Study on Isozyme Variation in *Pinus pinea* L.: Evidence for Low Polymorphism

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Abstract

The genetic diversity of *Pinus pinea* was studied by using isozymes. Seed tissues (megagametophytes and embryos) were analyzed by means of horizontal starch gel electrophoresis. A total of 37 enzyme systems were assayed from 10 populations of *Pinus pinea* from the northern Mediterranean. 27 enzyme systems were sufficiently revealed. They were coded by a minimum of 29 putative monomorphic loci and only 2 variable loci: menadione reductase (MNR-2) and malic enzyme (ME). Differentiation among 17 populations distributed around the Mediterranean basin was analyzed using only the ME allele frequencies due to the complex genetic control and poor quality zymograms found for MNR. Allele frequencies and genetic diversity parameters were highly variable between populations. Within population variation was less significant than between population variation. No relationship was found between the classification of the different populations according to allele frequencies of ME and the current geographic range of *Pinus pinea*.

Key words: *Pinus pinea*, isozymes, genetic diversity, population differentiation.

FDC: 165.3; 174.7 *Pinus pinea.*

Résumé

La diversité génétique du pin pignon a été étudiée à l'aide des marqueurs isoenzymatiques. La technique utilisée est l'électrophorèse sur gel d'amidon. Les analyses ont été effectuées sur les graines (endospermes et embryons). 37 systèmes enzymatiques au total ont été testés chez 10 populations de pin pignon du nord de la Méditerranée. 27 systèmes enzymatiques ont été correctement révélés, représentant un minimum de 29 locus monomorphes hypothétiques et seulement deux locus variables: la ménadione réductase (MNR-2) et l'enzyme malique (ME). La différenciation de 17 populations du pourtour méditerranéen a ensuite été analysée sur la base des fréquences alléliques de ME uniquement, MNR présentant un déterminisme complexe et une révélation difficile. Les fréquences alléliques et les paramètres de diversité génétique sont apparus très variables d'une population à l'autre. La variabilité de ME est essentiellement due à la composante interpopulation plutôt qu'à la composante intrapopulation. Le regroupement des différentes populations en fonction des fréquences alléliques de ME n'a pas permis d'établir un lien avec la répartition géographique actuelle du pin pignon.

Introduction

Pinus pinea is widely distributed around the Mediterranean basin. It was probably widely disseminated by man as early as the period of the first explorations. The esthetic shape, shade, wood and particularly the fruit of this conifer have been highly valued since antiquity. Some authors (EIG, 1931; RIKKLI, 1943, in: AGRIMI and CIANCIO, 1994) believe that *Pinus pinea* originates from the western part of the Mediterranean basin. The largest populations can be found in Spain and Portugal. However, FEINBRUN (1959) and QUEZEL (1980) have situated the origin of this pine in the eastern Mediterranean countries of Turkey and Lebanon, where some *Pinus pinea* stands form a climax structure surrounded by a characteristic vegetation. Populations in North Africa are artificial and of recent origin: they were planted during the last century. In the south of France, the spontaneity of *Pinus pinea* has long been disputed; however, borings near Fos-sur-Mer (PONS, 1964; TRIAT, 1975) revealed the presence of *Pinus pinea* pollen from a period (4.000 years B. P.) when anthropic introduction could not have occurred according to these authors.

This Mediterranean pine is the focus of a genetic diversity study particularly for reforestation of disturbed areas in North Africa. In order to assess the genetic variability of this species, a provenance trial was set up in France, Italy, Morocco, Tunisia and in Turkey within the framework of the F.A.O. Silva Mediterranea Network. At the same time, a genetic study using molecular markers was initiated. Isozyme markers were chosen because conifers, as a rule, display a high level of allozyme diversity and this technique has been widely used to study genetic structure of tree populations (HAMRICK and GODT, 1989; EL-KASSABY, 1991).

The goal of this work was to analyze the level and structure of allozyme diversity in *Pinus pinea*.

Materials and Methods

Plant material and sampling

Analyses were performed on seed tissues. Seeds were stratified and germinated. After the radicle emerged about 3 mm to 5 mm from the seed coats, megagametophytes and embryos were separated for extraction. Extraction (0.2 M phosphate buffer), migration on a 12.5% starch gel and staining were performed according to CONKLE *et al*. (1982) and LENGSIRI *et al*. (1990) with slight modifications (increase or decrease of substrate or coenzyme concentrations) for *Pinus pinea*. A total of 37 enzyme systems were tested: ACO (E.C.4.2.1.3), ACP (E.C.3.1.3.2), ADH (E.C.1.1.1.1), ALD (E.C.4.1.2.13), CAT (E.C.1.11.1.6), DIA (E.C.1.6.4.3), aEST (E.C.3.1.1.1), βEST (E.C.3.1.1.1), FDP (E.C.3.1.3.11), FUM (E.C.4.2.1.2), GDH (E.C.1.4.1.3), GluDH (E.C.1.1.1.47), GlyDH (E.C.1.1.1.29), GOT (or A.A.T., E.C.2.6.1.1), G3PD (E.C.1.2.1.12), G6PD (E.C.1.1.1.49), HK (E.C.2.7.1.1), IDH (E.C.1.1.1.42), LAP (E.C.3.4.11.1), MDH (E.C.1.1.1.37), ME (E.C.1.1.1.40), MNR (E.C.1.6.99.2), MPI (E.C.5.3.1.8), NADHDH (E.C.1.6.99.3), NADPDH (E.C.1.6.99.1), PEP (E.C.3.4.13.11), PER (E.C1.11.1.7.), 6PGD (E.C.1.1.1.44), PGI (E.C.5.3.1.9), PGM (E.C.2.7.5.1), PPO (E.C.1.14.18.1), SkDH (E.C.1.1.1.25), SOD (E.C.1.15.1.1), SrDH (E.C.1.1.1.14), SUDH (E.C.1.3.99.1), UGPP (E.C.2.7.7.9), XDH (E.C.1.2.1.37).

For the study of the inheritance of enzyme systems, a total of 39 mother trees were collected in 6 French populations from

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Table 1. – Survey of studied provenances of *Pinus pinea*.

Code	Country	Provenance name	Latitude	Longitude	Alt. (m)	Rainfall (mm)	Substratum
1	Spain	Andalucia occidental	36°20 N	6°5 W	50	631	calcic, loose
4	Spain	Cordillera Central	40°30 N	4°20 W	900	1007	acid, loose
5	Greece	Chalcidiki Agios Nikolaos	40°14 N	23°34 E	50	439	acid, compact
6	Greece	Metochi Patras	38°06 N	21°30 E	38		
7	Italy	Fenignlia 67	42°25 N	11°17 E	2-5	680	neutral, loose
8	Italy	Migliarino 76	43°47 N	10°17 E	5-10	966	neutral, loose
9	Lebanon	Beit Monzer	34°29 N	35°91 E	1300-1400		acid, loose
10	Lebanon	Kornael	33°84 N	35°77 E	1200-1400		acid, loose
13	Portugal	Norte do Tejo	40°40 N	7°54 W	400	1296	
14	Portugal	Sul do Tejo	38°23 N	8°31 W	40	589	
16	Turkey	Izmir	39°12 N	26°57 E	460	756	
19	Turkey	Artvin	41°11 N	41°51 E	225	654	
20	Turkey	Yatagan	37°22 N	28°03 E	660	1202	
22	France	Villeneuvette (Hérault)	43°37 N	3°24 E	130-220	800 - 900	calcic, compact
23	France	Saintes-Maries (B.-du-Rhône)	43°27 N	4°26 E	5-10	543	acid, loose
24	France	Puget-sur-Argens (Var)	43°27 N	6°41 E	20	920	acid, loose
25	France	Le Treps (Var)	43°16 N	6°24 E	600	1230	acid, compact

the Massif des Maures. For each mother tree, a minimum of 6 megagametophytes was analyzed, which yielded a probability of 0.97 $(1-1/2^{6-1})$ of detecting a heterozygous mother tree.

The same enzyme systems were also tested in 4 northern Mediterranean provenances (Spain 1, Greece 5, Portugal 13, Turkey 16, *Tab. 1*) in order to compare results obtained from French populations. 20 seeds (megagametophytes and embryos) were analyzed for each seedlot of these provenances.

Genetic diversity and its structure were analyzed for 17 provenances. Open pollinated seeds from 15 provenances were collected around the Mediterranean basin (*Tab. 1*). Depending on the rate of germination, between 20 to 40 seeds (megagametophytes and embryos) were analyzed for each provenance. In addition to these 15 provenances, the results from 2 French populations (France 24, France 25, *Tab.1*) scored for segregation study were reused. These 2 samples consisted of 20 individuals from the "Le Treps" population (France 25) and 10 individuals from the "Puget-sur-Argens" population (France 24). Each individual was represented by one out of the 6 open pollinated seeds.

Statistical analysis

Homogeneity of segregation among heterozygous mother trees was verified by a chi-square test. All megagametophytes were then pooled to test the goodness of fit of allele segregation with the expected 1:1 Mendelian ratio by a chi-square test. Genetic structure of the 17 Mediterranean provenances (embryo genotypes) was analyzed using GENEPOP (version 1.2, RAYMOND and ROUSSET, 1995) by computing allele frequencies, observed and expected heterozygosities (with correction according to LEVENE, 1949), FISHER's exact test for HARDY-WEINBERG equilibrium and WRIGHT's F-statistics (WRIGHT's, 1965).

FISHER's exact test for differentiation was used for all 17 populations taken together as well as on a pairwise basis according to allele frequencies at the ME locus.

Results

Polymorphism study

Among the 37 enzyme systems analyzed in the 6 French populations and the 4 northern Mediterranean populations, 10 showed insufficient or poor resolution: ALD, FUM, GDH, GluDH, GlyDH, G3PD, HK, PER, SOD et XDH. Only malic enzyme (ME) and menadione reductase (MNR) appeared to be polymorphic, the 25 other enzyme systems were monomorphic and have been described elsewhere (FALLOUR, 1994).

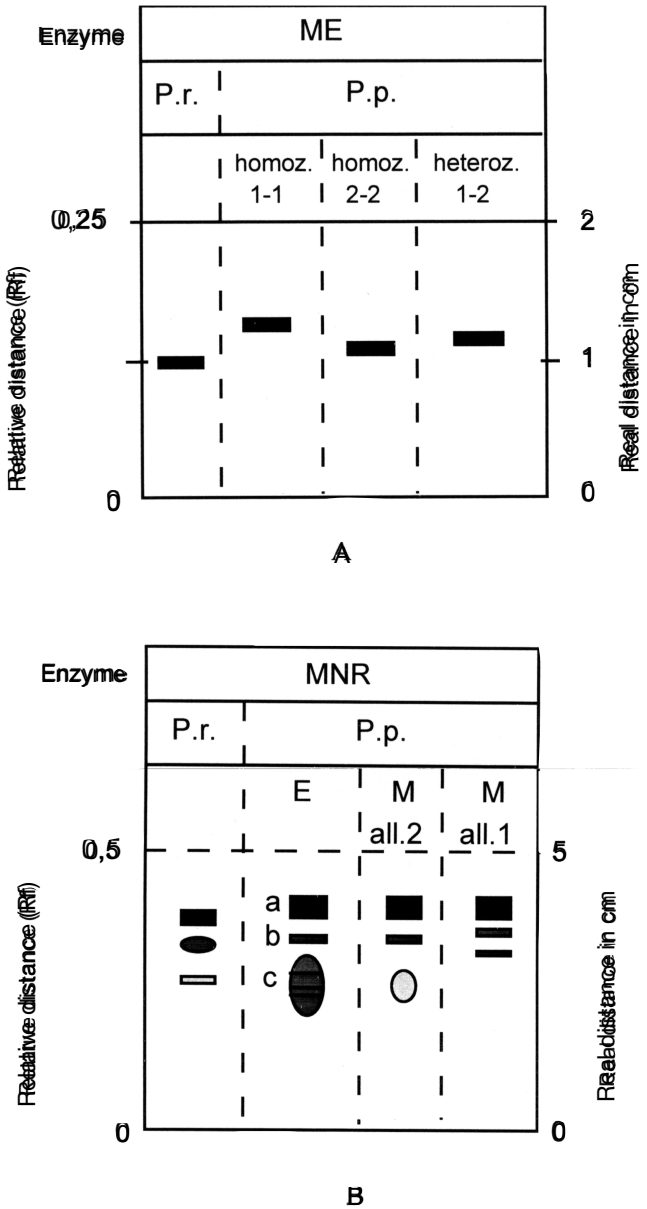


Figure 1. – Zymograms of polymorphic enzyme systems in embryo (E) and megagametophyte (M) of *Pinus pinea* (P.p.) and a reference species (P.r. = *Pinus resinosa*). A: Malic Enzyme (ME). B: Menadione Reductase (MNR).

The monomorphic enzyme systems in *Pinus pinea* were compared with those in other Pinaceae species. The number of putative loci was thus estimated to be 19 for the 12 following monomorphic enzyme systems: ACO, ACP, CAT, FDP, G6PD, IDH, LAP, MPI, 6PGD, PGI, PGM, SkDH. In the 9 remaining monomorphic enzyme systems (ADH, EST, GOT, MDH, PEP, PPD, SrDH, SUDH, UGPP), the number of coding loci was estimated to be at least one per system. Thus, with MNR-1, a minimum of 29 putative monomorphic loci were observed in these *Pinus pinea* populations. Only 2 enzyme systems appeared to be variable and are described below.

Malic enzyme

A single band was observed for this enzyme in *Pinus pinea* (*Fig. 1A*) as in *Pinus resinosa* (used as a migration reference) and in *Pinus halepensis* (studied at the same time, TEISSEIRE *et al.*, 1995). Two alleles were detected in *Pinus pinea*. Heterozygous embryos yielded one intermediate band. The chi-square test of homogeneity indicated that the data scored for each allelic pair were homogeneous over the 5 heterozygous mother trees, and the segregation fitted the expected 1:1 ratio (*Tab. 2*).

Table 2. – Segregation of allozymes: chi-square tests of heterogeneity among heterozygous mother trees and chi-square tests of deviation to the expected 1:1 MENDELIAN ratio.

heterozygous	ME		MNR	
mother trees	allele 1	allele 2	allele 1	allele 2
G1			3	3
G2	4	2	1	5
G3			3	3
G4			3	3
G5			1	5
G7			2	4
G8			5	1
G9			1	5
G10			2	4
P1			3	3
P2			3	3
P3	4	2	3	3
P5			1	5
P6			4	2
P7			2	4
P8			1	5
P9			2	4
P10			5	1
R1a			5	2
R1b			3	4
R2			5	1
R3	6	5		
R4			3	3
R5			5	6
R6			3	3
R7			7	4
R11	6	5		
L1			5	6
L3			2	7
L4	3	3	4	2
L5			4	2
Cap			5	1
GR			1	4
total	23	17	97	108

Results of tests		ME	MNR
Heterogeneity	Chi2 (df)	0.63 (4)	35.18 (30)
	p-value	0.96	0.24
Segregation	Chi2 (df)	0.90 (1)	0.49 (1)
	p-value	0.34	0.48

Menadione reductase

This enzyme system was difficult to stain and to interpret. Three bands appeared in *Pinus pinea* (*Fig. 1B*). No variation was found among the zymograms from embryos (E), whereas megagametophytes (M) yielded either a pattern similar to embryos, or a different one where "patch c" was replaced by a narrower, faster band with lower intensity. This pattern was also characterized by a faster migration of the "b" band. These 2 bands "b" and "c" were assumed to be coded by a single locus (MNR-2) and the invariable band "a" by another locus (MNR-1). To infer on the genetic control of this enzyme, we assigned 2 alleles to the 2 megagametophytes phenotypes: the first when the pattern is similar to that of the embryo and the second when the pattern is different. Homogeneity of segregation over the 31 heterozygous mother trees and segregation according to Mendelian 1:1 ratio were verified (*Tab. 2*). However, as genetic control of MNR was not clear enough, we focused the study of genetic differentiation on the single ME polymorphic locus.

Genetic differentiation according to malic enzyme variation

Allele frequencies of malic enzyme varied considerably between populations (*Tab. 3*): the frequency of allele 2 ranged from 0.012 ("Turkey 19") to 1 ("Greece 5").

Expected heterozygosities (*Tab. 3*) ranged from 0.009 ("Grece 5") to 0.5 ("Greece 6", "Portugal 13") with a mean heterozygosity of 0.344 for the populations under study.

If all 31 loci are considered, mean expected heterozygosity was 0.009 with a variation from 0.000 to 0.016.

Deviation from HARDY-WEINBERG equilibrium was not significant in most of the populations except for "Portugal 13", "Turkey 16", "Turkey 20" and "France 23" which showed a deficiency in heterozygotes (*Tab. 3*).

Differentiation parameters indicated a total diversity (F_{IT}) of 0.384, with a within-population component (F_{IS} = 0.146) lower than the between-population component (F_{ST} = 0.279).

In conclusion, the populations significantly differed for ME allele frequencies (p-value = 0). Results from this test performed by population pairs are represented on *figure 2*. We could distinguish 3 main groups: the "Turkey 19" population was isolated with an almost fixed allele 1, another group (Greece 5, France 22, 23 and 24, Lebanon 10 and Turkey 16) was characterized by a high frequency for allele 2 (> 0.80) and a third group (France 25, Greece 6, Spain 1 and 4, Portugal 13 and 14, Italy 7 and 8, Lebanon 9, Turkey 20) presented an intermediate frequency for allele 2, ranging from 0.25 to 0.65.

Discussion

Genetic control of ME and MNR

Malic enzyme

The malic enzyme is rarely studied because of its monomorphism or its very low polymorphism. One locus has been observed in several conifers (JACOBS *et al.*, 1984; NIEBLING and CONKLE, 1990). Two loci have been reported in *Abies pinsapo* (PASCUAL *et al.*, 1993), *Pinus contorta* (YEH, 1979) and *Pseudotsuga menziesii* (YEH and O'MALLEY, 1980). The structure of this enzyme appeared to be tetrameric in some studies reported by WEEDEN and WENDEL (1989), however, this enzyme behaves like a dimeric protein in *Thuja occidentalis* (PERRY and KNOWLES, 1989) and in *Pinus pinea* with an intermediate band for heterozygous embryos.

Table. 3. – Genetic diversity and deviation from HARDY-WEINBERG equilibrium.

			for malic enzyme (ME)			for 31 putative loci		for ME	
Code	**Country**	N	all. 2 fqc	H_o	H_e	mean H_o	mean H_e	H-W	F_{IS}
1	**Spain**	22	0.614	0.500	0.474	0.016	0.015	NS	
4	**Spain**	40	0.250	0.350	0.375	0.011	0.012	NS	
5	**Greece**	35	1.000	0.000	0.000	0.000	0.000		
6	**Greece**	20	0.500	0.600	0.500	0.019	0.016	NS	
7	**Italy**	40	0.425	0.500	0.489	0.016	0.016	NS	
8	**Italy**	35	0.529	0.486	0.498	0.016	0.016	NS	
9	**Lebanon**	40	0.587	0.425	0.485	0.014	0.016	NS	
10	**Lebanon**	39	0.833	0.282	0.278	0.009	0.009	NS	
13	**Portugal**	25	0.500	0.280	0.500	0.009	0.016	0.042	0.456
14	**Portugal**	35	0.357	0.314	0.459	0.010	0.015	NS	
16	**Turkey**	45	0.811	0.156	0.306	0.005	0.010	0.003	0.501
19	**Turkey**	41	0.012	0.024	0.024	0.001	0.001	NS	
20	**Turkey**	45	0.544	0.333	0.496	0.011	0.016	0.035	0.338
22	**France**	40	0.938	0.075	0.117	0.002	0.004	NS	
23	**France**	41	0.817	0.171	0.299	0.006	0.010	0.014	0.439
24	**France**	10	0.950	0.100	0.095	0.003	0.003	NS	
25	**France**	20	0.650	0.400	0.455	0.013	0.015	NS	
	mean	34	0.607	0.294	0.344	0.009	0.011		

N: number of analysed individuals for ME; H_o: observed heterozygoty; H_e: expected heterozygoty (with correction by LEVENE, 1949); H-W: p-values of FISHER's exact test for deviation from HARDY-WEINBERG equilibrium; F_{IS}: fixation index.

Menadione reductase

To explain the invariability of the embryo patterns, we can imagine that only heterozygous embryos are viable. But this would not explain presence of adult heterozygous trees at MNR locus. The difference in the observed patterns could possibly correspond to different stages of enzyme differentiation between some megagametophytes (we were not able to analyze all megagametophytes at the same stage due to germination problems) and, between embryos and megagametophytes as has been observed in *Picea abies* for 6PGD (GIANNINI *et al.*, 1988).

It is impossible to resolve this question without additional analyses and without better knowledge of this enzyme's functioning.

Moreover, it is interesting to note the diversity of the patterns observed for MNR in conifers with 1, 2, 3 or 4 activity zones according to species (CONKLE *et al.*, 1988; LEWANDOWSKI *et al.*, 1992; STRAUSS and CONKLE, 1986; LEDIG and CONKLE, 1983). The structure appears to be either dimeric (FADY and CONKLE, 1992) or tetrameric (PAPAGEORGIOU *et al.*, 1993).

In addition, we observed that NADHDH, NADPHDH and DIA yielded patterns which were superimposed on MNR (FALLOUR, 1994). These specificity problems have also been reported by other authors (WENDEL et al., 1988; ERNST *et al.*, 1987; YING and MORGENSTERN, 1989; LEWANDOWSKI and MEJNARTOWISZ, 1988). Based on the similarity of substrates, YI (1992) proposed that MNR and DIA be grouped in NADHDH (quinone: E.C. 1.6.5._.), at least in conifers.

For the above reasons, we preferred not to utilize MNR for studies on genetic differentiation in *Pinus pinea*.

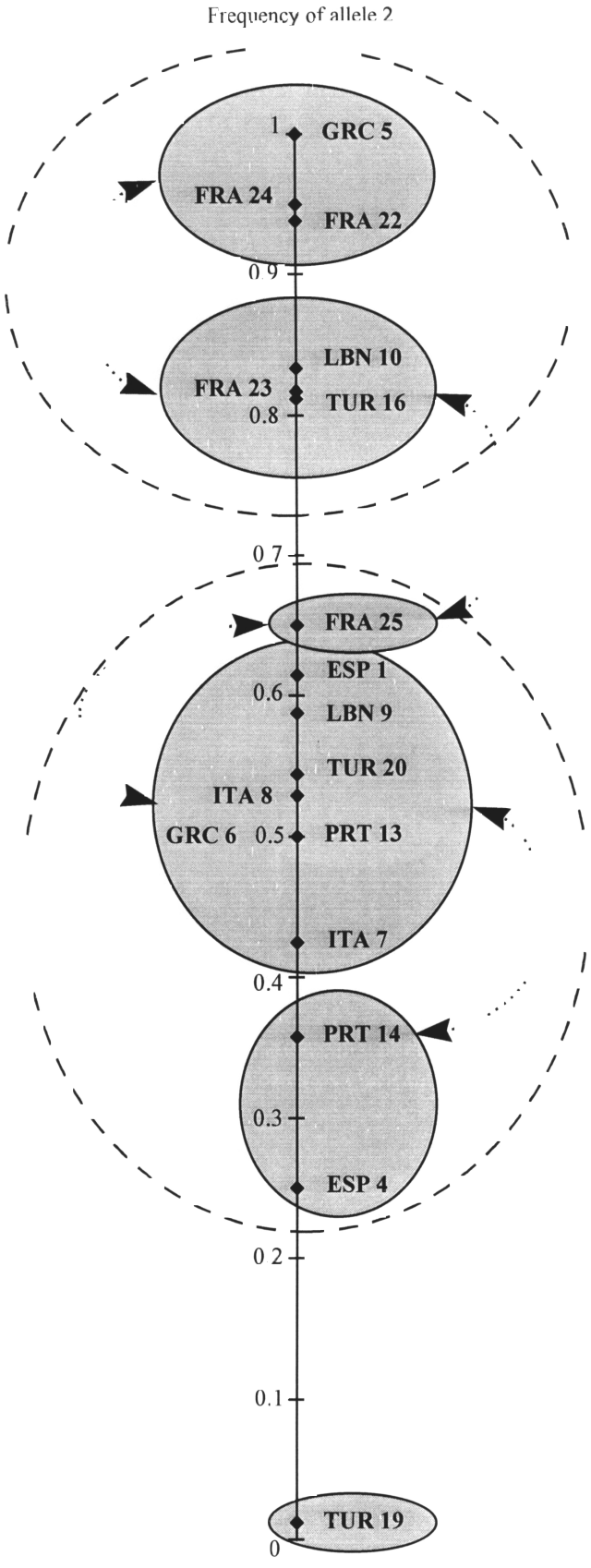
Level of diversity

The level of polymorphism observed for the 10 northern Mediterranean populations in the first part of this study was very low. The proportion of polymorphic loci was 6.45% at maximum although this amount is on average 50% in higher plants and is especially high (almost 70%) in conifers (HAMRICK and GODT, 1989; HAMRICK *et al.*, 1992). This low allozyme diversity resulted in a correspondingly low mean expected heterozygosity. It amounted to over 1%, although HAMRICK *et al.* (1992) have reported a heterozygosity of 15% on average in plants, with higher values in conifers. HAMRICK et al. (1979) observed a mean heterozygosity of 21% with values up to 33% in 20 conifer species. Our study is not really comparable with other isozyme studies on conifer species since it is based on only one locus which is rarely analyzed. If we consider only loci which are usually studied in population genetic of forest trees (reviewed by HAMRICK *et al.*, 1979, 1992), the mean expected heterozygosity is zero.

Pinus pinea could be added to the list of the few conifers species with extremely low allozyme diversity; along with *Pinus resinosa* (FOWLER and MORRIS, 1977; ALLENDORF *et al.*, 1982; MOSSELER *et al.*, 1991), *Thuja plicata* (COPES, 1981) and *Pinus torreyana* (LEDIG and CONKLE, 1983).

Low genetic diversity can generally be explained by a drastic reduction in the effective population size during Quaternary glaciations ("bottleneck effect" e.g. NEI, 1974). A few small populations surviving in refugia – often located near the Mediterranean sea for European species – had offspring which recolonized the area with a reduced genetic pool when climatic conditions improved. Nevertheless, other species which supported genetic drift during glaciations are currently differentiated. Thus, another explanation could be found with a wide anthropogenic diffusion of genetically homogeneous reproductive material as early as the first explorations: many European populations of *Pinus pinea* could originate from one or a few of the Lebanese populations introduced by the Etruscans (THIRGOOG, 1981).

It is interesting to report results of inbreeding experiments performed on *Pinus pinea* by AMMANATI (1988; in AGRIMI and CIANCIO, 1994) where no depreciation in vigor was observed. This could be explained by a previously low heterozygosity in



Results of the FISHER's exact test of differenciation by population pairs: populations grouped in a same colored ellipse are not significantly different; connection between groups indicates a significant differentiation between some populations but an absence of differentiation with other populations.

Figure 2. – Grouping of populations according to their allele frequencies at the locus ME.

this pine: if heterozygosity is very low, a decrease of this heterozygosity by inbreeding will not be appreciable, and thus, depression will not become evident. CONKLE (in LEDIG, 1986) found such a relationship between diversity and inbreeding effects: conifer species with high heterozygosity show a more important depression when they are inbred than species which have low heterozygosity.

Inbreeding effects can be linked to recessive lethal genes. LEDIG (1986) reported that conifers are characterized by high embryonary and post-embryonary mortality due to a high genetic load. As *Pinus pinea* survives inbreeding well and has a very low level of polymorphism, it can be postulated that it has been purged from its deleterious alleles as *Pinus resinosa* and *Pinus torreyana* (LEDIG, 1986).

At present, it is essential to analyze the level of *Pinus pinea*'s diversity by using standard adaptive traits (e.g. survival, growth, phenology). The first results from provenance tests will be available in a few years.

Concurrently, the low allozyme diversity should be verified by DNA markers. Some techniques are more efficient than isozymes for detecting polymorphism, such as chloroplast and mitochondrial DNA amplification using universal primers (DEMESURE *et al.*, 1995) or methods based on microsatellites (POWELL *et al.*, 1995). The few test runs using chloroplast microsatellites performed on some *Pinus pinea* individuals indicated that polymorphism could be found in this species (VENDRAMIN, pers. comm.).

Structure of Diversity

Four populations out of the 17 studied were significantly deficient in heterozygotes at the ME locus. Such deficiency was rarely observed in coniferous trees. This could have been caused by a WAHLUND effect if the studied populations were in fact complex mixtures of different origins.

Despite low enzyme polymorphism, *Pinus pinea* showed high differentiation between populations (F_{ST}/F_{IT} = 73%) although this diversity component is low in most conifers (HAMRICK *et al.*, 1992). This level of structure has also been found in *Pinus attenuata* (STRAUSS and CONKLE, 1986).

No clear evidence of a relationship was found between the distribution of ME alleles and the ecological characteristics of the provenances (elevation, substratum).

The chance to monitor geographical genetic variation with only one locus is very low but not zero. LEINEMANN (1996) found a clear differentiation among Douglas-fir provenances according to frequencies of the two more frequent alleles (and some rare alleles) at the 6PGD locus. Since the ME locus is the only available allozyme information for *Pinus pinea,* we analyzed the allele distribution of this locus. No clear geographic structure was found around the Mediterranean basin (*Fig. 3*): provenances from the same country were classified in different groups represented on *figure 2* (Greece), some with high differentiation (Turkey). It can also be noted that the 3 French populations were grouped into geographically distant populations (Lebanon, Turkey) which are different from the Spanish, Portuguese and Italian population groups. Oddly, these 3 French populations are presumed to be natural while the fourth one, which is nearer to the western European populations, is artificial (planted by the Forest Service). This suggests 2 hypotheses: either these western European populations are also non-indigenous, which appears to be very improbable, or, all the French populations which are assumed to be natural are in fact non-indigenous and have different origins.

The particular situation of the "Turkey 19" population is the only case which can be associated with its geographic isolation.

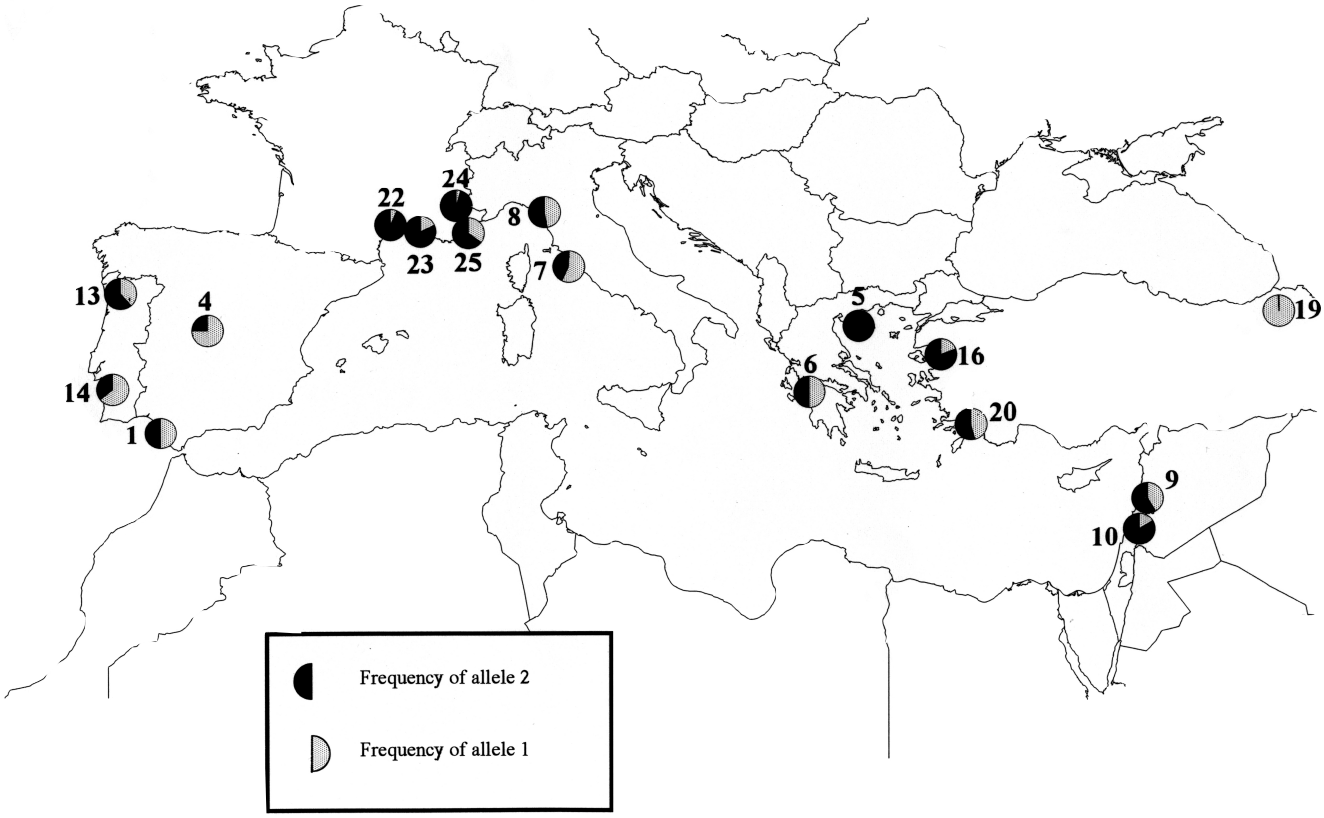


Figure 3. – Localization of the studied populations with their allele frequencies at the ME locus.

This small population is located about 50 km east of the Black Sea (it is the easternmost population): it could be a relic population genetically isolated from other populations by natural barriers or it could be an artificial population formed from a reduced seedlot brought by navigators over the Black Sea.

The relatively high differentiation among populations could be explained by a separation of these populations which occurred a long time ago. Given the late succession status of conifers, this would have been caused by genetic drift during glaciations which separated several genetically differentiated populations into several refugia. However, this does not explain the large differentiation between closely settled populations growing under the same ecological conditions which are not separated by natural barriers. This particularity could be explained by the anthropic origin of some populations among natural populations, as is the case for the "France 25" (artificial) and "France 24" (presumed natural) populations. The lack of available historical documents on the natural or artificial origins of the tree stands sampled did not enable us to draw further conclusions and this initial study did not make it possible to distinguish artificial populations from natural ones. This goal seems impossible to achieve by using exclusively isozyme markers. More results are expected from chloroplast microsatellites.

References

AGRIMI, M. and CIANCIO, O.: Le pin pignon (*Pinus pinea* L.). Monographie. Provisional proceedings, 16° C.F.F.S.A./C.E.F./C.F.P.O. meeting, Silva Mediterranea, F.A.O., Larnaca, Cyprus, 115 p. (1994). — ALLENDORF, W., KNUDSEN, K. L. and BLAKE, G. M.: Frequencies of null alleles at enzyme loci in natural populations of ponderosa and red pine. Genetics **100**: 497–504 (1982). — CHELIAK, W. M. and PITTEL, J. A.: Inheritance and linkage of allozymes in *Larix laricina*. Silvae Genetica **34** (4–5): 142–148 (1985). — CONKLE, M. T., HODGSKISS, P. D., NUNNALY, L. B. and HUNTER, S. C.: Starch gel electrophoresis of conifer seeds: a laboratory manual. Pacific Southwest Forest and Range Experiment Station, Berkeley, California, 18 p. (1982). — CONKLE, M. T., SCHILLER, G. and GRUNWALD, C.: Electrophoretic analysis of diversity and phylogeny of *Pinus brutia* and closely related taxa. Systematic Botany **13** (3): 411–424 (1988). — COPES, D. L.: Isozyme uniformity in western red cedar seedlings from Oregon and Washington. Can. J. For. Res. **11**: 451–453 (1981). — CRITCHFIELD, W. B. and LITTLE, E. L.: Geographic distribution of the pines of the world. U.S.D.A. Washington, Forest Service, 97 p. (1966). — DEMESURE, B., SODZI, N. and PETIT, R.: A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology **4**: 129–131 (1995). — EL KASSABY, Y. A.: Genetic variations within and among conifer populations: review and evaluation of methods. In: FINESCHI, S., MALVOLTI, M. E., CANNATA, F. and HATTEMER, H. H. (eds).: Biochemical markers in the population genetics of forest trees. Proceedings of the joint meeting of the I.U.F.R.O., Porano, Italy, October 1988. SPB Academic Publishing bv, The Hague, The Netherlands: 61–76 (1991). — ERNST, S. G., KEATHLEY, D. E. and HANOVER, J. W.: Inheritance of isozymes in seed and bud tissues of blue and ENGELMANN spruce. Genome **29**: 239–246 (1987). — FADY, B. and CONKLE, M. T.: Segregation and linkage of allozymes in seed tissues of the hybrid greek fir *Abies borisii regis* MATTFELD. Silvae Genetica **41** (4–5): 273–278 (1992). — FALLOUR, D.: Etude isoenzymatique du pin pignon (*Pinus pinea* L.): mise en evidence d'un faible polymorphisme. Mémoire de D.E.A., Univ. d'Aix-Marseille III, 51 p. (1994). — FEINBRUN, N.: Spontaneous pineta in the Lebanon. Israel Res. Council Bul. **70**: 132–153 (1959). — FOWLER, D. P. and MORRIS, R. W.: Genetic diversity in red pine: evidence for low genic heterozygosity. Can. J. For. Res. **7**: 343–347 (1977). — GIANNINI, R., MORGANTE, M. and VENDRAMIN, G. G.: A putative gene duplication in Norway spruce for 6PGD and its phylogenetic implications. In: FINESCHI, S., MALVOLTI, M. E., CANNATA, F. and HATTEMER, H. H. (eds).: Biochemical markers in the population genetics of forest trees. Proceedings of the joint meeting of the I.U.F.R.O., Porano, Italy, October 1988. SPB Academic Publishing bv, The Hague, The Netherlands: 23–29 (1991). — HAMRICK, J. L. and GODT, M. J. W.: Allozyme diversity in plant species. In: "Plant population genetics, breeding, and genetic resources." Edited by BROWN, A. H. D., CLEGG, M. T., KAHLER, A. L. and WEIR, B. S.. Sunderland, Massachusetts: 43–63 (1989). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — HAMRICK, J. L., MITTON,

J. B. and LINHART, Y. B.: Levels of genetic variation in trees: influence of life history characteristics. In: Proceedings of the Symposium of North American Forest Trees and Forest Insects. Berkeley, California: 35–41 (1979). — JACOBS, B. F., WERTH, C. R. and GUTTMAN, S. I.: Genetic relationships in *Abies* (fir) of eastern United States: an electrophoretic study. Can. J. Bot. **62**: 609–616 (1984). — LEDIG, F. T.: Heterozygosity, heterosis, and fitness in outbreeding plants. In: Conservation biology: the science of scarcity and diversity. Ed. by SOULE, M. E.: The Regents of the University of Michigan: 77–105 (1986). — LEDIG, F. T. and CONKLE, M. T.: Gene diversity and genetic structure in a narrow endemic, torrey pine (*Pinus torreyana* PARRY ex CARR.). Evolution **37** (1): 79–85 (1983). — LEINEMANN, L.: Genetic differentiation of damaged and healthy Douglas-Fir stands in Rheinland-Pfalz with respect to their origin. Silvae Genetica **45** (5–6): 250–256 (1996). — LEVENE, H.: On a matching problem arising in genetics. Ann. Math. Stat. **20**: 91–94 (1949). — LEWANDOWSKI, A., BURCZYK, J. and MEJNARTOWICZ, L.: Inheritance and linkage of some allozymes in *Taxus baccata* L.. Silvae Genetica **41** (6): 342–347 (1992). — LEWANDOWSKI, A. and MEJNARTOWICZ, L.: Inheritance of allozymes in *Larix decidua* MILL. Silvae Genetica **39** (5–6): 184–188 (1988). — LIENGSIRI, C., PIEWLUANG, C. and BOYLE, T. J. B.: Starch gel electrophoresis of tropical trees. A manual. A.S.E.A.N. Canada Forest Tree Seed Centre, Saraburi, Thailand, 52 p. (1990). — MOSSELER, A., INNES, D. J. and ROBERTS, B. A.: Lack of allozymic variation in disjunct Newfoundland populations of red pine (*Pinus resinosa*). Can. J. For. Res. **21**: 525–528 (1991). — NEI, M., MARUYAMA, T. and CHAKRABORTY, R.: The bottleneck effect and genetic variability in populations. Evolution **29** (1): 1–10 (1974). — NIEBLING, C. R. and CONKLE, M. T.: Diversity of Washoe pine and comparisons with allozymes of ponderosa pine races. Can. J. For. Res. **20** (3): 298–308 (1990). — PAPAGEORGIOU, A. C., BERGMANN, F., GILLET, E. and HATTEMER, H. H.: Genetic analysis of isozyme variation in mediterranean cypress (*Cupressus sempervirens* L.). Silvae Genetica **42** (2–3): 109–111 (1993). — PASCUAL, L., GARCIA, F. J. and PERFECTTI, F.: Inheritance of isozyme variations in seed tissues of *Abies pinsapo* BOISS. Silvae Genetica **42** (6): 335–340 (1993). — PERRY, D. J. and KNOWLES, P.: Inheritance and linkage relationships of allozymes of eastern white cedar (*Thuja occidentalis*) in north western Ontario. Genome **32**: 245–250 (1989). — PONS, A.: Contribution palynologique à l'étude de la flore et de la végétation pliocènes de la région rhodanienne. Thèse de l'Univ. de Montpellier, 548 p. (1964). — POWELL, W., MORGANTE, M., VENDRAMIN, G. G., RAFALSKI, J. A. and MCDEVITT, R.: Polymorphic simple sequence repeat regions in chloroplast genomes: applications to the population genetics of pines. Proc. Natl. Acad. Sci. **92**: 7759–7763 (1995). — QUEZEL, P.: Biogéographie et ecologie des conifères sur le pourtour méditerranéen. In: PESSON: Act. Ecol. Forest. Gauthier-Villars, Paris: 201–255 (1980). — RAYMOND, M. et ROUSSET, F.: GENEPOP (V 1.2): A population genetics software for exact tests and eucumenicism. Journal of Heredity **86**: 248–249 (1995). — STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72:** 483–493 (1986). — TEISSEIRE, H.: Etude de la variabilité génétique des peuplements naturels de *Pinus halepensis* en France. Représentativité des peuplements classés. Mémoire de M. S. T., Université de Lyon I (1994). —TEISSEIRE, H., FADY, B. and PICHOT, C.: Allozyme variation in five French populations of Aleppo pine (*Pinus halepensis* MILLER). Forest Genetics **2** (4): 225–236 (1995). — THIRGOOD, J. V. J.: Man and the Mediterranean forest. Academic Press, London, N.Y. VIII. 194 p. (1981). — TRIAT, H.: L'analyse pollinique de la tourbière de Fos sur Mer (Bouches du Rhône). Ecol. Medit. **I**: 109–121 (1975). — WEEDEN, N. F. and WENDEL, J. F.: Genetics of plant isozymes. In: "Isozymes in plant biology". SOLTIS, D. E. and SOLTIS, P. S. (eds). Advances in Plant Sciences Series, vol.4. Dioscorides Press, Portland, Oregon: 46–72 (1989). — WENDEL, J. F., GOODMAN, M. M., STUBER, C. W. and BECKETT, J. B.: New isozyme systems for maize (*Zea mays* L.): aconitase hydratase, adenylate kinase, NADH dehydrogenase. Biochem. Genet. **26** (5/6): 421–445 (1988). — WRIGHT, S.: The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution **19**: 395–420 (1965). — XIE, C. Y., DANCIK, B. P. and YEH, F. C.: Inheritance and linkage of isozymes in *Thuja orientalis*. Journal of Heredity **82**: 329–334 (1991). — YEH, F. C.: Analyses of gene diversity in some species of conifers. Symposium on Isozymes of North American Forest Trees and Forest Insects, Berkeley, California (1979). — YEH, F. C. and O'MALLEY, D.: Enzyme variations in natural populations of Douglas fir, *Pseudotsuga menziesii* (MIRB.) FRANCO, from British Columbia. I. Genetic variation patterns in coastal populations. Silvae Genetica **29** (3–4): 83–92 (1980). — YI, CHEONG-HO: A pitfall in utilization of isozyme markers caused by the complexities of staining reactions: case of *Pinus densiflora* and *Pinus thunbergii*. Thesis for the Degree of Master of Agriculture in Forestry, Korea University, 75 p. (1992). — YING, L. and MORGENSTERN, E. K.: Inheritance and linkage relationships of some isozymes of *Larix laricina* in New Brunswick, Canada. Silvae Genetica **39** (5–6): 245–251 (1989).

The Genetic Control of Flowering Precocity in *Eucalyptus globulus* ssp. *globulus*

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Abstract

The genetic control of flowering precocity was examined in 4 base population trials of *Eucalyptus globulus* ssp. *globulus* in Tasmania, which include nearly 600 open-pollinated families from 46 collection localities. Flowering precocity (measured as the presence/absence of capsules and or flower buds at age 4) was found to be highly heritable (h^2_L = 0.47 or 0.59; averaged across 3 or 4 trials respectively), and exhibit little genotype by environment interaction. Significant differences in the propensity for flowering precocity were observed between the 4 trials and also between different collecting localities. Progenies from localities on the Furneaux Group of islands showed significantly earlier flowering than those from other localities. Flowering precocity was generally not genetically correlated with growth (average r_g = 0.04), indicating that selection for this trait alone would have little impact on early growth. In addition precocious flowering was not significantly correlated with pilodyn penetration (average r_g = –0.07), although the consistent negative genetic correlation across 4 trials may be indicative of a weak positive genetic relationship with wood density. The potential of selecting for precocious flowering as a means of decreasing the generation interval in breeding programs is discussed.

Key words: REML, heritability, genetic correlation, generation interval, provenances, binomial traits.

FDC: 165.3; 181.521; 232.1; 176.1 *Eucalyptus globulus;* (946).

Introduction

From a breeding perspective, the rate of incorporation of new genetic material into commercial planting stock depends on both the gains made per generation, and the rate of generation