Genetic Structure of *Fagus sylvatica* L. Populations in Southeastern Europe

By K. Hazler[2], B. Comps[1][6], I. Šugar[2], L. Melovski[3], A. Tashev[4] and J. Gračan[5]

(Received 4th March 1997)

Abstract

Genetic structure of 38 populations from southeastern Europe has been studied using 11 enzymatic gene loci (PX-1, PX-2, GOT-1, 6-PGD-1, SOD-1 ACP-1, MDH-1, IDH-1, PGM-1, PGI-1 and MNR-1). Eight loci are polymorphic with gene diversity ranging from 0.179 to 0.461. Principal component analysis (PCA) based on allelic frequencies extracted 4 groups of populations: Rhodopes; Shars-Pindus range; Dinaric Alps and low Pannonic mountain ranges; Biokovo Mountain and Istrian Peninsula, well associated with geographical and climatic conditions. The statistical significance of this grouping was tested by discriminant analysis (DA). Although all 4 derived groups have high and similar values of the total diversity (respectively 0.250; 0.228; 0.282; 0.264), the share of loci which are more polymorphic varies from group to group. Presence of some rare alleles also indicates the difference between them. The correlations between estimated genetic differences and presumed occurrences during the glaciation and the postglaciation periods have been discussed, especially microgeographical conditions and evolutionary history of beechwoods from isolated regions of Biokovo and Istria. Multilocus F-statistics have also been computed. Positive F_{IT} values for all groups of populations indicate some deficit of heterozygotes, while high F_{ST} values prove certain differentiation among them.

Key words: *Fagus sylvatica*, isozymes, genetic structure, geographic variations, recolonization.

FDC: 165.3; 181.1; 182.1; 176.1 *Fagus sylvatica*; (4–12).

Introduction

Beech survived intense climatic and geological changes during the Quaternary because some of its populations lived in areas not reached by ice. These remaining populations are the origin of all present European beech populations. After extending from its main refugia in Southeastern Europe (Huntley and Birks, 1983; Bennett *et al.*, 1991; Willis, 1994) and Italy (Demesure, 1996), and possible secondary refugia in Southwestern Europe (Oldfield, 1960; Beug, 1967; Paquereau, 1974; Jalut, 1976; Jalut *et al.*, 1975; Šercelj, 1996), its current spacious area contains territories with very different ecological conditions.

Many analyses of genetic structure by protein markers carried out so far (Thiébaut, 1984; Barrière *et al.*, 1985; Comps *et al.*, 1987, 1988, 1990, 1991a and b; Gömöry *et al.*, 1992; Leonardi and Menozzi, 1995; Müller-Starck and Ziehe, 1991; Vyšny *et al.*, 1995), established a high diversity of beech populations in Europe as well as a strong connection of some alleles with geographical and ecological conditions. Müller-Starck (1985) describes the influence of man, or rather of the polluted atmosphere, on beech genetic structure. An adaptable group of beech is the most polymorphic one. A rather high degree of cpDNA diversity is also established (Demesure, 1996). The most polymorphic populations are generally the ones from the former refugia (Comps *et al.*, 1990, 1991b; Leonardi and Menozzi, 1995). However, values gained by Nei's formula, which expresses the expected heterozygosity, are not always higher in the refugia areas. Thus this parameter is not the best, in this case, to account for the diversity (Coulaud, 1994; Demesure, 1996). The originality of these refugia populations may be rather due to the presence of some fixed alleles absent in all other regions. As a reason for the specificity of refugia Comps *et al.* (1990) quotes the age of those beechwoods that have been in existence over numerous generations. Palynological analyses established beech in those areas 12 000 – 9 000BP (Huntley and Birks, 1983). Populations of the rest of the Europe, younger by half (5 000 – 2 500BP, Huntley and Birks, 1983), acquired, as Comps *et al.* (1990) presumes, only part of that genetic wealth accumulated over generations. The following evolutionary factors are responsible for occurrences in the genetic material: gene flow, gene drift and selection. Gene flow in mostly monospecific beech forests is described by Wright's (1943, 1946) "isolation by distance model" (Cuguen, 1986; Cuguen *et al.*, 1988). Gregorius *et al.* (1986) also established a very small genetic distance and low diversity between seed productions of two successive years, although each tree does not blossom and bear fruits every year. Beech is a long-living plant (> 100 years), which matures late (40 to 50 years old) and bears heavy nuts. It is also a wind pollinated, allogamous species with a low rate of self-fertilization (0.05 on average, Merzeau *et al.*, 1994).

In this study the genetic structure of 38 beech populations from Southeastern Europe has been analysed. A large part of that area has not yet been examined although there were potential refugia during the last glaciation and sources for extending during the postglaciation period. Pollen analyses in that area established the presence of beech in the Rhodope Mountains at the beginning of the Holocene, ~ 10 000BP (Huttinen *et al.*, 1992), although at a low percentage. In the area of Dinaric Alps (Lakes of Plitvice) beech pollen was found in Boreal sediments, ~ 7000 to 8 000BP (Culiberg and Šercelj, 1981; Srdoč *et al.*, 1985). As there are signs of an even earlier presence of beech on some of these territories, above all in the Istrian Peninsula (Culiberg and Šercelj, 1995), they were specially examined with the purpose of evaluating the microclimate and relief specifities as important factors in establishing the global schemes of vegetation structure today and in the past, as well as of vegetation migrations in the postglacial period. This study is also a supplement to the description of the genetic structure of this prevalent European species.

Materials and Methods

Sampling

Beech twigs with dormant buds were collected from 38 autochtonous locations in Southeastern Europe (*Fig. 1*). Each sample included approximately 50 individuals which were

---

[1] Université Bordeaux I, Département de Biologie Végétale, Avenue des Facultés, 33405 Talence Cedex, France

[2] Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Botany, Schrott-ova 39, 10 000 Zagreb, Croatia

[3] Faculty of Natural Sciences, Institute of Biology, 91 000 Skopje, R. of Macedonia

[4] Higher Forestry Institute, Bul. Kl. Ohridski 10, Sofia 1156, Bulgaria

[5] Institute of Forestry, Cvjetno naselje 41, 10 420 Jastrebarsko, Croatia

[6] Correspondence

selected randomly at least over a 3 ha to 4 ha area with a homogeneous environment. The samples cover a large geographical area from the Rhodopes in the East over Dinaric Alps to the slopes of Eastern Alps in the West, from the lower mountain ranges in the Pannonic Plain in the North to the high Shar-Pindus massif in the South. In this large area beechwoods cover different climatic conditions and a wide altitudinal range.

Electrophoretic Analysis

Eleven polymorphic enzyme loci were assayed: PX-1, PX-2 (peroxidases, EC 1.11.1.7), GOT-1 (glutamate oxaloacetate transaminase, EC 2.6.1.1), 6-PGD-1 (6-phosphogluconate dehydrogenase, EC 1.1.1.44), SOD-1 (superoxide dismutase, EC 1.15.1.1), ACP-1 (acid phosphatase, EC 3.1.3.2), MDH-1 (malate dehydrogenase, EC 1.1.1.37), IDH-1 (isocitrate dehy-

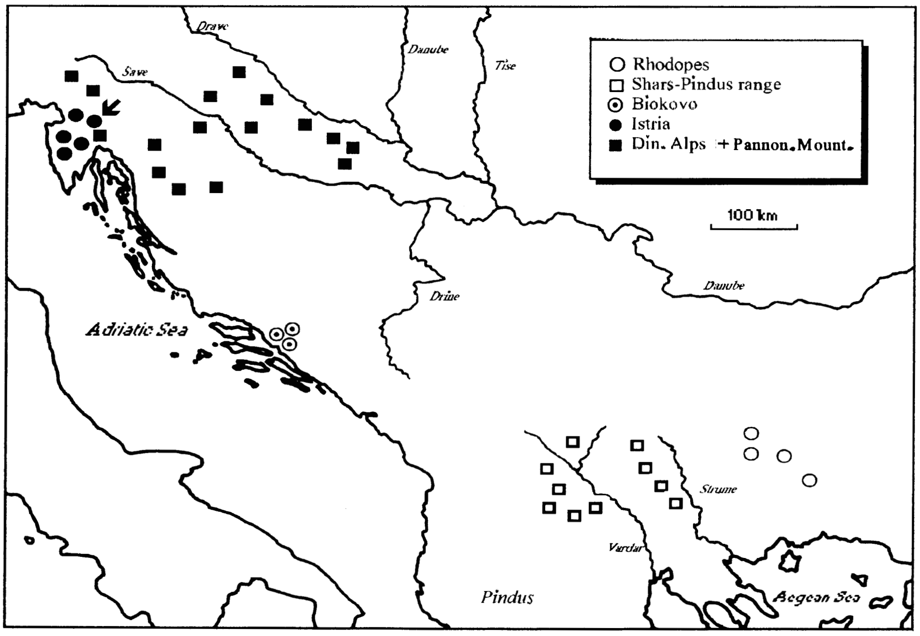

Fig. 1. – Beech populations sampled.
↙ Zbevnica, population from Istria which is included in the populations from the Dinaric Alps and the low Pannonic mountain ranges.

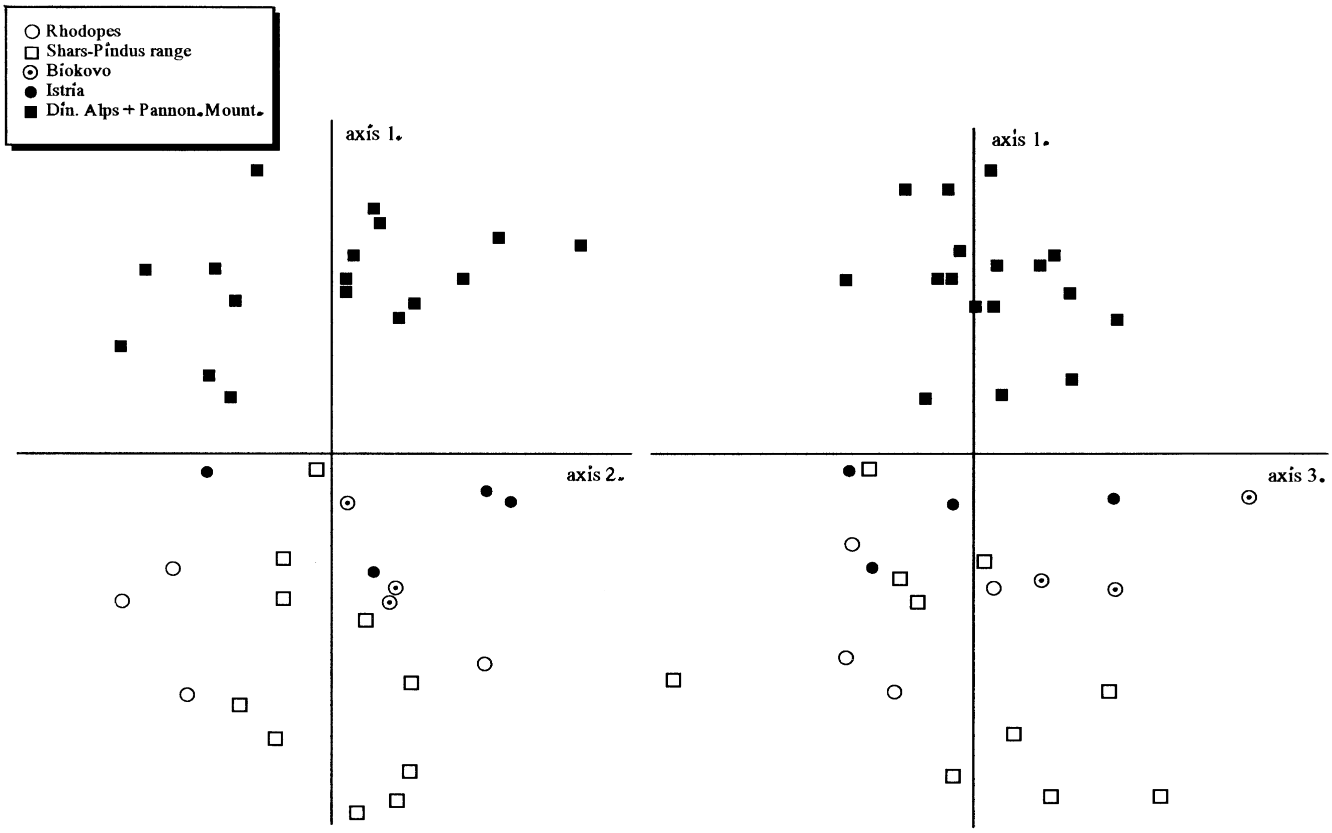

Fig. 2. – Principal component analysis (PCA) including 11 loci calculated from allele frequencies. Total variance explained by axis 1. is 35.4 %, axis 2. – 12.8 %, axis 3. – 10.1 %.

drogenase, 1.1.1.42), PGM-1 (phosphoglucomutase, EC 2.7.5.1), PGI-1 (phosphoglucose isomerase, EC 5.3.1.9) and MNR-1 (menadione reductase, EC 1.6.99.2). The method used for the extraction of enzymes from buds and cortical tissues of twigs, electrophoretic conditions and staining procedures were previously described by THIEBAUT *et al.*, 1982, and MERZEAU *et al.,* 1989.

Data Analysis

Each beech population is described by the means of allelic frequency calculated on the basis of diploid genotypes. The total gene diversity, Ht, was estimated using NEI's method (1973, 1977):

$$Ht = 1 - \Sigma p_i^2$$

where p_i is the mean frequency of the i-th allele, weighted by the sample size.

Mean allelic frequencies of each population of the examined region are compared using a PCA (principal component analysis) to find out how they are related. The groups of populations obtained by PCA were verified by discriminant analysis (DA). Nonparametric MANN-WHITNEY test based on total gene diversity of each population was also carried out.

Genotypic differentiation was analysed using the F-statistics (WRIGHT, 1951, 1965) corrected by WEIR and COCKERHAM (1984) in order to minimize the effect due to population size variation. Total genotypic differentiation – F_{IT} – is the sum of 2 components: F_{ST} – an estimation of inter-populations and F_{IS} – an estimation of intra-population component.

Results

Multivariate Analyses

Nineteen alleles from 11 loci were analysed by multilocus analyses. Frequency of 1 allele for each diallelic locus and frequency of 2 alleles for each triallelic locus were subjected to PCA. The first 3 axes derived from PCA account for 58.3% of the variation between samples (axis 1:35.4%; axis 2:12.8% and axis 3:10.1%). The analysis extracted 5 groups of populations (*Fig. 2*) which are well associated with geographic and climatic conditions. The analysis described 66% of samples with $\cos^2 \alpha \geq 0.4$.

The groups are: Rhodopes (4 populations); Shars-Pindus range (10 populations); Dinaric Alps and low Pannonic mountain ranges (17 populations, one of them from Istria); Biokovo Mountain (3 populations) and Istrian Peninsula (4 populations). Populations from Dinaric Alps and low Pannonic mountain ranges are situated on the positive side of axis 1 because of the highest frequencies of alleles PX-2-13 and PGM-1-106 (*Table 2*). These populations are also nearly monomorphic at locus GOT-1, which distinguishes them from all the other groups. The 4 other groups have settled down on the negative side of axis 1. Populations from Rhodopes are characterized by the highest polymorphism of locus GOT-1 and a low level of polymorphism at locus IDH-1 (*Table 2*). A strong difference of the populations from Shars-Pindus range is obvious in the great polymorphism of locus ACP-1 (*Table 2*), as well as in the highest frequency of allele PGI-1-87 (*Table 2*). Populations from Biokovo and Istria are distinguished by a high gene diversity of locus IDH-1 (*Table 2*, populations from

Table 1. – Investigated beech populations.

	Population	Geographic range	Coordinates		Altitude, m	Country
1	PETKOVO	Rhodopes	24° 54' E	41° 34' N	1000	Bulgaria
2	YUNDOLA	Rhodopes	23° 52' E	42° 05' N	1500	Bulgaria
3	OBORIŠTE	Rhodopes	24° 08' E	42° 30' N	750	Bulgaria
4	ŠIPKA	Rhodopes	25° 15' E	42° 40' N	1050	Bulgaria
5	PELISTER	Shars-Pindus range	21° 08' E	41° 02' N	1100-1300	Macedonia
6	PLAČKOVICA	Shars-Pindus range	22° 27' E	41° 46' N	1300-1500	Macedonia
7	OSOGOVO	Shars-Pindus range	22° 23' E	42 °12' N	1000-1150	Macedonia
8	SKOPSKA PLANINA	Shars-Pindus range	21° 27' E	42° 09' N	1050-1300	Macedonia
9	BELAŠICA	Shars-Pindus range	22° 46' E	41° 22' N	700-900	Macedonia
10	MAVROVO	Shars-Pindus range	20° 49' E	41° 39' N	1250-1400	Macedonia
11	LEUNOVO	Shars-Pindus range	20° 49' E	41° 42' N	1220-1300	Macedonia
12	GALIČICA	Shars-Pindus range	20° 50' E	40 °59' N	1100-1300	Macedonia
13	OGRAŽDEN	Shars-Pindus range	22° 52' E	41° 35' N	1050-1200	Macedonia
14	DEŠAT	Shars-Pindus range	20° 34' E	41° 37' N	950-1300	Macedonia
15	ZBEVNICA	Istrian Peninsula	14° 02' E	45° 29' N	900	Croatia
16	MEDVEDOVICA	Istrian Peninsula	14° 00' E	45° 15' N	300	Croatia
17	GURA	Istrian Peninsula	13° 46' E	45° 33' N	300	Slovenia
18	BRAVARI 2	Istrian Peninsula	13° 52' E	45° 17' N	400	Croatia
19	BRHAJI	Istrian Peninsula	13° 58' E	45° 16' N	430	Croatia
20	BIOKOVO I	Dinaric Alps	17° 06' E	43° 18' N	1330-1362	Croatia
21	BIOKOVO II	Dinaric Alps	17° 05' E	43° 19' N	1448	Croatia
22	BIOKOVO III	Dinaric Alps	17° 03' E	43° 20' N	1590-1670	Croatia
23	BILOGORA W	Pannonic Mountain	16° 51' E	45° 55' N	180-220	Croatia
24	BILOGORA E	Pannonic Mountain	17° 28' E	45° 30' N	220	Croatia
25	PAPUK W	Pannonic Mountain	17° 40' E	45° 28' N	350	Croatia
26	PAPUK E	Pannonic Mountain	17° 52' E	45° 25' N	520	Croatia
27	DILJ	Pannonic Mountain	17° 56' E	45° 15' N	320	Croatia
28	KALNIK	Pannonic Mountain	16° 28' E	46° 10' N	580	Croatia
29	MOSLAVAČKA GORA	Pannonic Mountain	16° 42' E	45° 36' N	160-340	Croatia
30	MEDVEDNICA	Pannonic Mountain	16° 05' E	45° 52' N	500-1000	Croatia
31	VUKOMERČKE GORICE	Dinaric Alps	15° 52' E	45° 38' N	150-180	Croatia
32	VELEBIT I	Dinaric Alps	15° 03' E	44° 49' N	800-1300	Croatia
33	VELEBIT II	Dinaric Alps	14° 58' E	45° 10' N	800	Croatia
34	UČKA	Dinaric Alps	14° 13' E	45° 22' N	760-1140	Croatia
35	VELIKA KAPELA	Dinaric Alps	15° 01' E	45° 18' N	500-600	Croatia
36	MALA KAPELA	Dinaric Alps	15° 13' E	45° 01' N	970	Croatia
37	HOTEDRŠICA	Dinaric Alps	14° 04' E	46° 00' N	600	Slovenia
38	POSTOJNA	Dinaric Alps	14° 15' E	45° 46' N	700	Slovenia

Table 2. – Mean allelic frequencies and mean total gene diversities according to the group of populations.

	Rhodopes		Shars-Pindus range		Istria + Biokovo		Din.Alps+ Pan.mount.		Ht - locus
	frequency	Ht	frequency	Ht	frequency	Ht	frequency	Ht	
PX-1-100	0.574	0.467	0.643	0.454	0.590	0.467	0.639	0.448	0.461
PX-2-13	0.005	0.335	0.038	0.329	0.031	0.294	0.077	0.455	0.353
PX-2-26	0.781		0.800		0.824		0.692		
GOT-1-100	0.793	0.315	0.837	0.260	0.829	0.246	0.933	0.123	0.236
6-PGD-1-84	0.060	0.296	0.021	0.091	0.058	0.110	0.125	0.258	0.189
6-PGD-1-100	0.816		0.952		0.943		0.848		
SOD-1-100	0.926	0.131	0.919	0.146	0.925	0.132	0.934	0.123	0.133
ACP-1-84	0.178	0.433	0.219	0.510	0.202	0.419	0.157	0.369	0.433
ACP-1-100	0.709		0.645		0.728		0.770		
MDH-1-18	0.901	0.177	0.928	0.130	0.839	0.259	0.743	0.371	0.234
IDH-1-100	0.840	0.268	0.821	0.290	0.704	0.396	0.721	0.362	0.329
IDH-1-116	0.160		0.179		0.293		0.275		
MNR-1-63	0.033	0.111	0.026	0.076	0.040	0.103	0.061	0.134	0.106
MNR-1-100	0.941		0.960		0.948		0.928		
MNR-1-126	0.015		0.011		0.014		0.005		
PGI-1-87	0.015	0.135	0.064	0.114	0.034	0.068	0.018	0.041	0.090
PGI-1-100	0.922		0.934		0.966		0.979		
PGM-1-100	0.961	0.076	0.943	0.107	0.896	0.190	0.760	0.342	0.179
PGM-1-106	0.032		0.050		0.102		0.240		
Ht - group		0.250		0.228		0.282		0.264	

Table 2a. – Total gene diversities of loci in which the MANN-WHITNEY test shows significant differences between populations from Istria and Biokovo, $0.01 < P < 0.05$.

locus	Ht Istria	Ht Biokovo
GOT-1	0.146	0.379
PGI-1	0.108	0.015
PGM-1	0.261	0.097

Biokovo and Istria are represented together because of their similar characteristics confirmed later with DA), but they are also very closely associated with the populations from Rhodopes (loci PX-1, ACP-1, SOD-1, MNR-1, allele 6-PGD-1-84, *Table 2*) as well as with the populations from Shars-Pindus range (loci PX-2, SOD-1, GOT-1 and 6-PGD-1, *Table 2*).

The first DA to which the populations from Istria and Biokovo were subjected separately had 95.8% good grouping. The second one, in which the populations from Biokovo and Istria were joined in one group (*Fig. 3*) had a 100% good grouping. The first 3 axes describe 100% of the variability (axis 1:81.1%; axis 2:12.8% and axis 3:6.1%). Populations from the Dinaric Alps and low Pannonic mountain ranges are discriminated by axis 1 ($\cos^2 \alpha = 0.9124$). The alleles PX-2-13, GOT-1-100, MDH-1-18, PGI-1-100, PGM-1-100 and PGM-1-106 are mostly correlated to axis 1 (correlation$^2 > 0.9$). Populations from Rhodopes are mostly discriminated by axis 2 ($\cos^2 \alpha = 0.6174$). The alleles 6-PGD-1-100, ACP-1-84 and PGI-1-87 are well correlated to this axis. Populations from Shars-Pindus range, Biokovo and Istria are described by axis 3 (respectively $\cos^2 \alpha = 0.6637$; $\cos^2 \alpha = 0.4085$). Loci PX-1 and IDH-1 are well correlated to this axis.

Of the 11 loci examined in these 4 groups of populations, 8 loci are polymorphic and 3 are almost monomorphic – SOD-1, MNR-1 and PGI-1 (*Table 2*). There are also rare alleles which more or less distinguish these groups (*Table 4*).

Gene Diversity (Tables 2 and 3)

The MANN-WHITNEY test allowed us to compare the level of gene diversity between the 4 groups discriminated by the multivariate analyses and to determine whether the differences put forward by these analyses are significant or not.

All groups of populations have similar degree of diversity at locus PX-1, SOD-1, MNR-1 and PGI-1. At locus PX-2 all the groups are polymorphic, but the populations from the Dinaric Alps and low Pannonic mountains have the highest diversity value (significantly different from all other populations). At locus GOT-1, the level of diversity increases significantly from the Dinaric Alps and the low Pannonic mountain ranges towards the South. Polymorphism at locus ACP-1 has the same tendency and the populations from Shars-Pindus range are significantly different from those of the groups located in the North. At loci IDH-1, MDH-1 and PGM-1 there is an opposite trend, polymorphism increases from the South towards the North and there are a lot of significant differences among the groups. Polymorphism variations at the locus 6-PGD-1 do not show any clear tendencies.

Table 3. – MANN-WHITNEY test based on total gene diversities, ns = non significant; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$; G1- Rhodopes; G2- Shars-Pindus massif; G3- Biokovo + Istria; G4- Dinaric Alps and Pannonic Mountain.

	G1 -G2	G1 - G3	G1 - G4	G2 - G3	G2 - G4	G3 - G4
PX-1	ns	ns	ns	ns	ns	ns
PX-2	ns	ns	**	ns	***	***
GOT-1	ns	ns	**	ns	***	*
6-PGD-1	*	ns	ns	ns	***	***
SOD-1	ns	ns	ns	ns	ns	ns
ACP-1	ns	ns	ns	**	***	ns
MDH-1	ns	ns	**	**	***	**
IDH-1	ns	*	***	**	***	ns
MNR-1	ns	ns	ns	ns	*	ns
PGI-1	ns	ns	ns	ns	ns	ns
PGM-1	ns	*	***	*	***	***

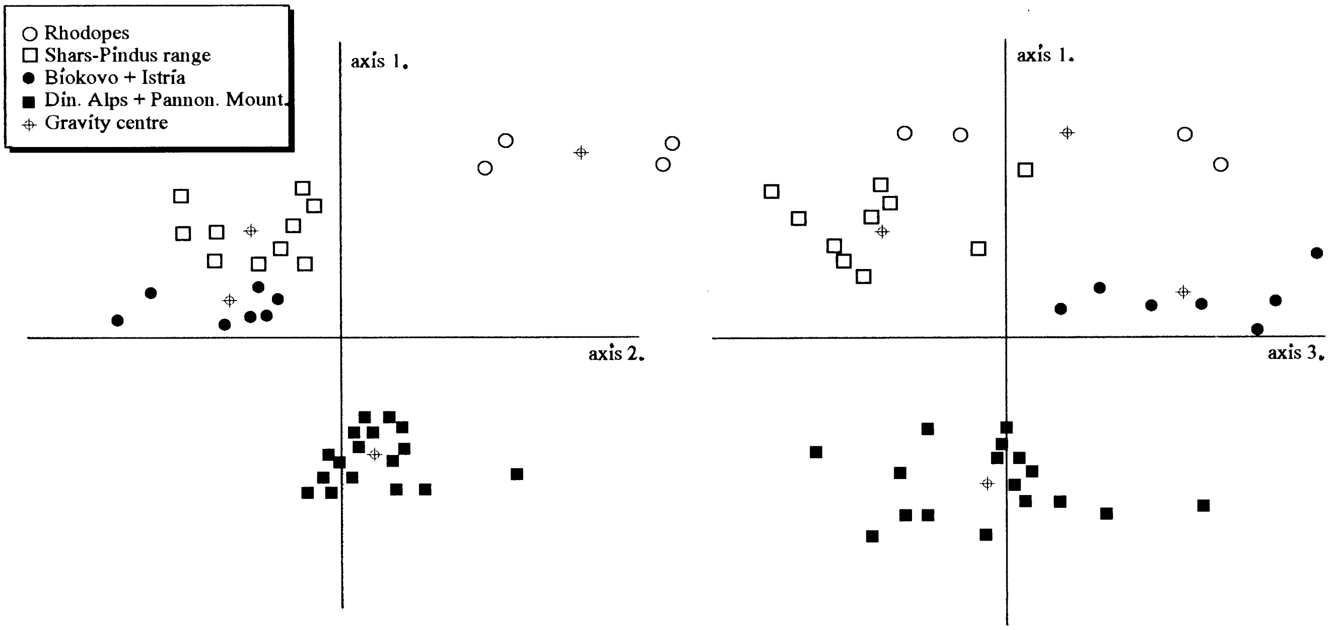

Fig. 3. – Discriminant analysis including 11 loci: comparison between groups obtained by PCA. Total variance explained by axis 1. is 81.1%, axis 2. – 12.8%, axis 3. – 6.1%.

Table 4. – Rare allele frequencies. In parenthesis are numbers of populations in which rare alleles occur.

	Rhodopes	Shars-Pindus range	Istria + Biokovo	Din.Alps+Pan.mount.
GOT-1-95	0.061 (3)	-	-	0.011 (1)
6-PGD-1-112	0.124 (4)	0.027 (10)	-	0.064 (7)
IDH-1-84	-	-	0.024 (1)	0.014 (5)
MNR-1-74	-	-	-	0.015 (7)
MNR-1-131	-	0.011 (2)	-	-
PGI-1-76	-	0.011 (2)	-	-
PGI-1-113	0.126 (2)	0.010 (2)	-	0.010 (2)
PGM-1-93	0.031 (1)	0.024 (3)	0.014 (1)	-
PGM-1-109	0.015 (1)	-	-	-

Rare Alleles (Table 4)

Nine rare alleles are present in 1 or several of 4 groups. The alleles GOT-1-95 and PGM-1-109 are characteristic of the Rhodopes populations and the alleles PGI-1-76 and MNR-1-131 of the Shars-Pindus range. Alleles 6-PGD-1-112 and PGI-1-113 are completely absent from the populations of Istria and Biokovo but present in the other 3 groups. Allele PGM-1-93 is absent and IDH-1-84 is present in the populations from the Dinaric Alps, the Pannonic mountain ranges and Istria, which differentiates them significantly from the populations located at the southern limit of beech and from the Biokovo populations. Allele MNR-1-74 is present only in the populations of Dinaric Alps and low Pannonic mountain ranges.

Altitudinal Gradient (Biokovo Mountain, Table 5)

It is possible to observe an altitudinal gradient of gene diversity along the slope of Biokovo Mountain for 2 loci, PX-1 and GOT-1. However, only one altitudinal transect is not enough to conclude any dependence between these loci and altitude.

Table 5. – Changes in allele frequencies along an altitudinal transect of Biokovo Mountain.

	1330 - 1362m	1448m	1590 - 1670m
PX-1-105	0.354	0.402	0.463
GOT-1-95	0.195	0.244	0.346

F-Statistics (Table 6)

Multilocus estimates of the F-statistics were carried out for the 4 regions at all loci. All F_{ST} are higher than F_{IS} values except for the populations from the Dinaric Alps and the low Pannonic mountain ranges. These populations are characterized by an intrapopulation component of genetic differentiation higher than that between populations. Intrapopulation component (F_{IS}) is close to zero only in the Rhodopes region, but that may be due to the low number of samples. F_{IS} values for populations from other regions are almost similar which proves an obviously deficit of heterozygotes.

Discussion and Conclusion

A high degree of polymorphism of beech populations has been found in the examined area. As for the majority of woody plants, this can be accounted for by the large number of polymorphic loci (HAMRICK *et al.*, 1992). Among 11 examined loci, 8 are polymorphic (Ht average 0.305). The other 3 are almost monomorphic – SOD-1, MNR-1 and PGI-1 (Ht average 0.110).

Multivariate analyses distributed the 38 populations into 4 groups. The distribution correlates with geographic distribution. The analyses clearly separated the populations from the Dinaric Alps and the low Pannonic mountain ranges from the other 3 groups, Rhodopes, Shars-Pindus range, Biokovo Mountain + Istrian Peninsula, which are placed on the opposite side of the first principal component. Gravity centres

Table 6. – Multilocus estimates of F-statistics according to the group of populations.

	FST	var	FIS	var	FIT	var
Rhodopes	0.0319	0.0001	-0.0037	0.0007	0.0283	0.0005
Shars-Pindus range	0.0260	0.0002	0.0204	0.0006	0.0459	0.0004
Istria + Biokovo	0.0396	0.0001	0.0195	0.0003	0.0583	0.0005
Din.Alps+Pan.mount.	0.0143	0.0001	0.0219	0.0001	0.0359	0.0001

in the discriminant analysis of all the groups are well separated.

At several loci polymorphism varies according to the latitude: from the North towards the South it increases at loci GOT-1 and ACP-1 and decreases at loci IDH-1, MDH-1 and PGM-1. Comparison with previous results concerning the geographical distribution of diversity is possible for the loci which have already been analyzed by previous studies. COMPS *et al.* (1990) pointed out an increase of gene diversity at GOT-1 locus from Central Europe towards the South (H_t = 0.104 in Poland, 0.122 in Croatia – that corresponds to the value found in Dinaric Alps and Pannonic mountains, 0.285 in Italy and 0.364 in Corsica). Values found in Shars-Pindus range and above all in the Rhodopes (H_t = 0.315) confirm this significant tendency, as does the result for the Istria + Biokovo group. The latter 2 populations, even if placed in the same group, differ significantly at loci GOT-1, PGI-1 and PGM-1 (*Table 2a*). Low polymorphism at locus GOT-1 and high at PGM-1 are characteristic of the Istrian populations, just as for the Dinaric Alps and the low Pannonic mountain population, while polymorphism at locus PGI-1 shows just opposite tendency. Polymorphism at locus GOT-1 in the Biokovo group is extremely high, H_t = 0.379, maybe due to the altitudinal range of the populations. Low polymorphism at locus PGM-1 of the Biokovo populations is the same as in populations from the Rhodopes and Shars-Pindus ranges. COMPS *et al.* (1990) also found a decrease of gene diversity at loci MDH-1 and IDH-1 (respectively 0.403 and 0.499 in Poland, 0.234 and 0.371 in Italy); in the Shars-Pindus range and Rhodopes gene diversity is even lower, respectively 0.130 and 0.177 at locus MDH-1 and 0.290 and 0.268 at locus IDH-1. Depending on the loci considered, gene diversity either increases or decreases from the South towards the North, so that mean diversity does not change much from one region to another. Accordingly, even if the Balkan ranges of Shars-Pindus and Rhodopes are generally considered as refuge areas, genetically different from the territories located more towards the North, they do not have a higher diversity.

Former examinations (COMPS *et al.*, 1990, 1991a and b) established relations between peroxidase loci (PX-1 and PX-2) and geographical and climatic features of the stands. These authors quoted higher polymorphism at locus PX-1 in areas where climatic changes were more intense. These authors suggested the possibility of a selection effect as they always observed the same allelic variations associated with the same climatic changes. Within the region analyzed in this study, there are extremes in climatic conditions due to the closeness of rather high mountains and low valleys and also due to the contrast between the Mediterranean and continental climates. A high degree of polymorphism at locus PX-1 is confirmed.

The group of populations from the Dinaric Alps and the low Pannonic mountain ranges has been distinguished from the others by significant differences of gene diversity at the loci GOT-1, MDH-1, IDH-1 and PGM-1. Diversities at some of these loci are comparable with Central European population values, for example with populations from Northwestern Italy (BELLETTI and LANTERI, 1996) at loci MDH-1 (H_t = 0.381) and IDH-1 (H_t = 0.407) and with populations from Bavaria (KONNERT, 1995) at loci GOT-1 (H_t = 0.087), MDH-1 (H_t = 0.407) and IDH-1 (H_t = 0.411). Lower mountain ranges in the Pannonic Plain (989 m) appeared during glaciation once the level of the fresh water Pannonic Sea lowered. Populations from this area show characteristics identical to those from the Dinaric Alps and Central Europe. Correlations in frequencies of alleles PX-2-13 (0.078), IDH-1-100 (0.727), IDH-1-116 (0.273) and MNR-1-63 (0.070) with populations from the Southwestern Carpathians as well as of GOT-1-100 (0.949) and 6-PGD-1-84 (0.122) with populations from Northwestern Carpathians (VYŠNY *et al.*, 1995) provide additional evidence that all these populations derived from the same refugium located in the Balkan Peninsula.

Some rare alleles, described by THIEBAUT *et al.*, 1982, and MERZEAU *et al.*, 1989, are present in all the 4 groups of beechwoods (*Table 4*). Most of them have not been included in multivariate analyses (except 6-PGD-1-112, IDH-1-84 and PGM-1-93). Yet their arrangement into groups often follows and confirms the obtained grouping, but indicates some new characteristics of the populations. Populations from the Rhodopes, Shars-Pindus range and Biokovo, although placed close to each other on the PCA and DA plots, differ substantially due to the presence of some rare alleles. Comparisons with the previously obtained data show some relations between different parts of Europe.

Rare alleles PGM-1-109 and GOT-1-95 are characteristics for the Rhodopes. Until now, PGM-1-109 was only found in 2 populations, Crimea and Northern Spain (COULAUD, 1994; VAQUIER, 1995). GOT-1-95 is present in 3 of the 4 analysed Rhodopes populations. Until now, this allele has been found only in few European populations, all of them in the East-Crimea, Carpathian Mountains, Bulgaria, Romania, Turkey (VAQUIER, 1995). LEONARDI and MENOZZI (1995) mentioned probably the same allele, GOT-A-97, in 1 Sicilian population.

In populations from the Shars-Pindus range there are 2 other very rare alleles, MNR-1-131 and PGI-1-76. As for allele PGI-1-76, this is its third location beside Crimea and Calabria as found by VAQUIER (1995). Previous examinations mentioned the presence of MNR-1-131 in Sicilian, South-Italian and Rhodopes populations (COULAUD, 1994; VAQUIER, 1995). These 4 alleles point to a peculiar nature of these beechwoods, as can be expected for the probably oldest European beech populations.

Apart from the populations in the Rhodopes and Shars-Pindus range, allele PGM-1-93 is present also in the populations from Biokovo. This allele has previously been found in Southwestern Carpathians, Rhodopes, Southern Italy, Sicily and also in Provence and Corsica (COULAUD, 1994; VAQUIER, 1995). This allele, as well as the above-mentioned MNR-1-131 places the populations from Rhodopes, Shars-Pindus range and Biokovo in affinity with populations from Southern Italy, Sicily, even Southern France. Perhaps terrestrial connection caused by the falling of the level of the Adriatic and Mediterranean Seas during glaciation (BÜDEL, 1960) may explain these similarities, especially between Southern Balkans and the

Southern Italian Beech populations whose presence at that time was confirmed by pollen analyses (Huntley and Birks, 1983).

Populations from Dinaric Alps have the alleles IDH-1-84 and MNR-1-74 that are somewhat more frequent and considerably extended in all European populations, the first one especially in the Alps and Apennines (Belletti and Lanteri, 1996; Leonardi and Menozzi, 1995), in the Pyrenees and the Central Massif of France (Vaquier, 1995). The other can be found more often in the Alps (Vaquier, 1995). Both alleles was found by Konnert (1995) in Bavarian populations. According to Coulaud (1994) and Vaquier (1995) both alleles are present in Istria, but only allele IDH-1-84 was found in 2 of our populations. One of them is a border population (marked by arrow on *Figure 1*). This population has elements of populations from Istria as well as of populations from the Dinaric Alps, which proves that gene flow might take place here. PCA and DA classified it among the group of populations from the Dinaric Alps.

Alleles 6-PGD-1-112 and PGI-1-113 are present in populations from the Dinaric Alps and low Pannonic mountain ranges, Shars-Pindus ranges and Rhodopes, and are according to Vaquier (1995) sporadically in all European populations. Leonardi and Menozzi (1995) found probably the same allele, 6-PGD-A-110, in one Italian population, Belletti and Lanteri (1996) allele PGI-B-1 in several populations from north-western Italy. The allele PGI-1-113 is also present in Istria (Coulaud, 1994) but has not been found in our examinations.

Differences between populations from the Dinaric Alps and the low Pannonic mountain ranges and the 3 other groups are observable also in genotypic differentiation. The populations from the Dinaric Alps and the low Pannonic mountain ranges have a lower interpopulation component (F_{ST}) than the rest of the populations, which proves a certain peculiarity of the local genetic structure. Positive values of F_{IS} show a deficit of heterozygotes, similarly as in other parts of Europe and a limited gene flow is the possible cause of that (Cuguen *et al.*, 1988). Yet, all the values are much lower than those quoted in the literature (Comps *et al.*, 1990; Leonardi and Menozzi, 1995) in spite of the same number of loci included (Coulaud, 1994).

Final Conclusions

In spite of the same degree of diversity of the four groups of populations derived by DA, the presence of some rare alleles in populations from Rhodopes and Shars-Pindus ranges proves their specific and original nature as the refugia populations during glaciations.

Populations from the Dinaric Alps and the low Pannonic mountain ranges, having all the characteristics of Central European beechwoods, are clearly separated from the others 3 groups.

In spite of the same latitude and geographical proximity populations from Istria and Biokovo are quite different from those of the Dinaric Alps and the low Pannonic mountain ranges. The Istria and Biokovo populations belong to the same group although there is no connection between the regions today. They are isolated by the sea and the slopes of the Dinaric Alps. Here beech populations show genetic characteristics of the Central European region. The deficit of heterozygotes proves that there is some degree of isolation. Beech pollen has been found in a Palaeolithic site in Šandalja in Istria dating from the period between 12 000 and 27 000 radiocarbon years (Culiberg and Šercelj, 1995). The particular nature of Istrian populations may also be accounted for by the merging of 2 postglacial recolonizations, one from the Balkans and the other from the Italian refugia (Vaquier, 1995).

Pollen analyses were not made in Biokovo. These populations are highly influenced by the Adriatic Sea on the one side (only 3000 m distant from the sea), and the nearness of Bosnian beechwoods, on the other, where the disputable species *Fagus moesiaca* (Domin, Maly) Czeczott also occurs. Analysis of the genetic structure of Bosnian beechwoods would make it easier to explain the particular features of the Biokovo beechwoods.

Acknowledgements

The authors are grateful to R. M. Guilbaud and J. Letouzey for their technical assistance.

References

Barrière, G., Comps, B., Cuguen, J., N'Tsiba, F. and Thiébaut, B.: The genetic ecological variability of Beech (*Fagus sylvatica* L.) in Europe, an alloenzymatic study: genetic isolations of beechwoods. IUFRO-Buchensymposium. H. J. Muhs (ed.), Hamburg. In: Mitteilungen der Bundesforschungsanstalt für Forst und Holzwirtschaft **150**: 24–50 (1985). — Belletti, P. and Lanteri, S: Allozyme variation among European beech (*Fagus sylvatica* L.) stands in Piedmont, North-Western Italy. Silvae Genetica **45**: 33–37 (1996). — Bennett, K. D., Tzedakis, P. C. and Willis, K. J.: Quaternary refugia of north European trees. Journal of Biogeography **18**: 103–115 (1991). — Beug, H. J.: On the forest history of the Dalmatian coast. Rev Paléobot Palynol **2**: 271–279 (1967). — Büdel, I.: Die Gliederung der Würmkaltzeit. Würzb. Geogr. Arbeiten, H.8 (1960). — Comps, B., Barrière, G., Merzeau, D. et Letouzey, J.: La variabilité alloenzymatique des hêtraies dans les sous-domaines médio- et eu-atlantiques d'Europe. Can. J. For. Res. **17**: 1043–1049 (1987). — Comps, B., Paule, L., Šugar, I., Thiébaut, B. and Trinajstić, I.: Genetic variability in beechwoods (*Fagus sylvatica* L.) over central Europe, allozymic variations in six enzyme systems: spatial differentiation among and within populations. In: Proc. 3rd Symp. Improvement and Silviculture of Beech. IUFRO-Project group P1, Zvolen (1988). — Comps, B., Thiébaut, B. and Merzeau, D.: Genetic variation in European beech stands (*Fagus sylvatica* L.). pp. 110–124. In: Müller-Starck, G. and Ziehe, M. (eds.): Genetic variation in European populations of forest trees. Sauerländer's Verlag, Frankfurt am Main (1991a). — Comps, B., Thiébaut, B., Paule, L., Merzeau, D. and Letouzey, J.: Allozymic variability in beechwoods (*Fagus sylvatica* L.) over central Europe: spatial differentiation among and within populations. Heredity **65**: 407–417 (1990). — Comps, B., Thiébaut, B., Šugar, I., Trinajstić, I. and Plazibat, I.: Genetic variation of the Croatian beech stands (*Fagus sylvatica* L.): spatial differentiation in connection with the environment. Ann. Sci. For. **48**: 15–28 (1991b). — Coulaud,Y.: Les hêtraies de l'arc méditerranéen occidental: évolution en fonction de la dynamique post-glaciaire et des facteurs de l'environnement. D.E.A. Université Bordeaux III, France. 84 p. (1994). — Cuguen, J.: Différenciation génétique inter- et intrapopulations d'un arbre forestier anémophile: le cas du hêtre (*Fagus sylvatica* L.). Thèse de Doctorat, Université des Sciences et Techniques du Languedoc, Montpellier, France (1986). — Cuguen, J., Merzeau, D. and Thiébaut, B.: Genetic structure of the European beech stands (*Fagus sylvatica* L.): F-statistics and importance of the mating system characteristics in their evolution. Heredity **60**: 91–100 (1988). — Culiberg, M. and Šercelj, A.: Pollen analyses of the sediments of Plitvička jezera (Lakes of Plitvice). Acta Bot. Croat. **40**: 147–154 (1981). — Culiberg, M. and Šercelj, A.: Anthracotomical and palynological research in the Palaeolitic site Šandalja II (Istria, Croatia). Razprave 4. razreda SAZU **36**: 49–57 (1995). — Demesure, B.: Analyse de la diversité chloroplastique en utilisant des fragments PCR chez des Fagacées: *Fagus sylvatica* L. et *Quercus spp*. Thèse de Doctorat, Université Bordeaux I, France (1996). — Gömöry, D., Vyšny, J., Comps, B. and Thiébaut, B.: Geographical patterns of genetic differentiation and diversity in European beech (*Fagus sylvatica* L.) populations in France. Biológia (Bratislava) **47**: 571–579 (1992). — Gregorius, H. R., Krauhausen, J. and Müller-Starck, G.: Spatial and temporal genetic differentiation among the seed in a stand of *Fagus sylvatica* L. Heredity **57**: 255–262 (1986). — 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). — Huntley, B. and Birks, H. J. B.: An Atlas of Past and Present Pollen Maps for Europe: 0–13 000 years ago. Cambridge University Press, Cambridge (1983). — Huttinen, A., Huttinen, R. L., Vasari,Y., Panovska, H. and Božilova, E.: Late-Glacial and Holocene history of flora and vegetation in the western Rhodopes Mountains, Bulgaria. Acta Bot. Fenn. **144**: 63–80 (1992). — Jalut, M.: La végétation pendant le Postglaciare dans les Pyrénées. In: La Préhistoire française. De Lumley, H. et Guilaine, J. (eds). CRNS, Paris. pp. 74–81 (1976). — Jalut, M., Sacchi, D. et Vernet, J. L.: Mise en évidence d'un refuge tardiglaciaire à moyenne altitude sur le versant nord-oriental des Pyrénées. (Berlirs, alt. 960 m, Aude). C.R. Ac. Sc., Paris, Série D, 280: 1781–1784 (1975). — Konnert, M.: Investigations of the genetic variation of beech (*Fagus sylvatica* L.) in Bavaria. Silvae Genetica **44**: 346–351 (1995). — Leonardi, S. and Menozzi, P.: Genetic variability of (*Fagus sylvatica* L.) in Italy: the role of postglacial recolonization. Heredity **75**: 34–44 (1995). — Merzeau, D., Comps, B., Thiébaut, B. and Letouzey, J.: Estimation of *Fagus sylvatica* L. mating system parameters in natural populations. Ann. Sci. For. **51**: 163–173 (1994). — Merzeau, D., Di Giusto, F., Comps, B., Thiébaut, B., Letouzey, J. and Cuguen, J.: Genetic control of isozyme systems and heterogeneity of pollen contribution in beech (*Fagus sylvatica* L.). Silvae Genetica **38**: 195–201 (1989). — Müller-Starck, G.: Genetic Differences between "Tolerant" and "Sensitive" Beeches (*Fagus sylvatica* L.) in an Environmentally Stressed Adult Forest Stand. Silvae Genetica **34**: 241–247 (1985). — Müller-Starck, G. and Ziehe, M.: Genetic variation in populations of *Fagus sylvatica* L., *Quercus robur* L. and *Quercus petraea* Liebl. in Germany. In: Müller-Starck, G. and Ziehe, M. (eds.): Genetic variation in European populations of forest trees. Sauerländer's Verlag, Frankfurt am Main. pp. 125–140 (1991). — Nei, M.: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**: 3321–3323 (1973). — Nei, M.: Statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. **41**: 225–233 (1977). — Oldfield, F.: Three pollen analyses from an interglacial mudbed on the foreshore near Biarritz, South-West France. Bull. Cent. Etude Rech. Sci. Biarritz **3**: 53–62 (1960). — Paquereau, M. M.: Le Würm ancien en Périgord. Quaternaria **18**, 67–115 et 117–159 (1974). — Srdoč, D., Obelić, B., Horvatinić, N., Culiberg, M., Šercelj, A. and Sliepčević, A.: Radiocarbon dating and pollen analyses of two peat bogs in the Plitvice National Park. Acta Bot. Croat. **44:** 41–46 (1985). — Šercelj, A.: The origins and development of forest in Slovenia. Academia scientiamum et artium Slovenica, Classis IV-Historia naturalis **35**: 106–125 (1996). — Thiébaut, B.: Variabilité génétique écologique du hêtre commun (*Fagus sylvatica* L.) dans les milieux montagnards et de haute altitude en Europe. Écologie des milieux montagnards et de haute altitude. Document d'Écologie Pyrénéenne, III–IV: 513–521 (1984). — Thiébaut, B., Lumaret, R. and Vernet, Ph.: The bud enzymes of beech (*Fagus sylvatica* L.) genetic distinction and analysis of polymorphism in several French populations. Silvae Genetica **31**: 51–60 (1982). — Vaquier, S.: Les hêtraies d'Europe: dynamique postglaciaire et évolution en relation avec les facteurs environnementaux. D.E.A. Université Bordeaux III, France (1995). — Vyšny, J., Shvadchak, B., Comps, B., Gömöry, D. and Paule, L.: Genetic diversity and differentiation of beech populations (*Fagus sylvatica* L.) in Western Ukraine: the Ukrainian Carpathians and Adjacent territories. Russian Journal of Genetics **31**: 1309–1319 (1995). — Weir, B. S. and Cockerham, C. C.: Estimating F-statistics for the analysis of population structure. Evolution **38**: 1358–1370 (1984). — Willis, K. J.: The vegetational history of the Balkans. Quat. Sci. Rew. **13**: 769–788 (1994). — Wright, S.: Isolation by distance. Genetics **28**: 114–138 (1943). — Wright, S.: Isolation by distance under diverse systems of mating. Genetics **31**: 39–59 (1946). — Wright, S.: The genetical structure of populations. Ann. Eugenics **15**: 323–354 (1951). — Wright, S.: The interpretation of population structure by F-statistics with spatial regard to system of mating. Evolution **19**: 358–420 (1965).

Genetic Variation for Growth and Wood Density in *Eucalyptus globulus* ssp. *globulus* in Tasmania (Australia)

By A. C. MacDonald, N. M. G. Borralho and B. M. Potts

Cooperative Research Centre for Temperate Hardwood Forestry, GPO Box 252–12, Hobart, TAS 7001, Australia

(Received 15th May 1997)

Summary

Genetic parameters for diameter and Pilodyn penetration were estimated for a 5 year-old *Eucalyptus globulus* ssp. *globulus* base population established across 5 sites in Tasmania (Australia). The model included race, family, plot, incomplete block and family-by-site interaction as random and replicate as fixed effects. Race effects were generally low for diameter and moderate for Pilodyn, with heritabilities across the 5 sites of $h^2 = 0.20$ and 0.30 respectively. For each trait, correlations between trials were generally high, suggesting that a combined analysis across sites should be appropriate. The genetic correlation between Pilodyn penetration and diameter was moderately unfavourable (averaged 0.25) whereas race correlations were low but favourable (–0.19).

Key words: heritability, genetic correlation, Pilodyn, REML.

FDC: 165.3; 232.1; 561.2; 812.31; 176.1 *Eucalyptus globulus*; (946).

Introduction

A number of genetic improvement programs using *Eucalyptus globulus* for pulpwood production have been initiated in the last few years in many countries, with breeding populations based mainly on ssp. *globulus* seed collected in native stands (*e.g.* Orme, 1977; Gardiner and Crawford, 1987, 1988). So far, published genetic parameters for a range-wide *E. globulus* ssp. *globulus* base population have included growth (Potts and Jordan, 1994b; Borralho *et al.*, 1995), frost tolerance (Volker *et al.*, 1994), leaf morphology (Potts and Jordan, 1994a), flowering time (Gore and Potts, 1995) and drought tolerance (Dutkowski, 1995). The genetics of wood density, albeit being identified as a key trait in pulpwood breeding (Borralho *et al.*, 1993; Greaves *et al.*, 1997a) has not been studied in great detail. Previous estimates were based on wood samples from a few families in a limited range of provenances (*e.g.* Dean *et al.*, 1990; Borralho et al., 1992b). The use of Pilodyn, a hand-held instrument which drives a steel pin into the wood with a known force, has been recommended as an effective method to measure wood density in standing trees (Sprague *et al.*, 1983; King *et al.*, 1988). In eucalypts, the relationship between Pilodyn penetration and wood density seems to be linear and relatively constant across