

# Allozyme Variation in Different Species of Deciduous Oaks From Northwestern Italy

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## Abstract

Allozyme variation was studied by electrophoresis at 11 loci in 14 populations of *Quercus petraea*, *Q. robur*, *Q. cerris* and *Q. pubescens* from Piedmont, northwestern Italy. The aim of the work was to characterize the genetic resources and to suggest effective measures for *in situ* preservation of biodiversity.

As expected, most of the genetic variability was found at the within population level, and genetic differentiation accounted for about 14% of it. The study confirmed the low level of genetic variability among the species studied: only *Q. cerris* populations could be clearly distinguished. *Q. pubescens* could be separated from *Q. petraea* and *Q. robur* using Principal Coordinate Analysis. The latter was also found to be useful for separating *Q. petraea* and *Q. robur* populations. However, in mixed populations, the differentiation between the latter species was similar to that scored among populations belonging to the same species. A significant deviation from random mating was observed, although it was variable among species: *Q. pubescens* had the highest value for the inbreeding coefficient  $F_{IS}$  (0.159).

**Key words:** Allozyme variation, genetic diversity, heterozygosity, population differentiation, *Quercus* spp.

## Introduction

Deciduous oaks are long-lived species producing timber of high value. In the past they were among the most common forest trees in the Po Valley (Northern Italy). At present, agricultural activities, environment deterioration and tree cutting have fragmented populations of *Quercus robur* L. (English oak) and *Quercus petraea* (Matt.) Liebl. (Sessile oak) into smaller and more isolated breeding groups with potentially restricted gene flow between them (KREMER et al., 2002a; PETIT et al., 2002; BRUSCHI et al., 2003; MUIR et al., 2004). The natural ranges of *Q. robur* and *Q. petraea* largely overlap, although the former is mainly found in rich and humid sites in the lowlands, while the latter is better suited to the dryer and warmer sites at medium altitude (KLEINSCHMIT, 1993). Moreover, *Q. robur* is considered to be a pioneer species that can be progressively replaced by *Q. petraea* (BACILIERI et al., 1996b). *Quercus cerris* L. (European Turkey oak) prefers the habitat of the hilly regions of Apennines chain, while *Quercus pubescens*

Willd. (Pubescent oak) is a more frugal species usually found on sunny and dry slopes at medium-high altitudes (FENAROLI and GAMBÌ, 1976).

According to MANOS et al. (1999) the species considered in this study belong to two phylogenetically distinct subgenera: subg. *Lepidobalanus* (*Q. petraea*, *Q. robur* and *Q. pubescens*, also known as white oaks) and subg. *Cerris* (*Q. cerris*). However, the systematic relationships between different species of the genus *Quercus* still have to be clarified, due to the frequent hybridisation between taxa (NIXON, 1993; MUIR et al., 2000; PETIT et al., 2002). In particular, hybrid introgression across species has been demonstrated, mainly by morphometric studies (RUSHTON, 1993; BRUSCHI et al., 2000), between *Q. petraea* and *Q. robur*, and extensive interspecific gene flow has also been reported in mixed stands of the same species. *Q. petraea* preferentially pollinates *Q. robur*, whereas the reciprocal crosses seldom occur (BACILIERI et al., 1993; BACILIERI et al., 1994; STREIFF et al., 1999). Isozyme analysis and DNA analysis could make an important contribution to improving our knowledge on this aspect, identifying markers useful for species differentiation. Results so far have been only partially encouraging (ZANETTO et al., 1994; KLEINSCHMIT et al., 1995; SAMUEL et al., 1995; BACILIERI et al., 1996; COART et al., 2002). Recently, several species-specific RAPD markers have been identified and proposed as a tool to detect interspecific hybridisation between *Q. robur* and *Q. petraea* (SAMUEL, 1999).

In oaks, isozymes have also been used to study the level and distribution of genetic variation in populations. In general, oaks were proven to be more variable than other broad-leaved and widespread forest species. In addition, genetic variation was found to be high within populations but low among populations, and a significant deficit of heterozygotes was observed in most populations studied (MÜLLER-STARCK and ZIEHE, 1991; KIM et al., 1993; MÜLLER-STARCK et al., 1993; ZANETTO et al., 1993; MATTILA et al., 1994; BACILIERI et al., 1995; SAMUEL et al., 1995; ZANETTO and KREMER, 1995; KREMER and ZANETTO, 1997; LE CORRE et al., 1998; GÓMÓRY et al., 2000; FINKELDEY and MÁTYÁS, 2003).

The present study was carried out to describe the genetic variation and differentiation at isozyme loci within and between 14 natural populations of four deciduous oak species from northwestern Italy. The effect of intraspecific gene flow on the genetic structure of *Q. petraea* and *Q. robur* has been evaluated, and general versus local differentiation between two locations where both species were sampled has been quantified. The data obtained are of basic importance for the characterisation of genetic resources and for the adoption of practical and effective measures for *in situ* preservation

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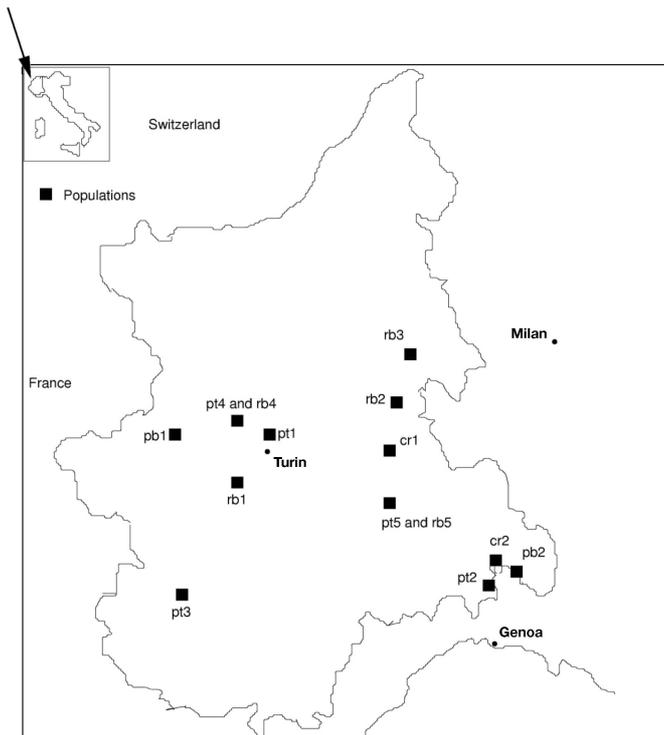
of biodiversity (BRUSCHI et al., 2003). Genetic structure could be used to understand mating system patterns, that can be influenced both by isolation of stands and low densities (LEDIG, 1988; 1992; ELLSTRAND and ELAM, 1993; DUTECH et al., 2005), and extent of genetic distinctness of populations. Furthermore, aim of the study was also to assess if our markers are useful to distinguish different oak species. This is particularly important for *Q. petraea* and *Q. robur*, giving the high levels of hybridisation between species and the difficulties to identify them by morphological and genetic characters (KREMER et al., 2002b; PONTON et al., 2004).

## Materials and Methods

### Plant material and sampling

Fourteen natural oak populations representative of the locations where the species grow in Piedmont were sampled, including 5 populations of *Q. petraea* and *Q. robur* and 2 of *Q. cerris* and *Q. pubescens*. Species identity of the collected material was verified on the basis of leaf characteristics, mainly length of peduncule and relative height of maximum width (DUPOUEY and BADEAU, 1993).

The collection sites are situated in Piedmont, north-western Italy. They are shown in *Figure 1*. The populations were chosen on the basis of preliminary observation regarding the possession of requisites for inclusion in the official Italian list of seed stands: they are native,



*Figure 1.* – Sites of studied oak populations. *Quercus petraea*: pt1 Castagneto Po, Torino; pt2 Capanne Marcarolo, Alessandria; pt3 Sampeyre, Cuneo; pt4 La Mandria, Torino; pt5 Rocchetta Tanaro, Asti. *Q. robur*: rb1 Stupinigi, Torino; rb2 Bosco della Partecipanza, Vercelli; rb3 Lame del Sesia, Vercelli; rb4 La Mandria, Torino; rb5 Rocchetta Tanaro, Asti. *Q. cerris*: cr1 Rolasco, Alessandria; cr2 Gavi Ligure, Alessandria. *Q. pubescens*: pb1 Chianocco, Torino; pb2 val Borbera, Alessandria.

large enough to prevent inbreeding phenomena and isolated from other stands of lower quality or artificial origin. The trees also possess good silvicultural and production characteristics. The populations contain different age classes and natural regeneration was observed in all of them. All the selected populations grow in mixed forest, with *Q. cerris*, *Q. pubescens* and *Q. petraea* representing the dominant species. *Q. robur* is present at a lower density. In two locations, the stands of *Q. petraea* are adjacent, and even overlapping with those of *Q. robur* (populations pt4 with rb4 and pt5 with rb5 respectively). However, in these locations material has not been sampled in the overlapping areas and only individuals with morphological characteristics peculiar to each species have been sampled.

Isozyme analysis was carried out on winter buds from at least 75 adult non-adjacent plants, chosen at random over a 2–5 ha area. Buds were collected in November to February, and were kept at 4°C until analysis (within a period no longer than two weeks).

### Electrophoretic methods

Enzymes were separated by horizontal gel electrophoresis in 11% hydrolysed potato starch (Sigma S4501). Eleven enzyme systems were studied (acronyms and Enzyme Commission number are reported between brackets): alcohol dehydrogenase (Adh, 1.1.1.1), NADH-diaphorase (Dia, 1.6.4.3), glutamate dehydrogenase (Gdh, 1.4.1.2), glutamate oxaloacetate transaminase (Got, 2.6.1.1), glyceraldehyde 3-phosphate dehydrogenase (G3pdh, 1.2.1.12), isocitric dehydrogenase (Idh, 1.1.1.42), malate dehydrogenase (Mdh, 1.1.1.37), phosphoglucuronate dehydrogenase (6Pgdh, 1.1.1.44), phosphoglucose isomerase (Pgi, 5.3.1.9), phosphoglucumutase (Pgm, 5.4.2.2), shikimic dehydrogenase (Skdh, 1.1.1.25). The number of scored alleles was 2 for G3pdh, 3 for Adh, Dia, Gdh, Got, Mdh, Pgm, Skdh and 4 for Idh, 6Pgdh, Pgi.

Two different buffer systems, differing only in pH, were used depending on the enzyme system to be analysed: tris-citrate 1 (TC1, Electrode buffer: tris 0.135 M and gel buffer: tris 0.056 M, titrated at pH 7.0 with HCl) for Adh, Gdh, Got, G3pdh, Pgm, Skdh and Tris-citrate 2 (TC2, pH 7.8) for Dia, Idh, Mdh, 6Pgdh, Pgi.

Approximately 100 mg of tissue from descaled buds was ground in 100 µl of extraction buffer (0.1 M Tris, 2.7 mM EDTA di-sodium salt, 0.1 M ascorbic acid, 6.5 mM dithiothreitol, supplemented with 1 mg/ml PVP-40 and 0.5% mercaptoethanol, and titrated at pH 7.2). The homogenate was absorbed onto filter-paper wicks and loaded onto gel slabs. Gels were run at 12 (TC1) or 8 (TC2) Vcm<sup>-1</sup> for 30 minutes before removing the wicks, and then left overnight.

Enzyme staining followed the procedures given by WENDEL and WEEDEN (1989), except for Dia (WEEDEN, 1984). In staining for Adh, Idh and Pgi, 5 mg of NADP were used instead of NAD, whereas in staining for Adh and Gdh, respectively, 2 ml of ethanol and 1 g of L-glutamate sodium salt were used.

The statistically identified loci were indicated by capital letters following the enzyme acronyms: the most

anodal zone was designated with A. Within a single zone of activity the allozyme variants were labelled according to their mobility relative to the most common allele, which was labelled 100 (Rm).

*Data analysis*

Population genetics parameters were estimated using the Biosys-1 program (SWOFFORD and SELANDER, 1981). Deviations from Hardy-Weinberg equilibria were tested by  $\chi^2$  and Fisher exact tests, pooling genotypes whenever expected frequencies were less than 0.05. The following multilocus measures of gene diversity were calculated: mean observed and expected heterozygosity, average number of alleles per locus, percentage of polymorphic loci (by convention, loci were considered polymorphic if the frequency of the most common allele did not exceed 0.99). Genetic distances were calculated according to NEI (1978).

Genetic differentiation among populations was measured as  $\theta$ , according to WEIR and COCKERHAM (1984).

Standard deviation of  $\theta$  was estimated by a jackknife procedure over loci. Principal Coordinate Analysis (PcoA) was carried out only on the matrix of Nei genetic distances using the *cmdscale* (VENABLES and RIPLEY, 1997) function of the R statistical (R DEVELOPMENT CORE TEAM, 2004). Linkage disequilibrium was calculated with a permutation test (WEIR, 1996) of 1000 times each by the GDA computer package program. Distinction between general ( $C_g$ ) and local differentiation ( $C_l$ ) between *Q. robur* and *Q. petraea* was calculated according to BODENES et al. (1997). Local species differentiation represents the variation of species differentiation from one region to another, and it is seen as an interaction between species and region.

**Results**

Eleven enzyme systems were studied, each with one region of activity, and all loci were found to be polymorphic. In general, the observed banding patterns were in

Table 1. – Allele frequencies and expected heterozygosity ( $H_e$ ) in the 14 oak populations under study.

Locus	Allele	Population													
		pt1	pt2	pt3	pt4	pt5	rb1	rb2	rb3	rb4	rb5	cr1	cr2	pb1	pb2
Adh	A-124	.076	.053	.203	.136	.200	.000	.000	.030	.064	.025	.000	.043	.125	.104
	A-100	.822	.856	.608	.864	.757	.758	.896	.948	.779	.950	.640	.761	.833	.885
	A-88	.102	.091	.189	.000	.043	.242	.104	.022	.157	.025	.360	.196	.042	.010
	$H_e$	<b>.311</b>	<b>.258</b>	<b>.461</b>	<b>.423</b>	<b>.232</b>	<b>.388</b>	<b>.370</b>	<b>.188</b>	<b>.101</b>	<b>.368</b>	<b>.097</b>	<b>.466</b>	<b>.385</b>	<b>.291</b>
Dia	A-122	.397	.613	.701	.300	.133	.066	.199	.048	.110	.133	.000	.000	.074	.067
	A-100	.532	.387	.201	.700	.867	.934	.801	.935	.884	.867	.866	1.000	.657	.767
	A-76	.071	.000	.098	.000	.000	.000	.017	.006	.000	.134	.000	.269	.167	
	$H_e$	<b>.559</b>	<b>.478</b>	<b>.461</b>	<b>.423</b>	<b>.232</b>	<b>.124</b>	<b>.321</b>	<b>.123</b>	<b>.209</b>	<b>.232</b>	<b>.235</b>	<b>.000</b>	<b>.495</b>	<b>.384</b>
Gdh	A-120	.167	.212	.025	.129	.008	.042	.000	.110	.068	.091	.000	.000	.144	.000
	A-100	.778	.636	.900	.550	.719	.842	.770	.797	.636	.773	.287	.364	.615	1.000
	A-86	.056	.152	.075	.321	.273	.116	.230	.093	.296	.136	.712	.636	.240	.000
	$H_e$	<b>.367</b>	<b>.532</b>	<b>.185</b>	<b>.582</b>	<b>.412</b>	<b>.279</b>	<b>.357</b>	<b>.348</b>	<b>.507</b>	<b>.379</b>	<b>.415</b>	<b>.468</b>	<b>.548</b>	<b>.000</b>
Got	B-122	.286	.125	.144	.112	.114	.082	.000	.038	.045	.174	.314	.107	.075	.000
	B-100	.714	.773	.856	.851	.886	.820	.919	.962	.932	.758	.686	.750	.925	1.000
	B-78	.000	.102	.000	.037	.000	.098	.081	.000	.023	.068	.000	.143	.000	.000
	$H_e$	<b>.411</b>	<b>.379</b>	<b>.249</b>	<b>.264</b>	<b>.203</b>	<b>.314</b>	<b>.150</b>	<b>.073</b>	<b>.130</b>	<b>.394</b>	<b>.436</b>	<b>.410</b>	<b>.141</b>	<b>.000</b>
G3pdh	A-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.974	.967
	A-94	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.026	.033
	$H_e$	<b>.000</b>	<b>.052</b>	<b>.065</b>											
ldh	A-136	.032	.000	.000	.115	.000	.000	.000	.000	.007	.000	.146	.000	.000	.000
	A-122	.145	.058	.235	.269	.097	.271	.234	.310	.197	.230	.134	.151	.010	.000
	A-100	.815	.758	.647	.615	.903	.681	.766	.627	.553	.738	.720	.849	.960	1.000
	$H_e$	<b>.317</b>	<b>.391</b>	<b>.516</b>	<b>.540</b>	<b>.176</b>	<b>.464</b>	<b>.362</b>	<b>.511</b>	<b>.600</b>	<b>.405</b>	<b>.448</b>	<b>.260</b>	<b>.078</b>	<b>.000</b>
Mdh	B-124	.151	.187	.101	.150	.153	.061	.097	.048	.013	.061	.310	.112	.048	.143
	B-100	.825	.806	.855	.707	.822	.932	.879	.798	.981	.931	.488	.888	.731	.847
	B-86	.024	.007	.044	.143	.025	.007	.024	.154	.006	.008	.202	.000	.221	.010
	$H_e$	<b>.298</b>	<b>.318</b>	<b>.259</b>	<b>.460</b>	<b>.303</b>	<b>.128</b>	<b>.219</b>	<b>.339</b>	<b>.037</b>	<b>.129</b>	<b>.633</b>	<b>.201</b>	<b>.419</b>	<b>.265</b>
6Pgdh	B-132	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.073	.065	.010	.000
	B-124	.156	.331	.217	.111	.000	.042	.234	.197	.147	.059	.293	.022	.029	.049
	B-100	.844	.645	.768	.794	.970	.888	.766	.803	.853	.846	.634	.913	.923	.951
	$H_e$	<b>.265</b>	<b>.478</b>	<b>.365</b>	<b>.351</b>	<b>.058</b>	<b>.120</b>	<b>.361</b>	<b>.319</b>	<b>.252</b>	<b>.276</b>	<b>.513</b>	<b>.163</b>	<b>.147</b>	<b>.094</b>
Pgi	B-124	.059	.031	.053	.033	.111	.021	.075	.008	.069	.033	.000	.000	.054	.023
	B-100	.864	.930	.909	.943	.841	.883	.895	.886	.925	.942	.000	.000	.902	.955
	B-92	.077	.039	.038	.024	.048	.096	.030	.106	.006	.025	.329	.153	.045	.023
	$H_e$	<b>.246</b>	<b>.134</b>	<b>.171</b>	<b>.111</b>	<b>.280</b>	<b>.211</b>	<b>.193</b>	<b>.205</b>	<b>.140</b>	<b>.112</b>	<b>.447</b>	<b>.262</b>	<b>.184</b>	<b>.089</b>
Pgm	A-124	.058	.083	.188	.104	.075	.189	.185	.109	.140	.081	.000	.040	.020	.239
	A-100	.904	.841	.754	.799	.884	.750	.781	.841	.760	.845	.610	.420	.880	.761
	A-82	.038	.076	.058	.097	.041	.061	.034	.051	.100	.074	.390	.540	.100	.000
	$H_e$	<b>.180</b>	<b>.282</b>	<b>.396</b>	<b>.345</b>	<b>.213</b>	<b>.401</b>	<b>.357</b>	<b>.281</b>	<b>.395</b>	<b>.275</b>	<b>.482</b>	<b>.536</b>	<b>.217</b>	<b>.368</b>
Skdh	A-128	.048	.042	.047	.033	.065	.016	.068	.000	.007	.022	.225	.000	.000	.000
	A-100	.912	.845	.773	.917	.892	.937	.873	.971	.926	.897	.663	.813	.973	.700
	A-92	.040	.113	.180	.050	.043	.047	.059	.029	.067	.081	.112	.188	.027	.300
	$H_e$	<b>.164</b>	<b>.273</b>	<b>.370</b>	<b>.157</b>	<b>.201</b>	<b>.120</b>	<b>.232</b>	<b>.057</b>	<b>.139</b>	<b>.190</b>	<b>.504</b>	<b>.308</b>	<b>.054</b>	<b>.425</b>

Table 2. – Measures of genetic variability and mean  $F_{IS}$  values for the oak populations studied. Standard error in brackets.

Population	Mean number of alleles per locus	Percentage of polymorphic loci	$F_{IS}$	Mean heterozygosity	
				Direct-count	expected
pt1	2.7 (.2)	90.9	-.025	.290 (.045)	.283 (0.044)
pt2	2.7 (.2)	90.9	.059	.301 (.042)	.320 (0.047)
pt3	2.7 (.2)	90.9	.053	.304 (.044)	.321 (0.050)
pt4	2.6 (.2)	90.9	.022	.309 (.051)	.316 (0.055)
pt5	2.5 (.2)	81.8	.089	.204 (.033)	.224 (0.037)
<b>Q. petraea mean</b>	<b>2.6 (.0)</b>	<b>89.1 (1.8)</b>	<b>.040</b>	<b>.282 (.002)</b>	<b>.293 (0.019)</b>
rb1	2.6 (.2)	90.9	.055	.225 (.041)	.238 (0.043)
rb2	2.3 (.2)	90.9	.108	.222 (.031)	.249 (0.035)
rb3	2.5 (.2)	72.7	.103	.192 (.041)	.214 (0.048)
rb4	2.8 (.2)	81.8	.111	.225 (.050)	.253 (0.058)
rb5	2.8 (.2)	90.9	.080	.208 (.039)	.226 (0.040)
<b>Q. robur mean</b>	<b>2.6 (.1)</b>	<b>85.4 (3.6)</b>	<b>.091</b>	<b>.214 (.001)</b>	<b>.236 (0.001)</b>
cr1	2.3 (.2)	90.9	.079	.383 (.052)	.416 (0.050)
cr2	2.2 (.2)	81.8	.077	.251 (.052)	.272 (0.053)
<b>Q. cerris mean</b>	<b>2.3 (.1)</b>	<b>86.4 (4.6)</b>	<b>.078</b>	<b>.317 (.066)</b>	<b>.344 (0.072)</b>
pb1	2.8 (.2)	72.7	.126	.209 (.040)	.239 (0.053)
pb2	2.1 (.3)	45.5	.192	.139 (.041)	.172 (0.050)
<b>Q. pubescens mean</b>	<b>2.5 (.4)</b>	<b>59.1 (13.6)</b>	<b>.159</b>	<b>.174 (.035)</b>	<b>.206 (0.034)</b>
<b>General mean</b>	<b>2.5 (.1)</b>	<b>83.1 (3.4)</b>	<b>.081</b>	<b>.247 (.017)</b>	<b>.267 (0.016)</b>

accordance with the reported allelism (MÜLLER-STARCK and ZIEHE, 1991; ZANETTO et al., 1993; ZANETTO et al., 1994; BACILIERI et al., 1996a; SAMUEL et al., 1995; HERZOG, 1996). The fastest zones of activity for Got, Mdh, 6Pgdh and Pgi stained faintly, or were absent from many gels, and were thus excluded from the study.

Allele frequencies and expected levels of heterozygosity for the 11 polymorphic loci are given in Table 1. In 7 loci, the most frequent allele was the same in all populations: exceptions are Dia-A, Gdh-A, Pgi-B and Pgm-A, where the allelic frequencies varied among species and/or populations. *Q. cerris* and *Q. pubescens* stands showed alleles not present in other populations, respectively Pgi-B<sup>76</sup> and G3pdh-A<sup>94</sup>. Allele 6Pgdh-B<sup>132</sup> was absent from *Q. petraea* populations, alleles Dia-A<sup>122</sup>, Gdh-A<sup>120</sup>, Idh-A<sup>94</sup>, 6Pgdh-B<sup>84</sup>, Pgi-B<sup>124</sup>, Pgi-B<sup>100</sup> from *Q. cerris* and alleles Got-B<sup>78</sup>, Idh-A<sup>136</sup> and Skdh-A<sup>128</sup> from *Q. pubescens*.

Genetic diversity values are given in Table 2. The mean number of alleles per locus showed little variation among populations, and in general the mean was 2.5. *Q. pubescens* showed a substantially lower percentage of polymorphic loci than the other species. A general excess of homozygotes was found in every population, except in population pt1 of *Q. petraea*. *Q. pubescens* populations showed the higher values of  $F_{IS}$ , with an average of 0.159. *Q. cerris* was the species with the higher expected heterozygosity, but it also showed the lowest number of alleles per locus. The heterozygosity level of *Q. pubescens* was the lowest of all the populations examined (0.206).

As expected, most of the genetic variability was accounted for by the within population component (85.6%), and  $\theta$  computed among all the populations was 0.144 ( $sd = 0.040$ ). Of this inter-population variability, the among species component was by far the most important and accounted for 13.1% ( $\theta = 0.131$ ;  $sd = 0.05$ ) of the total genetic variability. The Pgi and Dia loci were

responsible for most of the variation ( $\theta = 0.500$  and 0.200 respectively). *Q. cerris* had a very different array of frequencies for these two loci compared with the other species. Excluding the two *Q. cerris* populations, the percent of variability among species was less than half ( $\theta = 0.050$ ;  $sd = 0.027$ ).

Figure 2 shows results of the Principal Coordinate Analysis carried out on the matrix of Nei genetic distances. The first discriminant dimension (PcoA-1) accounts for the 75% of the variance and distinguishes very well *Q. cerris* group of populations from the others. Differentiation between *Q. petraea*, *Q. robur* and *Q. pubescens* is better seen on the second and third dimension plot, which account for 15% and 5% of the variance, respectively.

Differentiation between *Q. robur* and *Q. petraea* is around 5% ( $\theta = 0.048$ ;  $sd = 0.033$ ), but locus Dia ( $\theta = 0.233$ ) accounts for most of the variability. Dia is the only locus whose  $\theta$  is greater than 0.04. Excluding Dia,  $\theta$  calculated among these two species drops to 0.013 ( $sd = 0.004$ ). Considering only the two locations (La Mandria and Rocchetta Tanaro) where both *Q. robur* and *Q. petraea* were sampled, "geographic" and "species" differentiation can be compared. Differentiation among these 4 populations (2 species x 2 locations) is around 5% ( $\theta = 0.046$ ;  $sd = 0.011$ ) and is split in approximately equal parts between species ( $\theta = 0.025$ ;  $sd = 0.010$ ) and between locations ( $\theta = 0.026$ ;  $sd = 0.010$ ). Values of  $\theta$ , and thus percent of variability, are not exactly additive because they are calculated on different total bases of genetic diversity (i.e. Rocchetta Tanaro populations have less expected heterozygosity than La Mandria populations, and *Q. petraea* have slightly less expected heterozygosity than *Q. robur*). When both species are collected in every location, according to BODENES et al. (1997), it is possible to distinguish between general differentiation among species ( $C_g$ ) and local differentiation among species ( $C_l$ ).  $C_g$  averages around 52% of the

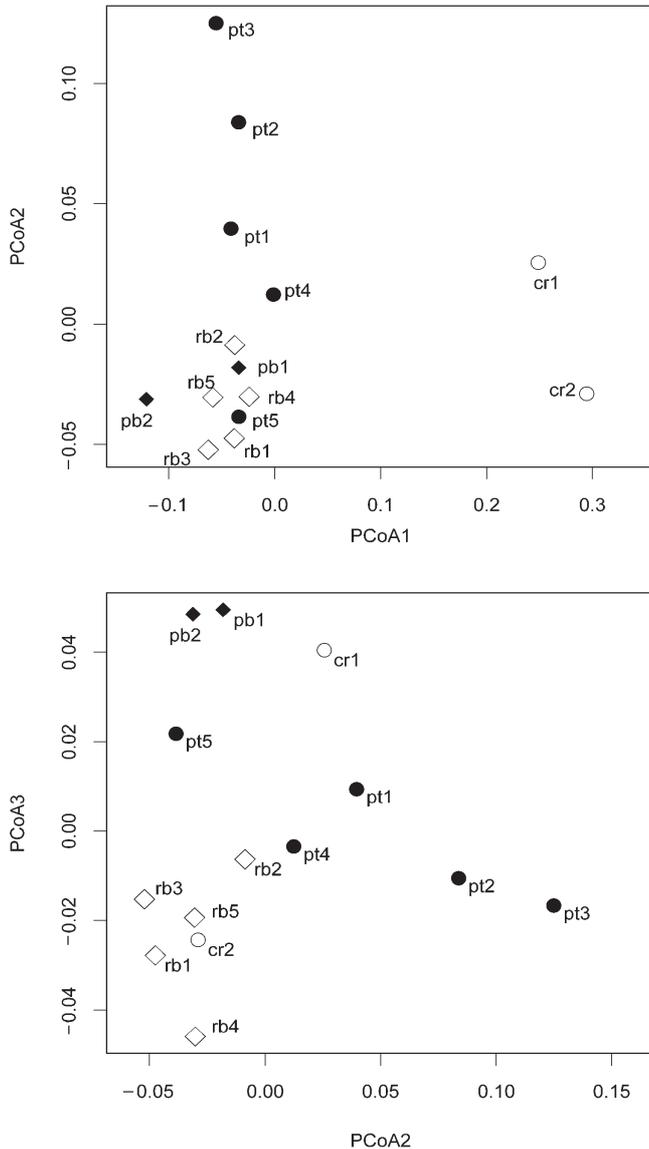


Figure 2. – Plot of Principal Coordinate Analysis computed on matrix of Nei genetic distance. Top: first principal coordinate vs. second. Bottom: second principal vs. third. Symbols indicate species: (• for *Q. petraea*, ◊ for *Q. robur*, o for *Q. cerris*, ◆ for *Q. pubescens*).

species differentiation, meaning that half of species differentiation is constant over all locations, but the pattern is quite different among loci. For Pgm, Mdh and Skdh general differentiation is more than 83%, while for Got 93% of differentiation is local.

Figure 3 shows the genetic differentiation averaged across loci among the four populations. Geographic differentiation is very similar to species differentiation.

Variation among populations within *Q. cerris* species accounts for 8.5% ( $\theta = 0.085$ ;  $sd = 0.025$ ) of variability, while  $\theta$  computed among *Q. pubescens* populations is 0.101 ( $sd = 0.042$ ). *Q. petraea* has more than twice the population differentiation level than *Q. robur* ( $\theta = 0.077$ ;  $sd = 0.028$  and  $\theta = 0.031$ ;  $sd = 0.005$  respectively).

In total, 550 permutation tests have been calculated to test for linkage disequilibrium, and in 61 cases probability values have been less or equal to 0.05 (alpha). The

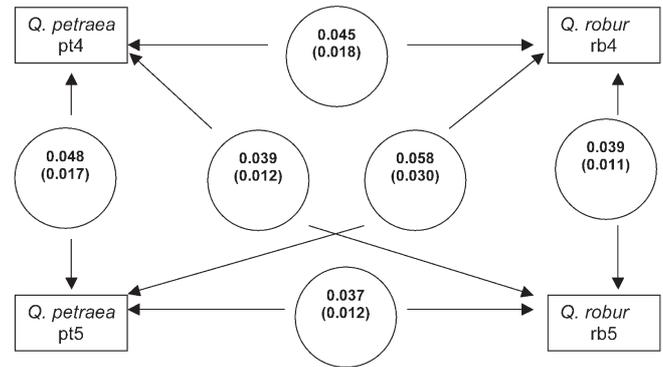


Figure 3. – Genetic differentiation averaged across loci among two populations of *Q. petraea* and two of *Q. robur*, sampled in two stands where both species are present. Standard error in brackets.

number of disequilibrium cases are significantly more numerous for *Q. petraea* (48 cases with  $P \leq 0.05$  out of 275) than for *Q. robur* (13 cases out of 275) ( $\chi^2 = 21.3$ ;  $df = 1$ ;  $P \leq 0.001$ ), and potential hybrid populations have a slightly greater proportion of significant cases of linkage disequilibrium than pure populations – but this result is not significant.

**Discussion**

The values of genetic diversity found in this study are substantially similar to those reported in the literature and scored through isozyme variation (MÜLLER-STARCK and ZIEHE, 1991; MÜLLER-STARCK et al., 1993; KIM et al., 1993; ZANETTO et al., 1993; MATTILA et al., 1994; ZANETTO and KREMER, 1995). Differences among species have been clearly observed: *Q. pubescens* showed the lowest values of heterozygosity as well as of percentage of polymorphic loci, while *Q. cerris* had the highest heterozygosity level. *Q. robur* and *Q. petraea* showed similar values of genetic diversity but the latter exhibited more heterozygosity.

Lower levels of heterozygosity for isozyme markers in *Q. pubescens* as compared to *Q. robur* and *Q. petraea* have been described in Switzerland oak population (FINKELDEY and MÁTYÁS, 2003). Heterozygosity level observed in our *Q. pubescens* populations is even lower than that observed in swiss populations (0.206 vs 0.215).

The deviation from random mating, expressed as the inbreeding coefficient  $F_{IS}$ , was significant and quite different among species: *Q. pubescens* had the highest value (0.159), suggesting high levels of inbreeding and reduced gene exchange, while *Q. petraea* showed observed heterozygosity quite similar to Hardy-Weinberg expectations ( $F_{IS} = 0.040$ ).

In the choice of stands to be used for production of high quality seeds, the homozygosity level should be taken into consideration, due to the negative effect of self-fertilization on seed quality.

Generally, in forest species levels of homozygosity (and thus  $F_{IS}$ ) tend to decrease in mature ages classes because of the effect of selection against homozygotes (ROSSI et al., 1996). These results in adults could suggest even higher values in younger classes, with the

probable presence of mating between relatives, but these result is different to what expected for *Q. pubescens*, because the sampled populations for this species are quite huge with high density of adult individuals (potential reproducers). This situation should favour high levels of pollen flow with consequently high outcrossing rates that should limit homozygote excess. So, these results need further investigations. In particular, an analysis of genetic variability in seeds should clarify if high levels of homozygotes are effectively present in younger age classes. In fact, only very high values of  $F_{IS}$  should be dangerous, because even moderate levels of homozygote excess can be purged by selection during the growth of the plants (MÜLLER-STARCK and ZIEHE, 1991). Heterozygotes are in fact more resistant to environmental stresses (HERTEL et al., 1994). Although all the stands included in the study are adult, none of them is really old and, in general, young individuals are present. Of course, in the choice of stands to be used for production of high quality seeds, the homozygosity level should be taken into consideration due to the negative effect of self-fertilization on seed quality.

Caution is needed in the interpretation in terms of adaptability of the population studies of our results, because the relation between variability at neutral loci and adaptive ones has been extensively discussed during recent years. REED and FRANCKHAM (2001) performed a metanalysis of the correlation between molecular and quantitative measures and find only a weak correlation between molecular and quantitative traits ( $r=0.217$ ), with a completely absence of relationship between neutral variation and life history traits ( $r=-0.11$ ) and with heritability, that is considered the best indicator of adaptive potential. Recently other studies confirmed this trend, both in experimental studies (BEKESY et al., 2003) and in computer simulations (CARVAJAL-RODRIGUEZ et al., 2005). So to assess the amount of adaptive variability present in our populations further studies on quantitative traits are needed.

The study confirmed the low level of genetic variability among the studied species: only *Q. cerris* populations can be distinguished on the basis of isozyme genotype. On the contrary, species belonging to the subg. *Lepidobalanus* are genetically very similar. Principal Coordinate Analysis showed a good separation of *Q. cerris* and *Q. pubescens* while *Q. robur* and *Q. petraea* still remain closer. These results are in agreement with those of other authors, where only 2% out of 2,800 RAPD tested loci differentiated the two species (BODENES et al., 1997). In other studies, non-overlapping ranges of allele frequencies for *Q. petraea* and *Q. robur* populations have been observed at particular isozyme gene loci (e.g. Idh-B, Pgm-A: ZANETTO et al., 1994; HERTEL and DEGEN, 2000; FINKELDEY, 2001) confirming that the observed morphological differentiation coincides with genetic differentiation between the species. Furthermore the locus Got-2 has been reported as a good species-specific marker (SAMUEL et al., 1995), while in the present study this feature was not proved. Probably, our Got-B locus corresponds with the little polymorphic Got-B locus reported by MÜLLER-STARCK et al. (1993) and not with Got-2 locus of Samuel and collaborators. The difference in the Got

results can be explained by the different gel system used: while SAMUEL and collaborators separated Got isozymes in a strongly basic gel, we used a gel system at pH 7.0. Among the isozymes tested in this study, Pgi-B and Dia-A proved to be the most efficient in discriminating among all the species tested, while differentiation between *Q. robur* and *Q. petraea* could be better pursued using allelic frequencies at locus Dia-A. Principal Coordinate Analysis was found to be more useful for separating species, namely *Q. robur* and *Q. petraea*. In such way it was possible to get an optimal discrimination, notwithstanding the scarce level of differentiation between species, which, in mixed populations, is similar to that scored among populations belonging to the same species. Quantification of general and local differentiation confirms that only about 50% of the genetic differences between *Q. robur* and *Q. petraea* are constant, while the remaining 50% is due to variation among populations. These values are very similar to those reported by BODENES et al. (1997).

Microsatellites seem to be more informative than isozymes to discriminate between *Q. robur* and *Q. petraea*. MUIR et al. (2000) were able to discriminate between these two species using 20 microsatellite markers, while PETIT et al. (2003) using chloroplast markers were not able to discriminate between different white oak species.

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