GENETIC DIFFERENTIATION IN *ABIES ALBA* MILL. POPULATIONS FROM SOUTHEASTERN FRANCE

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ABSTRACT

Horizontal starch gel electrophoresis was used to study isozyme polymorphism in *Abies alba* Mill populations from southeastern France. A total of 23 populations from the southwesternmost region of the Alps was compared with 5 control populations from selected French seed stands. Ten isozyme loci were consistently scored. Three loci (*Pgi2*, *Pgm1*, *Skdh*) appeared to be monomorphic while the remaining 7 were polymorphic: *Got(Aat)2*, *Got(Aat)3*, *Idh2*, *Lap1*, *Mnr2*, *6Pgd1* and *6Pgd2*. Mating system parameters were calculated in one population (Ventoux) to justify the use of pollen contributions to estimate population diversity: the multilocus outcrossing rate was 0.854 and the fixation index –0.034. Mean expected heterozygosity varied between 0.063 and 0.217 for 10 loci. Between population diversity was high with no clear geographical structure and *Abies alba* populations from Mediterranean France did not appear to be genetically different from other populations. This suggests a common genetic origin for all the southern and northern Alps, Corsica, Massif Central and Vosges population studied. The Pyrenean population analyzed appeared to be genetically different from all the others indicating that at least two different Würmian refugia are responsible for the current distribution of isozyme polymorphism in the *Abies alba* populations tested.

Key words: *Abies alba*, France, Mediterranean, isozymes, genetic diversity, paleoecology.

INTRODUCTION

Fir populations in France are thought to originate from different refugia and migratory pathways after the end of the last ice age (Würm). *Abies alba* populations currently found in the Pyrenees are believed to originate from an isolated Pyrenean refugium. French Alps *Abies alba* populations probably emerged from Italian Glacial refugia. Northeastern French (Jura and Vosges) and Massif Central populations are also presumed to stem from Italian refugia, with possible genetic influence from other undetected French refugia. These migratory pathways have been described at the range-wide level by KONNERT & BERGMANN in 1995. However, southeastern French *Abies alba* populations were never included in such isozyme studies. These populations are at the southwesternmost end of the distribution range of *Abies alba* in Europe and are in the middle of the colonization routes described above. They are today isolated from one another and can colonize sites with atypical Mediterranean-type climates (QUÉZEL 1985). Paleoecologists also believe that refugia may have existed locally in the Maritime Alps and in Corsica (NICOL-PICHARD & DUBAR 1998).

In this study, isozymes were used to examine: (1) regional structure of genetic diversity in southeastern French *Abies alba* populations and (2) possible genetic origin of these populations compared with non-native reference populations from presumably partially different genetic origins.

MATERIAL AND METHODS

Open-pollinated seeds were collected in September, 1994, from 16 French and 2 Italian forest stands in the southwesternmost region of the Alps (Figure 1). Between 20 to 40 single tree progenies were sampled per population. Harvested trees were separated by at least 30 meters to prevent consanguineous sampling. Bulk seeds from 5 selected seed stands (CEMAGREF 1996) were purchased from seed companies and used as controls (Table 1). Control stands were chosen to represent seed sources outside Mediterranean southeastern France. They were also chosen based on original stock figures from the seed company which indicated that collection had been made on more than 30 trees per population, except for the Savoie (SAV) population where figures indicated that approximately 20 trees had been sampled.

Seeds were dehydrated at 25 °C to 8% relative

Table 1. Description of sampled stands.

Population	Abbreviation	Coordinates		Altitude	Number of trees sampled
		Latitude	Longitude		
Southeastern French and Italian Stands					
Continental France					
Bayons	BAY	44° 16' N	6° 10' E	1350–1390	33
Beuil	BEU	44° 06' N	4° 40' E	1400–1700	40
Bleyne	BLE	43° 49' N	6° 49' E	1500	43
Boscodon	BOS	44° 32' N	6° 28' E	1340–1540	39
La brigue	BRG	44° 03' N	5° 18' E	1500–1600	39
Cheiron	CHE	43° 49' N	4° 35' E	1250–1290	40
Entraunes	ENT	44° 11' N	4° 24' E	1300–1880	40
Lambruisse	LAB	44° 03' N	6° 27' E	950–1560	40
Lachens	LAC	43° 44' N	6° 40' E	1110–1480	40
Lure	LUR	44° 07' N	5° 50' E	1300–1380	31
Saint Etienne de Tinee	SET	44° 14' N	4° 35' E	1500	41
Saint Martin Vesubie	SMV	43° 49' N	5° 02' E	1450–1750	40
Tartonne	TAR	44° 03' N	6° 25' E	1200–1500	29
Turini	TUR	43° 58' N	5° 02' E	1350–1650	35
Mnt Ventoux	VTX	42° 12' N	5° 15' E	1000–1440	40
Corsica					
Punteniellu	PUN	41° 57' N	9° 06' E	1510–1570	40
Italy					
Val Pesio	PES	44° 13' N	7° 40' E	1200	20
Valle Stura	STU	44° 19' N	7° 07' E	1100	19
Reference stands					
Aude, Pyrenees	AUD	42° 52' N	2° 07' E	900–1000	bulk
Velay, Masif Central	VEL	45° 20' N	4° 25' E	860–1270	bulk
Geradmer, Vosges	GER	48° 04' N	6° 53' E	650–1070	bulk
Savoie, Alps	SAV	46° 02' N	6° 21' E	860–1020	bulk
Dauphine, Alps	DAU	44° 30' N	5° 34' E	1270–1600	bulk

Table 2. Observed single locus segregation of allozymes from heterozygous mother trees in the Ventoux (VTX) population: χ^2 tests of "goodness of fit" to the 1:1 ratio and of heterogeneity of mother trees.

Locus	Number of megagametophytes				Segregation			Heterogeeity		
	Allele 1	Allele 2	Allele 3	Total	χ^2	df	*p*	χ^2	df	*p*
Got2	8	3	/	11	2.273	1	0.132	0.016	1	0.898
Got3	/	31	32	63	0.016	1	0.900	6.040	1	0.871
Idh2	40	38	/	78	0.051	1	0.821	10.267	15	0.803
Lap1	10	8	/	18	0.222	1	0.640	1.333	4	0.856
Mnr2	/	16	10	26	1.385	1	0.239	1.354	9	0.998
6Pgd1	17	16	/	33	0.030	1	0.862	2.250	7	0.945
6Pgd2	15	9	/	24	1.490	1	0.220	1.322	3	0.724

df: degrees of freedom.
Segregation: *p* is the probability of deviation from Mendelian expectations due to chance alone.
Heterogeneity: *p* is the probability of heterogeneity between trees due to chance alone.

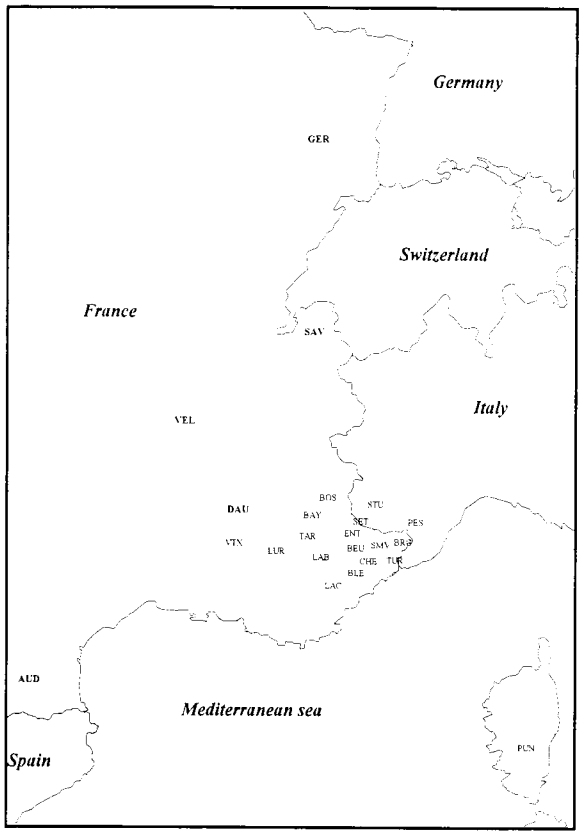

Figure 1. Location of sampled stands.

water content and stored at 4 °C in plastic bags until used. They were stratified before electrophoresis on moist filter paper at 4 °C for 3 weeks, then placed at room temperature for germination. Embryos and megagame-tophytes were extracted separately when the root emerged approximately 3 mm from the seed coats using the homogenization buffer described by HUSSENDÖRFER *et al.* (1995), slightly modified by adding 0.02% Bovine Serum Albumin. Horizontal starch gel electrophoresis was performed according to the methods of CONKLE *et al.* (1982) and KONNERT & MAURER (1995).

Ten enzyme loci consistently resolved and were subsequently used to study population diversity: *Got(Aat)2*, *Got(Aat)3*, *Idh2*, *Lap1*, *Mnr2*, *6Pgd1*, *6Pgd2*, *Pgi2*, *Pgm1* and *Skdh*. Banding patterns, isozyme names and allozyme numbers were consistent with those of KONNERT & MAURER (1995). Two other loci (*Gdh* and *Ndh*) were found to be monomorphic in a sample subset and eliminated from the analysis as they did not consistently resolve.

Mendelian inheritance of enzyme loci was presumed to be identical to that demonstrated by HUSSENDÖRFER *et al.* (1995) and was further verified on heterozygous mother trees from the Ventoux (VTX) population using the χ^2 goodness-of-fit method (Table 2). Between and within population diversity estimations were based on paternal (pollen) gene frequencies derived from megagametophyte (maternal haploid tissue) contributions to the embryo (diploid tissue). These data are well suited to study stand and between population diversity for predominantly allofecundated wind-pollinated species when few trees are available (STAUFFER & ADAMS 1993) and samples have different structures (bulk seeds in control populations compared to single tree progenies in southeastern French stands). Biased results could be expected if self-pollination and deviations from panmixia were high. This was checked for the Ventoux (VTX) population, where genotypes of both maternal trees and pollen contributions to the zygote were available, using RITLAND's (1990) MLTF mating system estimation program. The multilocus outcrossing rate t_m for 7 polymorphic loci (*Idh2*, *Got2*, *Got3*, *Lap1*, *Mnr2*, *6Pgd1* and *6Pgd2*) was equal to 0.855 (standard deviation SD = 0.043) indicating that this population is predominantly outcrossed. This was later assumed to be true for all studied *Abies alba* populations. As pollen data could not be used to calculate heterozygote tree frequency in the population but only yielded expected heterozygosity values, potential deviations from panmixia were also verified for the Ventoux (VTX) population using MLTF. F_{is} was found to be equal to –0.034 (SD = 0.073) and panmixia was assumed to control mating events in all populations studied.

Allele frequencies, mean number of alleles per locus, percentage of polymorphic loci and mean expected heterozygosity were calculated using the BIOSYS package (SWOFFORD & SELANDER 1981). Both NEI's genetic distance (1972) and CAVALLI-SFORZA & EDWARD's chord distance (1967) were used to compare populations and the latter was also used for cluster analysis using the UPGMA (SNEATH & SOKAL 1973) method. NEI's distance may provide a better estimate of between population evolution although CAVALLI-SFORZA & EDWARD's distance is a true distance without any genetic assumption.

RESULTS

Three loci were found to be monomorphic: *Pgi2*, *Pgm1*, *Skdh*. Allele frequencies of polymorphic loci are listed in Table 3. Mean number of alleles ranged between 1.4 and 1.8. Percentage of polymorphic loci varied between 30 and 70%. Mean estimated heterozygosity (H_e) was between 0.063 for the Pyrenean (AUD) population and 0.217 for the Corsican (PUN) population (Table 4). H_e ranged from 0.127 (AUD) to 0.392 (PUN) using the 5 most polymorphic loci (*Got3*, *Idh2*,

Table 3. Allele frequency estimates of 7 polymorphic loci in 23 *Abies alba* populations. *N* = sample size per locus (number of pollen contributions).

Locus	Southeastern French and Italian stands											
	BAY	BEU	BLE	BOS	BRG	CHE	ENT	LAB	LAC	LUR	SET	SMV
Got3												
(*N*)	49	51	51	52	94	50	47	43	50	48	41	56
Allele 1	0.000	0.000	0.078	0.038	0.000	0.020	0.000	0.047	0.000	0.021	0.000	0.018
Allele 2	0.735	0.588	0.765	0.827	0.638	0.780	0.766	0.767	0.580	0.708	0.805	0.768
Allele 3	0.265	0.412	0.157	0.135	0.362	0.200	0.234	0.186	0.420	0.271	0.195	0.214
Got2												
(*N*)	36	45	41	36	60	21	37	34	39	37	24	47
Allele 1	1.000	0.978	1.000	0.917	0.950	1.000	0.919	1.000	0.974	1.000	0.958	0.957
Allele 2	0.000	0.022	0.000	0.083	0.050	0.000	0.081	0.000	0.026	0.000	0.042	0.043
Idh2												
(*N*)	61	51	62	59	102	49	51	55	58	46	39	68
Allele 1	0.459	0.392	0.387	0.475	0.353	0.388	0.471	0.327	0.310	0.435	0.282	0.265
Allele 2	0.541	0.608	0.613	0.525	0.647	0.612	0.529	0.673	0.690	0.565	0.718	0.735
Lap1												
(*N*)	65	58	65	38	85	68	34	53	59	47	75	53
Allele 1	0.969	0.914	0.938	0.816	0.906	1.000	0.941	0.962	0.983	0.872	0.907	0.887
Allele 2	0.031	0.086	0.062	0.184	0.094	0.000	0.059	0.038	0.017	0.128	0.093	0.113
Mnr1												
(*N*)	63	58	55	50	103	40	44	53	57	59	63	71
Allele 1	0.048	0.017	0.036	0.020	0.068	0.025	0.023	0.000	0.000	0.034	0.079	0.014
Allele 2	0.952	0.983	0.964	0.980	0.932	0.975	0.977	1.000	1.000	0.966	0.921	0.986
6pgd1												
(*N*)	36	40	22	37	76	49	27	28	34	45	30	59
Allele 1	0.333	0.225	0.091	0.189	0.211	0.510	0.037	0.179	0.176	0.256	0.300	0.220
Allele 2	0.667	0.775	0.909	0.811	0.789	0.490	0.963	0.821	0.824	0.756	0.700	0.780
6pgd2												
(*N*)	31	43	24	42	68	42	26	40	41	37	24	63
Allele 1	0.161	0.000	0.083	0.119	0.074	0.095	0.115	0.125	0.024	0.027	0.125	0.032
Allele 2	0.839	1.000	0917	0.881	0.926	0.905	0.885	0.875	0.976	0.973	0.875	0.968

Lap1, *6Pgd1* and *6Pgd2* where frequency of the least frequent alleles was regularly greater than 10%).

Contingency χ^2 analysis performed on all populations indicated that all loci could be used to discriminate between populations ($p < 0.001$). Contingency χ^2-analysis performed on all population groups using a cluster analysis (Figure 2) revealed very few significant ($p < 0.05$) clusters: Beuil-Velay (BEU-VEL); Lure-Turini (LUR-TUR); Bleyne-Lambruisse (BLE-LAB); St Martin Vésubie-Dauphiné (SMV-DAU); Ventoux-Gérardmer (VTX-GER). These clusters included both southeastern French and reference populations. The greatest genetic distance was found between the Corsican (PUN) and Pyrenean (AUD) populations (Table 5). The Pyrenean population (AUD) was also the population with the highest distance from any other population (Table 5). It had the lowest frequencies for the least common alleles of loci *Got3* and *Idh2* although they were relatively frequent in the other populations (Table 3). The population from Savoie (SAV) had the highest frequency for the least common allele of *Idh2*. A cluster analysis using these 2 loci showed that the Corsican population was well-situated within the other population groups and only the reference populations from the Pyrenees (AUD) and Savoie (SAV) were clearly outgrouped populations (Figure 3).

DISCUSSION

This study detected fewer loci per enzymatic system

Table. 3 (continued).

Locus	Southeastern French and Italian stands						Reference populations				
	TAR	TUR	VTX	PUN	PES	STU	AUD	DAU	GER	SAV	VEL
Got3											
(*N*)	40	33	204	59	54	50	48	28	35	13	16
Allele 1	0.000	0.000	0.000	0.000	0.056	0.060	0.000	0.036	0.000	0.000	0.000
Allele 2	0.825	0.667	0.828	0.542	0.630	0.660	0.938	0.786	0.800	0.923	0.688
Allele 3	0.175	0.333	0.172	0.458	0.315	0.280	0.063	0.179	0.200	0.077	0.313
Got2											
(*N*)	6	7	197	56	43	35	37	25	22	9	16
Allele 1	1.000	1.000	0.975	1.000	0.884	0.829	1.000	0.960	0.955	1.000	1.000
Allele 2	0.000	0.000	0.025	0.000	0.116	0.171	0.000	0.040	0.045	0.000	0.000
Idh2											
(*N*)	44	37	259	70	47	49	41	48	56	22	28
Allele 1	0.318	0.486	0.336	0.500	0.404	0.245	0.146	0.375	0.411	0.591	0.536
Allele 2	0.682	0.514	0.664	0.500	0.596	0.755	0.854	0.625	0.589	0.409	0.464
Lap1											
(*N*)	58	35	185	52	36	43	38	34	52	24	25
Allele 1	0.983	0.943	0.930	0.827	0.944	0.837	0.947	0.882	0.904	0.833	0.920
Allele 2	0.017	0.057	0.070	0.173	0.056	0.163	0.053	0.118	0.096	0.167	0.080
Mnr1											
(*N*)	40	35	45	59	49	55	40	44	54	26	25
Allele 1	0.000	0.000	0.022	0.119	0.041	0.073	0.000	0.023	0.037	0.077	0.000
Allele 2	1.000	1.000	0.978	0.881	0.959	0.927	1.000	0.977	0.963	0.923	1.000
6pgd1											
(*N*)	49	35	153	43	43	42	23	19	36	13	24
Allele 1	0.163	0.371	0.118	0.279	0.372	0.119	0.087	0.105	0.111	0.077	0.167
Allele 2	0.837	0.629	0.882	0.721	0.628	0.881	0.913	0.895	0.889	0.923	0.833
6pgd2											
(*N*)	41	27	151	59	44	46	12	21	30	12	11
Allele 1	0.049	0.037	0.126	0.153	0.159	0.022	0.000	0.048	0.067	0.000	0.000
Allele 2	0.951	0.963	0.874	0.847	0.841	0.978	1.000	0.952	0.933	1.000	1.000

and alleles per locus than HUSSENDÖRFER *et al.* (1995) using *Abies alba* populations from Germany. This was probably due both to experimental procedures (failure to detect all IDH or MDH isozymes) and use of different samples. However, low polymorphism or absence of variation in enzyme systems GDH, PGI, PGM and SkDH has been confirmed by several studies for western Alps populations (HUSSENDÖRFER *et al.* 1995, KONNERT & MAURER 1995, SCHROEDER 1989)

Within population diversity values estimated by H_e were in the lower value range compared to other *Abies alba* studies: KORMUŤÁK (1987) found H_e values between 0.321 and 0.496 for 5 polymorphic loci (*Est1*, *Est2*, *Got*, *Lap1* and *Lap2*); BREITENBACH-DORFER *et al.* (1995) reported H_e values ranging from 0.359 to 0.540 for 4 polymorphic loci (*Idh1*, *Idh2*, *Lap1* and *6Pgd1*); SCALTSOYIANNES *et al.* (1991) found H_e values between 0.197 and 0.300 for 5 polymorphic loci (*Acp*, *Idh2*, *Per*, *6Pgd1* and *6Pgd2*). Part of the difference could be explained by our use of male contribution data (generation $n + 1$) rather than tree genotypes (generation n) as in the 3 above-mentioned references. When both kinds of data were available, as in the Ventoux (VTX) population, H_e increased from 0.139 (pollen value) to 0.161 (tree genotype value). Another explanation could be the use of fewer, different and exclusively polymorphic loci in the 3 references (H_e increased when only polymorphic loci were used in this study). These differences in estimate evaluation made it difficult to compare heterozygosity values from differ-

Table 4. Genetic diversity estimated of 23 *Abies alba* populations at 10 loci using pollen allele frequencies. Standard deviations are in parentheses.

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of polymorphic loci*	Mean expected heterozygosity (under Hardy-Weinberg equilibrium)**
Southeastern French and Italian stands				
Continetnal France				
BAY	43.3 (4.7)	1.6 (0.2)	40.0 (0.065)	0.177
BEU	40.0 (5.3)	1.6 (0.2)	40.0 (0.065)	0.156
BLE	40.5 (5.2)	1.7 (0.2)	50.0 (0.054)	0.138
BOS	38.3 (4.4)	1.8 (0.2)	60.0 (0.055)	0.182
BRG	66.8 (10.0)	1.7 (0.2)	70.0 (0.057)	0.179
CHE	41.1 (4.4)	1.6 (0.2)	40.0 (0.067)	0.156
ENT	31.5 (4.3)	1.7 (0.2)	50.0 (0.054)	0.145
LAB	39.1 (3.9)	1.6 (0.2)	40.0 (0.056)	0.142
LAC	42.6 (4.4)	1.6 (0.2)	30.0 (0.061)	0.135
LUR	38.1 (4.3)	1.7 (0.2)	40.0 (0.063)	0.164
SET	38.1 (5.6)	1.7 (0.2)	60.0 (0.052)	0.178
SMV	48.2 (6.2)	1.8 (0.2)	40.0 (0.052)	0.148
TAR	35.8 (4.6)	1.5 (0.2)	30.0 (0.051)	0.114
TUR	29.4 (2.8)	1.5 (0.2)	40.0 (0.070)	0.161
VTX	159.9 (18.7)	1.7 (0.2)	50.0 (0.047)	0.139
Corsica				
PUN	46.6 (5.8)	1.6 (0.2)	60.0 (0.066)	0.217
Italy				
PES	35.7 (5.1)	1.8 (0.2)	60.0 (0.066)	0.213
STU	35.2 (5.1)	1.8 (0.2)	60.0 (0.055)	0.182
Reference populations				
AUD	33.4 (3.2)	1.4 (0.2)	40.0 (0.029)	0.063
DAU	32.4 (3.2)	1.8 (0.2)	40.0 (0.052)	0.145
GER	40.4 (3.8)	1.7 (0.2)	50.0 (0.050)	0.148
SAV	15.0 (2.2)	1.5 (0.2)	50.0 (0.052)	0.122
VEL	20.3 (1.8)	1.4 (0.2)	40.0 (0.064)	0.138

* A locus is considered to be polymorphic if the frequency of the most common allele does not exceed 0.95.
** Unbiased estimate (NEI 1978).

ent studies. However, our H_e values seemed to be typical of *Abies* species as well as predominantly outcrossed, wind-pollinated and relatively widespread Gymnosperms in general, although the lowest values (e.g. AUD) might indicate some endemism (HAMRICK *et al.* 1992).

Genetic distances did not reveal any clear geographical pattern between populations or groups of populations within the southeastern French population group. Genetic distances were comparable, for example, to those mentionned by VICARIO *et al.* (1995) for Italian *Abies alba* populations, which also have a scattered range. Current geographic isolation in southeastern French populations did not seem to induce any pattern for genetic distances. Genetic distances did not reveal any clear separation either between southeastern French Alps populations and reference Alps, Massif Central and Vosges populations. In addition, statistically significant clusters included both "Mediterranean" and "non-Mediterranean" populations, indicating that both population types share a common gene pool regardless of regional differences.

Historical data indicate that the earliest development of *Abies* forests in western Europe dates from approximately 12 000 years BP (before present) during the late Glacial interstadial (PONNEL & LOWE 1992). It

Table 5. Matrix of genetic distance coefficients between 23 *Abies alba* populations using 10 loci. Below diagonal: CAVALLI-SFORZA & EDWARDS (1967) chord distance. Above diagonal: NEI (1978) unbiased genetic distance.

Pop.	Southeastern French and Italian stands											
	BAY	BEU	BLE	BOS	BRG	CHE	ENT	LAB	LAC	LUR	SET	SMV
BAY	–	.006	.007	.006	.004	.003	.009	.003	.009	.002	.003	.007
BEU	.102	–	.007	.010	.000	.015	.009	.003	.000	.001	.008	.005
BLE	.093	.110	–	.003	.004	.020	.001	.000	.007	.003	.005	.003
BOS	.102	.112	.082	–	.007	.017	.003	.004	.014	.003	.005	.006
BRG	.073	.065	.094	.082	–	.113	.075	.003	.001	.001	.049	.002
CHE	.066	.123	.114	.103	.014	–	.027	.012	.018	.006	.006	.012
ENT	.105	.105	.089	.080	.006	.146	–	.004	.009	.006	.011	.009
LAB	.079	.108	.053	.089	.095	.091	.098	–	.085	.003	.091	.001
LAC	.095	.057	.105	.118	.072	.111	.093	.005	–	.004	.099	.005
LUR	.070	.064	.065	.081	.066	.096	.102	.072	.084	–	.078	.002
SET	.067	.097	.096	.077	.003	.098	.094	.001	.008	.003	–	.000
SMV	.093	.069	.077	.067	.058	.109	.089	.072	.072	.059	.060	–
TAR	.080	.090	.079	.108	.091	.094	.093	.054	.054	.076	.091	.073
TUR	.066	.070	.109	.111	.086	.083	.119	.084	.084	.058	.097	.086
VTX	.075	.096	.069	.069	.061	.114	.052	.066	.066	.176	.057	.060
PUN	.072	.104	.114	.112	.004	.126	.014	.013	.009	.005	.013	.015
PES	.088	.111	.109	.079	.074	.098	.109	.099	.099	.093	.075	.007
STU	.141	.101	.107	.086	.081	.160	.101	.124	.124	.103	.097	.069
AUD	.146	.120	.112	.140	.134	.151	.132	.107	.107	.117	.128	.097
DAU	.100	.085	.052	.050	.068	.125	.064	.070	.070	.060	.077	.043
GER	.084	.079	.074	.062	.049	.123	.043	.083	.083	.067	.062	.056
SAV	.132	.113	.104	.114	.122	.161	.114	.132	.132	.092	.128	.114
VEL	.106	.056	.104	.117	.098	.127	.108	.100	.100	.064	.122	.091

Pop.	Southeastern French and Italian stands						Reference populations				
	TAR	TUR	VTX	PUN	PES	STU	AUD	DAU	GER	SAV	VEL
BAY	.006	.000	.007	.006	.000	.018	.023	.008	.006	.016	.005
BEU	.007	.002	.009	.004	.005	.007	.020	.005	.005	.019	.001
BLE	.000	.011	.000	.014	.011	.007	.008	.000	.000	.006	.004
BOS	.005	.009	.003	.012	.009	.010	.015	.001	.001	.003	.004
BRG	.004	.004	.005	.072	.003	.004	.015	.003	.003	.017	.003
CHE	.013	.004	.018	.018	.004	.028	.028	.019	.018	.031	.017
ENT	.004	.014	.002	.119	.013	.009	.015	.001	.000	.006	.003
LAB	.000	.008	.000	.119	.006	.007	.007	.000	.001	.013	.006
LAC	.005	.007	.008	.119	.007	.006	.016	.006	.007	.025	.005
LUR	.003	.001	.005	.077	.004	.008	.016	.002	.002	.009	.000
SET	.002	.007	.003	.089	.003	.007	.008	.004	.004	.018	.011
SMV	.001	.008	.002	.110	.083	.003	.005	.001	.002	.016	.008
TAR	–	.009	.000	.126	.121	.132	.004	.000	.001	.011	.006
TUR	.074	–	.013	.096	.105	.143	.028	.010	.009	.018	.002
VTX	.061	.096	–	.103	.097	.100	.006	.000	.000	.010	.007
PUN	.017	.005	.017	–	.006	.018	.038	.014	.012	.022	.006
PES	.009	.001	.011	.105	–	.013	.026	.010	.010	.025	.008
STU	.007	.018	.007	.135	.097	–	.010	.003	.005	.021	.012
AUD	.077	.133	.103	.177	.171	.141	–	.104	.109	.118	.113
DAU	.080	.102	.052	.112	.092	.068	.006	–	.000	.005	.003
GER	.074	.095	.032	.098	.095	.085	.008	.042	–	.090	.002
SAV	.115	.129	.108	.136	.164	.139	.021	.095	.004	–	.006
VEL	.078	.063	.101	.118	.136	.135	.022	.092	.088	.093	–

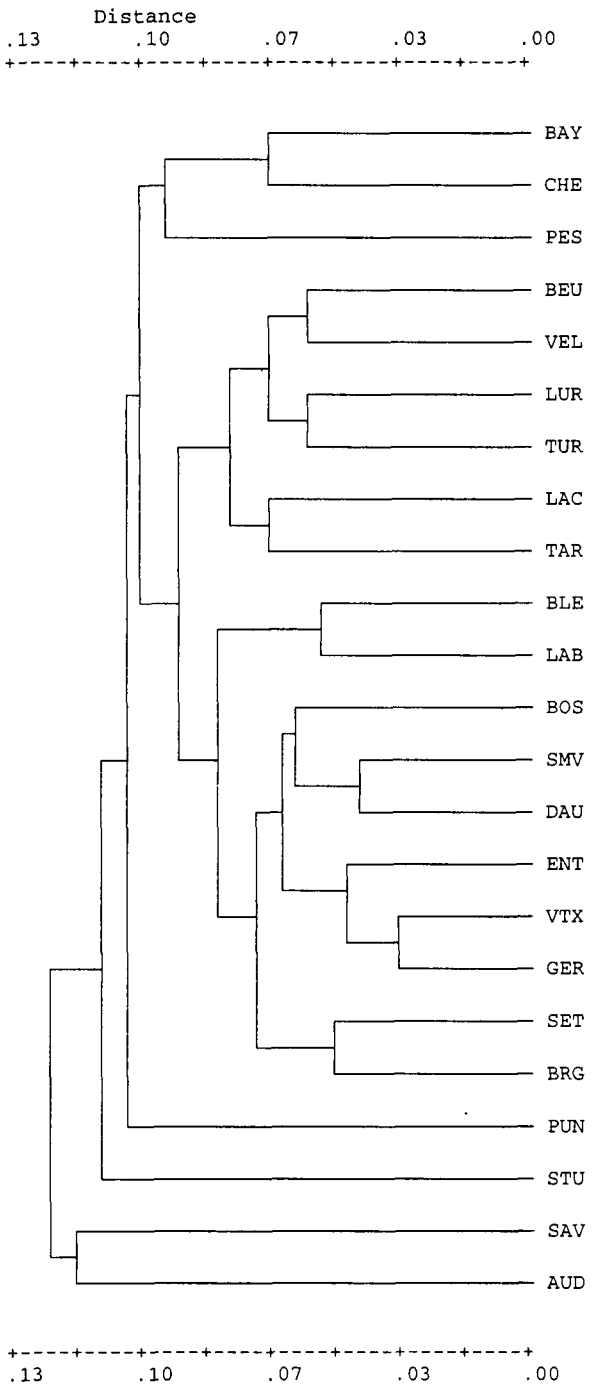

Figure 2. Cluster analysis using unweighted pair group (UPGMA) method for 23 *Abies alba* populations. Coefficient used: CAVALI-SFORZA & EDWARDS (1967) chord distance. All 10 loci used.

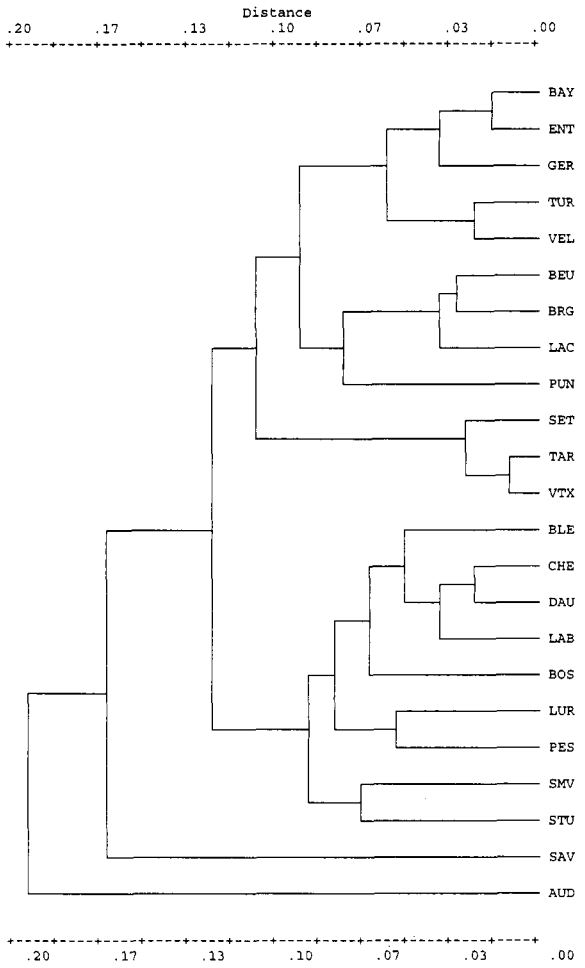

Figure 3. Cluster analysis using unweighted pair group (UPGMA) method for 23 *Abies alba* populations. Coefficient used: CAVALI-SFORZA & EDWARDS (1967) chord distance. Two loci used: *Got3* and *Idh2*.

occured in the Apennine range, which clearly demonstrates the existence of *Abies* refugia in Italy. Later events in the French Alps are still controversial as radiocarbon dating techniques are often biased and have yielded contradictory dates for the appearance of the first forests. For example, dates from Lac Long Inférieur and Lac Mouton in the Maritime Alps differ by approximately 1000 years although the two sites are located very close to each other. These discrepancies have resulted in different authors defending widely different paleoecological hypotheses using the same data (BEAULIEU 1977, CLERC 1988, BRUGIAPAGLIA 1996, NAKAGAWA 1998). The existence of local refugia in the Maritime Alps (NICOL-PICHARD & DUBAR 1998), the Dévoluy (BEAULIEU 1977) and the Vercors mountains (CLERC 1988) has been hypothesized. Along with fossil-pollen data which indicate that *Abies alba* pollen reached the Jura and Vosges mountains well after the Alps (BEAULIEU *et al.* 1994), our data support the following recolonization hypothesis: *Abies alba* migrated northwards from the Apennines along the Ligurian mountains into the Alps (8000 to 7500 years

BP), then colonized the Jura and Vosges mountains (7500 to 7000 years BP). This Alps-Jura-Vosges continuity was also revealed by BEYHAUT (1990) through terpene analyses and corresponds to the "West Alpine route" mentioned in the isozyme study of KONNERT & BERGMANN (1995). Our data did not yield any proof of the existence of local refugia in southeastern France.

Velay (VEL) was the only Massif Central population included in the study and is clearly well within the "Alps" population group, indicating that this mountain could have been populated from Alpine stands. Palynological data (BEAULIEU *et al.*, 1988) have excluded the Pyrenees but not the Alps as a source for *Abies* populations in the Massif Central and suggested the possible existence of local refugia, as did isozyme data from KONNERT & BERGMANN (1995). A specific study using more Massif Central populations is needed to better understand the origin of *Abies alba* in this region.

The Corsican population (PUN) appeared to be relatively isolated when all loci were examined. However, when only the 2 loci which most significantly differentiated between the Pyrenean and the Alpine populations were analyzed, the position of the Corsican population was shown to be well within the general "Alpine" population cluster. This seems to indicate a common Glacial origin with Alpine populations.

Two populations consistently stood out in this study: the ones from the Pyrenees (AUD) and from Savoie (SAV). The Savoie population is located in the northern French Alps and its position outside the group was unexpected. Several reasons could explain this position, such as the low number of harvested trees when seed stock was constituted (see material and methods), but also possibly random genetic drift or other undetected non-genetic factors (human pressure is known to have been strong on *Abies alba* forests as early as 5000 years BP in some regions, REILLE 1990). Local origin cannot be ruled out either as early pollen occurrences have been found in the Alps in at least one area indicating possible population development from a local refugium (BEAULIEU *et al.* 1994). The Aude population's situation outside the cluster confirms isozyme data from KONNERT & BERGMANN (1995) and terpene data from BEYHAUT (1990). Its particular gene pool could be the result of isolation in the Pyrenees during the last glacial periods at the latest. In addition, pollen analyses demonstrated that *Abies alba* appeared at approximately the same time (around 8000 years BP) in both the Alps and Pyrenees mountains (*e.g.*, BRESSET 1986 and BEAULIEU *et al.* 1994). All these data point to the existence of at least one *Abies alba* refugium in the Pyrenees, different from the *Abies alba* refugia from which southeastern French Alps, and probably northern Alps and Vosges, populations emerged.

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