

## Genetic variation of the Croatian beech stands (*Fagus sylvatica* L): spatial differentiation in connection with the environment

B Comps <sup>1\*</sup>, B Thiebaut <sup>2</sup>, I Sugar <sup>3</sup>, I Trinajstić <sup>4</sup>, M Plazibat <sup>3</sup>

<sup>1</sup> Université de Bordeaux I, Département de Biologie Végétale,  
Avenue des Facultés, 33405 Talence Cedex;

<sup>2</sup> Centre Louis Emberger, CNRS, BP 5051, 34033 Montpellier Cedex;  
Institut de Botanique, Université des Sciences et Techniques du Languedoc,  
rue Auguste Broussonnet, Montpellier, France;

<sup>3</sup> Botanicki Zavod, Fakulteta Sveucilista u Zagrebu, Marulićev trg 20/11, 41000 Zagreb;

<sup>4</sup> Sumarski Fakultet, Simunska Cesta, 4100 Zagreb, Yugoslavia

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**Summary** — Thirty-five beech stands located in Croatia, (Yugoslavia) have been analysed using 6 polymorphic enzymatic loci. Three of them (PX-1, PX-2, GOT-1) seem to be more or less influenced by selection since their allelic frequencies are related to climatic conditions. The total gene diversity is higher for only 1 out of 6 loci in the continental region. Discriminant analyses on allelic frequencies show that the Mediterranean *Seslerio-Fagetum* which grows in rather dry conditions, is an association apart; and a significant difference exists between 2 groups of Mediterranean beechwoods located in the highlands and on the plain, respectively. A general tendency towards a heterozygotic deficit occurs with the same significance in both regions. Multilocus *F*-statistics reveal that the total genotypic differentiation and its 2 components (intra- and interpopulations) do not differ between the 2 regions.

genetic differentiation / beech population / Croatia / geographic variation

**Résumé** — La variation génétique des hêtrales croates (*Fagus sylvatica* L) : différenciation spatiale en relation avec l'environnement. Trente-cinq hêtraies localisées en Croatie (Yougoslavie) ont été étudiées à l'aide de 6 marqueurs enzymatiques polymorphes. Trois d'entre eux (PX-1, PX-2, GOT-1) semblent plus ou moins soumis à la sélection, en particulier parce que leurs fréquences alléliques varient parallèlement aux conditions climatiques. La diversité allélique totale est plus élevée pour 1 locus sur 6 seulement, en région continentale. Deux analyses discriminantes sur les fréquences alléliques montrent :

– que le *Seslerio-Fagetum* méditerranéen qui caractérise des stations relativement sèches représente une association originale;

– une différence significative entre 2 groupes de hêtraies méditerranéennes en fonction de leur localisation soit en montagne, soit à basse altitude. Une tendance générale se manifeste vers un déficit en hétérozygotes de même importance dans les deux régions. Les *F*-statistiques montrent que la différenciation génotypique totale, aussi bien que ses 2 composantes (intra- et interpopulations) ne sont pas différentes d'une région à l'autre.

différenciation génétique / hêtraie / Croatie / variation géographique

\* Correspondence and reprints

## INTRODUCTION

The genetic structure of beech stands depends on selection and the mating system, in addition to gene flow and genetic drift: these factors induce inter- and intra-population genetic differentiation over space and time (Kim, 1979; 1980; Müller-Starck, 1985, 1989; Cuguen, 1986; Gregorius *et al*, 1986; Cuguen *et al*, 1988).

Beech is a climax species in most of western Europe where it grows under various ecological conditions. Particularly close to the Mediterranean sea, neighbouring beechwoods may develop in very different climates, *ie* Mediterranean or continental climates, depending on whether they are located in the lowlands or in the highlands (Misic, 1957; 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; N'Tsiba, 1984; Thiébaud, 1984; ; Barrière *et al*, 1985; Cuguen *et al*, 1985; Comps *et al*, 1987).

Beech is an anemophilous and mostly allogamic species characterized by a low self-fertilization rate (Nielsen and Schaffalitzky-de-Muckadell, 1954) which can nevertheless produce some heterozygotic deficit. In addition, gene flow may be generally limited within populations in the optimal beech range due to the high density of beechwoods: it is therefore likely that mating occurs between closely spaced individuals. According to Cuguen (1986) and Cuguen *et al* (1988), genetic structures should be approximately described by the "isolation by distance model" (Wright, 1943, 1946). This model induces a relatedness between individuals and therefore a genetic differentiation within and among populations (Cuguen, 1986). Since Wright's theoretical works, other

methods and models have been proposed, which can best be applied to actual plant populations (Malécot, 1969; Gregorius, 1975a, b; Van Dijk, 1987).

Historical factors could also play an important role in the genetic structure of beechwoods. After the last glacial period, current beech stands spread out from a principal source located in the Balkans and from several secondary sources in southwestern Europe (Paquereau, 1965; Beug, 1967; Sercelj, 1970; Jalut *et al*, 1975; Triat-Laval, 1978; Pons, 1983). It appears that beech colonized its present areas at various periods: southern countries have been colonized since 5 000 or 4 000 BP (approximately 40–50 generations) and northern plains since only 3 000 or 2 500 BP (25–30 generations) (Vernet, 1981). Thus there has been a higher number of generations in the south than in the north.

Genetic differentiation within and among beech stands seems to be higher in the south where ecological conditions are more heterogeneous, the stands are older and their sources more numerous (Comps *et al*, 1989). Our purpose was to examine the genetic differentiation of beechwoods in Croatia characterized both by the Mediterranean and continental climates. Preliminary results have shown that this genetic differentiation is higher in Croatia than in other countries of central Europe (Comps *et al*, 1989).

## MATERIAL AND METHODS

### *Sampling*

Sampling was carried out in 35 beech stands representing the various climatic conditions, the various soils and topographic locations where beech grows in Croatia (fig 1, table I). In each beech stand, plant material (buds and twigs)

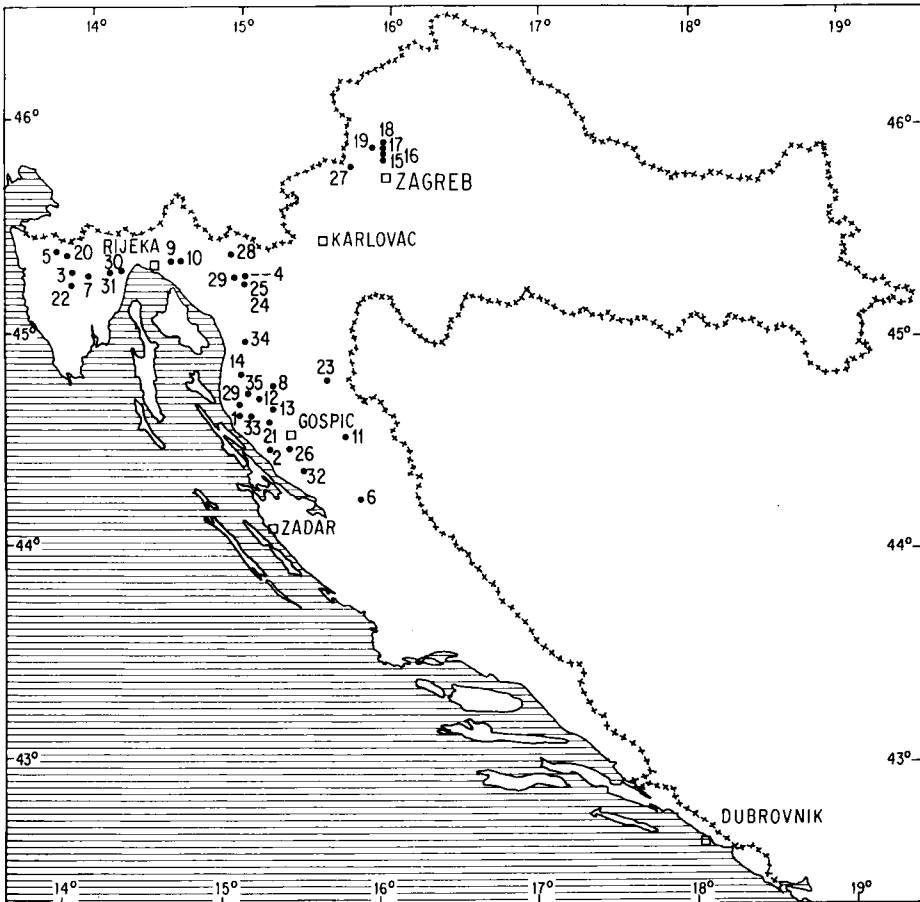


Fig 1. Location of the beechwoods studied in Croatia.

was sampled from about 50 trees chosen at random over a 3–4-ha area and in as homogeneous an environment as possible.

With regard to the climate, we compared 2 regions located on either side of the Dinaric Alps, in Mediterranean and continental climates, respectively. In the Mediterranean region, we also distinguished the forests located near the littoral at low altitude from the highland forests: the former are generally oak–beech mixed forests characterized by the dominance of oak and mostly located below 500 m in altitude (only

one of them located at 800 m was included in this group due to the dryness of the station); the latter are beechwoods which are always located above 900 m. The continental region was not subdivided: it includes all the forests located along the northern slope of the Dinaric Alps, on the plain and in the highlands of Croatia. With regard to soil factors, we could only define 3 classes of pH (acid, neutral and basic); we preferred to carry out a synthetic characterization of environment by analysing the plant associations to which the forests under study belong.

**Table I.** Croatian beechwoods studied.

	<i>Beechwood locations</i>	<i>Regional climate</i>	<i>Plant associations</i>	<i>Altitude (m)</i>	<i>Soil pH</i>
1	Alancic	Ms	<i>Fagetum-subalpinum</i>	1 440	Neutral
2	Baske-Ostarije	Ms	<i>Seslerio-Fagetum</i>	920	Basic
3	Bravari	Mp	<i>Seslerio-Fagetum</i>	300	Basic
4	Brezovac	C	<i>Fagetum-montanum</i>	850	Neutral
5	Cepici	Mp	<i>Seslerio-Fagetum</i>	410	Basic
6	Crnopac	C	<i>Seslerio-Fagetum</i>	780	Basic
7	Dausi	Mp	<i>Seslerio-Fagetum</i>	445	Basic
8	Gacko Polje	C	<i>Abieti-Fagetum</i>	600	Neutral
9	G Jelenje N	C	<i>Abieti-Fagetum</i>	840	Neutral
10	G Jelenje S	Ms	<i>Seslerio-Fagetum</i>	900	Neutral
11	Gorica	C	<i>Seslerio-Fagetum</i>	802	Basic
12	Gromovaca	Ms	<i>Fagetum-subalpinum</i>	1 620	Neutral
13	Kalinovaca	C	<i>Luzulo-Fagetum</i>	600	Acid
14	Krasno	C	<i>Abieti-Fagetum</i>	750	Acid
15	Medvednica Sb	C	<i>Luzulo-Fagetum</i>	400	Neutral
16	Medvednica SM1	C	<i>Fagetum-montanum</i>	500	Acid
17	Medvednica SM2	C	<i>Fagetum-montanum</i>	500	Acid
18	Medvednica H	C	<i>Aceri-Fraxinetum</i>	1 020	Neutral
19	Medvednica N	C	<i>Abieti-Fagetum</i>	500	Neutral
20	Oprtalj	Mp	<i>Seslerio-Fagetum</i>	260	Basic
21	Pazariste	C	<i>Abieti-Fagetum</i>	850	Neutral
22	Pazin	Mp	<i>Anemone-Carpinetum</i>	300	Basic
23	Plitvice	C	<i>Fagetum-montanum</i>	600	Neutral
24	Ponikve J	C	<i>Fagetum-montanum</i>	350	Basic
25	Ponikve V	C	<i>Fagetum-montanum</i>	350	Basic
26	Rizvanula	C	<i>Luzulo-Fagetum</i>	600	Acid
27	Samoborsko	C	<i>Luzulo-Fagetum</i>	450	Neutral
28	Skrad	C	<i>Blechno-Fagetum</i>	550	Acid
29	Turinski Krc	Ms	<i>Seslerio-Fagetum</i>	1 130	Basic
30	Ucka E	Ms	<i>Seslerio-Fagetum</i>	1 000	Basic
31	Ucka W	Mp	<i>Seslerio-Fagetum</i>	800	Basic
32	Velika Pak	Mp	<i>Seslerio-Fagetum</i>	680	Neutral
33	Veliki Koz	Ms	<i>Fagetum-subalpinum</i>	1 400	Neutral
34	Vratnik	Ms	<i>Seslerio-Fagetum</i>	900	Neutral
35	Vucjak	Ms	<i>Fagetum-subalpinum</i>	1 645	Neutral

### **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* (1989). Genetic variability was studied using 6 polymorphic loci: PX-1, PX-2 (peroxidases), GOT-1 (gluta-

mate oxaloacetate transaminase), PGI-1 (phosphogluco-isomerase), MDH-1 (malate dehydrogenase) and IDH-1 (isocitrate dehydrogenase). Three of these loci (PX-1, GOT-1 and MDH-1) possess 2 codominant alleles while the others have 3 codominant alleles (PX-2, IDH-1 and PGI-1) (Thiébaud *et al*, 1982; Merzeau *et al*, 1989).

## Mathematical analyses

### Allelic differentiation

The total gene diversity ( $H_T$ ) was estimated using Nei's method (1973, 1977) (\*):

$$H_T = 1 - \sum p_i^2$$

where  $p_i$  is the mean frequency of the  $i$ th allele, weighted by the sample size. We also estimated  $H_S$  and  $D_{ST}$  which are the weighted average gene diversities within and among populations, respectively, with  $H_T = H_S + D_{ST}$ .

Allelic frequencies of the different groups of beechwoods characterized according to climate, soil or plant associations were compared for each locus using an analysis of variance and the Mann-Whitney test. Gene diversities were compared using only the non parametric Mann-Whitney test because they did not fit a normal distribution. We then carried out a discriminant analysis including only the loci for which previous comparisons displayed significant differences. Only one allele was taken into account for each diallelic locus and only 2 for each triallelic locus.

### Genotypic differentiation

Since the theoretical works of Wright (1965), genotypic structures have often been analysed using  $F$ -statistics.  $F_{IT}$  is an estimation of the total genotypic differentiation, and allelic diversity is partitioned into intra- ( $F_{IS}$ ) and inter- ( $F_{ST}$ ) population components. Estimates of the 3  $F$ -statistics were made according to the method of Weir and Cockerham (1984). They were weighted by the stand sample size and its variance and by the number of stands studied. For each index, a variance was estimated using a jack-knife procedure (Miller, 1974; Reynolds *et al*, 1983). This variance allowed us to determine whether each value was significantly different from 0, and to test differences between 2 regions.

In populations which are not very polymorphic, negative  $F_{IS}$  values are generally more frequent than within stands characterized by a

high level of polymorphism: the corresponding heterozygote excess is connected with a statistical effect due to the low probability of encountering homozygotes of a rare allele in the sampling (Cuguen, 1986; Cuguen *et al*, 1988).

## RESULTS

### Allelic differentiation

At each locus, one allele generally appeared more frequently than the others in the beechwoods under study (table II). However for PX-1, the rarer allele (PX-1-105) appeared more frequently in some Mediterranean forests located in the highlands (6 out of 7 stands) and only 2 out of 7 at low altitude.

### Allelic differentiation according to regions

Gene diversity varied from one locus to another and according to the region. It was relatively high for PX-1, PX-2, IDH-1 and MDH-1, whereas it was lower for GOT-1 and PGI-1 (table III). It was higher for PX-1 in the Mediterranean region ( $P < 0.1$ ) and for MDH-1 in the continental region ( $P < 0.05$ ) (table IV). In the Mediterranean region, the gene diversity was higher for 2 out of 6 loci in the highlands: PX-2 ( $P < 0.001$ ) and GOT-1 ( $P < 0.01$ ) and was higher on the plain for MDH-1 and PGI-1 ( $P < 0.10$ ).

Gene diversity was distributed in the same manner throughout, the greater part being within the forests (86.9–99.1%, table III) as observed in mostly allogamic species (Tigerstedt, 1973; Rudin *et al*, 1974; Lundkvist and Rudin, 1977; Hamrick *et al*,

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

**Table II.** Mean allelic frequencies according to regions and associations.

<i>Beechwood No</i>		<i>All</i>	<i>Mediterranean region</i>			<i>Continental</i>	<i>Seslerio</i>	<i>Other</i>
		<i>beechwoods</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>region</i>	<i>Fagetum</i>	<i>associations</i>
		35	7	9	16	19	13	22
<i>Loci, alleles</i>								
PX-1	<i>N</i>	1 897	349	556	905	992	752	1 145
	105	0.41	0.40	0.49	0.46	0.36	0.45	0.37
	100	0.59	0.60	0.51	0.54	0.64	0.55	0.63
PX-2	<i>N</i>	1 908	350	559	909	999	756	1 152
	39	0.23	0.18	0.29	0.25	0.23	0.28	0.21
	26	0.72	0.80	0.66	0.71	0.72	0.70	0.73
	13	0.05	0.02	0.05	0.04	0.05	0.02	0.06
GOT-1	<i>N</i>	1 886	350	551	901	985	755	1 131
	105	0.06	0.04	0.08	0.07	0.05	0.07	0.05
	100	0.94	0.96	0.92	0.93	0.95	0.93	0.95
MDH-1	<i>N</i>	1 784	346	547	893	891	744	1 040
	120	0.23	0.22	0.18	0.20	0.26	0.21	0.24
	100	0.77	0.78	0.82	0.80	0.74	0.79	0.76
IDH-1	<i>N</i>	1 641	348	542	890	751	740	901
	116	0.31	0.30	0.31	0.31	0.31	0.28	0.35
	100	0.69	0.69	0.69	0.69	0.69	0.71	0.65
	84	0.00	0.01	0.00	0.00	0.00	0.01	0.00
PGI-1	<i>N</i>	1 903	350	557	907	996	757	1 146
	113	0.00	0.01	0.00	0.00	0.00	0.00	0.01
	100	0.98	0.93	0.98	0.96	0.98	0.98	0.97
	87	0.02	0.06	0.02	0.04	0.02	0.02	0.02

*N*, Tree number; <sup>1</sup> lowlands; <sup>2</sup> highlands; <sup>3</sup> whole of the Mediterranean region.

1979; Loveless and Hamrick, 1984). However, the inter-population component varied somewhat according to region and locus.

Comparison of allelic frequencies between regions confirmed the contrast between the Mediterranean and the continental regions for PX-1 (table IV); we also found  $0.05 < P < 0.10$  for MDH-1. In the Mediterranean region, the deviation between the lowlands and the highlands was confirmed for PX-2 and GOT-1.

A discriminant analysis was carried out including only PX-1, PX-2 and GOT-1 allelic frequencies for which the previous comparisons had shown significant deviations ( $P < 0.05$ , table IV, fig 2). It confirmed the difference between the 2 groups of Mediterranean beechwoods (highland and lowland) for which discrimination was complete. Continental beechwoods constituted an intermediate group between the other two. GOT-1 and PX-2 loci were more responsible than PX-1 for this discrimination between these climatic groups.

**Table III.** Mean gene diversities according to regions and associations (Nei's procedure, 1973, 1974).

Loci		Whole area	Mediterranean region			Continental region	Seslerio-Fagetum	Other associations
			1	2	3			
PX-1	$H_T$	0.482	0.478	0.500	0.496	0.460	0.497	0.465
	$H_S$	94.6	94.2	96.1	94.5	96.7	95.6	95.5
PX-2	$H_T$	0.430	0.332	0.476	0.428	0.431	0.447	0.415
	$H_S$	96.3	98.3	97.6	96.1	96.8	95.9	97.7
GOT-1	$H_T$	0.116	0.084	0.144	0.122	0.108	0.129	0.104
	$H_S$	93.1	87.6	97.1	98.5	89.6	98.1	91.2
MDH-1	$H_T$	0.348	0.347	0.298	0.317	0.376	0.329	0.362
	$H_S$	95.7	97.9	98.0	97.9	94.5	97.4	94.5
IDH-1	$H_T$	0.434	0.433	0.435	0.434	0.434	0.412	0.450
	$H_S$	94.7	92.3	98.7	97.4	91.4	98.8	92.0
PGI-1	$H_T$	0.050	0.110	0.037	0.066	0.035	0.043	0.053
	$H_S$	94.0	92.0	94.2	91.9	95.9	99.1	86.9
Mean values	$H_T$	0.310	0.297	0.315	0.311	0.307	0.310	0.308
	$H_S$	(0.047) 94.7	(0.067) 93.7	(0.078) 97.0	(0.013) 96.0	(0.027) 94.2	(0.014) 97.5	(0.014) 93.0

$H_T$ , Total gene diversity;  $H_S$ , weighted average gene diversity within populations (%). <sup>1</sup> Lowland; <sup>2</sup> highland; <sup>3</sup> whole of the Mediterranean region. Numbers in parentheses represent standard error.

### **Allelic differentiation according to associations**

The originality of Croatian beechwood associations is mostly related to the existence of rather dry stands. This ecological character has induced the settlement of a special association, the *Seslerio-Fagetum*, whereas other beechwoods are more akin to those of Central Europe. This explains why we only compared this association with the others taken as a whole.

Gene diversity varied from one locus to another (table III). In *Seslerio-Fagetum* it was only found to be higher for PX-1

( $P < 0.10$ ) (table IV). The greatest part of gene diversity was always found to be within the populations, whatever the association group and the locus.

Comparisons of allelic frequencies between the 2 groups of associations showed significant differences for PX-1, PX-2-39 and IDH-1 (table IV). Thus, there was a greater contrast between associations than between Mediterranean and continental regions.

A discriminant analysis including PX-1, PX-2 and IDH-1 allelic frequencies (fig 3) confirmed the previous differences between the 2 groups of associations; but

**Table IV.** Comparisons of allelic frequencies and diversities.

	<i>Loci</i> <i>Alleles</i>	<i>PX-1</i> 100	<i>PX-2</i> 26 39	<i>GOT-1</i> 100	<i>MDH1-</i> 100	<i>IDH-1</i> 100 116	<i>PGI-1</i> 100		
Comparisons between :									
Mediterranean and Continental regions	1	*	ns	ns	ns	+	ns	ns	ns
	2	*	ns	ns	ns	+	ns	ns	ns
	3	+		ns	ns	*		ns	ns
Mediterranean highland and lowland	1	ns	**	*	*	ns	ns	ns	ns
	2	ns	**	*	**	ns	ns	ns	ns
	3	ns		***	**	+		ns	+
<i>Seslerio-Fagetum</i> and other associations	1	+	ns	*	ns	ns	*	*	ns
	2	*	ns	*	ns	ns	*	*	ns
	3	+		ns	ns	ns		ns	ns

1, ANOVA on allelic frequencies; 2 and 3, Mann-Whitney test, respectively on allelic frequencies and diversities; ns, non significant; +,  $0.05 < P < 0.10$ ; \*  $0.01 < P < 0.05$ ; \*\*,  $0.01 < P < 0.001$ ; \*\*\*,  $P < 0.001$ .

their discrimination was not quite complete. The PX-2 locus was the most responsible for this discrimination.

### Genotypic differentiation

For the whole of Croatia, all multilocus estimates of  $F$ -statistics were different from

0, which implies the existence of genotypic differentiation both intra- and interpopulations (table V).  $F_{IS}$  value was positive, which reveals a general tendency towards a heterozygote deficit.

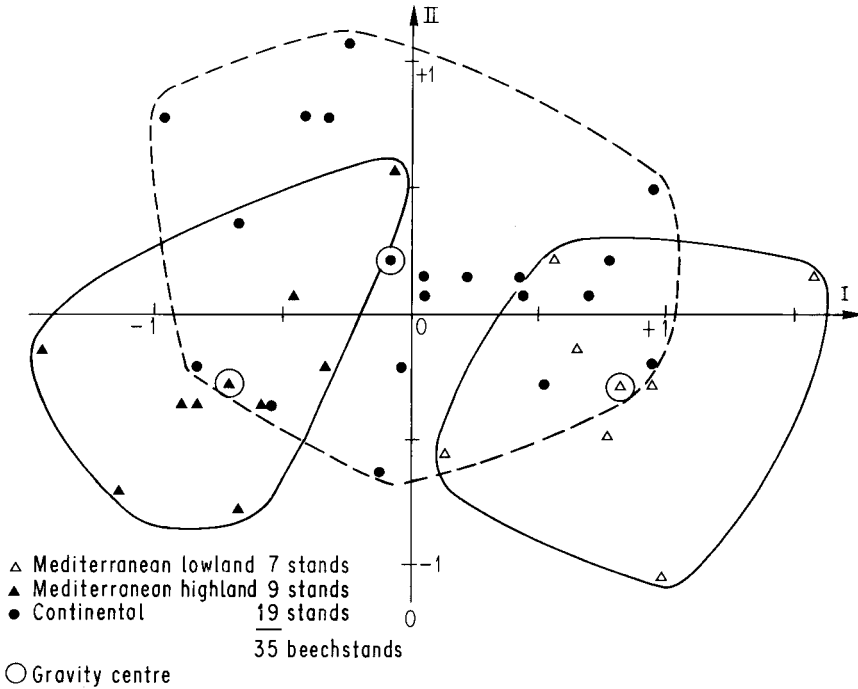
The number of beechwoods and trees studied within each region were very alike, which allowed the comparison of  $F$ -statistics values. As for the whole of Croa-

**Table V.** Multilocus estimates of  $F$ -statistics according to regions and associations (Weir and Cockerman, 1984).

	$F_{IS} \pm I$		$F_{ST} \pm I$		$F_{IT} \pm I$	
Whole of Croatia [35]	0.115	0.059	0.046	0.019	0.156	0.048
Continental region [19]	0.090	0.052	0.036	0.028	0.123	0.034
Mediterranean region [16]	0.068	0.065	0.055	0.048	0.120	0.068
<i>Seslerio-Fagetum</i> [13]	0.085	0.081	0.037	0.019	0.119	0.091
Other associations [22]	0.086	0.039	0.052	0.051	0.134	0.000

I, Confidence interval ( $P < 0.05$ ); the number of stands appears in parentheses.





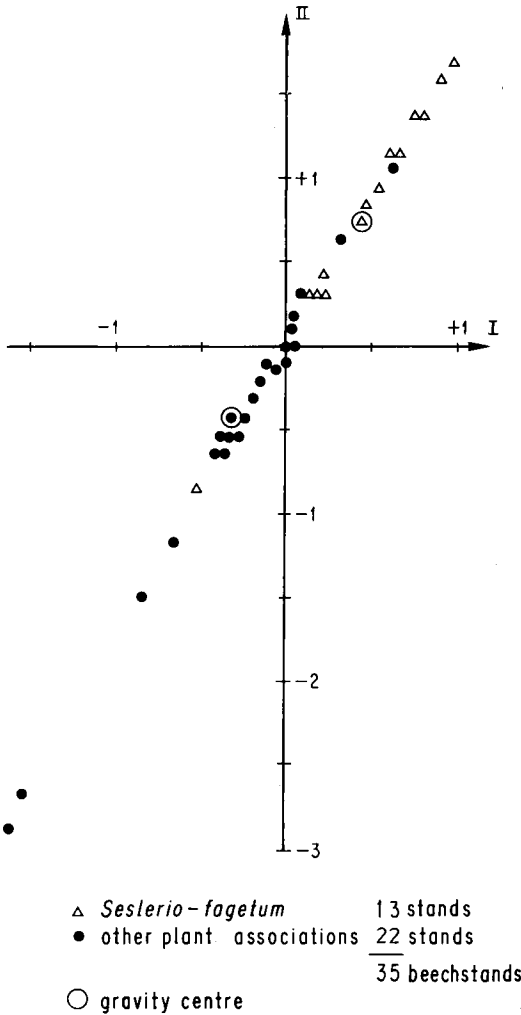
**Fig 2.** Discriminant analysis including PX-1, PX-2 and GOT-1 loci: comparison between climatic regions. Variance axis I: 69.97%, axis II: 30.13%. Variable discriminant values for GOT-1:  $-0.144$ , PX-2:  $-0.100$ , PX-1:  $-0.052$ .

tia, all multilocus estimates differed from 0, and  $F_{IS}$  values were positive. Differences between the 2 regions were never significant. However, to similar  $F_{IT}$  values tended to correspond lower  $F_{IS}$  and higher  $F_{ST}$  values in the Mediterranean region; but these differences were not significant. In the Mediterranean region, the comparison between lowland and highland beechwoods became impossible due to the low number of stands in each group.

Within each association group, all multilocus estimates of  $F$ -statistics were different from 0. As for the regions, differences between any pair of homologous values taken from each group of associations respectively were never significant.

## DISCUSSION AND CONCLUSIONS

The number of beechwoods studied was rather low as there is a variety of climate in Croatia. We could have sampled a greater number of stands on the mountain summits and within the continental region where beech is very frequent, but not along the littoral where beechwoods are rare. A lack of balance in the sampling which would have favoured the number of beechwoods studied belonging to the first group could have introduced a bias in our analysis. However, as the total number of trees analysed was great in both cases, the previous disadvantage was partially compensated. It would be interesting to



**Fig 3.** Discriminant analysis including PX-1, PX-2 and IDH-1: comparison between the 2 groups of associations. Variable discriminant values for PX-1: -0.151, PX-2: 0.025, IDH-1: 0.022. Coordinates are the same on the 2 axes (only 2 groups of stands).

sample over a wider area along the Dinaric chain as far as Macedonia, particularly in the lowlands.

In Croatia, 4 loci are regularly polymorphic (PX-1, PX-2, MDH-1 and IDH-1) and the other 2 less so (GOT-1, PGI-1) (table II). Allelic variations could be connected with climate for PX-1, PX-2, GOT-1 and with plant associations for PX-1, PX-2 and IDH-1 (table IV, figs 2 and 3). There is a greater difference between the 2 groups of associations than between Mediterranean and continental regions. This may be due to the existence in the Mediterranean region of an association, *Fagetum subalpinum* (4 stands) very different from the *Seslerio-Fagetum* and more hygrophilous, which tends to reduce deviations between the 2 regions. This result confirms the originality of the *Seslerio-Fagetum* which characterizes the driest stands of the Croatian beechwoods.

On this scale, either PX-1 or PX-2 appear to be subject to genetic selection following environmental changes. This observation confirms some previous results (Thiébaud *et al*, 1982; Thiébaud, 1984; Barrière *et al*, 1985; Comps *et al*, 1987): in other southern countries, we found a positive correlation between PX-2 allelic diversity and the extent of climatic variations connected with the altitudinal range of the stands. For PX-1, a positive correlation was shown between extreme climatic conditions for beech and the highest values of PX-1-105 frequency. The dry conditions to which *Seslerio-Fagetum* is subject seem to be the most unfavourable to the growth of the beech in Croatia and also correspond to the highest frequencies of the same allele in this country.

On the other hand, in Southwestern Europe where GOT-1 polymorphism is high, the frequency of the GOT-1-105 allele is significantly higher under severe climate conditions at high altitude (Comps *et al*, 1987). In Croatia, in spite of the low polymorphism of this locus, its allelic frequency

is also connected with altitudinal climatic changes (higher diversity in the highlands).

Taking into account all these observations, it should be noted that the 3 mentioned loci may be subject to selection. Koehn (1978) considered it is necessary to display the interrelations between phenotypic diversity, physiological reaction and variations of environmental factors in order to prove the adaptative signification of an enzymatic polymorphism. However, the connections between climate and allelic variations of PX-1, PX-2 and GOT-1 have been exhibited in different regions, each apart from the others. Even if the repetition of such a phenomenon does not constitute an indisputable proof of a selection effect, it is nevertheless a favourable argument (Clarke, 1975; Bergmann, 1978).

By using a greater number of loci, this study complements our previous results: in Croatia, allelic frequencies of 2/3 newly used loci (MDH-1, PGI-1) do not vary substantially as a function of environmental factors, even if MDH-1 gene diversity differs according to the region. IDH-I locus does not seem to be connected with regional climate; however, its polymorphism is significantly lower in *Seslerio-Fagetum* subject to dry conditions than in the other more mesophilous associations.

Multilocus  $F$ -statistics reveal a total genotypic differentiation which does not differ whatever the climate (table V). The intra- is higher than the inter-component, as observed by various authors (Sakai and Park, 1971; Mitton *et al*, 1977, 1981; Linhart *et al*, 1981; Knowles, 1984; Knowles and Grant, 1985; Shea, 1985; Cuguen, 1986). All  $F_{IS}$  values are positive, which implies a general tendency towards a heterozygotic deficit. These observations confirm previous results obtained throughout Europe (Cuguen, 1986). As the self-fertilization rate of beech is low (from 0 to 0.05); isolation by distance within each

population (*ie* a Wahlund effect) can mostly explain the observed deficits.

The arguments put forward in the *Introduction* suggest that there has been more opportunity for the development of genetic differentiation among and within populations close to the Mediterranean sea. To what extent do our results confirm this hypothesis?

Only the allelic frequencies of PX-1 locus differ significantly between the continental and the Mediterranean regions (table IV); in the latter the corresponding gene diversity is higher, but only at the 0.10 level. The discriminant analysis does not allow us to entirely separate one region from the other (fig 2). Allelic differentiation is not clearly higher within *Seslerio-Fagetum* which is chiefly a Mediterranean association than in the other mostly continental associations (table III). However, comparisons of allelic frequencies between the 2 groups of associations display a greater contrast than between regions (table IV).

Over the whole of Croatia, a genotypic differentiation within and among populations is clearly apparent. However, comparisons of the corresponding  $F$ -statistics values between the Mediterranean and continental regions do not display any significant deviations. Comparisons of  $F$ -statistic values between the 2 groups of associations lead to the same result. However, the lack of genotypic differences between 2 regions is probably increased for 2 reasons : 1) the methods employed may be limiting (jackknife); 2) due to a biological reality which is more complex and less perceptible at the genotypic than at the allelic level.

Finally, very few arguments confirm previous hypotheses, at least on the Croatian scale. Of course, on this scale, the time which has elapsed between the settlement

of Mediterranean and that of continental beechwoods may be insufficient. To test the validity of our hypothesis, we are carrying out a study to compare the beech stands of Croatia, a part of Yugoslavia located close to the Mediterranean, with the Slovak beech stands in central Europe. Our hypothesis will be confirmed if the genetic differentiation is found to be higher in Croatia.

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