populations (*He*) varied from 0.117 to 0.168, with a mean value of 0.158. The higher values were found in southern Iberian populations

Table 3. – Diversity parameters (mean and *standard deviation*). N: sample size; Na: Observed number of alleles; Ne: Effective number of alleles (KIMURA and CROW, 1964); Obs. Het: Observed heterozygosity; Exp. Het.: Expected heterozygosity computed using LEVENE (1949); F_{is}: Fixation index (WRIGHT, 1978); Pl-99/95: number of polymorphic loci at 99/95% criterium; % Pl: percentage of polymorphic loci.

Pop	N	Na	Ne	Obs. Het.	Exp. Het.	F_{is}	Pl-99	% Pl-99	Pl-95	% Pl-95
Sp-1	185	1.78	1.25	0.115	0.140	0.174	8	57.1	4	28.6
		0.801	*0.427*	*0.173*	*0.204*					
Sp-2	225	1.86	1.25	0.150	0.155	0.076	8	57.1	6	42.8
		0.770	*0.345*	*0.193*	*0.186*					
Sp-3	223	1.71	1.27	0.147	0.153	0.061	6	42.8	6	42.8
		0.825	*0.408*	*0.212*	*0.211*					
Sp-4	229	1.78	1.27	0.146	0.159	0.138	9	64.3	5	35.7
		0.699	*0.384*	*0.193*	*0.202*					
Sp-5	230	1.93	1.29	0.155	0.166	0.058	8	57.1	7	50.0
		0.829	*0.428*	*0.200*	*0.207*					
Sp-6	181	1.78	1.30	0.161	0.165	0.023	7	50.0	6	42.8
		0.699	*0.439*	*0.214*	*0.216*					
Sp-7	223	1.71	1.24	0.144	0.148	0.009	8	57.1	6	42.8
		0.611	*0.332*	*0.180*	*0.183*					
Sp-8	194	1.86	1.25	0.156	0.150	0.029	8	57.1	6	42.8
		0.770	*0.369*	*0.213*	*0.194*					
Sp-9	228	1.86	1.27	0.139	0.152	0.045	8	57.1	5	35.7
		0.770	*0.420*	*0.186*	*0.204*					
Sp-10	220	1.78	1.29	0.157	0.163	0.072	9	64.3	6	42.8
		0.699	*0.419*	*0.210*	*0.211*					
Sp-11	210	1.93	1.31	0.145	0.167	0.108	9	64.3	7	50.0
		0.829	*0.453*	*0.188*	*0.216*					
Sp-12	216	1.78	1.31	0.156	0.169	0.093	9	64.3	6	42.8
		0.699	*0.459*	*0.213*	*0.223*					
Sp-13	217	1.64	1.25	0.131	0.141	0.111	6	42.8	5	35.7
		0.745	*0.384*	*0.196*	*0.203*					
Sp-14	206	1.78	1.29	0.140	0.150	0.136	7	50.0	4	28.6
		0.801	*0.479*	*0.222*	*0.227*					
Po-1	225	1.86	1.25	0.143	0.145	0.056	7	50.0	5	35.7
		0.770	*0.385*	*0.206*	*0.199*					
It-1	215	1.71	1.26	0.150	0.148	0.004	8	57.1	4	28.6
		0.726	*0.394*	*0.208*	*0.201*					
It-2	196	1.64	1.19	0.110	0.117	0.031	7	50.0	4	28.6
		0.633	*0.315*	*0.171*	*0.180*					
Mo-1	251	1.93	1.28	0.160	0.164	0.115	9	64.3	5	35.7
		0.829	*0.388*	*0.211*	*0.199*					
Ov.Pop		2.07	1.28	0.145	0.158	0.118	9	64.3	5	35.7
		0.917	*0.413*	*0.196*	*0.206*					

Table 4. – Mean comparison for number of alleles (Na), expected heterozygosity (He), fixation index (F_{is}) and minimum genetic distance (D), for each of central and marginal groups. * denotes significant differences at 95% confidence level.

	Na	He	F_{is}	D
Central range	1.84	0.155	0.071	0.0013
Unique large stand (Sp-12, Sp-13)	1.71	0.155	0.102	0.0016
Several small stands (Sp-5, Sp-6, Sp-4, Sp-2)	1.84	0.161	0.074	0.0023
Unique small stand (Sp-1, Sp-3, It-1, It-2)	1.71	0.139	0.067	0.0027
Mean marginal	1.76	0.151	0.081	0.0023

Difference:	Na	He	F_{is}	D
Central – Marginal stands	n.s.	n.s.	n.s.	n.s.
Central – Unique large stands	*	n.s.	n.s.	n.s.
Central – Unique small stands	*	*	n.s.	n.s.
Central – Several small stands	n.s.	n.s.	n.s.	n.s.
Unique large – Several small stands	n.s.	n.s.	n.s.	n.s.
Unique large – Isolated small stands	n.s.	n.s.	n.s.	n.s.
Several small – Unique small stands	*	*	n.s.	n.s.

(Sp-12, Sp-11, Mo-1, and Sp-10) and in some populations from central Spain (Sp-5, Sp-6) (*Table 3*). Lower levels were found in two small-sized isolated populations: the Italian It-2 (0.117) and the Spanish Sp-1 (0.140).

The mean number of alleles per locus was 2.1, ranging from 1.64 to 1.93 between populations. The species presents a low level of polymorphic loci: 64% (at the 99% criteria), with a range of 43% to 64% between populations. Less polymorphic loci were found in marginal populations from northern and eastern Spain (Sp-3 and Sp-13, at the 99% criteria and Sp-1, Sp-14, It-1 and It-2 at the 95% criteria).

Table 4 shows the LSD means comparison for number of alleles per locus and expected heterozygosity for marginal and central populations. No significant differences were found between central and marginal groups, but a lower level of diversity in allopatric populations was apparent depending on relative size and isolation. Single small-sized stands displayed significantly (at the 95% level) lower levels of heterozygosity than central group populations and populations formed by several small stands. Three comparisons revealed that the number of alleles (*Na*) decreases when isolation degree was higher. *Na* was higher in central populations when compared with both large and small marginal single stands. Also, marginal populations formed by several small stands had a greater number of alleles than unique small ones. No significant differences were found comparing F_{is} (a measure of deviation from HARDY-WEINBERG equilibrium) or minimum genetic distance (means of the lowest genetic distance of each population with the others).

Maximum values of F_{is} were found in Sp-1 and Sp-4, while minimum indexes were displayed by It-1 and Sp-7. WRIGHT's coefficients of diversity showed that inter-population differentiation is 3% of total diversity. The loci contributing most to inter-population differences were Pgi-B (6%) and Skdh (3%) (*Table 5*).

The minimum genetic distance was found between Sp-8 and Po-1 (0.0005) and the maximum was 0.0311, between Sp-1 and It-2. The Italian populations separate clearly from the Iberian and Moroccan ones. The bottleneck test (CORNUET and LUIKART, 1996) (*Table 6*) indicated that Sp-3 and Sp-13 were the populations where more probably (p = 0.055) a size reduction had occurred recently.

Table 5. – WRIGHT's F-statistics for each locus. Number of migrants (Nm) is estimated as $(1-F_{st})/4F_{st}$.

Locus	N	F_{is}	F_{it}	F_{st}	Nm
ACPH-C	4120	0.136	0.159	0.027	9.14
ADH	4116	0.060	0.085	0.026	9.47
CAT	3800	****	****	0.000	****
GOT-B	3606	0.161	0.185	0.029	8.29
GOT-C	3768	****	****	0.000	****
LAP-A	3714	0.009	0.028	0.018	13.30
MDH-A	3368	0.173	0.188	0.018	13.56
MNR	3606	****	****	0.000	****
6PGD-A	4020	0.122	0.134	0.013	19.05
6PGD-B	4122	0.138	0.146	0.009	27.77
PGI-A	4080	0.035	0.094	0.060	3.88
PGM	4074	****	****	0.000	****
SKDH	3750	0.018	0.051	0.034	7.06
SOD	4092	****	****	0.000	****
Mean	3874	0.048	0.080	0.033	7.31

Table 6. – Bottleneck.– WILCOXON test: probability (one tail for heterozygosity excess). All loci are assumed to fit infinite allele model.

Population	Probability	Population	Probability
Sp-1	0.32031	Sp-10	0.21289
Sp-2	0.21289	Sp-11	0.28516
Sp-3	0.05469	Sp-12	0.21289
Sp-4	0.10156	Sp-13	0.05469
Sp-5	0.28516	Sp-14	0.19141
Sp-6	0.12500	Po-1	0.36719
Sp-7	0.21289	It-1	0.15625
Sp-8	0.28516	It-2	0.52734
Sp-9	0.41016	Mo-1	0.21289

Discussion

Diversity and genetic structure

The results are comparable to the studies on *Q. robur* and *Q. petraea* by ZANETTO *et al.* (1994) and ZANETTO and KREMER (1995): 8 loci are common, offspring is considered in both cases and sample size is similar. The expected heterozygosity recorded in *Q. suber* (0.158) is lower than in these temperate and widespread oaks: 0.252 for *Q. robur* (ZANETTO *et al.,* 1994) and

0.245 to 0.257 for *Q. petraea* (ZANETTO *et al.,* 1994; ZANETTO and KREMER, 1995). The effective number of alleles follows a similar pattern: 1.28 (*Q. suber*), 1.49 (*Q. robur*) and 1.47 (*Q. petraea*). Some different life-history traits can explain the higher heterozygosity in white oaks. First, distribution range is positively correlated with diversity (HAMRICK *et al.,* 1979, 1992; KREMER and PETIT, 1993). Second, post-glacial history can also be a causal agent, as the range reduction had to be more severe in a thermophilous species such as cork oak than in temperate taxa.

Previous works on genetic variation of *Quercus suber* (ELENA-ROSSELLÓ and CABRERA, 1996; TOUMI and LUMARET, 1998) report different levels of heterozygosity: *He* = 0.29 (13 loci, including 3 monomorphic) and *Hs* = 0.28 (with 7 polymorphic loci; 0.18 when considering the monomorphic ones), respectively. These studies used similar methodology, but are hardly comparable with the present study. Few loci were in common with the present study: two loci (Adh-2 and Lap-1) in the ELENA-ROSSELLÓ and CABRERA (1996) study, or three loci (Adh-1, Lap-1 and Pgi-1) in the TOUMI and LUMARET (1998) study. In addition, both studies considered two enzymatic systems, peroxidases and esterases, which show high variability between different developmental stages and tissues, and were excluded in the present study for this reason. These systems are considered as non-neutral loci (ELENA-ROSSELLÓ and CABRERA, 1996) and the genetic interpretation is not demonstrated in the case of peroxidases. These factors could also explain the very high levels of interpopulation differentiation detected in these studies. For Spain, ELENA-ROSSELLÓ and CABRERA (1996) indicate a mean coefficient of diversity (F_{st}) of 16%, but due to three loci (23% for Per-1, 36% for Per-3, 28% for Est-1). This value is of 11% for the whole range (TOUMI and LUMARET, 1998) due to the study of two loci (11% for Per-1, 18% for Acph-1).

In the present study, cork oak has similar levels of interpopulation differentiation (F_{st} = 3.3%) to those of *Q. robur* (G_{st} = 2.4 %) and *Q. petraea* (G_{st} = 3.2-2.5%) (ZANETTO *et al.,* 1994; ZANETTO and KREMER, 1995). The fact that most of the variation is distributed within populations is typical of widely distributed outcrossing tree species – such as oaks – for which an important gene flow between populations also exists (HAMRICK *et al.*, 1979, 1992). In most of the range of *Q. suber,* the distance between stands is not enough to prevent gene exchanges, which can quickly homogenise the genetic structure of the species. A low differentiation can also indicate a recent expansion from the glacial refugia. Palynological records in southern Spain (PONS and REILLE, 1988) date the beginning of the post-glacial expansion of the species at 8.000 BP, 4.000 years later than deciduous oaks. This means fewer generations for such a long-lived species and, subsequently, a shorter time for differentiation.

Comparison between central and marginal populations

Although the least diverse populations are the marginal ones, a clear correspondence between heterozygosity and population isolation and size was not found since some of the most variable populations (Sp-12, Sp-5) were separated from the sympatric populations. If a marginal population is compared with the proximal central populations, a loss of diversity is not observed. Therefore, we can not conclude that a small size or strong isolation necessarily leads to a reduced variability.

Other factors must have had effects in determining the genetic structure of marginal stands. Post-glacial migration could be an important factor. The results showed a loss of heterozygosity in the most scattered populations. These occur in the regions where suitable habitats are limited because of the predominance of limestone or arid climate. Colonisation in these areas had to rely in long-distance dispersal events, involving founding effects with a low number of individuals. In northwest and central Spain where soils are mostly siliceous and drought is less marked, the distribution is more continuous. Even in central Spain (province of Valladolid), where only two small populations exist nowadays, there are archaeological evidences – charcoal and wood remains – of a more continuous presence of *Quercus suber* in the past (UZQUIANO, 1995). Our results show that the easternmost marginal populations are less diverse (Sp-1, Sp-3, Sp-13), while those from southern or western Spain are comparable to central forests (Sp-12, Sp-5, Sp-6, Sp-4, Sp-2). Two populations from Spain (Sp-1 and Sp-3) and the Italian ones, which are the most isolated and small-sized, present a significantly lower number of alleles per locus and lower *He* values than the central Iberian populations. This suggests the existence of founder effects in the origin of these stands, in addition of a lack of gene flow. NEI *et al.* (1975) showed how alleles are lost when a population reduction occurs. Fixation indices, on the other hand, display the highest values in isolated forests, but without any relationship to the actual population characteristics. Marginal stands have the highest deficiency of heterozygotes (Sp-1, Sp-4), but also the lowest (It-2). F_{is} is considered as a measure of inbreeding, but population substructuring can originate an overall deficit of heterozygotes although each subpopulation displays HARDY-WEINBERG equilibrium (BERG and HAMRICK, 1997). Data on within-population structure would be needed before concluding that inbreeding is linked or not to allopatric stands.

Gene flow prevents genetic erosion even in small stands. In the isolated populations, where such exchange can not exist, they must have a size sufficient to avoid the risk of genetic erosion. An effective number of 500 trees is enough to guarantee the conservation of alleles with frequencies higher than 0.01 (VARELA and ERIKSSON, 1995), and none of the populations analysed has less than 5.000 trees. In addition, the main features of the species' mating system (allogamy, wind-pollination and asexual propagation) favor the maintenance of diversity. Only in case when there is a combination of isolation and a small population size, a loss of diversity is detected. This loss is manifested in different ways: even when heterozygosity can be rapidly recovered after reduction of population size, some rare alleles can be permanently lost (NEI *et al.,* 1975). In this study, the number of alleles is too small to allow a comparison, but some loci have become monomorphic in certain marginal populations (Acph-C in Sp-14, Mdh-A in It-2 and Sp-3, 6Pgd-A in It-1 and Sp-13, and 6Pgd-B in Sp-1, Sp-3 and Sp-13). A similar pattern was obtained by CWYNAR and MACDONALD (1987) in the most recently established populations of *P. contorta.* Similarly, MICHAUD *et al.* (1995) found a lower number of alleles in marginal versus central populations of *Q. ilex* (1.87 to 2.50). EL MOUSADIK and PETIT (1996) also described a reduced allelic richness in isolated populations of *Argania spinosa* in Morocco. Results of the bottleneck test applied in the present study indicate a probable reduction in two populations (Sp-3 and Sp-13). These two stands also present a higher number of invariant loci (i.e., some alleles have been lost) suggesting the reduction of the effective size. The bottleneck test only detects recent effects; such events can have occurred in other populations and not be detected if number of generations after reduction is greater than 2.5 N_e (N_e being effective size of the population after reduction) (CORNUET and LUIKART, 1996).

Selection relating to cork exploitation has been suggested to explain the genetic structure of *Quercus suber* (ELENA-

ROSSELLÓ and CABRERA, 1996; TOUMI and LUMARET, 1998). From our results, no evidence was found to support this hypothesis, since similar levels of diversity were found in managed and non-managed populations. Additionally, the most diverse stands (Sp-11, Sp-12) have been intensively exploited while, in the least diverse populations (Sp-1, Sp-3, Sp-13), cork is generally of minor importance. Moreover, no relationship exists between isoenzymatic polymorphism and cork quality (NÓBREGA, 1997a,b). Even if cork exploitation effectively led to a reduction in diversity, this may have occurred only since the last two centuries, a very short time in terms of cork oak generations. The first cork industries arose in Spain at the end of the 1700s (ARTIGAS, 1895; DÍAZ-FERNÁNDEZ *et al.,* 1995). There are no historical references on the importance of cork oak plantations before the 1800s. Previously, cork was only locally used for handcraft uses while acorns, grazing and fuel wood were the most valuable forest products. *Quercus suber* (together with other species) may have actually been eliminated by man from mixed stands to favour *Q. ilex,* a more valuable tree in most of Spain at that time (BLANCO *et al.,* 1997).

Quercus suber appears to be genetically comparable to the European white oaks, with their own peculiarities. It can not be considered as a homogenous group of populations, as different levels of diversity exist within central as well as marginal populations. As some of the marginal stands have as much variation as the central populations, thus they can be highly desirable for inclusion in conservation and breeding programs.

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Effects of Site and Intensive Culture on Family Differences in Early Growth and Rust Incidence of Loblolly and Slash Pine

By J. LÓPEZ-UPTON[1]), T. L. WHITE[2]) and D. A. HUBER[2])

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Summary

Eleven field tests with two silvicultural treatments (intensive and less intensive) with open-pollinated families of improved *P. taeda,* and improved and unimproved *P. elliottii* were established by the Cooperative Forest Genetics Research Program in the Lower Coastal Plain of the southeastern USA. Results for third-year fusiform rust infection indicated highly consistent family rankings across sites ($r_{B\text{-}site}$=0.90) and across management intensities ($r_{B\text{-}treat}$=0.97). Single-site heritabilities for rust in the binary scale ($h^2_{B\ 0,1}$) and in the underlying scale ($h^2_{B\ und}$=0.29) were not affected by site nor by the increase in management intensity.

For third-year height growth, family rankings were less influenced by environmental differences among treatments ($r_{B\text{-}treat}$=0.87) than by differences among sites ($r_{B\text{-}site}$=0.57). These early results imply that stable rankings for height may be expected as cultural intensity increases. However, a few families were more responsive to culture than others. Differences in susceptibility to fusiform rust and seedling quality caused some instability in height rankings across sites. The intensive culture had a smaller site by family interaction (higher $r_{B\text{-}site}$ value) than less intensive culture, meaning family ranking for height were more stable across sites for intensive culture.

In the intensive treatment, additive variance was reduced by 6% and environmental error decreased by 25%. This resulted in higher heritability for the intensive treatment as compared to non-intensive treatment (h^2_B = 0.3 *vs.* 0.2, averaged over all three taxa and all sites). The higher heritability for height growth in the intensive management treatment implies that genetic gains from progeny testing are higher in intensive culture. Further, the higher heritability in the intensive culture along with little GxE between treatments ($r_{B\text{-}treat}$=0.87) implies that progeny testing with intensive culture could have advantages for operational deployment in either culture.

Key words: Pinus elliottii, Pinus taeda, cultural intensity, heritability, GxE, type B genetic correlation, early growth, rust resistance, genetic gain.

1. Introduction

Loblolly pine (*P. taeda* L.) and slash pine (*Pinus elliottii* ENGELMAN var. *elliottii*) are the two most important commercial timber species in the southeastern United States (BORDERS and HARRISON, 1989). Several studies have examined the response of loblolly and slash pine to cultural practices at levels considered „operational" by forest industries, but few have included intensive silviculture (e.g., HAINES and GOODING, 1983; BLAKESLEE *et al.,* 1987; COLBERT *et al.,* 1990). Such comparisons are important, because of the increasing interest in intensive culture to produce larger volumes of wood per unit area (HAGLER, 1996).

Moreover, genetic improvement has occasionally been incorporated into studies with both fertilization and weed control to compare genotypes of both species (BORDERS and HARRISON, 1989; SWINDEL *et al.,* 1988; NEARY *et al.,* 1990). Fertilization and competition affect growth and can increase susceptibility to physical damage and pest incidences on trees in both loblolly and slash pine, e.g., fusiform rust, caused by *Cronartium quercuum* (BERK.) MIYABE ex SHIRAI f. sp. *fusiforme* (BLAKESLEE *et al.,* 1987; SWINDEL *et al.,* 1988; SHOULDERS *et al.,* 1990). Genotypes may respond differentially to these challenges in disparate silvicultural treatments.

Genetic parameters such as family variances, heritabilities and the interaction of family with environment may also be affected by cultural practices. For example, heritabilities are greatly influenced by environmental homogeneity of the test, and homogeneity may be impacted by management activities. Further, since heritabilities are population-specific, studies with intensive and less intensive culture may provide a good opportunity to explore how genotypes within species respond to culture by examining genetic expression and genotype by environment interaction.

[1]) Especialidad Forestal, IRENAT, Colegio de Postgraduados en Ciencias Agrícolas, Montecillo, Mex. 56230, Mexico
Corresponding author: 52(595) 2-0200 ext. 1463, fax: 52(595) 2-0256, uptonj@colpos.colpos.mx

[2]) School of Forest Resources and Conservation, IFAS, University of Florida, Gainesville, Fl. 32611-0420, USA