GENETIC VARIABILITY AMONG EUROPEAN LARCH (*LARIX DECIDUA* MILL.) POPULATIONS IN PIEDMONT, NORTH-WESTERN ITALY

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ABSTRACT

Genetic diversity and differentiation of 12 native populations of European larch (*Larix decidua* Mill.) from Piedmont (North-Western Italy) were estimated by means of variation detected in 11 enzyme systems encoded by 20 loci. The trees showed relatively large genetic variability but small interpopulational variation. The expected heterozygosity ranged from 0.239 to 0.279 (mean 0.254); on average, more than 66% of the loci per population were polymorphic and the number of alleles per locus was 2. Only 3.3% of the observed genetic diversity was due to differentiation among populations, and the mean value of genetic distances, as measured by pairwise F_{ST}, was 0.033, with a maximum of 0.087. A positive correlation was found between genetic and geographic distances. The low interpopulational variability suggests the presence of a single gene pool and a lack of barriers to gene flow between the populations. Principal components analysis showed that the first two principal components accounted respectively for 27 and 18% of the total variation of allele frequencies. The Navette population was clearly different from the others, while, unexpectedly, the Formazza population appeared to be genetically close to other populations in spite of its geographical isolation. The results of the study provide useful information for *in situ* preservation of genetic variability and for identification of the most valuable stands for production of high quality seed.

Key words: *Larix decidua*, allozyme variation, genetic diversity, population differentiation

INTRODUCTION

European larch (*Larix decidua* Mill.) is an important long-lived tree species producing timber of high value. Furthermore, the tree is a prominent element of mountain ecosystems which provide substantial benefits for human society as regards soil protection and amenity functions. Larch is widespread in the Alps, from about 800 m a.s.l. up to the subalpine vegetation zone. The species tolerates different climate and soil conditions (FENAROLI & GAMBI 1976). This might in part be an explanation for the large genetic variability shown by larch, also characteristic of most coniferous species (LOVELESS & HAMRICK 1984; HAMRICK & GODT 1990).

In spite of its importance as forest tree species, the population genetics of larch received relatively little attention. For this purpose, biochemical markers have proved more suitable than morphological and phenological ones, which are subjected to environmental influence. LEWANDOWSKI and MEJNARTOWICZ (1991) studied levels and patterns of genetic variation in some European populations employing isozymes. Isozyme analysis has also been used to assess the mating system of the species (LEWANDOWSKI *et al.* 1991; GÖMÖRY & PAULE 1992) and to clarify the genetic relationship between European and Siberian larch (LEWANDOWSKI 1996).

Very little is known about the genetic structure of populations in Italy, where larch covers more than 241,000 ha (ISAFA 1985). In Piedmont, in the north-west of the country, larch is the most widespread coniferous tree and covers, as pure stands, about 45,000 ha (REGIONE PIEMONTE 1981). In the region, in spite of its importance, only two stands for seed production, officially recognized by Italian law are present (MORANDINI & MAGINI 1975). They are considered in this study (numbers 6 and 12).

Due to increasing afforestation it is expected that in future the need of forest regeneration material will be higher than at present. It therefore seems important to improve our knowledge on the genetic structure of the stands where seed is collected, and obtain information on other populations, as possible candidates to join the official list of seed-producer stands.

The present study is aimed at describing intra- and interpopulation genetic variation in larch populations from Piedmont through isozyme analysis, contributing to the characterization of genetic resources and to practical and effective measures for *in situ* preservation

of biodiversity. Genetic variability, which is essential for the adaptability of tree populations to variable environmental conditions, is of extreme importance. It is foreseeable that in the near future new stress factors (pollution, increasing carbon dioxide levels, global warming, etc.) will be even more severe to the point of threatening the survival of populations. This work also contributes to identification of the most valuable stands for production of high quality seed: it is well-known that high levels of genetic variation and heterozygosity of populations are associated with adaptative ability and therefore with survival capacity of the populations (SCHOLZ *et al.* 1989; GREGORIUS 1991; MÜLLER-STARCK 1991; HERTEL 1992). Therefore, the genetic characteristics of the populations should be considered, together with ecological, phytosanitary and sylvicultural aspects, in choosing the stands to be used as high quality seed-producers.

Materials and Methods

Plant material and sampling

Samples of 12 natural adult larch populations, representing the locations where the species grows in Piedmont, were collected (Fig. 1 and Table 1). The populations were chosen on the basis of preliminary observations regarding the possession of the requisites for inclusion in the official Italian list of seed stands: they are native, free from disease, large enough to prevent inbreeding phenomena and isolated from other stands of lower quality. The trees also possess good sylvicultural and production characteristics. The populations contained different age classes, and natural regeneration was observed in almost all of them, with the exception of stands 6 (Saucheres) and 12 (Navette), where young trees are very limited in number. These two stands were however included in the study because they are in the official list of seed stand and, at present, seed is collected from them.

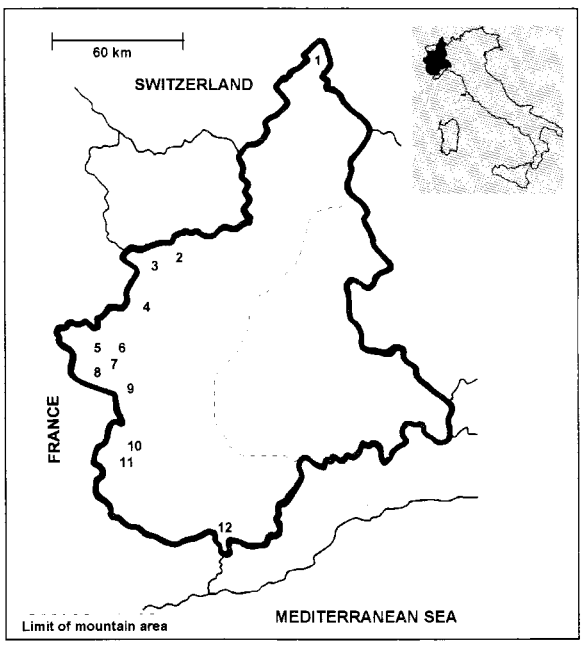

Figure 1 The larch population studied in North-Western Italy

Isozyme analysis was carried out on haploid macrogametophyte tissue extracted from seeds germinated at 20 °C and then stored at 4 °C. The genotype of each individual tree was inferred from at least six macrogametophytes, so reducing to about 3% the chance of misclassification of the heterozygosity. In each stand, material was collected from at least 30 non-adjacent plants, with the exception of stand no. 9 (Bobbio), where it was possible to obtain germinated seeds from only 25 plants. The plants, at least 30 year aged, were chosen at random over a 5 to 10 ha area.

Electrophoretic methods

Enzymes were separated by horizontal electrophoresis, in 11% hydrolysed potato starch (Sigma S4501) gels, using the Tris-citrate buffer system (LEWANDOWSKI & MEJNARTOWICZ 1990). Eleven enzyme systems were studied (acronyms and Enzyme Commission number are reported between brackets): esterase (EST, 3.1.1.2), glutamate oxaloacetate transaminase (GOT, 2.6.1.1), glyceraldehyde 3-phosphate dehydrogenase (G3PDH, 1.2.1.9), isocitrate dehydrogenase (IDH, 1.1.1.42), malate dehydrogenase (MDH, 1.1.1.37), menadione reductase (MNR, 1.6.99.2), 6-phosphogluconate dehydrogenase (6PGDH, 1.1.1.44), phosphoglucose isomerase (PGI, 5.3.1.9), phosphoglucomutase (PGM, 5.4.2.2), shikimate dehydrogenase (SKDH, 1.1.1.25), triose-phosphate isomerase (TPI, 5.3.1.1.). The number of scored *loci* was one for EST, IDH, PGM, SKDH, 2 for GOT, G3PDH, MNR, 6PGDH, PGI, TPI, 4 for MDH.

Macrogametophytes were homogenized in 30 l of extraction buffer (0.1 M Tris-HCl, pH 7.2, plus 0.5% mercaptoethanol). Gels were run at 12 $V \cdot cm^{-1}$ for 30 minutes before removing the wicks, and then overnight. Staining was done according to WENDEL and WEEDEN (1989), except for MNR (CONKLE *et al.* 1982). In staining for IDH and PGI, 5 mg of NADP were used instead of NAD.

The loci were identified using capital letters following the enzyme acronyms. The band closest to the anode was labelled A. Within a single zone of activity the different alleles were designated with numbers, assigning the lowest number to the band migrating fastest.

Table 1 Survey of site and stand characteristics of larch populations

Population	Valley	Province	Latitutde (°N)	Longitude (°E)	Altitude (m)	Exposure	Slope (°)
1 Formazza	Formazza	Verbania	46.35	8.45	1,300–1,600	West	5–15
2 Campiglia	Soana	Torino	45.55	7.60	1,500–1,600	South	5–15
3 Ceresole	Orco	Torino	45.45	7.25	1,600–1,900	South	20–30
4 Pian della Mussa	Lanzo (Ala)	Torino	45.30	7.20	1,700–1,800	East	0–10
5 Gran Bosco di Salbertrand	Susa	Torino	45.10	6.80	1,700–1,900	North, West	15–25
6 Saucheres basses	Chisone	Torino	45.00	7.00	1,500–1,700	North	20–30
7 Val Troncea	Chisone	Torino	44.95	6.95	1,600–1,950	North, West	15–25
8 Thures	Susa	Torino	44.85	6.75	1,700–1,900	North, West	20–30
9 Bobbio	Pellice	Torino	44.75	7.15	900–1,100	North	25–35
10 Sampeyre	Varaita	Cuneo	44.55	7.20	1,500–1,800	North	10–20
11 Elva	Maira	Cuneo	44.50	7.15	1,600–1,800	East	20–30
12 Navette	Tanaro	Cuneo	44.10	7.80	1,300–1,650*	West	10–20

* Two separate populations: 1,300–1,400 m, and 1,550–1650 m

Data analysis

Population genetics parameters were estimated using the BIOSYS–1 program (SWOFFORD & SELANDER 1981). Deviations from Hardy-Weinberg equilibrium were tested by the 2 and Fisher exact tests, pooling genotypes whenever expected frequencies were less than five. The following multilocus measures of gene diversity were used: mean observed and expected heterozygosities, 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. Wright's *F*-statistics (F_{IT}, F_{ST} and F_{IS}) as proposed by WEIR and COCKERHAM (1984) were used to estimate homozygote excess/defect and genetic differentiation among populations; sample variances were estimates by a jackknife procedure over loci. F_{ST} (equivalent to θ in WEIR & COCKERHAM 1984) was also calculated for each pair of populations and considered as a measure of genetic distance; it was then used to construct a dendrogram of populations by the unweighted pair-group method using arithmetic means (SNEATH & SOKAL 1973).

The SAS statistical package was used for statistical computations (SAS INSTITUTE 1985). Non-metric Multidimensional Scaling (PROC MDS) procedure was computed using matrix of F_{ST} distances.

Results

Three loci of the 20 found for 11 enzyme systems were monomorphic in all the populations (*Mdh–C*, *Pgi–A* and *Tpi–A*). In general, the observed banding patterns were in accordance with the allelism reported in the literature (LEWANDOWSKI & MEJNARTOWICZ 1990; GÖMÖRY & PAULE 1992; LEWANDOWSKI 1996). The mode of inheritance of the isoenzymes *Got–B*, *Got–C*, *G3pdh–A*, *G3pdh–B*, *Pgi–B* and *Tpi–B*, not found in literature, was verified by studying segregation among the offspring of heterozygous plants at the seed stage (analysis of macrogametophyte and corresponding embryo), while the monomorphic nature of *Pgi–A* and *Tpi–A* prevented us from doing such an analysis. Therefore *Pgi–A* and *Tpi–A* should be considered as tentative loci designations. Seven zones of activity stained faintly or were absent on many gels and thus were excluded from the study: *Est–B*, *Est–C*, *Got–A*, *Mnr–A*, *Mnr–B*, *Pgm–B* and *Skdh–A*.

Allele frequencies and expected heterozygosity for the 17 polymorphic loci are given in Table 2. In 11 loci the most frequent allele was the same in all popula–tions: exceptions are *Est–A*, *G3pdh–A*, *Mnr–C*, *Pgm–A*, *6Pgdh–A* and *Tpi–B*, where the most common allele varied among populations. Campiglia, Saucheres and Sampeyre populations showed alleles ($Mdh–A_1$, $G3pdh–B_1$ and $Got–B_4$ plus $Mdh–B_1$ respectively), not present in other populations; all other alleles were present in at least 5 populations. At locus *Mnr–C* it was possible to score a null allele.

Genetic diversity values are given in Table 3. The mean number of alleles per locus was on average 2: Pian della Mussa and Elva had the lowest values (1.9) and Bobbio the highest (2.2). The percentage of polymorphic loci ranged from 60 (Sampeyre and Elva) to 70 (six populations) with an average of 66.7. The mean expected heterozygosity per population ranged from 0.239 (Pian della Mussa) to 0.279 (Bobbio) with an average of 0.254.

A good fit to Hardy-Weinberg expectations was found; significant differences between observed and

Table 2. Allele frequencies and expected heterozygosity (H_e) in 12 larch populations from Piedmont, North-Western Italy

Locus	Allele	Population											
		1	2	3	4	5	6	7	8	9	10	11	12
Est–A	1	.364	.712	.568	.548	.767	.667	.712	.761	.760	.707	.583	.646
	2	.636	.288	.432	.452	.233	.333	.288	.239	.240	.293	.417	.354
	H_e	**.470**	**.416**	**.498**	**.503**	**.364**	**.453**	**.416**	**.368**	**.372**	**.419**	**.494**	**.463**
Got–B	1	.000	.030	.041	.000	.000	.019	.000	.000	.060	.024	.000	.024
	2	.964	.939	.905	.968	.983	.963	.985	.967	.900	.854	.919	.952
	3	.036	.030	.054	.032	.017	.019	.015	.033	.040	.110	.081	.024
	4	.000	.000	.000	.000	.000	.000	.000	.000	.000	.012	.000	.000
	H_e	**.070**	**.117**	**.178**	**.063**	**.033**	**.073**	**.030**	**.065**	**.189**	**.262**	**.151**	**.093**
Got–C	1	.250	.309	.311	.138	.300	.352	.318	.367	.340	.159	.367	.457
	2	.750	.691	.689	.862	.700	.648	.682	.633	.660	.841	.533	.543
	H_e	**.380**	**.433**	**.434**	**.242**	**.427**	**.465**	**.441**	**.470**	**.458**	**.270**	**.472**	**.504**
G3pdh–A	1	.112	.188	.149	.097	.167	.154	.227	.218	.200	.207	.113	.179
	2	.587	.578	.662	.597	.533	.462	.545	.474	.540	.305	.500	.333
	3	.225	.234	.176	.306	.300	.365	.227	.308	.200	.427	.387	.452
	4	.075	.000	.014	.000	.000	.019	.000	.000	.060	.061	.000	.036
	H_e	**.593**	**.585**	**.515**	**.549**	**.608**	**.642**	**.608**	**.641**	**.638**	**.687**	**.597**	**.659**
G3pdh–B	1	.000	.000	.000	.000	.000	.019	.000	.000	.000	.000	.000	.000
	2	1.000	1.000	1.000	1.000	1.000	.981	1.000	1.000	1.000	1.000	1.000	1.000
	H_e	**.000**	**.000**	**.000**	**.000**	**.000**	**.037**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Idh–A	1	.015	.029	.014	.000	.017	.000	.029	.000	.020	.000	.000	.149
	2	.985	.971	.986	1.000	.983	1.000	.971	1.000	.980	1.000	1.000	.851
	H_e	**.030**	**.056**	**.028**	**.000**	**.033**	**.000**	**.058**	**.000**	**.040**	**.000**	**.000**	**.257**
Mdh–A	1	.000	.029	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
	2	1.000	.971	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	H_e	**.000**	**.056**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Mdh–B	1	.000	.000	.000	.000	.000	.000	.000	.000	.000	.037	.000	.000
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.963	1.000	1.000
	H_e	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.071**	**.000**	**.000**
Mdh–D	1	.048	.000	.041	.016	.033	.000	.071	.068	.060	.000	.000	.024
	2	.952	1.000	.946	.984	.967	.963	.914	.932	.920	1.000	1.000	.929
	3	.000	.000	.014	.000	.000	.037	.014	.000	.020	.000	.000	.048
	H_e	**.092**	**.000**	**.105**	**.032**	**.066**	**.073**	**.161**	**.128**	**.153**	**.000**	**.000**	**.137**
Mnr–C	null	.280	.191	.311	.290	.250	.135	.115	.289	.240	.280	.323	.205
	1	.451	.750	.541	.419	.433	.615	.436	.303	.380	.415	.468	.449
	2	.268	.059	.149	.290	.317	.250	.449	.408	.380	.305	.210	.346
	H_e	**.654**	**.403**	**.105**	**.666**	**.660**	**.551**	**.603**	**.667**	**.667**	**.665**	**.644**	**.645**
Mnr–D	1	.726	.682	.568	.649	.667	.648	.554	.817	.580	.793	.806	.655
	2	.274	.318	.432	.306	.333	.352	.446	.183	.420	.207	.194	.345
	H_e	**.402**	**.441**	.498	**.432**	**.452**	**.465**	**.501**	**.303**	**.497**	**.333**	**.317**	**.458**
6pgdh–A	1	.429	.469	.446	.371	.750	.630	.579	.622	.560	.500	.500	.256
	2	.560	.391	.459	.629	.200	.278	.395	.322	.420	.420	.468	.628
	3	.012	.141	.095	.000	.050	.093	.026	.056	.020	.020	.032	.115
	H_e	**.509**	**.618**	**.589**	**.474**	**.402**	**.528**	**.515**	**.512**	**.520**	**.520**	**.539**	**.533**

Table 2 (continued)

Locus	Allele	Population											
		1	2	3	4	5	6	7	8	9	10	11	12
6Pgdh–B	1	.048	.094	.095	.367	.467	.278	.256	.167	.160	.122	.217	.064
	2	.952	.906	.905	.633	.533	.722	.744	.833	.840	.878	.783	.936
	H_e	**.092**	**.173**	**.174**	**.472**	**.506**	**.409**	**.386**	**.281**	**.274**	**.217**	**.345**	**.122**
Pgi–B	1	.048	.029	.000	.017	.033	.000	.091	.021	.080	.000	.016	.086
	2	.952	.971	1.000	.983	.967	1.000	.909	.979	.920	1.000	.984	.914
	H_e	**.092**	**.056**	**.000**	**.033**	**.066**	**.000**	**.168**	**.042**	**.150**	**.000**	**.032**	**.159**
Pgm–A	1	.083	.143	.122	.000	.017	.037	.029	.056	.040	.049	.000	.000
	2	.357	.257	.365	.419	.250	.315	.279	.444	.380	.317	.417	.175
	3	.452	.514	.473	.468	.533	.519	.529	.375	.440	.573	.517	.563
	4	.107	.086	.041	.113	.200	.130	.162	.125	.140	.061	.067	.262
	H_e	**.657**	**.651**	**.635**	**.602**	**.623**	**.625**	**.624**	**.652**	**.654**	**.572**	**.564**	**.591**
Skdh–B	1	.146	.186	.108	.167	.117	.185	.283	.183	.300	.087	.177	.226
	2	.094	.029	.041	.017	.017	.056	.017	.061	.040	.100	.032	.048
	3	.805	.786	.851	.817	.867	.759	.700	.756	.660	.813	.790	.726
	H_e	**.332**	**.352**	**.265**	**.310**	**.239**	**.393**	**.437**	**.396**	**.482**	**.326**	**.348**	**.424**
Tpi–B	1	.619	.400	.432	.274	.433	.537	.441	.500	.600	.598	.613	.720
	2	.381	.600	.568	.726	.567	.463	.559	.500	.400	.402	.387	.280
	H_e	**.477**	**.487**	**.498**	**.405**	**.499**	**.507**	**.500**	**.506**	**.490**	**.487**	**.482**	**.409**

Table 3. Sample sizes, measure of genetic variability and mean F_{IS} values for the studied larch populations (standard error in brackets)

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of polymorphic loci	F_{IS}	Mean heterozygosity	
					direct-count	expected
1 Formazza	40.8 (0.6)	2.0 (0.2)	70.0	.078	.224 (.054)	.243 (.056)
2 Campiglia	34.2 (0.3)	2.0 (0.2)	70.0	.091	.221 (.049)	.242 (.054)
3 Ceresole	37.0 (0.1)	2.1 (0.2)	65.0	.052	.238 (.055)	.251 (.056)
4 Pian della Mussa	30.8 (0.1)	1.9 (0.2)	65.0	–.068	.255 (.061)	.239 (.056)
5 Gran Bosco Salb.	30.0 (0.0)	2.0 (0.2)	70.0	.118	.220 (.052)	.249 (.056)
6 Saucheres basses	26.9 (0.1)	2.0 (0.2)	65.0	.098	.236 (.054)	.261 (.057)
7 Val Troncea	34.5 (0.5)	2.0 (0.2)	70.0	.082	.251 (.052)	.272 (.056)
8 Thures	41.5 (0.9)	2.0 (0.2)	65.0	.040	.242 (.054)	.251 (.057)
9 Bobbio	25.0 (0.0)	2.2 (0.2)	70.0	.121	.246 (.050)	.279 (.057)
10 Sampeyre	41.0 (0.1)	2.0 (0.2)	60.0	.005	.243 (.057)	.244 (.056)
11 Elva	30.8 (0.1)	1.9 (0.2)	60.0	–.030	.258 (.059)	.250 (.056)
12 Navette	40.0 (0.6)	2.1 (0.2)	70.0	.056	.258 (.051)	.273 (.055)
Mean	**34.4 (1.7)**	**2.0 (0.0)**	**66.7 (1.8)**	**.056 (.019)**	**.241 (.004)**	**.254 (.004)**

* Unbiased estimate (see NEI, 1978)

** A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99

expected genotype frequencies were found only for the following isozymes: *Est–A* (Gran Bosco di Salbert rand), *Got–B* (Formazza), *Got–C* (Elva), *Mnr–D* (Pian della Mussa), *6Pgdh–B* (Saucheres basses) and *Skdh–B* (Ceresole). A total of 6 out of 204 (less than 3%) significant deviations from equilibrium was in accordance with type I statistical error. All deviations, except two cases (*Got–C* in Elva and *Mnr–D* in Pian della

Table 4. *F*-statistics according to Weir and Cockerham (1984) for the 12 larch populations

Locus	F_{IS}	F_{IT}	F_{ST}
Est–A	0.044	0.087	0.045
Got–B	0.007	0.018	0.011
Got–C	0.053	0.075	0.023
G3pdh–A	0.113	0.133	0.022
G3pdh–B	–0.003	–0.001	0.003
Idh–A	0.026	0.090	0.065
Mdh–A	–0.010	–0.001	0.010
Mdh–B	–0.018	–0.001	0.016
Mdh–D	–0.044	–0.032	0.012
Mnr–C	0.021	0.053	0.033
Mnr–D	–0.021	0.005	0.025
6Pgdh–A	–0.029	0.024	0.051
6Pgdh–B	0.222	0.293	0.091
Pgi–B	–0.053	–0.032	0.020
Pgm–A	0.073	0.083	0.011
Skdh–B	0.103	0.108	0.006
Tpi–B	0.091	0.132	0.046
Jackknife mean	0.056	0.087	0.033
standard error	0.019	0.019	0.006

Mussa) were the result of heterozygote deficiencies. In all populations studied it was possible to observe, on average, a slight excess of homozygotes, with the exception of Pian della Mussa and Elva populations, which showed a negative value of F_{IS} (Table 3). Homozygote excess was mainly due to the locus *6Pgdh–B*, which had an F_{IS} value as high as 0.222 (Table 4).

Genetic differentiation among populations was low: only about 3% of the total genetic diversity resulted from differences among populations (mean F_{ST} = 0.033, Table 4). F_{ST} values among all possible pairwise populations are reported in Table 5, together with the geographic separations. The maximum value of F_{ST} (0.087) was found between Gran Bosco di Salbertrand and Navette. The F_{ST} index was positively correlated with geographical separation (r = 0.47; $p < 0.0001$). Significance was tested by the Mantel test with 1,000 randomization; in all cases the observed correlation was higher than the randomized one. The relationship between genetic and geographic distances was not linear, mainly due to the data from the Formazza stand, a stand that is genetically closer to other populations than would be expected from its geographical position. The population which showed the highest value of mean genetic distances was Navette.

No clear geographical distribution of the genetic variability could be detected. However, some significant correlations were found, namely between stand latitude and number of polymorphic loci (r = –0.63: p = 0.05) and between mean stand altitude and mean number of alleles per locus (r = –0.69; p = 0.01).

A dendrogram constructed ,using the UPGMA method is shown in Fig. 2. The Navette population is seen to be clearly differentiated from the others, and populations from the western part of Torino Province (Gran Bosco di Salbertrand, Saucheres Basses, Val Troncea, Thures and Bobbio) tended to group together, as did the populations from the Gran Paradiso National Park (Campiglia and Ceresole). Pian della Mussa and Elva appeared from the dendrogram to be very similar, although they are geographically quite far apart, they are the only two populations with an eastern exposure. In contrast, Sampeyre and Elva populations are very close geographically, being on opposite slopes of the same mountain, but they have different exposures and were cleraly differentiated genetically.

The dimensional scaling gave the results shown in Fig. 3. The first dimension was significantly correlated at 5% level with longitude (r = –0.63; P = 0.03), while

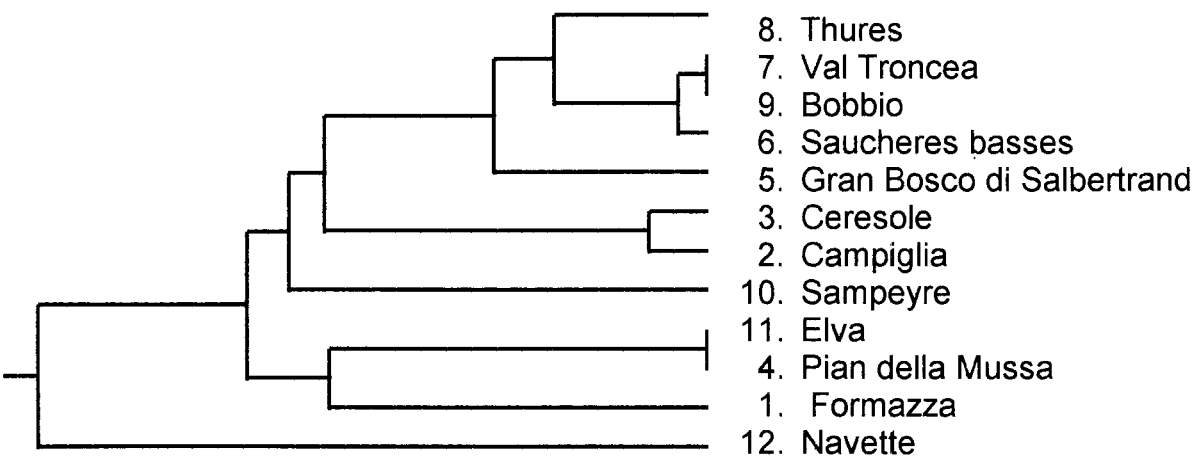

Figure 2 Dendrogram of genetic distances between the 12 larch populations obtained with the UPGMA method

Table 5. Pairwise F_{ST} values (above diagonal) and geographic separations (km, below diagonal) among the larch populations studied

Population	1	2	3	4	5	6	7	8	9	10	11	12
1 Formazza	–	.044	.015	.043	.084	.038	.047	.047	033	.037	.014	.042
2 Campiglia	115	–	.004	.047	.045	.008	.026	.040	.026	.037	.027	.056
3 Ceresole	140	24	–	.028	.049	.016	.023	.036	.017	.039	.019	.055
4 Pian della Mussa	151	38	18	–	.044	.039	.031	.049	.046	.051	.031	.085
5 Gran Bosco di Salbertrand.	184	73	51	29	–	.006	.012	.024	.026	.046	.035	.087
6 Saucheres basses	186	74	54	35	7	–	.001	.014	.003	.020	.005	.040
7 Val Troncea	189	75	56	38	10	4	–	.017	.006	.037	.028	.043
8 Thures	199	87	67	49	21	21	17	–	.004	.019	.013	.052
9 Bobbio	203	91	73	59	35	28	24	28	–	.023	.014	.027
10 Sampeyre	224	112	94	84	56	49	49	48	24	–	.011	.039
11 Elva	228	116	98	87	59	52	52	49	28	3	–	.029
12 Navette	259	157	154	143	126	119	115	116	91	66	67	–

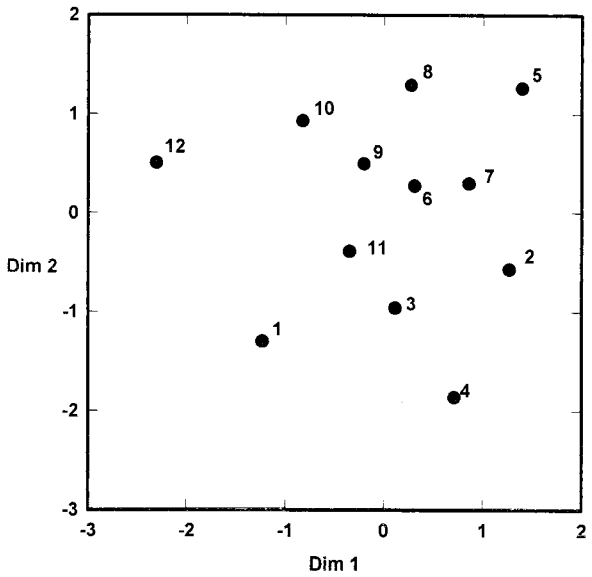

Figure 3. Plot of first (DIM1) and second (DIM2) dimensions calculated according to Non-metric Multidimensional Scaling procedure.

the second dimension was correlated with latitude ($r = -0.59$; $p = 0.04$): the eastern populations showed higher values for the first dimension and northern populations showed higher values for the second component.

Discussion

The values of mean expected heterozygosity per population obtained in this study are in good agreement with those characteristic of coniferous species (MÜLLER-STARCK 1991) but higher than those reported in the literature for the European larch (LEWANDOWSKI & MEJNARTOWICZ 1991; LEWANDOWSKI 1996). Very good agreement was found between the mean number of alleles per locus and the percentage of polymorphic loci. However, comparisons between the present results and those in the literaure are hampered by differences in number and kind of loci studied and in the geographical scale considered. Furthermore LEWANDOWSKI and MEJNARTOWICZ (1991) also studied subspecies other than the one analysed by us. Good correlation was found among the values of mean expected heterozygosity, mean number of alleles per locus and percentage of polymorphic loci obtained in our study; all three of Spearman's rank correlation coefficients were positive and significant ($p < 0.05$), notwithstanding the presence of rare alleles, which contribute little to the mean expected heterozygosity but are relevant both for the mean number of alleles per locus and the percentage of polymorphic loci.

The good fit found to Hardy-Weinberg expectations confirms the allelic nature of the observed electromorphs. In all the populations except Elva and Pian della Mussa a slight excess of estimated homozygotes was found. The average F_{IS} was as low as 0.056, but significantly different from zero. Ten out of the 12 populations showed positive values of F_{IS}, and Bobbio and Gran Bosco di Salbertrand showed the highest values (respectively 0.121 and 0.118). The homozygotes excess could be due to inbreeding or mating between closely located trees. Moreover, these results may reflect the tendency, already observed in other conifers, towards a lack of heterozygosity at the juvenile stage, which tends to disappear in more mature populations, probably as a consequence of selection against selfed individuals (YAZDANI 1985; MUONA *et al.* 1987). Although all the stands evaluated in our study could be considered adult, none of them is really old and, in general, young individuals were present. Of course, in the choice of stands for production of high quality seed, the level of homozygote excess should be kept into consideration, due to the negative effect of

self-fertilization on seed quality (LEWANDOWSKI *et al.* 1991).

Most of the genetic diversity was localized within populations, since only 3.3% of the total was found between populations. This value is slightly lower than the 4.1% found by LEWANDOWSKI and MEJNARTOWICZ (1991), where indeed the investigated area was much larger than ours. Our figure was however higher than the 1% reported by LEWANDOWSKI (1996) for larch populations from the Alps. The small amount of isozyme diversity between populations from Piedmont which grow under different ecological conditions proves the presence of a single gene pool and the lack of barriers to gene flow between populations. The existence of effective outcrossing is confirmed by the good fit to Hardy-Weinberg expectations. The lack of large genetic variation among the populations studied is probably due to a common refugium during glacial periods and to a common and continuous migration path following glacial retreat. The short period passed after the last post-glacial invasion (about 10,000 years) probably prevented significant selection and genetic drift among populations. On the other hand, a low level of differentiation between populations is typical of species such as European larch and other conifers, characterized by large stands, wide pollen dispersion and high outcrossing rate.

Among the loci studied, *6Pgdh–B* showed the highest relative degree of genetic differentiation (F_{ST} = 0.091) and the highest homozygote excess (F_{IS} = 0.222). This isozyme was also found to be the most variable in a study on European beech (*Fagus sylvatica*), where a significant correlation was found between the frequency of the allele *6Pgdh–B*$_1$ and the altitude where the plant was growing (BELLETTI *et al.* 1996). In the present study no such correlation was found, so making uncertain a possible adaptative role of the allele.

The lack of a clear geographical distribution of the allelic frequencies could be due to limited sample size, to the low level of differentiation among populations and to the small geographic separations. Notwithstanding this, some patterns could be found. In general, geographically closed populations are grouped on the multidimensional scaling graph: the Navette population is clearly differentiated, while the Formazza population, although geographically isolated, group with the other populations (Fig. 3). These data are confirmed by the dendrogram, where it is evident that, although the genetic distance values are very low, the Navette population stands out from the others, while, in general, the other populations group according to their geographic separations.

The results of our study give a practical guide on measures for preservation of biodiversity in European larch in North-Western Italy. They could also be used in the choice of stands for production of high quality seed. The two stands from Piedmont where seeds are at present collected show very limited regeneration, making uncertain their future inclusion in the official list of seed-producer stands. This could be a problem mainly for the Navette stand, which we have shown is clearly differentiated from the others. The Navette stand is at a particular site, where proximity of the sea modifies the climate. Therefore, it seems important to make efforts to maintain this stand among those officially recognized for seed production or, if this turns out not to be possible, to look for other stands with similar genetic characteristics.

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ERRATA

Belletti, P., Lanteri, S. & Leonardi, S. 1997: Genetic variability among European larch (Larix decidua Mill.) populations in Piedmont, North-Western Italy. Forest Genetics 4(3):113-121.

Abstract
The third sentence from the end (Principal components analysis ....) should read:
Non-metric multidimensional scaling showed that the first dimension was significantly correlated with longitude and the second with latitude.

p. 114, 2nd column, 3rd paragraph, 5th line:
acronyms and Enzyme Commission numbers
p. 114, 2nd column, 4th paragraph, 1st line:
30 µl
p. 115, 1st column, 1st paragraph, 4th line:
by the χ^2 and .....
p. 115, 1st column, 1st paragraph, 15th line:
were estimated ....

p. 116, Table 2,
population 10, the allele frequencies should read: .500, .415, .085, and H_e = .578
p. 117, Table 3,
Percentage of polymorphic loci**
expected*
p. 118, 2nd column, 3rd paragraph, 2nd line
The Navette population was seen
p. 118, 2nd column, 3rd paragraph, 9th line
similar; although
p. 118, 2nd column, 3rd paragraph, 15th line
were clearly differentiated.....
P. 120, 1st column, 1st pragraph, 8th line:
BELLETTI & LANTERI 1996

Kjær, E. D. & Wellendorf, H., 1997: Variation in flowering and reproductive success in a Danish *Picea abies* (Karst.) seed orchard. Forest Genetics 4(4):181-188.

p. 182, 2nd column, 2nd paragraph, 4th line:
instead of s^2_{sg} should be σ^2_{sg}

p. 183, 1st column, 5th paragraph, 8th line:
instead $P = (f_i p_i)$ should be $P = \Sigma (f_i p_i)$

p. 183, 1st column, 5th paragraph, 17th line
the equation should read

$$P = (1-t) + t\,\Sigma (f_i p_i)$$

p. 183, 2nd column, 1st paragraph, 15th line
instead of $S(f_i p_i)$ should be $\Sigma(f_i p_i)$

p. 183, 2nd column, 1st equation should read:

$$P = (1-t) + w\,t\,\Sigma (f_i p_i)$$

p. 183, 2nd column, 2nd equation should read:

$$N_e^{(i)} = 1/P \approx 1/(\Sigma (1-t) + 1/3\, t\, \Sigma(f_i p_i)) = 1/(0.05+0.32\, \Sigma(f_i p_i)),\ for\ i=1...100$$

p. 183, 2nd column, 3rd equation should read:

$$N_e^{(v)}(infinite) = \frac{N_{t-1} - 1/2}{N_{t-1}\Sigma r_i^2 - 1},\ for\ i = 1,....,100$$

p. 184, 1st column, 1st equation should read:

$$N_s = 1/\Sigma r_i^2,\ \text{for i} = 1...100$$

p. 187, 2nd column, 17 line from bottom:
KJÆR, E. D., GRAUDAL, L. & MYRTHUE, Å. K. 1995:

p. 187, 2nd column, 6th line from bottom:
Forest Genetics **4**(2):69–76.