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Morphological and Molecular Diversity Among Italian Populations of *Quercus petraea* (Fagaceae)

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Quercus petraea (sessile oak) has a scattered distribution in southern and central Italy. The objective of this work was to evaluate the level and distribution of diversity in five Italian populations of *Q. petraea* by using morphological markers and hypervariable molecular markers such as microsatellites. Forty-eight morphological traits and six nuclear and three plastid loci were scored for each population. Evidence for differentiation in both sets of traits was found, but patterns of differentiation of morphological traits did not coincide with microsatellite differentiation. Morphological variation was correlated with ecological conditions at the site of origin. Analysis of molecular variance revealed significant genetic variation among populations ($P < 0.001$), both at the nuclear and plastid levels. There was a slight, but significant, correlation between nuclear genetic distance and geographic distance. The relatively high genetic diversity in the populations analysed indicates that the maintenance of their evolutionary potential is possible if population sizes are maintained or increased. Low levels of haplotype diversity found within the small southernmost population (Piano Costantino) indicates that genetic erosion may increase the extinction risk for this population.

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Key words: *Quercus petraea*, morphological traits, microsatellites, adaptation, gene flow.

INTRODUCTION

Analysis of the amount and distribution of genetic variation within and among populations of a species can increase understanding of the historical processes underlying the genetic diversity (Dumolin-Lapegue *et al.*, 1997) and can also provide important basic information for breeding programmes and for the establishment of programmes to conserve genetic resources.

The distribution of genetic diversity within and among populations is a function of the rate of gene flow between populations. The extent of gene flow in a species depends on the distribution of the habitats it occupies, the size and degree of isolation of its populations, and the movement of pollen and seeds between populations.

Sessile oak [*Quercus petraea* (Matt.) Liebl.] is a late-successional species of great ecological and silvicultural importance in Europe. Its wide-ranging genetic variability allows it to live in different climatic and edaphic environments and to adapt to extreme ecological conditions (Kleinschmit, 1993). Its natural range extends from Spain to the Ukraine, and from Scotland to Turkey. Within this range, the distribution of *Q. petraea* is continuous, except in the southern limits where stands are scattered.

Palynological data suggest a southern origin for European oaks (Huntley and Birks, 1983). More recently, phylogeographic investigations (Dumolin-Lapegue *et al.*, 1997) carried out using plastid DNA markers have demonstrated

the existence of three glacial refugial areas localized in the three peninsulas: Iberia, Italy and the Balkans. Italian populations are at the southern end of the European distribution range of *Q. petraea*, and their study is of great interest in understanding and reconstructing the recolonization processes in the post-glacial period and in investigating the effects of fragmentation on genetic structure of the species. The fragmentation of habitats should affect the size and degree of isolation of individual populations, thus contributing to genetic impoverishment and adaptive flexibility of populations.

Population fragmentation assumes particular importance in central and southern Italy, where the distribution of *Q. petraea* is scattered owing to its replacement in many locations by crops of higher agricultural value. Further populations have been lost through habitat destruction and fragmentation, logging and adverse land-use practices. Extant populations continue to be threatened by these practices. This ecological risk can also translate into an economic risk given the high timber value of *Q. petraea* (Nepveu, 1993). Moreover, the genetic integrity of certain populations has probably been disturbed by interspecific hybridization with *Q. pubescens*. In Italy, *Q. petraea* often coexists with *Q. pubescens* Willd., and morphologically intermediate individuals, presumed hybrids, have often been reported. The morphological and molecular differentiation between *Q. petraea* and *Q. pubescens* in mixed stands was analysed by Bruschi *et al.* (2000). There is thus a need to gather more information about the genetic architecture of

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TABLE 1. Location and principal abiotic characteristics of the five study sites

Label	Locality	Latitude and longitude	Altitude (m)	Precipitation* (mm)	Temperature*(°C)	Insolation (hm year ⁻¹)	Parent-rock
CR	Carrega	44°43'N 10°80'E	200	860	12.3	2255	Clay
TT	Tatti	43°21'N 10°56'E	550	870	13.0	2264	Sandstone
MR	Monterufoli	43°16'N 10°45'E	400	880	13.5	2270	Serpentine
MC	Monte Corona	43°13'N 12°17'E	600	825	13.5	2280	Sandstone
PSM	Piano Costantino	37°53'N 14°40'E	1417	687	18.8	2332	Schist

hm, normal sun hours.

* Mean annual value.

this species (e.g. amount and distribution of genetic variation, degree of local adaptation) and to identify sources of genetic diversity among natural stands.

Levels of genetic diversity in *Q. petraea* have been estimated using morphological traits (Dupouey and Badeau, 1993; Bacilieri *et al.*, 1995), biochemical markers such as allozymes (Bacilieri *et al.*, 1995; Zanetto and Kremer, 1995; Kremer and Zanetto, 1997; Le Corre *et al.*, 1998), DNA markers such as plastid DNA PCR/RFLP-based polymorphism (Dumolin-Lapegue *et al.*, 1997) and random amplified polymorphic DNA (RAPDs). These studies have shown that *Q. petraea* is characterized by high within-population diversity associated with little differentiation among populations. Recently, the analysis of nuclear simple sequence repeats (SSRs, microsatellites) revealed a larger polymorphism than that previously found in this species (Streiff *et al.*, 1998).

Of all the marker systems, microsatellites are considered highly efficient markers for population genetic studies because they are widely abundant, highly polymorphic, are usually inherited in a codominant manner and are randomly dispersed in the genome (Tautz, 1989). The purpose of this study was to examine the genetic and morphological variation of sessile oak throughout its range in Italy, based on microsatellites and leaf morphology. Morphological traits and nuclear and plastid microsatellite loci were examined in a preliminary survey of five populations. Several population studies have shown that plastid DNA variation is geographically structured (Petit *et al.*, 1993; Sewell *et al.*, 1996; Dumolin-Lapegue *et al.*, 1997), mainly due to the fact that the plastid genome is maternally inherited in oak and is therefore transmitted by seeds (Petit *et al.*, 1993).

MATERIALS AND METHODS

Collection of the material

Five stands were sampled, three from central Italy (TT, Tatti; MR, Monterufoli; MC, Monte Corona), one from the northern Apennines (CR, Carrega), and one from the southern disjunct Madonie mountains (PSM, Piano Costantino) (Table 1; Fig. 1). TT, MR and MC are mixed *Q. petraea*–*Q. pubescens* stands, while CR and PSM are pure *Q. petraea* stands. The sampled populations of *Q. petraea* encompass the Italian range of the species and, except for Tatti and Monterufoli, are isolated from each



FIG. 1. Location of populations studied.

other. Most of the additional (unsampled) populations of *Q. petraea* are currently represented by small numbers of highly scattered individuals. The sites at MR and PSM are dry owing to climatic factors and edaphic factors, respectively. Stand MR is located on ultrabasic (serpentine) soil. The main characteristics of such soils are infertility due to the low level of macronutrients, a Ca : Mg ratio <1, and the high concentration of heavy metals; moreover, these soils are shallow, well drained and prone to erosion (Brooks, 1987). Thirty individual trees were sampled at random for each population. Individuals were initially classified in the field at the time of sampling by visual determination based on bark structure, branch architecture, leaf and acorn characters, and pubescence of twigs, following published descriptions of *Q. petraea* (Schwartz, 1993). Within-tree variation in morphological characters was minimized by collecting a total of four shade branches (shade sub-sample)

TABLE 2. Morphological characters examined

Abbreviation and units	Definition
Macromorphological characters	
LL (cm)	Length of lamina
LP (cm)	Length of petiole
MWL (cm)	Maximal width of lamina
HMW (cm)	Height of maximal width (length of lamina from terminal lobe to widest part)
MDS (cm)	Maximal depth of sinus
WHL (cm)	Width of the most handing lobe
DVL (cm)	Distance of principal vein to top of the most handing lobe
DS (cm)	Distance of the principal vein to the sinus (placed below the most handing lobe)
WTL (cm)	Width of the terminal lobe
LTL (cm)	Length of the terminal lobe
NLR	Number of lobes on right side
NLL	Number of lobes on left side
NVR	Number of intercalary veins on right side
NVL	Number of intercalary veins on left side
TLL (cm)	Total leaf length (LL + LP)
LLW (cm)	Length of lamina from base to widest part (LL – HMW)
P%	Length of petiole \times 100/total leaf length
HW%	Height of maximal width \times 100/total leaf length
DW%	Distance of maximal width \times 100/total leaf length
MWL/MDS	
LL/MWL	
HMW/MWL	
LTL/WTL	
LLW/MWL	
Thickness	
TTL (μm)	Total thickness of lamina
TUE (μm)	Thickness of upper epidermal cells
TP (μm)	Thickness of palisade cells
TS (μm)	Thickness of spongy cells
TLE (μm)	Thickness of lower epidermal cells
Productivity parameters	
AREA (cm^2)	Leaf area
DW (mg)	Dry weight
SDW (g cm^{-2})	Specific dry weight (dry weight/leaf area)
V (cm^3)	Volume (leaf area \times total thickness)
DMC (mg cm^{-3})	Dry matter concentration (dry weight/volume)
Micromorphological characters	
SD (number cm^{-2})	Stomatal density
LS (μm)	Length of stoma
WS (μm)	Width of stoma
FR (μm)	Freedom of rim (width of stomatal opening uncovered from waxes)
NST	Number of stellate trichomes
NGT	Number of glandular trichomes
NR	Number of rays in stellate trichomes
LRS (μm)	Length of rays of stellate trichomes
SAI	Stomatal area index (stomatal density \times stomatal length)
Pubescence density parameters	
DOR	Midrib on abaxial surface (dorsal)
AXI	Midrib on abaxial surface (axillar)
PET	Petiole
TSH	Terminal shoot
TW	Twig

and four light branches (light sub-sample) from the crown of each plant. Twenty leaves and four twigs were chosen at random from each sub-sample after the elimination of broken, incomplete or damaged units. To verify that the sampled trees had been correctly identified as *Q. petraea*, we sampled, for each mixed stand, trees classified as *Q. pubescens* Willd. For molecular analysis, young leaves of each tree were collected and stored at -80°C pending DNA extraction.

Morphological analysis

In total, 48 characters were scored for the five populations (Table 2). For each individual tree, the score for each character was the mean value of 20 (macromorphology and productivity parameters) or ten measurements (thickness, pubescence and micromorphology), and the score for each character for each population was the mean value for all individuals in that population.

TABLE 3. Sequences of the nuclear (from Steinkellner et al., 1997) and plastid (Weising and Gardner, 1999) microsatellite loci analysed

Locus	Repeat units	Sense primer	Antisense primer	Annealing temperature (°C)
AG 15	(GT) ₅ (GA) ₉	GCTTGAGAGTTGAGATTTGT	GCAACACCCTTTAACTACCA	59
AG 16	(AC) ₂₁	CTTCACTGGCTTTTCTCCT	TGAAGCCCTTGCAACATGC	59
AG 364	(AC) ₁₉	TAGAAAGCCCAAAACAAAACC	CTTTTTGGAAGCCGCTTCCGTA	59
AG 362	(AC) ₄	CTTGAGCATGGAATCCTATG	TCTAGAGGAGCTTCTTTACAC	58
AG 9	(AC) ₁₂	GCAATTACAGGCTAGGCTGG	GTCTGGACCTAGCCCTCATG	59
AG 36	(AC) ₁₉	GATCAAAATTTGGAATTAAGAGAG	ACTGTGGTGGTGAATCAACATGTTG	50
ccmp3	(T) ₁₂	CAGACAAAAGCTTGACATAG	GTTCATTCGGCTCCTTTA	52.5
ccmp4	(T) ₁₃	AATGCTGAATCGAYGACCTA	CCAAAATATTBGGAGGACTCT	55.5
ccmp7	(A) ₁₃	CAACATATACCACTGTCAAG	ACATCATTATTGTATACTCTTTC	45

AG, Nuclear loci; ccmp, plastid loci.

Pubescence was assessed using a binocular microscope. Six classes of pubescence were established, ranging from absent to full [0, absent; 1, occasional (1–20 % of the organ surface); 2, poor (21–40 %); 3, abundant (41–60 %); 4, highly abundant (61–80 %); and 5, full (81–100 %)].

To measure leaf thickness, two 0.5-cm² strips were taken from the middle portion of the lamina across the midrib from each of five leaves of each tree. Measurements were carried out following Ashton and Berlyn (1994).

Total surface area was measured using a leaf area meter (LICOR LI-3100) Afterwards, these leaves were dried at 70 °C for 72 h, and specific weights were calculated as the dry weight divided by lamina area. Micromorphological observations were carried out using a scanning electron microscope (Philips XL 20) on two pieces of leaf material measuring about 10 mm² that had been removed from between the veins of five leaves of each plant.

Molecular analysis

DNA was extracted from approx. 100 mg leaf material using a DNAeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), following manufacturer's instructions, and quantified by fluorometric assay. Six nuclear microsatellite loci developed by Steinkellner *et al.* (1997) were used. These loci (ssrQpZAG1/5, ssrQpZAG1/6, ssrQpZAG3/6, ssrQpZAG 3/64, ssrQpZAG3/62 and ssrQpZAG9) amplify (AG)_n dinucleotide repeats (Table 3). PCR amplifications were carried out following Streiff *et al.* (1998). In addition, three primers designed from the plastid genome of *Nicotiana tabacum* L. were used to amplify three plastid microsatellite regions following the procedures reported in Weising and Gardner (1999) (Table 3). One of the primers in each reaction was 5' fluorescently labelled.

PCR-amplified fragments were detected on a denaturing 7M polyacrylamide gel (6 %) by means of an automated laser (ALF) fluorescence sequencer (Pharmacia, Piscataway, NJ, USA). Fragment sizes were calculated using the computer program Fragment Manager version 1.2 (Pharmacia) by comparison with internal and external standards.

Data analysis

In the first stage of the analysis, a study of correlation coefficients between all pairs of the initial 48 morphological variables allowed elimination of 12 completely redundant variables. ANOVA with post-hoc LSD mean comparisons was used for univariate analysis of all morphological characters except HW% and DW%. Data for NLR, NLL, NVR and NVL were square-root transformed before analysis. Due to extreme non-normal distributions of sample values for HW% and DW%, a Kruskal–Wallis test was used to assess differences among *Q. petraea* populations for these characters. Discriminant analysis was used to assess the degree of separation of the populations by multivariate measurements and to test the impact of individual variables on the discrimination (Sokal and Rohlf, 1995). Cluster analysis was used to determine the clustering of populations in multivariate space. A separate discriminant analysis was also performed to verify how *Q. petraea* was delimited taxonomically from *Q. pubescens* in the mixed stands.

Genetic diversity parameters for each population were computed for both nuclear and plastid data according to Nei (1987). Genetic differentiation among populations was assessed by R_{st} (Slatkin, 1995) based on the stepwise-mutation model (SMM). Following this model, alleles similar in size are less different in terms of mutational steps than alleles showing greater difference in size (Jarne and Lagoda, 1996). A hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was also performed. The significance of the values obtained was inferred by bootstrap analysis (Excoffier *et al.*, 1992). Average linkage clustering using an unweighted pair-group method (UPGMA) was performed for study populations using R_{st} genetic distance in order to portray genetic dissimilarity among populations graphically.

Mantel tests (Mantel, 1967) were performed on matrices of dissimilarity between populations considering morphological characters, genetic distances, geographic distances and climatic variables. The matrix of geographic distances was calculated from Euclidean distances between populations using coordinates of latitude and longitude. Matrices

TABLE 4. Means and standard deviation (s.d.) for leaf characters

Character	PSM			MC			TT			CR			MR			F [4, 145]
	Mean	s.d.		Mean	s.d.		Mean	s.d.		Mean	s.d.		Mean	s.d.		
LP	1.54	0.29	ac	1.79	0.33	b	1.58	0.45	ac	1.66	0.45	ab	1.43	0.33	c	3.97
LL	7.86	0.98	a	9.85	0.99	b	9.52	1.02	bc	9.19	1.15	c	9.74	1.60	bc	14.26
MWL	4.48	0.99	a	6.01	0.61	b	5.04	1.02	c	5.41	0.68	c	5.15	0.79	c	16.26
HMW	4.20	0.91	a	4.23	0.45	a	3.54	0.62	b	3.85	0.62	c	3.70	0.55	bc	5.73
LSP	1.96	0.82	a	2.32	0.31	b	2.14	0.28	b	1.97	0.40	b	1.98	0.30	b	10.56
MDS	1.48	0.45	a	1.35	0.19	b	1.31	0.24	b	1.30	0.22	b	1.31	0.27	b	9.21
DVL	2.68	0.38	a	3.03	0.32	b	2.63	0.42	a	2.81	0.34	a	2.75	0.71	a	4.13
WHL	1.53	0.42	a	1.11	0.11	b	1.22	0.24	b	1.11	0.15	b	2.77	0.61	c	8.16
WTL	0.95	0.23	a	0.69	0.17	b	0.84	0.19	a	0.89	0.19	a	0.85	0.38	a	10.90
LTL	0.58	0.21	a	0.45	0.10	b	0.46	0.13	b	0.50	0.10	ab	0.46	0.24	a	10.97
NLR	5.11	0.87	a	6.76	0.63	b	6.52	1.03	b	6.60	0.59	b	6.70	0.86	b	21.79
NLL	5.17	0.82	a	6.66	0.80	b	6.60	1.11	b	6.60	0.76	b	6.60	0.65	b	17.73
NVR	6.37	0.99	a	9.31	1.15	b	7.47	0.69	b	8.60	0.97	b	7.73	0.83	b	43.16
NVL	6.39	1.07	a	9.46	0.97	b	7.53	0.82	c	8.51	1.31	d	7.57	0.76	c	39.40
TTL	198.47	19.4	a	159.8	9.48	b	175.6	15.16	c	159.14	15.92	b	174.53	18.0	c	42.12
TUE	22.88	3.61	a	21.03	1.50	b	22.56	1.33	a	22.85	1.82	a	23.36	2.18	a	4.71
TP	82.76	25.6	a	79.56	5.89	ab	75.16	11.52	b	56.74	12.34	c	67.52	9.42	d	15.29
TS	72.03	9.01	a	45.52	3.09	b	64.52	7.93	c	66.36	9.95	cd	69.19	9.92	ad	46.74
AREA	23.93	5.33	a	31.67	5.45	b	30.08	4.67	b	33.24	5.65	b	32.79	5.80	b	20.26
SDW	0.010	0.002	a	0.0078	0.001	b	0.0086	0.001	c	0.0077	0.001	b	0.0117	0.02	a	21.98
V	4749	1108	a	5052.4	881.2	b	5325	1082	b	5320.5	1218	b	5637.5	2271	c	8.76
DOR	2.38	0.72	a	1.53	0.43	b	2.21	0.54	a	1.33	0.99	b	1.43	0.40	b	16.16
AXI	3.01	0.64	a	1.10	0.30	b	1.32	0.33	b	1.33	0.92	b	2.03	0.85	c	41.95
PET	1.86	0.70	a	0.82	0.68	bc	0.96	0.39	b	0.60	0.56	b	0.88	0.24	c	23.91
TSH	2.53	0.57	a	2.10	0.68	bc	2.35	0.63	ab	1.73	0.78	d	1.81	0.72	c	7.44
TW	2.05	1.19	a	0.82	0.91	bc	1.10	1.08	b	0.56	0.56	c	0.98	0.60	bc	11.51
SD	376.42	63.9	a	335.58	40.02	bc	306.7	48.32	b	350.7	40.85	c	422.21	59.2	d	76.40
LS	23.11	2.39	a	25.01	1.32	b	25.29	1.73	b	24.70	1.74	b	22.80	1.69	a	81.20
WS	17.37	1.98	a	18.76	0.73	bc	20.30	1.51	d	19.06	1.39	b	18.29	0.80	c	65.40
FR	5.64	0.94	a	7.11	0.77	b	10.29	1.07	c	8.77	1.41	d	6.00	1.02	a	100.19
NST	75.26	17.6	a	40.24	15.5	b	43.58	18.0	b	41.19	14.8	b	45.72	14.0	b	25.09
NGT	67.55	9.74	a	125.08	16.9	b	62.86	19.5	a	56.82	14.6	a	61.77	14.9	a	100.50
LRS	109.16	17.2	a	113.11	9.26	a	112.7	14.2	a	80.26	18.8	b	92.40	11.8	c	29.65

Means followed by the same letter in the same row are not significantly different at $P < 0.001$ according to LSD test.

of dissimilarity in climatic data among populations were derived from taxonomic distances of annual mean temperatures, annual mean precipitation, mean temperatures of the warmest and coldest months, and the index of normalized insolation, which was used as an index of heat. Based on the number of hours per year of overhead sunlight, the index of normalized insolation is a measure of the radiation received by a site in a single year, given its aspect, slope and latitude (Bartorelli, 1967). All matrices were transformed to zero mean and unit variance before performing Mantel tests.

RESULTS

Morphological analysis

A preliminary discriminant analysis performed on trees sampled from mixed stands showed that *Q. petraea* and *Q. pubescens* are morphologically differentiated, both at the inter- and intrapopulation level.

The leaf characters measured and their ratios were highly variable. There were significant differences among *Q. petraea* populations (ANOVA, $P < 0.001$) for 31 characters (Table 4). LSD tests indicated that leaves of PSM trees were significantly smaller and thicker, and had higher specific leaf mass, than leaves of trees in the other populations. Trees from both PSM and MR populations had

TABLE 5. Standardized coefficients for the first three discriminant functions of population means morphological traits

Variables	Function 1	Function 2	Function 3
MWL			0.444
LS	0.320		
FR	0.414	0.476	-0.573
SD	-0.338		
NST		0.347	
NGT		0.498	0.415
AXI	-0.432		
TTL	-0.456	1.319	0.706
TP	0.271		
SDW	0.338		
V	1.103	2.381	0.883
AREA	-0.655	-1.678	-0.572

smaller leaves and denser stomata than those from more mesic areas (Table 4).

Discriminant analysis revealed that the three functions represented 91 % of the total variation in the data set (Table 5). The first discriminant function accounted for 39 % of the total variance, and separated the PSM and MR populations from the others (Fig. 2). The standardized coefficients of function 1 were highest for micromorphological, pubescence and thickness traits and for productivity

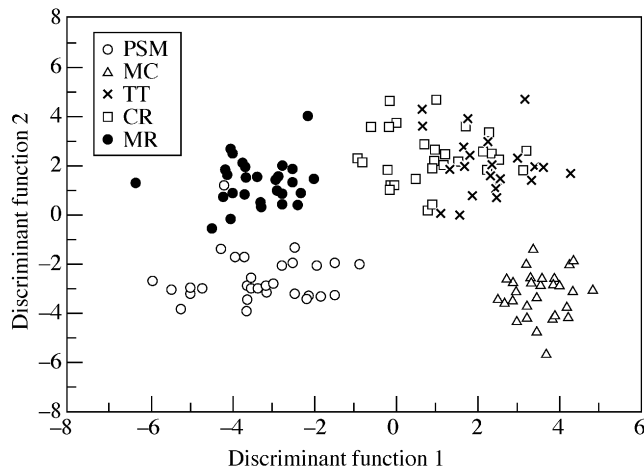


FIG. 2. Discriminant scores for the first three functions of population morphological trait means.

parameters. Function 2 represented another 30 % of the total variance and separated the MR, TT and CR populations from the MC and PSM populations (Fig. 2). This function was related to micromorphology, thickness and productivity traits. Function 3 accounted for 22 % of the total variation and was a productivity and a freedom of rim susceptibility function. It separated MC from CR and TT. Cross validation of the original data suggested that the classification scheme of five populations provided robust discrimination. Overall misclassification was low (1 %), with values of 100, 100, 100, 96 and 100 % for populations PSM, MC, TT, CR and MR, respectively.

Cluster analysis was used to investigate further the inter-relationships of these populations. The unweighted pair-group arithmetic average method (UPGMA) and the complete-linkage method were carried out on the taxonomic distance matrix (Fig. 3). Relationships of populations were similar to the relationships observed in the discriminant analysis using the first three functions. The PSM population represents a distinct group in both the cluster and discriminant analysis. The second group represents MC. Another group is composed of the TT and CR populations, while the final group represents the MR population.

The multivariate taxonomic distance matrix for all traits showed no significant association with geographic distance. A significant correlation ($P = 0.039$; $r = 0.753$) was found between the multivariate morphological matrix and the multivariate climatic distance matrix when the MR population was omitted from the data set. Given the pedological characteristics of this site, xeric morphological adaptation may be attributed to edaphic factors rather than climatic variables.

Molecular analysis

Quercus petraea shows remarkably high levels of diversity. A total of 81 alleles was scored from the six nuclear loci across five populations. In particular, loci AG 36 and AG 9 were highly polymorphic with 21 and 14

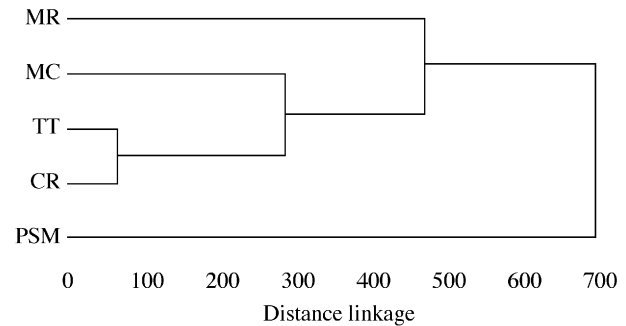


FIG. 3. UPGMA dendrogram based on average taxonomic distances among population means of morphological traits.

alleles, respectively. Locus AG 362, characterized by a smaller number of repetitions, showed only six alleles. Three plastid microsatellite loci produced 11 variants combined in 16 haplotypes. No haplotype was common to all populations, while eight of them were found in single populations (i.e. population-specific haplotypes). Genetic variability parameters for the nuclear markers are presented in Table 6. Differentiation among populations based on R_{st} was 18 % for nuclear loci. AMOVA confirmed this result: 80 % of the diversity resided within populations, while the proportion of total genetic diversity among populations was about 20 % (still highly significant; $P < 0.001$) (Table 7). A UPGMA phenogram constructed on R_{st} values to examine patterns of relatedness among populations showed that the close populations (TT and MR) tended to be paired, whereas the southernmost population (PSM) appeared highly differentiated from the others (Fig. 4). Genetic distance and geographic distance were significantly correlated ($P = 0.0$, $r = 0.530$).

At the plastid level, the population that showed the lowest value of within-population diversity was PSM, as revealed by the estimated parameters that are based on haplotype frequencies (effective number of haplotypes, n_e , and genetic diversity, H).

The proportion of genetic differentiation among populations, estimated using R_{st} , was 0.393, thus indicating that more than 39 % of haplotype variation was due to differences among populations. The higher level of haplotype divergence among populations is confirmed by the analysis of molecular variance (Table 8).

The UPGMA phenogram revealed three groups: TT–MR, MC–CR and PSM (Fig. 5). A Mantel randomization test showed no significant relationship between geographic and genetic distance ($P = 0.405$, $r = -0.0686$).

DISCUSSION

In recent years there has been much research into the distribution of genetic diversity in *Q. petraea*. A common characteristic of such studies is their large sampling of populations covering most of the natural range (Zanetto and Kremer, 1995). Results from these studies have indicated clinal trends of variation. In Italy, the number of sessile oaks has been drastically reduced in the last two centuries due to

TABLE 6. Genetic diversity statistics

Population	H_o (s.d.)	H_e (s.d.)	A	n_e	H	n_e^*
CR	0.445 (0.22)	0.737 (0.25)	7.83	3.80	0.633 (0.24)	2.51
TT	0.558 (0.31)	0.777 (0.28)	9.80	4.48	0.755 (0.27)	3.46
MR	0.549 (0.24)	0.740 (0.32)	8.00	3.84	0.855 (0.27)	5.34
MC	0.571 (0.27)	0.770 (0.33)	8.00	4.34	0.871 (0.28)	5.81
PSM	0.587 (0.27)	0.750 (0.28)	9.50	4.00	0.585 (0.21)	2.23

H_o , Observed heterozygosity; H_e , gene diversity, or expected heterozygosity; A, mean number of alleles per locus; n_e , effective number of alleles; H, haplotypic diversity; n_e^* , effective number of haplotypes. Values in parentheses are standard deviations.

TABLE 7. Nuclear markers

Variance component	d.f	SS	Variance	% Total	Φ -stats	P
Among populations	4	128 226	0.50100	20.00	0.201	<0.001
Within populations	295	587 717	1.99226	80.00		
Total	299	715 943	2.49326			

Hierarchical analysis of molecular variance (AMOVA): d.f., degree of freedom; SS, sum of squares; P, relative probability.

human activity, and *Q. petraea* is currently distributed in discontinuous and widely spaced patches. It is impossible to sample intensively along a latitudinal gradient to determine whether the variation is a cline. In this study, samples were obtained from five locations at various latitudes in order to study the general pattern of morphological and molecular variation and to determine whether the variation is related to geographic factors.

Morphological analysis

Several studies have indicated that morphological variation is apparently the result of an adaptive response to the environment; for example, variation of some traits is associated with a latitudinal and altitudinal range (Kleinschmit, 1993). Our study suggests that some characters were variable among populations without showing any geographic trend. The observed trend of morphological variation suggests adaptation to the contrasting climatic conditions prevailing for these groups, and this was supported by the significant correlation with climatic parameters and not with interpopulation distance.

Multivariate analyses revealed a structure in which PSM and MR populations were differentiated from those in the more mesic localities. Trees sampled in PSM and MR populations showed morphological differences (e.g. leaves with numerous but smaller stomata) that have been noted for xeric vs. mesic populations of *Q. petraea* and that may influence leaf water balance, resulting in each genotype being better adapted to its own environment (Abrams, 1990). The PSM trees, which are subjected to higher light intensity, had smaller leaves and a high volume : leaf surface area ratio.

Although trees in the neighbouring populations of TT and MR experienced the same climate, many morphological differences were found, probably due to edaphic factors. As suggested by Carter *et al.* (1987) for the serpentine soils of

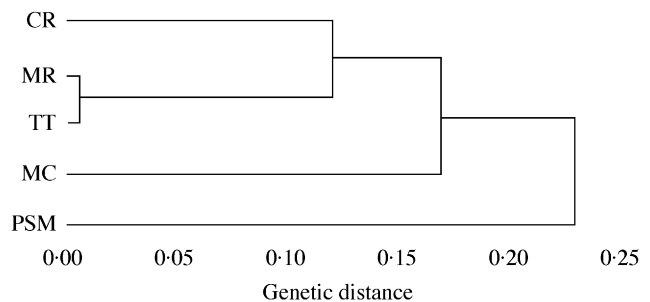


FIG. 4. Dendrogram of *Q. petraea* populations based on R_{st} values (Slatkin, 1995) calculated at nuclear loci.

the Shetland Islands, summer droughts of 7 or more consecutive days may exhaust the water reserves in this type of soil. Thus, water stress, together with soil nutritional deficiencies, may have led to the development of xeromorphic adaptations in the MR population compared with the TT population.

Molecular analyses

The total genetic diversity found among *Q. petraea* populations (Table 6) is in the range for long-lived woody perennials, wind-outcrossing species, and late-successional species (Hamrick and Godt, 1990). Genetic diversity is greater within populations than between them. However, although only populations from a restricted geographical area were analysed, significant differences in R_{st} were observed at both the nuclear and plastid levels (18 and 39 %, respectively). In particular, the differentiation value for nuclear microsatellites was much higher than that for other markers: the mean coefficient of genetic differentiation for 11 isoenzyme loci in *Q. petraea* was 0.03 (Zanetto and Kremer, 1995).

TABLE 8. *Plastid markers*

Variance component	d.f.	SS	Variance	% Total	Φ -stats	<i>P</i>
Among populations	4	78 367	0.61384	34.29	0.3428	<0.001
Within populations	145	170 588	1.17646	65.71		
Total	149	248 955	1.79030			

Hierarchical analysis of molecular variance (AMOVA): d.f., degree of freedom; SS, sum of squares; *P*, relative probability.

The higher level of subdivision observed for plastid DNA polymorphism is in agreement with theoretical expectations (Birky *et al.*, 1989; Petit *et al.*, 1993) and with that reported in earlier studies of oak (Dumolin-Lapegue *et al.*, 1997) and other species (Ennos, 1994). The strong population differentiation in plastid DNA for *Q. petraea* is most likely due primarily to maternal inheritance, and restricted seed dispersal. The rate of gene flow by pollen dispersal of *Q. petraea* is considerably higher than that by seed dispersal (Ennos, 1994). Thus, differential rates of gene flow via seed and pollen dispersal should be an important factor in the high level of population differentiation found in plastid DNA compared with that in the nuclear genome.

The low, but significant, correlation between geographic and genetic distance, observed at nuclear loci, suggests that isolation by distance could have played a role in the genetic structure of these populations, with the more closely adjacent populations (TT and MR approx. 15 km apart) exposed to higher interpopulation pollen flow. The PSM population is the most disjunct (approx. 900 km away from any other) and it has the greatest genetic distance, as seen in the phenogram. According to Wright's (1943, 1946) isolation by distance models, mating is dependent on the distance between individuals and their ability to disperse propagules. Habitat fragmentation could prevent further gene flow among Italian populations of *Q. petraea*. Other factors, such as founder effects, bottlenecks and genetic drift could have affected genetic relationships among these populations, and the pattern that exists could be the result of past and current gene flow and other historical events. However, additional research is necessary to confirm this. Microsatellite variation should be reassessed in these and other populations to look at the long-term effects of human-induced habitat fragmentation and habitat degradation on genetic structure in this species.

Average gene heterozygosity (H_e) did not differ among the five populations at the nuclear level. A possible cause for this lack of differentiation is introgression of genes from *Q. pubescens* (hairy oak). Evidence for introgression in *Quercus* has been documented for a number of species, including *Q. petraea* and *Q. robur* (Bacilieri *et al.*, 1995). Introgression from *Q. pubescens* into *Q. petraea*, and vice versa, is also a possibility; artificial crosses have demonstrated that the two species are fully compatible (unpubl. res.). Moreover, in a previous study (Bruschi *et al.*, 2000) in which levels of genetic differentiation between *Q. petraea* and *Q. pubescens* in both mixed and pure stands were compared, we found a nuclear differentiation value in the TT mixed stand that was lower than that observed between

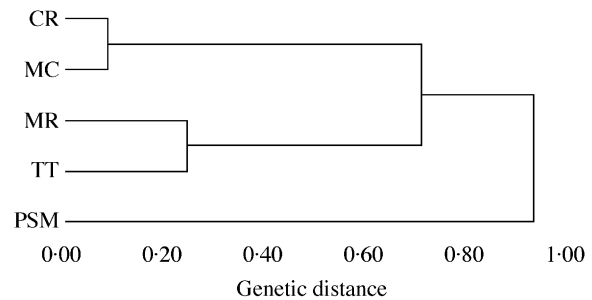


FIG. 5. Dendrogram of *Q. petraea* populations based on R_{st} values (Slatkin, 1995) calculated at plastid loci.

pure stands. This finding could confirm the high level of gene flow and indicate possible hybridization between the species.

At the plastid level, the highest value ($H = 0.871$) was found in the MC population, while the lowest ($H = 0.585$) was found in trees from the southern region—this may have been the result of genetic drift. The PSM population is situated at the southernmost point of the biogeographic range of *Q. petraea*, and isolation may have exacerbated genetic drift in this disjunct population. On the other hand, the uniparental inherited plastid genome appears to be a more sensitive indicator of the possible effects of genetic drift and bottleneck because the effective population size is half that of diploid, biparentally inherited markers (Dumolin-Lapegue *et al.*, 1997). For example, Morgante *et al.* (1997) detected a dramatic bottleneck among Greek and Italian populations of *Pinus halepensis* by means of plastid microsatellites, whereas this was not picked up when using allozyme markers.

Correlation between morphological and molecular markers

No significant correlation was detected between the observed patterns of molecular variation and leaf morphological variation ($P = 0.478$, $r = -0.0589$). There may be several reasons for the discrepancy between results based on morphology and microsatellites. First, microsatellites are considered to be neutral and thus to provide no direct assessment of fitness. The forces that cause differentiation for these markers would be the result of mutation, genetic drift and low gene flow and no selection. Conversely, morphological traits are generally believed to be subject to natural selection, and their expression is partially under the influence of environmental factors. Secondly, in contrast to

morphological traits, microsatellite variation is based directly on DNA sequence variation. A change in a nucleotide repetition can result in a change in microsatellite pattern. Despite these basic differences, low gene flow would allow accumulation of small (MC) or higher (PSM) adaptive differences, explaining some concordance between morphological and molecular traits.

Conservation implications

From a conservation perspective, the high genetic diversity observed within the populations tested is encouraging. Nevertheless, ecological management of *Q. petraea* sites will be necessary to preserve populations *in situ*. Four of the populations analysed (TT, MR, CR and PSM) are legally protected by public ownership; the fate of MC is dependent on a private landowner. If there were to be excessive logging, the consequent decline in population size could increase the risk of extinction due to demographic and environmental stochasticity (Gilpin and Soule, 1986). Increased risks of extinction could also be expected in the PSM population due to its small size, logging disturbance, fire and heavily grazed pastures. The diminished genetic diversity found within this population may be the result of genetic drift. In subsequent generations, the loss of heterozygosity that results from genetic drift and inbreeding may lead to diminished fitness in this typically outcrossing species. Moreover, Newman and Pilsen (1997) have shown that populations with small genetically effective population sizes have a higher probability of extinction compared with populations with a high effective size. Thus, given that the Sicilian populations are presumably one of the refugia and the origin of genetic diversity prior to post-glacial migration (Dumolin-Lapegue *et al.*, 1997), conservation efforts should focus on this population. Moreover, following Lessica and Allendorf (1995), peripheral or isolated populations that exist in habitats that differ with respect to soil, climate and competitors may be the source of new adaptations.

Ex situ efforts should also be undertaken to preserve *Q. petraea* genotypes. Restoration of extirpated populations should also be considered. *Ex situ* preservation could be achieved through seed or vegetative propagation, open-pollinated progeny, controlled crosses and cryopreservation. Propagation from seeds is preferred since it would be the least detrimental to the extant populations and would include the widest range of genetic variability.

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