

Allozymic variability in beechwoods (*Fagus sylvatica* L.) over central Europe: spatial differentiation among and within populations

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One hundred and forty beech stands located in central and Mediterranean Europe have been analysed using six enzymatic loci (*PX-1*, *PX-2*, *GOT-1*, *IDH-1*, *MDH-1* and *PGI-1*). They were investigated on allelic and genotypic levels with the aim of estimating the intra- and inter-population components of their genetic differentiation. Four loci (*PX-1*, *PX-2*, *MDH-1* and *IDH-1*) were found rather polymorphic whereas the remaining two (*GOT-1* and *PGI-1*) showed a lower degree of polymorphism. Significant association of climate and allelic diversity was found for two loci (*PX-1* and *PX-2*). Differences between the continental and the Mediterranean regions are sometimes significant at the allelic level, especially for *GOT-1*, *MDH-1* and *IDH-1* loci. In all cases, the inter-population component of the diversity is much lower than the intra-population one but it is higher in the Mediterranean region except in the *PGI-1* locus.

INTRODUCTION

The genetic structure of beech stands depends on selection and the mating system, in addition to gene flows and genetic drift; these factors induce an inter- and an intra-population genetic differentiation over space and time (Kim 1979, 1980; Müller-Starck, 1985; Gregorius *et al.*, 1986; Cuguen, 1986; Cuguen *et al.*, 1988).

Beech is considered as a climax species in most part of Europe where it grows in very different environments. Particularly in the southern part of its area, near the Mediterranean Sea, beech stands develop in very contrasted climates: Mediterranean or continental, and plain or mountain climates (Misić, 1957; Comps, 1972; Thiébaud, 1984). This environmental diversity favours the genetic differentiation of beechwoods by selection and genetic isolation due to phenological differences (Thiébaud *et al.*, 1982; Felber and Thiébaud, 1982, 1984; Thiébaud, 1984; N'Tsiba, 1984; Barrière *et al.*, 1985; Comps *et al.*, 1987).

Beech is a monoecious species which does not reproduce until it is at least 40 or 50 years old.

The species is anemophilous and mostly allogamic (Schaffalitzky de Muckadell, 1955). In natural conditions (wind-pollination), the beech outcrossing rate varies between 0.90 and 1.00, with 0.95 on average (Merzeau *et al.*, in preparation). This average is higher than the one (0.87) estimated in artificial conditions (controlled-pollination) by Nielsen and Schaffalitzky de Muckadell (1954). In addition to self-fertilization, another component of inbreeding can intervene within beech populations. Gene flows are generally limited in the optimal range of the Beech because of the high density of beechwoods, thus reproduction likely occurs between closely spaced individuals and genetic structures could best be described by the "isolation by distance model" of Wright (1943, 1946) (Cuguen, 1986; Cuguen *et al.*, 1988). This model induces an increasing relatedness between individuals among and within populations, therefore a genetic differentiation; it also contributes to this differentiation of beech populations by genetic drift. However, gene flows vary according to the age and the physiognomical aspects of the population (Thiébaud *et al.*, 1986).

The history of the Beech after the last glacial period is well known. Today's stands apparently spread out from an important source located in the Balkans and from several secondary sources in southwestern Europe (Oldfield, 1960; Paquereau, 1965, 1970, 1974; Beug, 1967; Serceelj, 1970; Jalut, 1974; Jalut *et al.*, 1975; Triat-Laval, 1978; Pons, 1983). One beech generation is at least 60 to 100 years. Therefore, recently established beech stands have passed through few generations at their present sites. It seems that Beech has reached the different countries of its present range at various periods: southern countries (close to the Mediterranean Sea) since 5000 or 4000 BP and central European ones only since 3000 or 2500 BP (Vernet 1981). Thus, a higher number of generations has passed in the south than in the north.

Genetic differentiation among and within beech stands seems to be much higher towards the south where climatic conditions are more heterogeneous, and the populations are older and their sources more numerous.

The allozyme variation of beech stands across Europe has already been analysed using only three loci. Allelic frequencies vary according to climatic changes for two peroxidase loci (*PX-1* and *PX-2*), particularly in the southern countries.* This fact may be partly interpreted as an effect of selection. For another locus (glutamate oxaloacetate transaminase, *GOT-1*), allelic frequencies do not vary as a function of environmental conditions but, are related to the history of the populations (Thiébaud *et al.*, 1982; Felber and Thiébaud, 1982, 1984; Thiébaud, 1984; N'Tsiba, 1984; Barrière *et al.*, Cuguen *et al.*, 1985; Comps *et al.*, 1987). Other authors have studied the effects of selection between several generations (Kim, 1979, 1980; Gregorius *et al.*, 1986) or as a consequence of pollution (Müller-Starck, 1985).

The aim of this paper is to investigate the genetic differentiation of beech stands in central and southern Europe.

MATERIAL AND METHODS

Sampling

The 140 sampled beech stands are distributed over a wide geographical range from Poland in the north

* Conventionally, we will use the term "regions" when talking about the northern and the southern parts of the studied area, respectively characterized by continental and Mediterranean climates; "country" will be used in its proper meaning but also to name administrative subdivisions as Corsica, Serbia and Croatia.

as far as Italy and Corsica in the south; it includes Rumania and Bulgaria towards the east. The number of stands sampled in each of these two last countries and in Serbia is rather low, so we pooled them considering their geographical and environmental similarity. In each beech stand, plant material (buds and twigs) was sampled from about 50 non-adjacent trees chosen at random over a 3-4 ha area and in as homogeneous an environment as possible.

Sampling was carried out according to the heterogeneity of environmental factors (fig. 1, table 1): climate, topographic location, aspect, soil pH. Only climate variation could be more or less connected with genetic structure of beechwoods; so, we are going to discuss only this.

Climate varies a great deal according to latitude and altitude.

From the north towards the south, the various types of climate encountered are continental in Poland, Czechoslovakia, Rumania, Bulgaria and Serbia, both continental and Mediterranean in Croatia, only Mediterranean in Italy and also in Corsica where climate is, however, more insular. Thus, we could distinguish two climatic regions, continental and Mediterranean.

Climate varies as a function of altitude; the altitudinal range of investigated beechwoods differs from one country to another: 100-500 m in Poland, 390-1400 m in Czechoslovakia, 600-1200 m in Bulgaria, Rumania and Serbia, 260-1645 m in Croatia, 800-1450 m in Italy and 1100-1450 m in Corsica.

Taking into account both latitude and altitude, the climate variety of sampling areas increases firstly from the north in Poland towards the south as far as Croatia; then it decreases from Croatia towards Italy and Corsica.

Biochemical analysis

Extraction from buds and cortical tissues of twigs, electrophoresis and staining were performed using the techniques described by Thiébaud *et al.*, (1982) and Merzeau *et al.* (In press). The genetic variation of beech populations was studied using six polymorphous loci: *PX-1*, *PX-2* (peroxidases), *GOT-1* (glutamate oxaloacetate transaminase), *PGI-1* (phosphoglucose isomerase), *MDH-1* (malate dehydrogenase) and *IDH-1* (isocitrate dehydrogenase). The first four loci have already been used in West European beech studies (Cuguen *et al.*, 1985; Comps *et al.*, 1987), the latter two are being used for the first time. Three loci (*PX-1*, *GOT-1* and *MDH-1*) possess two codominant alleles while the remainder (*PX-2*, *IDH-1* and

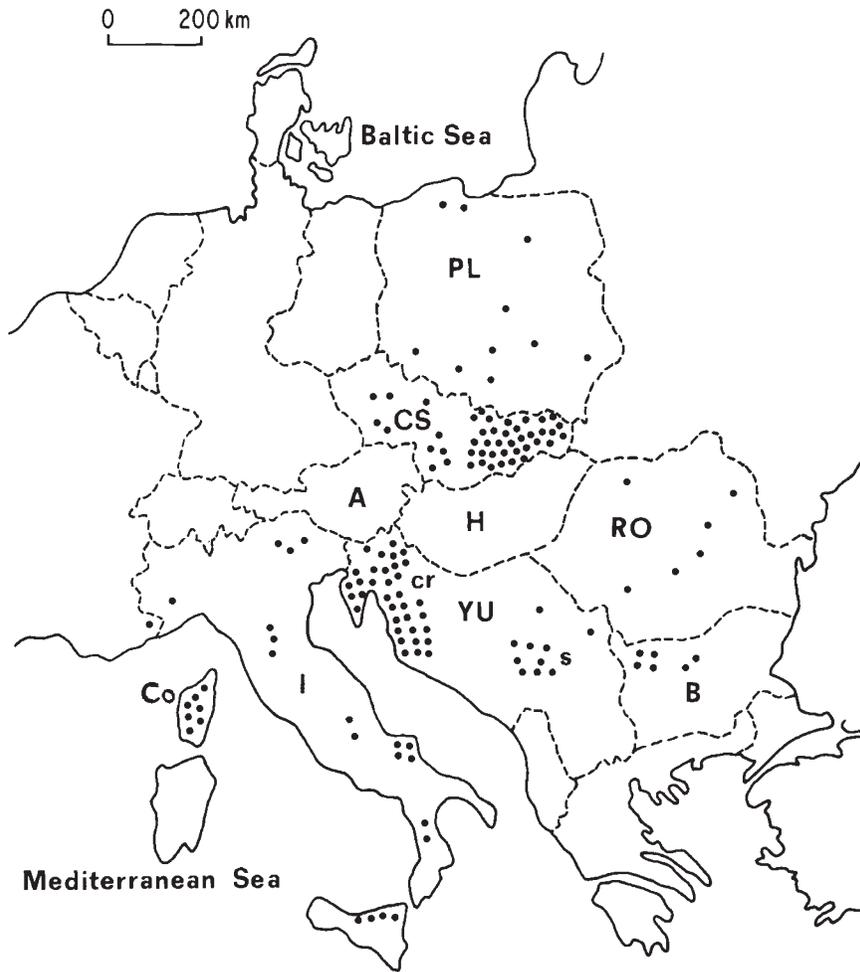


Figure 1 Location of beechwoods studied: PL, Poland; CS, Czechoslovakia; A, Austria; H, Hungary; Ro, Rumania; I, Italy; Yu, Yugoslavia; cr, Croatia; s, Serbia; B, Bulgaria; Co, Corsica.

PGI-1) possess three codominant alleles (Thiébaud *et al.*, 1982; Merzeau *et al.* In press).

Mathematical analyses

The total genetic differentiation among all the beechwoods studied is partitioned into inter- and intrapopulation components. They were estimated using two methods based on allelic and genotypic criterions.

Allelic differentiation

We used a nested anova with arcsine transformation to test both the inter-region and intercountry variabilities of allelic frequencies. Then, the studied countries were compared in pairs using Tukey-Kramer's test (Dunnet, 1980). However,

even if this test is well adapted to multiple comparisons among pairs in case of unequal sample sizes, it is very conservative and sometimes hides differences which nevertheless seem to tally with biological reality. So that we also carried out a single classification anova in order to compare both series of results, even if we have increased the risk of a type 1 error.

The total gene diversity (H_T) was estimated using Nei's procedure (1973, 1977):* $H_T = 1 - \sum p_i$, where \bar{p}_i is the mean frequency of the *i*th allele, weighted by the sample size. H_T is partitioned as $H_T = H_S + D_{ST}$ where H_S and D_{ST} are the weighted average gene diversity within and among populations, respectively.

* As recommended by Nei (1973), we will use the word "gene diversity" instead of heterozygosity.

Table 1 Beech stand (*N*) and tree (*n*) numbers studied according to the locus and the territory

Loci		PX-1	PX-2	GOT-1	MDH-1	IDH-1	PGI-1
No. of alleles		2	3	2	2	3	3
Whole area	<i>N</i>	143	143	143	121	99	114
	<i>n</i>	6795	7041	7289	5611	4646	5640
Continental region	<i>N</i>	97	97	97	85	64	79
	<i>n</i>	5200	4287	3899	3193	2313	4443
Mediterranean region	<i>N</i>	46	46	46	36	35	35
	<i>n</i>	1595	2754	3390	2418	2333	1197
Poland	<i>N</i>	10	10	10	6	6	—
	<i>n</i>	371	476	452	261	264	—
Czechoslovakia	<i>N</i>	46	46	46	44	30	43
	<i>n</i>	2239	2448	2671	2114	1530	2324
B + R + S	<i>N</i>	22	22	21	16	11	15
	<i>n</i>	1052	985	937	576	321	596
Croatia	<i>N</i>	35	35	35	35	33	35
	<i>n</i>	1896	1908	1896	1829	1643	1904
Italy	<i>N</i>	21	21	22	11	10	10
	<i>n</i>	953	892	963	483	504	420
Corsica	<i>N</i>	9	9	9	9	9	9
	<i>n</i>	274	332	370	348	384	396

B + R + S: Bulgaria + Rumania + Serbia.

Gene diversities were compared using only a non-parametric test because their distribution law is unknown. We tried to carry out multiple comparisons among pairs of countries, based on unequal sample sizes, using Noether's method (1976) which takes into account the experiment-wise error rate (Sokal and Rohlf, 1981); but we sometimes found inconsistent results probably due to type II errors, the risk of which is unknown. So for all comparisons among pairs we used the Mann-Whitney test, even if we have slightly (low number of comparisons) increased the risk of a type I error.

Finally, we carried out a discriminant analysis (Escouffier *et al.*, 1988) including all loci together (except *PGI-1*) to compare (1) the two climatic regions, (2) the different countries. Only one allele was taken into account for each diallelic locus and only two for each triallelic locus. The axis number equals the number of classes compared minus one, thus when comparing continental and Mediterranean regions, there is only one axis (fig. 2).

Genotypic differentiation

Genotypic structures are often analysed using *F*-statistics (Wright, 1965). A relationship can occur between *F*-statistic values and gene diversity, especially when the latter is very low (Wright, 1951 and 1978; Cuguen, 1986). One of the loci (*PGI-1*) generally reaches low diversities, thus it often transgresses the assumption of independence from

F-values. F_{IT} is an estimation of the total genotypic differentiation and as for gene diversity, it is partitioned into intra- (F_{IS}) and inter- (F_{ST}) population components.

Estimates of the *F*-statistics were computed according to the method of Weir and Cockerham (1984). They are weighted by the sample size, the variance and the number of stands studied. For each *F*-statistic, a variance was estimated using a jackknife procedure allowing us to determine whether each *F*-value is significantly different from 0 and to test deviations between two regions.

RESULTS

Allelic differentiation

Heterogeneity between regions and among countries within regions

Allelic frequencies differ significantly among countries, whatever the locus may be but not between the two climatic regions, except for *MDH-1* (table 2). In the whole area, gene diversities are rather high for four loci, not for *GOT-1* ($H_T = 0.175$) and for *PGI-1* ($H_T = 0.045$) (table 3). The intrapopulation component is always higher than 90 per cent. The gene diversity is significantly higher in the Mediterranean stands only for *GOT-1* locus ($P < 0.001$) and significantly lower for *IDH-1* and *MDH-1* loci ($P < 0.001$). This confirms results concerning the allelic frequencies of *MDH-1*

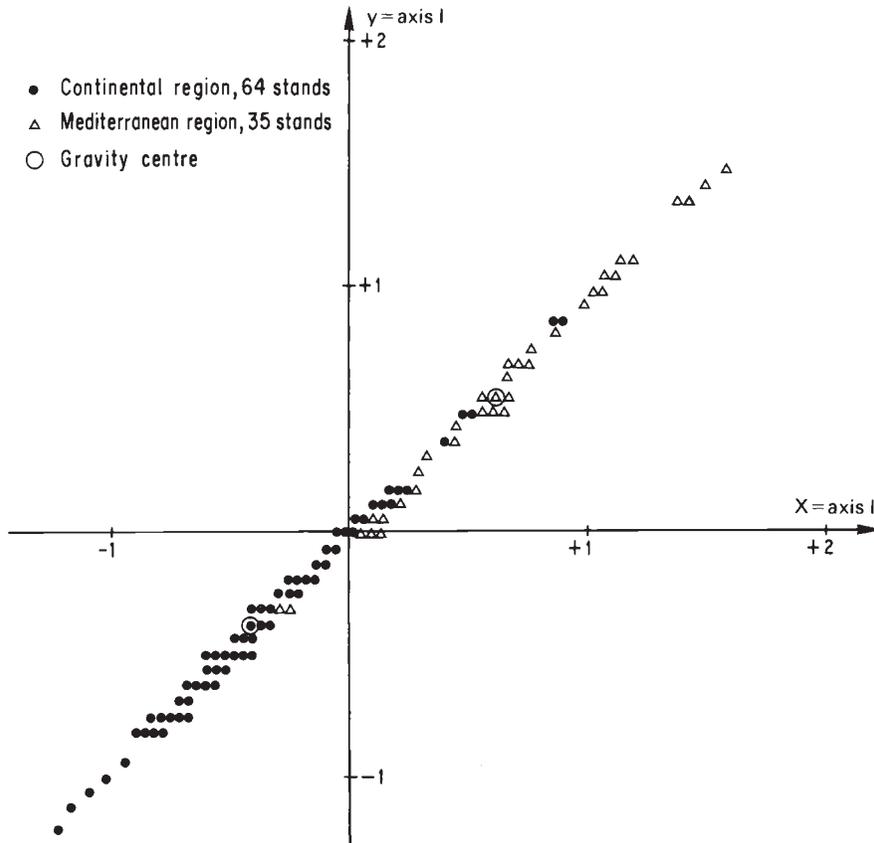


Figure 2 Discriminant analysis including *PX-1*, *PX-2*, *GOT-1*, *MDH-1* and *IDH-1* loci: comparison between climatic regions. Variance axis I: 100 per cent. Variable discriminant values for *IDH-1-84*: +0.315, *IDH-1-100*: +0.086, *PX-2-13*: -0.070, *IDH-1-116*: +0.063, *GOT-1-100* and -105: -0.030, *PX-1-100* and -105: -0.021, *MDH-1-100* and -120: +0.015, *PX-2-39*: -0.007, *PX-2-26*: -0.002.

locus; also we found $0.05 < P < 0.10$ for the two most frequent alleles of *IDH-1* (table 2). The discriminant analysis including all loci except *PGI-1* (fig. 2) shows a difference between the two climatic regions, however their discrimination is not quite complete. *IDH-1* locus is the most responsible for this discrimination.

Comparisons between countries

Results differ according to the locus considered:

PX-1. The allelic frequencies and the gene diversities vary according to the variety of climates (tables 2 and 3). Diversity is significantly lower in Poland than in all other countries (table 3). It increases in Czechoslovakia and reaches its highest values in the central part of the studied area (Croatia, Serbia, Bulgaria, Rumania). Then it

decreases towards the South, particularly in Corsica where it becomes significantly different from all other values.

PX-2. The gene diversity variations may be rather well related to the altitudinal range of the beechwoods: Croatia, 1385 m ($H_T = 0.431$ and 0.428); Czechoslovakia, 1060 m ($H_T = 0.404$); Italy, 650 m ($H_T = 0.365$); Serbia, Rumania and Bulgaria, 600 m ($H_T = 0.317$); Poland, 400 m ($H_T = 0.333$); Corsica, 350 m ($H_T = 0.265$).

GOT-1 and *IDH-1*. The gene diversity reveals a latitudinal opposition between the northern and the southern parts of the studied area (table 3); however, Mediterranean Croatia does not generally differ from the continental countries. These two locus diversities vary in an opposite way: *GOT-1* diversity increases from the north to the south whereas *IDH-1* one decreases.

Table 2 Mean allelic frequencies according to regions and countries

Loci and alleles	Whole area	Regions		Countries intra-regions (1)	Countries								
		Cont.	Medit.		PL	CS	BRS	CrC	CrM	I	Co		
<i>PX-1-100</i>	0.65	0.66	0.60	***		0.88	0.65	0.61	0.64	0.50	0.64	0.79	
		$P > 0.10$			(2)	b	a	a	a	a	a	a	b
					(3)	c	b	ab	b	a	ab	d	
<i>PX-2-39</i>	0.20	0.19	0.22	***		0.18	0.20	0.15	0.24	0.23	0.20	0.15	
		$P > 0.10$			(2)	ab	ab	a	b	b	ab	ab	
					(3)	abc	bc	a	d	cd	bd	ab	
<i>PX-2-26</i>	0.76	0.76	0.74	***		0.80	0.74	0.80	0.71	0.73	0.77	0.84	
		$P > 0.10$			(2)	ac	ac	bc	a	ab	ac	c	
					(3)	bc	ab	c	a	a	abc	c	
<i>GOT-1-100</i>	0.90	0.92	0.87	***		0.94	0.93	0.87	0.95	0.93	0.83	0.76	
		$0.05 < P < 0.10$			(2)	c	c	b	c	c	ab	a	
					(3)	c	c	b	c	c	a	a	
<i>MDH-1-100</i>	0.77	0.75	0.80	**		0.72	0.75	0.78	0.74	0.80	0.86	0.79	
		*			(2)	a	a	a	a	a	b	a	
					(3)	a	ab	bc	abc	c	d	abc	
<i>IDH-1-116</i>	0.31	0.35	0.27	**		0.34	0.34	0.43	0.33	0.31	0.24	0.18	
		$0.05 < P < 0.10$			(2)	bc	bc	c	bc	bc	ab	a	
					(3)	cd	c	d	bcd	c	b	a	
<i>IDH-1-100</i>	0.68	0.65	0.72	**		0.66	0.66	0.57	0.67	0.68	0.75	0.81	
		$0.05 < P < 0.10$			(2)	ab	ab	a	ab	ab	bc	c	
					(3)	b	b	a	abc	b	c	d	
<i>PGI-1-100</i>	0.98	0.98	0.98	*		—	0.99	0.97	0.98	0.96	0.99	0.96	
		$P > 0.10$			(2)	—	a	a	a	a	a	a	
					(3)	—	a	a	a	a	a	a	
<i>PGI-1-87</i>	0.02	0.02	0.02	*		—	0.01	0.02	0.02	0.04	0.01	0.04	
		$P > 0.10$			(2)	—	a	a	a	a	a	a	
					(3)	—	a	a	a	b	a	b	

(1) Nested Anova (Sokal and Rohlf 1981).

(2) Multiple comparisons among pairs of means based on unequal sample sizes: Tukey-Kramer's test (Dunnett 1980).

(3) Single classification Anova.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Any two countries not characterized by the same letter present allelic frequencies significantly different one from the other. PL, Poland; CS, Czechoslovakia; BRS, Bulgaria+Rumania+Serbia; CrC, continental Croatia; CrM, Mediterranean Croatia; I, Italy; Co, Corsica.

MDH-1. The gene diversity and partly allelic frequencies vary also as a function of latitude, in almost the same way as *IDH-1* ones, however Mediterranean and continental Croatia differ from one another.

PGI-1. Polymorphism and variations of allelic frequencies are very low. Only Italy seems to be apart from the other countries.

The discriminant analysis including *PX-1*, *PX-2*, *GOT-1*, *IDH-1* and *MDH-1* nearly reveals the

difference between four latitudinal groups of beechwoods for which discrimination is not complete (fig. 3): two southern groups (1) in Italy and (2) in Corsica, one intermediate group (3) in Bulgaria, Serbia, Rumania, continental and Mediterranean Croatia and Czechoslovakia, (4) one last group in Poland. It also shows that the two climatic regions of Croatia (continental and Mediterranean) are not different. As the two axes I and II only explain about 70 per cent of the variance, another analysis was carried out on axes I and III

Table 3 Mean gene diversity according to regions and countries—Mann-Whitney test

Loci	<i>H</i>	Whole area	Climatic regions		Countries						
			Cont.	Medit.	PL	CS	BRS	CrC	CrM	I	Co
<i>PX-1</i>	<i>H_T</i>	0.457	0.451	0.468	0.205	0.458	0.474	0.460	0.496	0.460	0.324
	<i>H_S</i>	94.4	95.9	91.6	98.1	98.9	97.1	96.7	94.5	93.8	98.5
			n.s.		a	c	de	cde	e	cd	b
<i>PX-2</i>	<i>H_T</i>	0.383	0.385	0.380	0.333	0.404	0.317	0.431	0.428	0.365	0.265
	<i>H_S</i>	95.6	96.4	92.1	98.5	96.9	96.1	96.8	96.1	94.7	97.1
			n.s.		b	cd	b	c	cd	bd	a
<i>GOT-1</i>	<i>H_T</i>	0.175	0.139	0.241	0.104	0.118	0.228	0.108	0.122	0.285	0.364
	<i>H_S</i>	90.5	93.1	89.9	96.9	94.9	94.4	89.6	98.5	92.0	93.9
			***		a	a	b	a	a	c	d
<i>MDH-1</i>	<i>H_T</i>	0.353	0.375	0.299	0.403	0.381	0.344	0.376	0.317	0.234	0.332
	<i>H_S</i>	94.5	96.2	92.4	99.3	95.6	97.2	94.5	97.9	78.5	95.1
			***		d	d	bc	cd	b	a	bc
<i>IDH-1</i>	<i>H_T</i>	0.433	0.451	0.395	0.449	0.449	0.489	0.434	0.434	0.371	0.314
	<i>H_S</i>	94.4	95.3	94.3	99.0	96.2	98.6	91.4	97.4	98.6	89.9
			***		cd	c	d	c	c	b	a
<i>PGI-1</i>	<i>H_T</i>	0.045	0.042	0.052	0.049	0.045	0.049	0.035	0.066	0.016	0.068
	<i>H_S</i>	91.1	88.0	93.1	100	83.4	81.6	95.9	91.9	90.5	97.3
			n.s.		(1)	b	bc	bc	c	a	c

H_T, total gene diversity; *H_S*, intra-component of the diversity (%). Cont., Continental region; Medit., Mediterranean region. PL, Poland; CS, Czechoslovakia; BRS, Bulgaria + Rumania + Serbia; CrC, continental Croatia; CrM Mediterranean Croatia; I, Italy; Co, Corsica.

ns = not significant.

*** $P < 0.001$.

Any two countries not characterized by the same letter present gene diversities significantly different one from the other. (1) No comparison including Poland was performed because of the small number of studied populations.

(the axis III explains 20.72 per cent of the variance): it completely confirms the discrimination between the two southern groups of beech populations (in Italy and in Corsica) and all the others.

Genotypic differentiation

Multilocus estimates of *F*-statistics required balanced data in all loci. Therefore, the *PGI-1* locus was not taken into account because of the low number of Polish beech stands examined for this locus. In addition, the procedure for the *IDH-1* locus has recently been improved, so it was not possible to analyse it for all the sampled stands. *F*-statistic estimations were therefore carried out on those 99 beechwoods for which results at all loci are complete.

All multilocus estimates differ from 0 ($P < 0.05$) over the whole area as well as in continental and Mediterranean regions and in most countries (table 4). All *F_{IS}* values are positive; also they are higher than *F_{ST}* values except in Czechoslovakia and Corsica. As for diversities, the intrapopulation

component of the genetic differentiation is higher than the inter-one.

The number of beech stands and trees we studied varies from one country to another; thus, it is better to only compare the genotypic differentiation (*F_{IS}*, *F_{ST}* and *F_{IT}*) of countries having similar sample sizes. In countries with lower stand numbers, intra- and interpopulation differentiation tends to be lower towards the north in Poland than towards the south in Italy (table 4). Beechwoods located in an intermediate area (Bulgaria, Rumania, Serbia) also show an intermediate genotypic differentiation. *F*-estimates do not differ from 0 in Corsica, an island located in the Mediterranean region. In countries with larger stand number, the intrapopulation differentiation is significantly lower in Czechoslovakia than in Croatia. In Croatia, comparisons between continental and Mediterranean regions do not reveal any significant differences. Thus, genotypic differentiation within populations seems to be similar in stands belonging to the same country and located near to each other even if they grow under different

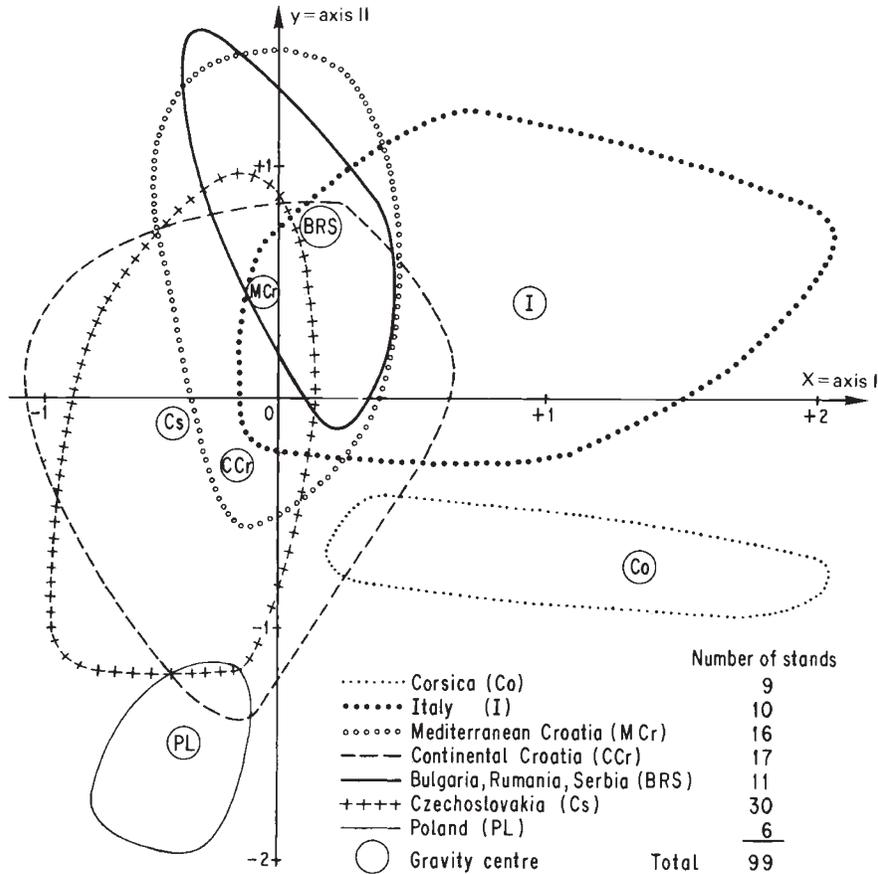


Figure 3 Discriminant analysis including *PX-1*, *PX-2*, *GOT-1*, *MDH-1* and *IDH-1* loci: comparison between countries. Variance axis I: 39.15 per cent, axis II: 30.51 per cent. Variable discriminant values (I+II) for *GOT-1-100* and *-105*: -0.089 , *PX-1-100* and *-105*: -0.058 , *IDH-1-84*: $+0.037$, *MDH-1-100* and *-120*: $+0.028$, *PX-2-13*: -0.026 , *PX-2-39*: -0.022 , *IDH-1-116*: -0.020 , *IDH-1-100*: -0.017 , *PX-2-26*: -0.012 .

Table 4 Multilocus F -statistic values according to regions and countries (*PX-1*, *PX-2*, *GOT-1*, *MDH-1* and *IDH-1*. Weir and Cockerham 1984)

Regions, countries and stand numbers	F_{is}	F_{st}	F_{it}
Whole area (99)	+0.065 (0.030)	+0.054 (0.000)	+0.115 (0.026)
Continental region (64)	+0.053 (0.026)	+0.038 (0.010)	+0.089 (0.026)
Mediterranean region (35)	+0.085 (0.034)	+0.071 (0.020)	+0.150 (0.036)
Poland (6)	+0.043 (0.017)	+0.003 (0.000)	+0.046 (0.017)
Czechoslovakia (30)	+0.013 (0.024)	+0.033 (0.010)	+0.046 (0.020)
B+R+S (11)	+0.095 (0.054)	+0.026 (0.014)	+0.119 (0.056)
Croatia (33)	+0.088 (0.026)	+0.046 (0.010)	+0.131 (0.020)
Continental stands (17)	+0.094 (0.026)	+0.037 (0.014)	+0.128 (0.014)
Mediterranean stands (16)	+0.083 (0.031)	+0.054 (0.022)	+0.133 (0.030)
Italy (10)	+0.125 (0.047)	+0.058 (0.030)	+0.176 (0.053)
Corsica (9)	+0.036 (0.044)	+0.036 (0.014)	+0.069 (0.047)

Standard errors appear in parentheses and were derived by jackknifing over stands $-B+R+S$: Bulgaria+Rumania+Serbia.

climatic conditions whereas this differentiation can differ between two countries within one climatic region.

DISCUSSION AND CONCLUSION

The arguments put forward in the introduction concerning the origins of the beech populations suggest that there has been more opportunity for the development of genetic differentiation within and among populations in the south than in the north of the range.

Allelic level

In the whole area, some loci (*PX-1*, *PX-2*, *MDH-1* and *IDH-1*) are very polymorphic; *GOT-1* locus is less polymorphic and *PGI-1* locus is nearly monomorphic.

Allelic variations of *PX-1* and *PX-2* loci were shown to be partially related to climatic changes. For *PX-2*, they are related to the altitudinal range of the beech stands. For *PX-1*, the gene diversity is lowest within countries where all beechwoods grow under optimal climatic conditions; it is highest in Croatia where both optimal and extreme climates for the Beech can be found. In some of our previous works about other southern countries far apart from each other, we also found variations of *PX-2* allelic diversity according to the altitudinal range of the stands (Thiébaud *et al.*, 1982; Thiébaud, 1984; Barrière *et al.*, 1985; Cuguen *et al.*, 1985; Comps *et al.*, 1987; Comps *et al.*, 1989). For *PX-1*, we showed a positive correlation between the climatic conditions extreme for the Beech (at high and low altitudes) and the highest values of *PX-1-105* frequency. These previous results suggested the possibility of a selection effect as we always observed the same allelic variations in connection with the same climatic changes, as for other species (Clarke, 1975; Bergmann, 1978). In another study (yet unpublished), we found a significant correlation between the highest frequencies of *PX-1-105* allele and the driest stands of the Mediterranean slope of the Dinaric Alps in Croatia. The discriminant analysis based on all loci confirms the significant differences between continental and Mediterranean regions.

Among countries, the discriminant analysis displays four pools of beechwoods for which discrimination is rather good but not complete: (1) in Poland, (2) in Czechoslovakia, Bulgaria +

Rumania + Serbia, Croatia, (3) in Italy and (4) in Corsica.

Considering other studies on genetic differentiation of anemophilous forest tree species, the inter-population component of the diversity is generally found lower than the intrapopulation one and varies between 0 and 15 per cent (see review in Gullberg *et al.*, 1985). In this study, it varies from 4.2 to 9.5 per cent over the whole area and it is lower than the intrapopulation component both in continental and Mediterranean regions. Nevertheless, it is the highest in the Mediterranean region for all loci except *PGI-1*. This suggests the following remarks:

(1) Almost all results are rather similar in spite of different calculation methods, population size and locus number. This homogeneity expresses a similar behaviour of allogamic and anemophilous forest tree species (conifers as well as deciduous species like the Beech) for which the intracomponent of the diversity is clearly higher than the inter-component because of important gene flows from one population to another.

(2) Differences between studied species in Eurasia as well as in America suggested to Gullberg *et al.* (1985) that populations that have settled within areas covered by ice during the glacial periods in the north have lower inter-population components of the diversity than those located in the south. One obvious explanation for lower differentiation in northern populations could be that the time that has passed after the last glaciation has been too short.

The results can be related to the hypothesis assumed for the Beech regarding the higher age of southern populations and it is known that these populations had been spread out from several secondary sources isolated from each other during glaciations. In addition, the three studied Mediterranean countries are rather separate from one another and more or less from other countries of central Europe: Corsica is entirely isolated by the sea, also Italy by the sea along most of the country and by mountains in the north; Mediterranean Croatia is separated from Italy by the Adriatic sea but much less from the continental Croatian slope of the Dinaric Alps. This may have reduced the postglacial exchanges between the three Mediterranean countries but not migrations from Mediterranean Croatia towards central Europe; so, it can explain the observed discriminations. The degree of isolation and postglacial migrations must be taken into account in Europe because they can also play an important role in the genetic differentiation of beech populations.

Genotypic level

Mostly multilocus estimates of F -statistics differ significantly from zero. F_{IS} values are always positive and generally higher than F_{ST} ones. These results imply:

(1) The existence of a total genotypic differentiation with intra- and interpopulation components. In comparison with the previous study of Cuguen *et al.* (1988), including 250 beech populations from Europe and using three loci, we have obtained similar results concerning F_{ST} . Multilocus estimate of F_{IS} is lower than that obtained by Cuguen *et al.* (1988) ($F_{IS} = 0.065$ and 0.120 , respectively); this may be due to the two new loci (*IDH-1* and *MDH-1*) being used in our study which possess similar variations but with a contradictory trend. In most studied species, values of F_{ST} tend indeed to be lower than 0.100 and provide evidence that genotypic heterogeneity is generally rather low. The Beech shows the highest estimates among the anemophilous forest tree species, with only two exceptions: *Pinus banksiana* and *Pinus halepensis* which reach values about 0.120 (Grunwald *et al.*, 1986; Dancik and Yeh, 1983; O'Reilly *et al.*, 1985; Plessas and Strauss, 1986). Higher values of F_{ST} could be due to the larger area and higher number of investigated stands but, however, any generalization is not possible regarding the lower number of loci.

(2) A general tendency towards a heterozygote deficit. As pointed out by Brown (1979), allogamic species often exhibit a heterozygote deficit. Among the different causes he listed, two can be applied to the Beech, selfing and isolation by distance: (a) referring to the low level of self-fertilization rate of the Beech (from 0 to 0.05 , Merzeau *et al.* in preparation), self-fertilization can explain only a small part of the observed heterozygote deficit. (b) Cuguen *et al.* (1986) formulated the "isolation by distance" hypothesis as the most important cause of heterozygote deficit in Beech.

According to our previous hypothesis, the genotypic differentiation within and among populations tends to be higher towards the south. The differences between continental and Mediterranean regions are, however, not significant. This may be due to the applied method (jackknife procedure) or the lower number of investigated populations in some countries with higher ecological differentiation (*e.g.*, Rumania, Bulgaria, Serbia, Poland).

Finally, three causes, selection, gene flows and the mating system have been proposed to explain the genetic differentiation of beech populations.

But, it is rather difficult to rank them according to their significance.

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