

seed produced, and the contribution of the top clones to the overall genetic makeup of the seed produced would be less than 58 percent.

The genetic correlations between number of female flowers and the number of sound seed per ramet produced from that particular flower crop were 0.86 for 1977 and 0.88 for 1978. Clones which produced female flowers were the ones which produced the seeds, despite all the hazards which occurred between formation of the female strobilus and harvest of full seed.

There was a very low correlation among years for each clone for either sound seed yields or conelet survival. Although the correlation coefficient between years for female flowering was high ($r = 0.63$) the correlations between years for conelet survival and between years for sound seed were low.

Conclusions

The number of clones which should be included in a seed orchard is a matter of controversy. This paper does not attempt an answer, but points out the importance of considering complex interactions as well as clonal variation.

The large clonal component of variation is an important factor because many clones never produce enough seed or pollen to justify the cost of their maintenance in an orchard. The "genetic base" of the average orchard, however, may not be as narrow as this clonal variation seems to indicate. If variation in pollen production as well as variation in seed production over a period of several years is considered, the genetic base includes a large proportion of the orchard.

The prospect for identifying the poorest clones at an early stage is not promising. Although clones which are fruitful early tend to continue to be fruitful, some that show little reproductive activity in early years become fruitful later on. Strong year \times clone interactions also make it imperative that fruitfulness, including pollen production, be assessed over a period of several years.

As a consequence of year \times clone interactions in pollen production, open-pollinated seed of orchard clones could vary genetically from year to year. What might appear as family \times site or family \times planting year interactions could be partly due to pollen crop \times year interactions, which cause large differences in male parentage from year to year. This undoubtedly accounts for the seed crop year \times genotype interaction observed by C. H. LEE (1978). The same kind of variation could be expected in various kinds of "check" lots collected in different years. A more realistic evaluation of gain could be obtained by mixing seed from several years production.

Acknowledgements

I am indebted to DRS. RAY GODDARD, A. E. SQUILLACE, W. L. PRITCHETT, E. S. HORNER, and C. A. HOLLIS for help in preparation of this manuscript, and to past and present tree improvement personnel of the Southern Region of the U. S. Forest Service, JIM MCCONNELL, JOHNSEY KING, and WILL SCHOWALTER, for their cooperation.

Literature Cited

- ANONYMUS, North Carolina State University.: Twentieth annual report cooperative tree improvement and hardwood research program. N. C. State Univ., Raleigh, 48 p. (1976). — BARNES, R. L., and BENGTON, G. W.: Effects of fertilization, irrigation, and cover cropping on flowering and on nitrogen and soluble sugar composition of slash pine. *For. Sci.* 14: 172–180. (1968). — BECKER, W. A.: Manual of procedures in quantitative genetics. 2nd ed. Wash. State Univ., Pullman. 130 p. (1967). — BEERS, W. L., JR.: Industry's analysis of operational problems and research in increasing cone and seed yields. In *Seed Yield from South. Pine Seed Orchards*. J. Kraus, ed. *Proc. Colloq. Ga. For. Cent.*, p. 86–96. (1974). — BERGMAN, A.: Variation in flowering and its effect on seed cost. N. C. State Univ., Sch. For. Resour. Tech. Rep. 38, 63 p. (1968). — DANBURY, D. J.: Economic implications of selection for seed production in *radiata* pine seed orchards. *Aust. For. Res.* 5: 37–44. (1971). — FORCELLA, F.: Cone predation by pinyon cone beetle (*Cenophthorus edulis*; *Scolytidae*): dependence on frequency and magnitude of cone production. *American Naturalist* 116: 594–598. (1980). — GREENWOOD, M. S., and SCHMIDLING, R. C.: Regulation of catkin production. In *Pollen Management Handbook*. U. S. Dep. Agric. For. Serv. Gen. Tech. Rep. 20–26. (1981). — LEE, C. H.: Seed crop \times genotype interaction in Ponderosa pine. In *Proc. Fifth North Am. For. Biol. Workshop*, p. 301–305. (1978). — McLEMORE, B. F.: Strobili and conelet losses in four species of southern pines. U.S.D.A. For. Serv., South. For. Exp. Stn. Res. Note SO-226, 5 p. (1977). — SCHMIDLING, R. C.: Fruitfulness in conifers: Nitrogen, carbohydrates, and genetic control. In *Proc. Third North Am. For. Biol. Workshop*. C. C. P. Reid and G. H. Fechner, eds. p. 148–164. (1974). — SCHMIDLING, R. C.: Fertilizer timing and formulation affect flowering in a loblolly pine seed orchard. In *Proc. 13th South. For. Tree Improv. Conf.*, p. 153–160. (1975). — SCHMIDLING, R. C.: Rootstock affects growth and flowering in inter- and intra-specific loblolly pine grafts. In *Proc. Fifth North Am. For. Biol. Workshop*. Abstr., p. 419. (1978). — SCHMIDLING, R. C.: The inheritance of precocity and its relationship with growth in loblolly pines. *Silvae Genet.* 30: 188–192 (1981). — SCHULTZ, R. P.: Stimulation of flower and seed production in a young slash pine orchard. U.S.D.A. For. Serv. Southeast. For. Exp. Stn. Res. Pap. SE-91, 10 p. (1971). — SHOULDERS, E.: Fertilizer application, inherent fruitfulness, and rainfall affect flowering of longleaf pine. *For. Sci.* 13: 376–383. (1967). — STERN, K., and GREGORIUS, H. R.: Schätzungen der effektiven Populationsgrösse bei *Pinus sylvestris*. *Theor. and Appl. Genet.* 42: 107–110. (1972). — VARNELL, R. J., SQUILLACE, A. E., and BENGTON, G. W.: Variation and heritability of fruitfulness in slash pine. *Silvae Genet.* 16: 125–128. (1967). — WEBSTER, S. R.: Nutrition of seed orchard pine in Virginia. Ph. D. Thesis, N. C. State Univ. Raleigh. 185 p. (1974).

Isoenzyme Variation within and among Populations of European Black Pine (*Pinus nigra* Arnold)

By D. NIKOLIĆ¹) and N. TUCIĆ²)

(Received 12th July 1982)

¹) Maize Research Institute, 1080 Belgrade-Zemun Polje, Yugoslavia.

²) Faculty of Science, University of Belgrade, 11000 Yugoslavia. Correspondence:

DR. NIKOLA TUCIĆ, Faculty of Science, Department of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Yugoslavia.

Summary

Genetic variation in 28 natural populations of European black pine (*Pinus nigra* ARNOLD) is studied at the enzyme level. Using techniques of gel electrophoresis, we have characterized the isoenzyme patterns of esterase (Est), acid phosphatase (Acph) and leucine aminopeptidase (Lap) in dormant seeds. The samples examined are from 19 loca-

lities in Yugoslavia and nine localities in other mediterranean countries.

The study concerns the organization of isoenzyme variation throughout the following subspecies of *P. nigra* (classification based on VIDAČOVIĆ 1974): ssp. *austriaca* (7 populations), *dalmatica* (5 populations), *pallasiana* (4 populations), *gočensis* (4 populations), *marocana*, *Fenzlii*, *corsicana*, *laricio*, *Villetta Barrea* (all with one population) and two populations from Cyprus.

The mean value for all samples of the average expected heterozygosity over four loci is 27.2%. The average number of alleles detected per locus is 3.02.

Analysis of population subdivision using NEI's (1973) statistics indicates that heterogeneity within populations is higher than heterogeneity between populations or subspecies.

The genetic distances (NEI 1972) associated with various stages of evolutionary divergence are calculated. The average genetic distance for all 378 pairwise population comparisons of *P. nigra* is 0.069 ± 0.004 . The average genetic distance between pairs of recognized subspecies ranges from 0.017 (e.g. for ssp. *Villetta Barrea* and *Fenzlii*) to 0.389 (for ssp. *marocana* and *laricio*) with the mean value for all 55 pairwise comparisons of 0.111 ± 0.01 .

Differences in allelic frequencies are statistically significant among the localities examined, but fail to show any decisive geographic patterning. ROYALTEY *et al.* (1975) tests and autocorrelation analyses, both based upon Gabriel connected-graphs for 19 localities from Yugoslavia, are used to determine whether the distribution of gene frequencies depart from a random pattern. Significant deviations are absent in Yugoslavian localities of *P. nigra*. Different models of population differentiation are discussed.

Key words: *Pinus nigra*, isoenzyme, geographic variation.

Zusammenfassung

In 28 natürlichen Populationen wurde die genetische Variation der europäischen Schwarzkiefer *Pinus nigra* ARNOLD auf dem Enzym-Niveau untersucht. Unter Verwendung der Gel-Elektrophorese-Technik haben wir die Isoenzym-Muster der Esterase (Est), der Acid-Phosphatase (AcpH), sowie der Leucin-Aminopeptidase (Lap) in ruhenden Samen charakterisiert. Die untersuchten Proben stammen von 19 Standorten in Jugoslawien und 9 Standorten in anderen Mittelmeerländern. Die Untersuchung bezieht sich auf die Ausbildung der Isoenzym-Variation in den folgenden Unterarten von *Pinus nigra* (Klassifikation nach VIDAČOVIĆ 1974): ssp. *austriaca* (7 Populationen), *dalmatica* (5 Populationen), *pallasiana* (4 Populationen), *gočensis* (4 Populationen), *marocana*, *Fenzlii*, *corsicana*, *laricio*, *Villetta Barrea* mit je einer Population und 2 Populationen aus Zypern.

Der Mittelwert für alle Proben für die im Mittel zu erwartende Heterozygotie über alle 4 Loci beträgt 27,2%. Die mittlere Anzahl der beobachteten Allele beträgt pro Locus 3,02. Die Analyse zur Unterteilung der Populationen nach Anwendung von NEI's (1973) statistischem Verfahren ergab, daß die Heterogenität innerhalb der Populationen höher ist, als die zwischen den Populationen oder Unterarten. Die genetischen Distanzen (NEI 1972), verbunden mit verschiedenen Stadien evolutionärer Abweichung, wurden berechnet. Die mittlere genetische Distanz für alle 378 paarweisen Populationsvergleiche von *Pinus nigra* beträgt 0.069 ± 0.004 . Der Rang für die mittlere genetische Distanz zwischen den Paaren bekannter Unterarten reicht von 0.017 (für ssp. *Villetta Barrea* und *Fenzlii*) bis 0.389 (für ssp. *marocana* und *laricio*) mit einem Mittelwert von 0.111 ± 0.01 für alle 55 paarweisen Vergleiche.

Unterschiede in den Allelhäufigkeiten sind zwischen den untersuchten Standorten statistisch signifikant, aber es

zeigte sich kein eindeutiges geographisches Muster.

Die Tests von ROYALTEY *et al.* (1975) und Autokorrelationsanalysen, die beide auf Gabriels graphischer Methode basieren und bei 19 Standorten in Jugoslawien benutzt wurden, dienen der Bestimmung der Abweichung der Genfrequenzen von einer Zufallsverteilung. Signifikante Abweichungen waren bei jugoslawischen Herkünften von *Pinus nigra* nicht vorhanden. Verschiedene Modelle zur Unterscheidung von Populationen werden diskutiert.

Introduction

European black pine (*Pinus nigra* ARNOLD) inhabits a discontinuous area which includes Southern Europe, North-Western Africa and Asia Minor. In Yugoslavia the distribution of black pine includes the Julian Alps, the Dinaric Alps (including the Dalmatian islands) and old mountains in central and south-east regions of Bosnia, Serbia, Montenegro and Macedonia. According to MIROV (1967) and VIDAČOVIĆ (1974) *P. nigra* is a Tertiary relict which during Pliocene inhabited the same localities where it occurs at present. Despite of its somewhat narrow geographic distribution, black pine is not an environmentally very restricted species. It grows in extreme xeric and humid habitats with considerable tolerance of temperature fluctuations.

Black pine is subdivided into several distinct subspecies which are largely allopatric or parapatric (BLEČIĆ 1967, VIDAČOVIĆ 1974 and references therein). According to VIDAČOVIĆ (1974) there is no general consensus on *P. nigra* taxonomy; especially on the variability and taxonomy in the Balkan Peninsula. Reproductive barriers to gene exchange are weak and there are transition forms between subspecies (VIDAČOVIĆ 1974). The integrity of the species seems to be due to ecogeographical isolation.

Despite of difficulties mentioned, our analyses are based on *a priori* identification of taxa. This study deals with the organization of isoenzyme variation throughout the

Table 1. — Localities of *P. nigra* collections.

Sample number	Locality	Number of trees	subspecies
1	Brač, dalmatian island, Yug.	51	dalmatica
2	Hvar, dalmatian island, Yug.	55	dalmatica
3	Pelješac I, Croatia, Yug.	50	dalmatica
4	Pelješac II, Croatia, Yug.	50	dalmatica
5	Senj I, Croatia, Yug.	50	dalmatica
6	Senj II, Croatia, Yug.	50	austriaca
7	Split, Croatia, Yug.	53	austriaca
8	Knin, Croatia, Yug.	55	austriaca
9	T.Korenica, Croatia, Yug.	52	austriaca
10	Vrhovine, Croatia, Yug.	50	austriaca
11	Višegrad, Bosnia, Yug.	50	gočensis
12	Kranj, Slovenia, Yug.	14	austriaca
13	Valjevo, Serbia, Yug.	50	gočensis
14	Kraljevo, Serbia, Yug.	57	gočensis
15	T. Užice, Serbia, Yug.	72	gočensis
16	Kavadarci, Macedonia, Yug.	53	pallasiana
17	Berovo, Macedonia, Yug.	51	pallasiana
18	Bitolj I, Macedonia, Yug.	52	pallasiana
19	Bitolj II, Macedonia, Yug.	50	pallasiana
20	prov. Talassentane, Morocco	++	marocana
21	Corsica, France	++	corsicana
22	Kles, South Tirol, Italy	++	austriaca
23	Anjone, Molize, Italy	++	Villetta Barrea
24	Lasila, Calabria, Italy	++	laricio (calabrica)
25	Cyprus I	++	?
26	Cyprus II	++	?
27	Gölcük, Dursunbey, Turkey	++	Fenzlii
28	Crimea, USSR	++	pallasiana (?)

** mass seed sample.

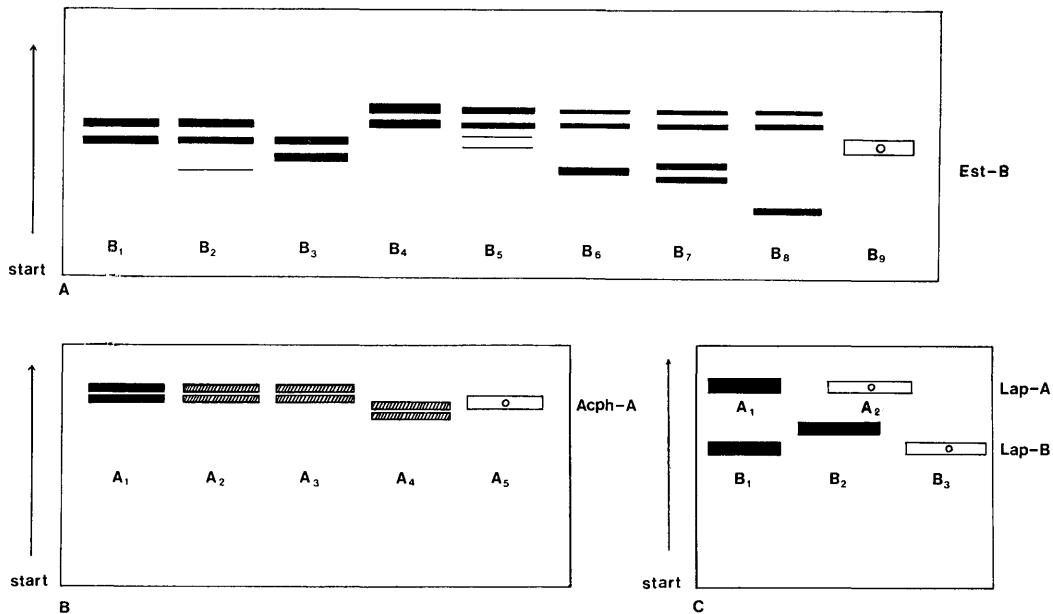


Figure 1. — Schematic diagram of electrophoretic phenotypes for esterase (A), acid phosphatase (B), and leucine aminopeptidase (C) in *Pinus nigra*.

following subspecies (classification based on VIDAKOVIĆ 1974, and VIDAKOVIĆ personal communication): ssp. *austriaca* (Austria, Northern Italy, and in Yugoslavia it inhabits Slovenia and western Croatia), ssp. *dalmatica* (Dalmatian seaboard and islands), ssp. *pallasiana* (Macedonia), ssp. *gočensis* (Serbia, Bosnia, Montenegro, Northern Macedonia, Croatia). According to VIDAKOVIĆ, in Morocco and Asia Minor, there are probably two subspecies: ssp. *marocana* and ssp. *Fenzlii*. Subspecies *corsicana* inhabits the island of Corsica (France), whereas the pine known as "Calabrian" (Italy) belongs to ssp. *laricio*. Italy is also inhabited by the Abruzzian pine known as *Villetta Barrea*, which is "between Calabrian and Austrian pine" (VIDAKOVIĆ 1974). In the present study two populations from Cyprus are also included, and one population sample from Crimea (in Flora Europea 1964, the Crimea population belongs to ssp. *pallasiana*).

The primary objectives of this study were: 1. to analyse the genetic variation in *P. nigra*, 2. to examine the extent of genetic diversity within and between recognized subspecies, 3. to describe geographical variation patterns for corresponding alleles and genetic diversity, 4. to determine the relationship between the patterns of micro- and macro-differentiation for isoenzymes. We hope that these kinds of information will be of use to both experimental population biologists trying to understand the generation and maintenance of genetic variability and to treebreeders and foresters involved in domestication efforts.

Material and Methods

Electrophoresis was carried out on dormant seed haploid endosperms of black pine. The seeds were gathered from individual trees of the representative localities of this species throughout Yugoslavia as well as a sample of some Mediterranean areas. The localities of black pine collections are shown in Table 1.

Isoenzyme patterns of *esterase* (Est), *acid phosphatase* (Acph) and *leucine aminopeptidase* (Lap) were examined on about 100 endosperm samples of the individual trees. The analyses of isoenzyme variability of black pine from

Yugoslav localities was done on 100 or more zymograms, based on single tree collections of seeds the sizes of the samples are given in Table 1. In the localities out of Yugoslavia these analyses were done on random samples of 100 or more macrogametophytes from mass collections.

Techniques of electrophoresis and protein staining were the same as those described in BERGMANN (1974, 1975) and NIKOLIĆ and BERGMANN (1974).

Since endosperm in conifers is haploid, it is possible to assess alleles directly to each isoenzyme phenotype in the zymogram. The distribution of the isoenzyme phenotype in the seed samples of individual trees was examined with regard to 1 : 1 segregation ratio assuming a one-locus, two-alleles system with randomly distributed gametes after meiosis. Chi-square tests for goodness of fit were used in the analysis of the segregation of alleles in endosperms. All chi-square tests comprising one degree of freedom were subjected to Yates' correction.

Genetic analyses of isoenzymes in *P. nigra* for each enzyme system were as follows:

Esterases (Est), in the endosperm of dry black pine seeds, could be identified as being determined by two polymorphic gene loci which on zymograms are designed as Est-A and Est-B (Figure 1a). However, only one of these loci (Est-B) shows a relatively high enzyme activity and sufficient banding repeatability. It was found that Est-B possesses nine different alleles, which are phenotypically expressed in one or several bands. Alleles Est-B₉ is a "null" allele. The inheritance of each of the observed phenotypes, checked by a chi-square analysis, gives a clear 1 : 1 segregation ratio in all cases (Table 2).

The *acid phosphatase* (Acph) enzyme system in black pine shows only one zone of activity, which correspond to one gene locus (Figure 1b). This locus has five alleles with two bands, or no band expresses as a phenotypic form on zymograms. Acph-A₅ has no bands, i.e. this is a "null" allele. Segregation of the different phenotypes also show a 1 : 1 ratio (Table 2).

Leucine aminopeptidase (Lap) as in earlier study of *P. nigra* (NIKOLIĆ and BERGMANN 1974), shows two zones of

Table 2. — Frequency distribution of different isoenzyme phenotypes in different loci in seed samples of individual trees.

Locus	tree no.	Frequency distribution of the isoenzyme phenotypes	chi-square	P
Est-B				
	D ₇	B ₁ : B ₂ - 39 : 43	.11	.7 - .8
	D ₁₇	B ₁ : B ₃ - 49 : 55	.24	.5 - .7
	172	B ₁ : B ₄ - 49 : 51	.01	.9 - .95
	D ₂₂	B ₁ : B ₅ - 48 : 41	.41	.5 - .7
	B ₃₈	B ₁ : B ₆ - 62 : 57	.13	.7 - .8
	D ₁₅	B ₁ : B ₇ - 57 : 44	1.43	.2 - .3
	182	B ₁ : B ₈ - 49 : 30	4.1	.01 - .05
	147	B ₁ : B ₉ - 29 : 29	.00	1.0
	154	B ₂ : B ₉ - 29 : 41	1.73	.2 - .1
	188	B ₆ : B ₈ - 27 : 39	1.83	.1 - .2
Acph-A				
	183	A ₁ : A ₂ - 39 : 41	.01	.9 - .95
	180	A ₁ : A ₃ - 51 : 41	.88	.3 - .5
	169	A ₁ : A ₄ - 39 : 43	.11	.7 - .8
	189	A ₁ : A ₅ - 49 : 41	.54	.3 - .5
Lap-A				
	K ₁	A ₁ : A ₂ - 39 : 23	.51	.3 - .5
Lap-B				
	K ₇	B ₁ : B ₂ - 28 : 23	.31	.5 - .7
	K	B ₁ : B ₃ - 22 : 26	.19	.5 - .7

activity with corresponding loci (Lap-A and Lap-B). Lap-A has two alleles (one banded Lap-A₁ and "null" allele Lap-A₂) and Lap-B has three alleles (Lap-B₁, Lap-B₂ and "null" allele Lap-B₃). Segregation analyses show that in individual heterozygous trees during meiosis, macrogame-

tophytes (endosperm) with alternative alleles were produced in 1 : 1 ratio (Table 2).

These analyses indicate that electrophoretically detectable bands actually are markers for mutant genes in corresponding gene loci.

In the present paper, estimates of genetic variability were obtained by calculating several common variability measures based on allele frequencies of four enzyme loci of black pine. The statistic most commonly used to assess genetic variation within population has been the expected proportion of heterozygosity (H_e) in a randomly mating population. An alternative way of representing genetic variation within population is, according to PIERCE and MITTON (1979), the allelic heterozygosity (h_a): $h_a = 2p(1 - p)$, where p equals the frequency of the allele in the population. If a locus possesses only two alleles, then the heterozygosity for those alleles will be equal and they will also equal the heterozygosity per locus.

Heterogeneity in allele frequencies and subdivision between populations was analysed using the H_T , H_S and G_{ST} statistics of NEI (1973, 1975). If the gene diversity is defined as the heterozygosity expected under Hardy-Weinberg proposals, genetic variation and differentiation between populations within subspecies and species can be analysed. Total gene diversity expected for the sampled populations of a subspecies or a species is $H_T = H_S + D_{ST}$, where H_S is the average gene diversity within subpopulations. H_S is calculated as the average heterozygosity of all subpopulations sampled, while D_{ST} is obtained as the difference $H_T - H_S$. The relative magnitude of gene differentiation of interpopulation gene diversity (NEI's "coefficient of gene differentiation") is obtained as $G_{ST} =$

Table 3. — Allele frequencies at the different loci in 28 populations of *P. nigra*.

Locality	Est-B									Total No.
	B ₁	B ₂	B ₃	B ₄	B ₅	B ₆	B ₇	B ₈	B ₉	
1	.680	.100	.090	.020	.060	.050				100
2	.582	.221	.074	.041		.074			.008	122
3	.800	.040	.070	.010		.050	.020		.010	100
4	.637	.118	.019	.098	.010	.118				102
5	.667	.176	.118	.020		.020				102
6	.570	.180	.130	.030			.090			100
7	.690	.267	.017	.090					.017	116
8	.555	.148	.180		.008	.039	.047		.023	128
9	.676	.120	.046			.139	.018			108
10	.703	.089	.040		.030	.049	.089			101
11	.564	.119	.218			.069	.020		.010	101
12	1.000									28
13	.574	.139	.129		.020	.059	.079			101
14	.652	.094	.080	.036		.044	.094			138
15	.584	.143	.113	.035		.017	.108			231
16	.580	.080	.170			.071	.098			112
17	.408	.223	.087	.049	.029	.136	.029	.039		103
18	.632	.226	.047	.009		.057	.019	.009		106
19	.580	.140	.053	.100		.050	.030	.050		100
20	.060	.070	.410	.440					.020	100
21	.311	.049	.602	.019					.019	103
22	.625	.048	.135	.048		.029	.115			104
23	.760	.070	.132	.031		.008				129
24	.743	.109	.010	.079		.020	.010			101
25	.214		.571	.214						126
26	.470		.500	.030						134
27	.598	.167	.069	.020		.108		.039		102
28	.604	.028	.245	.009		.057	.009		.047	106

Table 3. — Continued.

Locality	AcpH-A					Total no.	Lap-A		Lap-B			Total No.
	A ₁	A ₂	A ₃	A ₄	A ₅		A ₁	A ₂	B ₁	B ₂	B ₃	
1	.431	.392	.108		.069	102	1.000		1.000			106
2	.040	.337	.455		.168	101	1.000		1.000			104
3	.340	.340	.160		.160	100	.962	.038	.981	.020		104
4	.710	.290				100	.962	.038	.981	.020		104
5	.126	.146	.379	.019	.330	103	1.000		.990	.010		100
6	.846	.128	.029			102	1.000		1.000			104
7	.743	.188	.069			101	1.000		.980	.020		100
8	.382	.500	.118			102	.991	.009	1.000			108
9	.598	.284	.079	.039		102	1.000		1.000			101
10	.824	.088	.039	.049		102	1.000		.990	.010		100
11	.921	.079				101	.990	.010	.941	.059		101
12	1.000					28	1.000		1.000			28
13	.465	.396		.139		101	1.000		.970	.030		100
14	.880	.040	.040	.040		101	1.000		1.000			103
15	.710	.210	.040	.040		100	1.000		1.000			103
16	.436	.337	.168		.059	101	1.000		.932	.019	.05	103
17	.475	.277	.149	.040	.059	101	1.000		1.000			103
18	.873	.078	.020		.029	102	1.000		.932	.068		103
19	.860	.032	.108			93	1.000		1.000			100
20	.769	.154	.029		.048	100	.750	.250	.981	.020		104
21	.770		.170	.020	.040	100	1.000		.900	.060	.040	100
22	.504	.248	.248			101	1.000		1.000			104
23	.436	.386	.049	.079	.050	101	1.000		.952	.019	.030	104
24		.206	.451		.343	102	1.000		.943	.019	.040	104
25	.210	.290	.250	.080	.170	100	1.000		1.000			104
26			.460	.080	.460	100	1.000		1.000			104
27	.560	.250	.130		.060	100	.979	.021	.986		.014	141
28	.289	.538	.087	.038	.048	104	1.000		1.000			162

D_{ST}/H_T and can be used as an index of allele frequency heterogeneity.

Genetic relationships between local populations and subspecies were estimated using Nei's index (NEI 1972, 1975) of normalized genetic identity (I) and distance (D).

Genetic variation between populations within *P. nigra* species can be examined further by the variance in allelic frequencies between local populations (S^2_A ; PIERCE and MITTON 1979):

$$S^2_A = \frac{1}{n-1} \sum_{j=1}^n (p_j - \bar{p})^2$$

where p_j is the allelic frequency in population j , n is the number of local populations and \bar{p} is the unweighted average frequency for all populations. If only two alleles exist at a particular locus, then the variance for those alleles will be equal; but in the case of more than two alleles, the variance of allelic frequency will vary between alleles of a single locus.

An additional measure of population diversity which we use in the present paper is the standardized variance. The standardized allelic variance, based on the variance of allelic frequency, PIERCE and MITTON (1979), is calculated as follows:

$$F_{ST} = \frac{S^2_A}{p(1-p)}$$

According to PIERCE and MITTON (1979), the standardized and unstandardized variance of allelic frequency and allelic heterozygosity (h_a) can be employed in an analysis of the relationship between intra- and interpopulation genetic variation.

Results

Three enzyme systems encoded by four genetic loci were examined in 28 populations of several described subspecies of *Pinus nigra*. Estimates of allelic frequencies for each locus in the 28 populations are presented in Table 3.

Individual enzyme systems differed in their geographic variation and contribution to differentiation between local populations. Only the population from Kranj (sample # 12) was monomorphic for all analysed loci. At the Lap-A locus, variant alleles were present in substantial frequency only in the population from Morocco (sample # 20). In most of the remaining populations, Lap-A had only one allele. At the Lap-B locus only rare alleles were detected which contributed to the fixed differences between populations.

The Est-B and AcpH-A loci were highly polymorphic, and effectively differentiated among the populations of black pine. In the majority of the populations at the AcpH-A locus "null" alleles were present. At this locus only populations of the subspecies *austriaca* and ssp. *gočensis* did not contain any "null" alleles. Exceptionally high frequencies of "null" alleles were observed in populations of the subspecies *dalmatica*, two populations from Cyprus (samples # 25 and 26) and the subspecies *laricio* (sample # 24).

Samples of 28 populations of black pine have been assayed for genetic variation. We use four measures of genetic variability: 1. proportion of polymorphic loci (P); 2. average number of alleles detected per locus (A/L); 3. expected heterozygosity per locus (h_e) and 4. expected heterozygosity averaged over all loci (H_e). The results are summarized in Table 4.

Table 4. — Value of different measures of genetic variation in samples from 28 populations of *P. nigra*.

Locality	h_e				H_e	P	A/L
	Est-B	Acph-A	Lap-A	Lap-B			
1	.531	.664	.000	.000	.289	.50	3.00
2	.600	.650	.000	.000	.312	.50	3.00
3	.350	.718	.073	.037	.294	1.00	3.75
4	.509	.412	.073	.037	.258	1.00	3.00
5	.509	.710	.000	.020	.309	.75	3.25
6	.617	.267	.000	.000	.221	.50	2.50
7	.444	.408	.000	.039	.223	.75	2.75
8	.633	.590	.018	.000	.310	.75	3.25
9	.507	.554	.000	.000	.265	.50	2.75
10	.486	.309	.000	.020	.203	.75	3.25
11	.615	.146	.020	.111	.223	1.00	3.00
12	.000	.000	.000	.000	.000	.00	1.00
13	.624	.608	.000	.058	.322	.75	3.00
14	.548	.221	.000	.000	.192	.50	3.00
15	.612	.449	.000	.000	.265	.50	3.00
16	.613	.665	.000	.129	.351	.75	3.25
17	.752	.670	.000	.000	.356	.50	3.75
18	.543	.230	.000	.127	.225	.75	3.50
19	.626	.248	.000	.000	.218	.50	3.00
20	.629	.382	.375	.037	.356	1.00	3.25
21	.538	.376	.000	.185	.275	.75	3.25
22	.573	.623	.000	.000	.299	.50	2.75
23	.399	.650	.000	.093	.286	.75	3.50
24	.428	.754	.000	.109	.323	.75	3.25
25	.582	.774	.000	.000	.339	.50	2.50
26	.528	.570	.000	.000	.274	.50	2.00
27	.596	.603	.041	.028	.317	1.00	3.50
28	.569	.616	.000	.000	.296	.50	3.50
Mean	.534	.495	.021	.037	.272	.661	3.02
± S.E.	±.03	±.04	±.01	±.01	±.01	±.04	±.10

On the average, 66.1% of the analyzed loci are polymorphic (P) in populations of this species. The mean value for all samples of the average expected heterozygosity over all loci is 27.2%.

The average number of alleles detected per locus ranges from one in the Kranj population (sample # 12) to 3.75 in Pelješac I (sample # 3) and Berovo (sample # 17) with the mean value for all studied populations of 3.02 alleles per locus.

The mean values of P, H_e and A/L over the 28 populations of black pine indicate a relatively high degree of interpopulational genetic variability. However, roughly equivalent levels of genetic variation to those of black pine population have been found for other gymnosperm populations (HAMRICK *et al.* 1979; for eleven gymnosperm species: P = 67%, H_e = 0.27, A/L = 2.12).

Table 5 presents the normalized genetic distance for all possible pairs of 28 populations (i.e. 378 combinations). According to NEI (1972) the normalized genetic identity (I) is an estimate of the proportion of alleles that control electrophoretically identical proteins in pairs of populations. Genetic distance (D) estimate the average number of substitutions per locus since the divergence of two populations. The average genetic distance for all pairwise population comparisons of *P. nigra* is 0.069 ± 0.004 .

In order to estimate the amount of genetic differentiation among the described subspecies of *P. nigra*, we have averaged genetic distance over appropriate interpopulation comparisons. As can be seen from Table 6, the average genetic distance between pairs of recognized subspecies ranges from 0.017 for *Villetta Barrea* and *Fenzlii*

Table 5. — Genetic distances between 28 populations of *P. nigra*.

Population	Population													
	15	16	17	18	19	20	21	22	23	24	25	26	27	28
15	.02	.04	.01	.01	.13	.07	.02	.03	.16	.14	.22	.01	.06	
16	.02	.06	.05	.18	.10	.01	.01	.08	.09	.14	.01	.02		
17	.05	.04	.14	.10	.02	.04	.11	.09	.16	.01	.04			
18	.01	.15	.08	.05	.06	.21	.21	.28	.03	.12				
19	.12	.07	.04	.06	.19	.18	.25	.02	.11					
20	.09	.16	.21	.39	.13	.31	.16	.21						
21	.09	.13	.27	.09	.17	.10	.14							
22	.02	.09	.10	.12	.01	.03								
23	.03	.13	.05	.01	.11	.01	.08	.03	.15	.07	.10	.09		
24	.01	.06	.03	.04	.07	.06	.05	.09	.25	.07	.13	.08		
25	.01	.09	.02	.01	.07	.02	.01	.03	.10	.26	.17	.15		
26	.05	.17	.06	.01	.13	.01	.01	.07	.02	.11	.27	.04		
27	.07	.21	.10	.03	.17	.01	.02	.09	.04	.01	.12	.28		
28	.08	.24	.09	.04	.18	.03	.03	.13	.05	.02	.03	.13		
1	.01	.09	.03	.02	.09	.04	.03	.01	.02	.04	.06	.09	.14	
2	.06	.09	.08	.02	.14	.01	.02	.08	.03	.002	.01	.02	.05	
3	.03	.13	.05	.01	.11	.01	.01	.04	.01	.01	.02	.04	.02	.01
4	.01	.06	.02	.03	.06	.04	.04	.01	.01	.04	.06	.09	.01	.05
5	.02	.07	.04	.03	.07	.04	.03	.02	.02	.05	.06	.11	.02	.05
6	.06	.18	.08	.02	.15	.01	.01	.08	.03	.01	.01	.02	.05	.01
7	.06	.17	.08	.02	.14	.01	.01	.08	.03	.01	.01	.03	.06	.01
8	.20	.32	.25	.14	.30	.12	.16	.18	.18	.16	.12	.23	.18	.14
9	.13	.23	.16	.11	.18	.06	.10	.13	.11	.09	.05	.13	.12	.08
10	.01	.06	.02	.03	.06	.03	.03	.02	.01	.03	.05	.07	.03	.04
11	.01	.08	.01	.03	.07	.05	.03	.02	.01	.04	.07	.07	.01	.06
12	.08	.002	.05	.16	.01	.20	.15	.10	.12	.18	.24	.23	.13	.20
13	.12	.11	.13	.17	.11	.17	.18	.09	.15	.20	.18	.29	.11	.20
14	.16	.08	.13	.26	.05	.25	.25	.17	.21	.25	.27	.32	.19	.26
15	.01	.08	.002	.01	.07	.02	.01	.02	.003	.02	.04	.06	.02	.03
16	.02	.06	.02	.06	.05	.09	.08	.01	.04	.10	.11	.15	.02	.11

Table 6. — Mean genetic distance among subspecies of *P. nigra*.

	subspecies				
	Cyprus	Villette Barrea	laricio	Fenzlii	Crimea
corzicana	.133	.131	.269	.095	.143
subspecies	Cyprus	.148	.110	.149	.112
austriaca	Villette Barrea		.091	.018	.017
	dalmatica		laricio	.103	.094
dalmatica	.069		gočensis	Fenzlii	.039
gočensis	.029	.080		pallasiana	Crimea
pallasiana	.033	.067	.030		marocana
marocana	.169	.241	.140	.147	
corzicana	.102	.162	.078	.087	.085
Cyprus	.196	.132	.195	.175	.218
Villette Barrea	.036	.037	.041	.043	.209
laricio	.152	.061	.182	.147	.389
Fenzlii	.021	.033	.023	.018	.157
Crimea	.072	.039	.076	.072	.209

to 0.389 for *marocana* and *laricio*. Thus, several recognized subspecies, using the classical taxonomic approach, are not distinct with respect to electrophoretically detectable enzyme markers. This result is not uncommon (see e.g. AYALA 1975) but a very limited number of loci in our work (only 4) may have contributed to our observations.

Genetic relationships among the described subspecies, using the distance coefficients, are presented in a dendrogram (Figure 2). According to UPGMA analysis, the *P. nigra* subspecies complex can be divided into three major groups. Group I: *corsicana* and *marocana* form a related subspecies complex with an average genetic distance of 0.085. Group II: *laricio* clustered with populations which inhabit Cyprus with $\bar{D} = 0.11$. Group III: The average genetic distance between the remainder subspecies is only 0.06. It is remarkable that in the third group, populations classified as *austriaca*, *gočensis*, *pallasiana* and *Fenzlii* subspecies form one „subgroup“ (which inhabits inland mountains), whereas the *Villette Barrea*, population from Crimea and *dalmatica* form another “subgroup” of subspecies, inhabiting the central region of the Italian and Yugoslav seaboard, the neighbouring islands, as well as the Crimea.

Genetic variation and differentiation among populations within subspecies can be examined further by analysing intra- and interpopulation components of gene diversity, when the later is defined as the heterozygosity expected under Hardy-Weinberg proposals. Since a sufficient number of local populations was sampled only for *austriaca*, *dalmatica*, *pallasiana* and *gočensis* our interpopulation

Table 7. — Gene diversity parameters in *P. nigra* subspecies. The number of populations is in parentheses.

Taxa	H_S	H_T	D_{ST}	G_{ST}
<i>P.n. austriaca</i> (7)	.2174	.2433	.0259	.1064
<i>P.n. dalmatica</i> (5)	.2951	.3225	.0296	.0910
<i>P.n. pallasiana</i> (4)	.2877	.3085	.0208	.0673
<i>P.n. gočensis</i> (4)	.2507	.2662	.0155	.0583
All <i>P.nigra</i> populations (28)*	.2701	.3259	.0559	.1351

* unweighted mean over all loci.

analysis was limited to those subspecies groups which are presented in Table 7, along a comparison of all black pine populations.

Table 7 indicates that intrapopulation estimates of gene diversity (H_S) were relatively high for all analysed subspecies. Even when the species as a whole is considered (all 28 populations), intrapopulation gene diversity was still considerably high: 0.27 (or 86% of total gene diversity of the species). Evidently, heterogeneity within populations was higher than heterogeneity among population even when all populations were treated collectively. The coefficient of differentiation (G_{ST}) of about 0.14, observed for all populations, suggests that subdivision among *P. nigra* subspecies is not strong.

The above results concern the apportionment of gene frequencies within and among populations within the taxonomic framework. A complementary and equally important problem is analysis of the possible relationship between variation within and among populations. In order to determine whether significant correlation exists between intrapopulation and interpopulation variabilities, the procedure suggested by PIERCE and MITTON (1979) is employed. SOKAL (1978) has called such correlation the “Kluge-Kerfoot phenomenon”. To test for the Kluge-Kerfoot phenomenon in gene frequencies of black pine, we

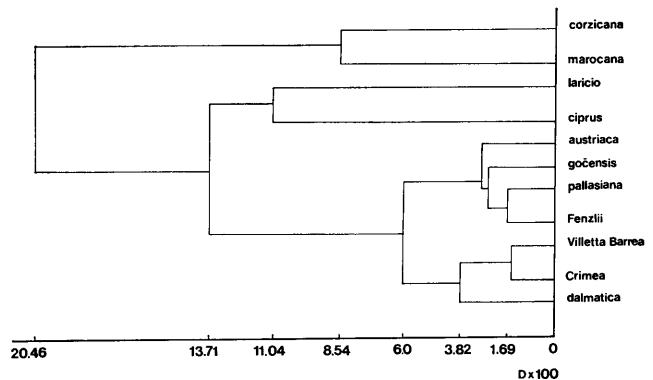


Figure 2. — A dendrogram, based on UPGMA clustering, of *Pinus nigra* subspecies using the genetic distances of NEI (1972).

have used two sets of statistics proposed by PIERCE and MITTON: 1. heterozygosity per allele (\bar{h}_a) and the variance of allelic frequency (S^2_A) and 2. heterozygosity per allele and standardized variance (F_{ST}).

In determining the pattern of relationship between the parameters of within population variation (\bar{h}_a , a weighted allelic heterozygosity over all populations) and the two sets of measures of between population variation, we have employed a Spearman's rank order correlation coefficient. The correlation coefficient between allelic heterozygosity and the unstandardized allelic variance is positive and highly significant ($r_s = 0.84$; $P < 0.01$). A similar trend exists between \bar{h}_a and F_{ST} . In these comparisons, the correlation coefficient is somewhat lower than those calculated with \bar{h}_a and S^2_A , but is still positive and highly significant ($r_s = 0.64$; $P < 0.01$).

The last point which could be, to some extent, analysed in black pine populations is the geographic variation pattern in gene frequencies. In this respect only populations from Yugoslavia (samples # 1—19) are analysed.

The statistical significance of the heterogeneity of gene frequencies of black pine from Yugoslavia was tested by

the chi-square test developed by WORKMAN and NISWANDER (1970; since the gene frequencies are determined in the seed samples, we use the formula $\chi^2 = N \sigma_p^2 / \bar{p} \bar{q}$, i.e. N insted of 2N). Chi-square values for the most common alleles at the Est-B and Acp-A loci are as follows: Est-B₁ 128.97 (P < 0.01); Est-B₂ 84.65 (P < 0.01); Est-B₃ 89.77 (P < 0.01); Acp-A₁ (P < 0.01); Acp-A₂ 241.63 (P < 0.01).

However, statistical heterogeneity of gene frequencies "does not necessarily imply geographic pattern" (SOKAL and ODEN 1978b pp 231). In order to determine whether spatial patterns between populations depart significantly from random, the statistical method of ROYALTEY *et al.* (1975) is employed. According to this method, population-localities are vertices in a graph the edge of which between sites are connections based on the Gabriel criterion of geographic contiguity (GABRIEL and SOKAL 1969). For each population, ranked frequencies of a given allele were assigned. For each edge, rank differences were calculated and observed and expected means of absolute differences between ranks along edges were compared with expectations of random patterning.

The results of this analysis are given in Table 8. As it can be seen from Table 8, for all analysed allele frequencies there are not statistically significant departures of mean length from the expected values. Significant deviation toward high value of mean length is evident only for the mean heterozygosities over all loci (H_e ; $t_s = 4.54$, P < 0.01). Since this is the only variable with significant departure of mean length it has been of considerable interest to consider these values in more refined way. The technique resembling ROYALTEY *et al.* (1975) statistics is spatial autocorrelation analysis (SOKAL and ODEN 1978a). Both statistics are based on the Gabriel criterion of geographic contiguity, but „autocorrelation seems to be a finer tool for picking up patterns, whereas the Royaltey-Astrachan-Sokal test seems to be a more conservative test for departure from randomness" (SOKAL and ODEN 1978a pp 222).

The autocorrelation coefficients for gene frequencies are Moran's I statistics. These statistics are analogous to the conventional product moment correlation coefficients and vary from -1 to +1. Positive autocorrelation predicts homogeneity or equality of gene frequencies; negative autocorrelation predicts a heterogeneity or dissimilarity.

The autocorrelation coefficients for 19 populations of

Table 8. — Statistics for expected and observed distribution of allele frequencies and heterozygosities (H_e) over Gabriel-connected graph. Only 19 populations from Yugoslavia (samples # 1–19) were analysed.

Allele	Statistics		
	\bar{L}	s	t_s
Expected	6.67	4.34	
Est-B ₁	6.72	2.23	.022
Est-B ₂	7.11	.68	.611
Est-B ₃	7.00	2.42	.125
Acp-A ₁	6.67	2.33	.012
Acp-A ₂	6.14	.66	.767
H_e	9.91	.71	4.54*

\bar{L} = mean edge length; s = standard deviation of edge length;
 t_s = is distributed as t with α df
 * = p < 0.01

Table 9. — Autocorrelation analysis. Moran's I coefficient for allele frequencies and heterozygosities (H_e) over 19 populations of *P. nigra* in Yugoslavia.

Alleles	I	I _{.05}	
Est-B ₁	-0.040	0.346	n.s.
Est-B ₂	-0.326	-0.397	n.s.
Est-B ₃	-0.311	-0.399	n.s.
Acp-A ₁	-0.065	-0.401	n.s.
Acp-A ₂	-0.007	-0.403	n.s.
H_e	-0.134	-0.477	n.s.

black pine from Yugoslavia are presented in Table 9. As we can see, all autocorrelation coefficients are negative, but statistically non-significant (for the formula of proper significance test see SOKAL and ODEN 1978a).

Discussion

Geographic patterns of protein variation in plant species have recently been the subject of considerable interest and analysis (see BROWN 1979, for recent review and references). It has been demonstrated that genetic variation in plants is distributed in nonrandom patterns within and between populations (SCHAAL 1975, ALLARD 1975, LEVIN 1977). There are also examples of clinal variation of isoenzymes in conifers (TIGERSTEDT 1974, BERGMANN 1978, LUNDKVIST 1979). But, several authors have failed to find any departure from random spatial patterns (LEVIN 1978, NEVO *et al.* 1979).

In the present study an attempt is made to discern the organization of isoenzyme variation throughout the natural range of the Mediterranean endemic conifer, *Pinus nigra*. The black pine that we have analysed is distributed in an area that has recently undergone considerable climatic and topographic change. According to VIDAKOVIĆ (1974), this pine comes from Southeast Asia, and during the Southward migration of glaciation towards the Mediterranean region most Tertiary pines of northern parts of Europe disappeared. In addition, black pine during postglacial periods was an important pioneer species in some parts of the Mediterranean region. These factors could be important determinants of the pattern of genetic differentiation observed in *P. nigra*.

Black pine exhibits a pattern of genetic variability (Table 7) characterized by rather high intrapopulation variation ($H_S = 0.27$) and a moderate degree of interpopulation genetic diversity ($D_{ST} = 0.06$), which results in a high total variability. The organization of gene diversity in black pine is quite comparable to that observed for other conifers. Our data suggest that about 86% of total variability has occurred within populations, as compared with 88% for ponderosa pine (O'MALLEY *et al.* 1979), 96% for lodgepole pine (YEH and LAYTON 1979), 97% for Douglas-fir (YEH and O'MALLEY 1980) and 97% for pitch pine (GURIES and LEDIG 1982). However, it should be kept in mind that our analyses covered only a small number of genes.

The considerable intrapopulation isoenzyme variation in *P. nigra* is consistent with the findings of HAMRICK *et al.* (1979) that gymnosperms, long-lived trees, species which are primarily outcrossed and plant species with high fecundities are associated with high intrapopulation genetic variability. All these factors are life-history of black pine.

Several studies on morphological traits (KLUGE and KERFOOT 1973, SOKAL 1976) as well as the more recent study on isoenzymes (PIERCE and MITTON 1979) have conclusively demonstrated the existence of significant positive correlation between variation within populations and variation among populations. There are also several other studies which indicate such relationships between intra- and interpopulation variation of isoenzymes (see PIERCE and MITTON 1979 for review and references).

We have been able to confirm the conclusion of PIERCE and MITTON that there is a positive correlation between h_a and S^2_A , as well as between h_a and F_{ST} . These authors suggest that because the intra/interpopulation correlation coefficient decrease as one goes from the S^2_A to the F_{ST} statistic that the remaining correlation is due to some form of selection. However, since F_{ST} is still probabilistic with a limited number of populations (see NEI and CHAKRAVARTI 1977), there is no way one can partition out possible selective effects from those due to drift. Thus, it is very difficult to ascertain the extent to which selection actually contributes to the Kluge-Kerfoot phenomenon.

As we have seen in the above discussion, the increased interpopulation differentiation for those isoenzymes that are most variable within populations could be in agreement with both selectionist and neutralist hypotheses. In order to see which of these two hypotheses could be the primary cause of population differentiation in isoenzymes of black pine, we have used two tests for determining a departure in allelic frequencies from random spatial arrangement, which are developed by ROYALTEY *et al.* (1975) and SOKAL and ODEN (1978a). These statistical procedures test whether the observed allele frequencies at one locality are independent of allele frequencies at neighbouring localities. The results of these tests for the most common alleles at Est-B and Acph-A loci are shown in Table 8 and 9. As noted earlier, despite statistical heterogeneity and negative autocorrelation coefficients, we do not find any significant departure from a random spatial arrangement of gene frequencies among the 19 localities of black pine in Yugoslavia.

SOKAL (1978) has considered four models for the origin of patterns of population differentiation, as well as whether different models of origin give rise to different relations between intra- and interpopulation variation. According to Sokal, the first two models consider selection as the most important factor in population differentiation: a trait is differentiated in response to an environmental gradient producing a cline (Model I) or to environmental variables which form "patches" that are heterogeneous between localities but homogeneous within (Model II). The second model does not necessarily imply that patches exhibited a spatial ordering. A third and fourth models propose stochastic differentiation; the classical isolation-by-distance (Model III) or differentiation due to different historical factors (Models IV).

Our results imply that population differentiation exists in black pine. However, the differences in gene frequencies are not followed by geographic patterning. Following Sokal's reasoning there are three plausible explanations: 1. The intensities of selection and migration are weak so that populations are differentiated as a consequence of genetic drift. This would be a classical isolation-by-distance model. 2. Founder effects could be a factor of population differentiation in the absence of a pattern. Historical factors such as accidental immigration and establishment of

populations with somewhat different gene frequencies coupled with relatively small colony size compared to average interlocality distances. Since historical information on population sizes, migration and past allele frequencies is unobtainable, this hypothesis cannot be refuted. 3. The most likely explanation for long-lived woody trees, such as black pine, would be heterogeneity of environments over the area where gene frequencies are controlled by the local environment through selection. In addition, black pine populations are built of representatives from many generations and, as pointed out by HAMRICK *et al.* 1979, if different alleles are favoured during the establishment phase of various generations, individuals surviving to maturity will maintain a genetic record of these past evolutionary events. Longevity (black pine trees live over 200 years) and high fecundity ensure large and stable populations resistant to chance fluctuations in gene frequencies. Since each generation may be faced with slightly different selection pressures, a wide variety of genotypes may be maintained.

To be sure, all mechanisms suggested above for maintaining genetic variation within and among black pine populations may have considerable influence on the magnitude and rate of speciation in this species.

Acknowledgements

We are grateful to Prof. R. MORANDINI (Italy), Dr. A. ZAKI (Morocco), Dr. E. F. MOLCHANOV (USSR), Dr. M. CH. IACOVIDES (Cyprus), Dr. N. UYAR (Turkey) and Dr. ARBEZ (France) for providing us with some of the materials on which the present work is based. We thank Prof. M. VIDAKOVIĆ for stimulating discussion; and Prof. F. J. AYALA, Prof. J. R. POWELL, Prof. D. MARINKOVIĆ and Prof. J. McDONALD for helpful suggestions and comments of the manuscript.

Literature

ALLARD, R. W.: The mating system and microevolution. *Genetics* 79: 115–126 (1975). — AYALA, F. J.: Genetic differentiation during speciation process. In *Evolutionary biology*, vol. 8. TH. DOBZHANSKY, M. K. HECHT, W. C. STEERE (eds.) Plenum Press, New York. Pp. 1–78 (1975). — BERGMANN, F.: Genetischer Abstand zwischen Populationen. II. Die Bestimmung des genetischen Abstands zwischen europäischen Fichtenpopulationen (*Picea abies*) auf der Basis von Isoenzym Genhäufigkeiten. *Silvae Genet.* 23: 28–32 (1974). — BERGMANN, F.: Adaptive acid phosphatase polymorphism in conifer seeds. *Silvae Genet.* 24: 175–177 (1975). — BERGMANN, F.: The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along similar climatic gradients. *Theor. Appl. Genet.* 52: 57–64 (1978). — BLEČIĆ, V.: *Gymnospermae* in *Catalogus Florae Yugoslaviae*, vol. 1–2, Ljubljana. Pp. 8–9 (1967). — BROWN, A. H. D.: Enzyme polymorphism in plant populations. *Theor. Pop. Biol.* 15: 1–42 (1979). — GABRIEL, K. R. and R. R. SOKAL: A new statistical approach to geographical variation analysis. *Syst. Zool.* 18: 259–278 (1969). — GURIES, R. P. and F. T. LEDIG: Genetic diversity and population structure in pitch pine (*Pinus rigida* MILL.). *Evolution* 36: 387–402 (1982). — HAMRICK, J. L., Y. B. LINHART, J. B. MITTON: Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Ann. Rev. Ecol. Syst.* 10: 173–200 (1979). — KLUGE, A. G., W. C. KERFOOT: The predictability and regularity of character divergence. *Amer. Natur.* 107: 426–442 (1973). — LEVIN, D. A.: The organization and genetic variability in *Phlox drummondii*. *Evolution* 31: 477–494 (1977). — LEVIN, D. A.: Genetic variation in annual *Phlox*: Self-compatible versus self-incompatible species. *Evolution* 32: 245–263 (1978). — LUNDEKVIST, K.: Allozyme frequency distributions in four Swedish populations of Norway spruce (*Picea abies*). I. Estimation of genetic variation within and among populations, genetic linkage and mating system parameter. *Hereditas* 90: 127–143 (1979). — MIROV, N. T.: The genus *Pinus*. The Ronald Press Comp. New York (1967). — NEI, M.: Genetic distance between populations. *Amer. Natur.* 106: 283–292 (1972). — NEI, M.: Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. Wash.* 70: 3321–3323 (1973). — NEI, M.: Molecular population genetics and evolution. North-Holland, Amsterdam (1975). — NEI, M. and CHAKRAVARTI, A.: Drift variances of F_{ST} and G_{ST} statistics obtained

from a finite number of isolated populations. *Theor. Pop. Biol.* 11: 307–325 (1977). — NEVO, E., D. ZOHARY, A. H. D. BROWN and H. HABER: Genetic diversity and environmental associates of wild Barley, *Hordeum spontaneum*, in Israel. *Evolution* 33: 815–833 (1979). — NIKOLIĆ, D. and F. BERGMANN: Genetic variation of leucine aminopeptidase isoenzyme in seeds of *Pinus nigra* ARN. *Genetika* 6: 361–365 (1974). — O'MALLEY, D. M., F. W. ALLENDORF and G. M. BLAKE: Inheritance of isoenzyme variation and heterozygosity in *Pinus ponderosa*. *Biochem. Genet.* 17: 233–250 (1979). — PIERCE, B. A. and J. B. MITTON: A relationship of genetic variation within and among populations: An extension of Kluge — Kerfoot phenomenon. *Syst. Zool.* 28: 63–70 (1979). — ROYALTEY, H. H., E. ASTRACHAN and R. R. SOKAL: Tests for patterns in geographic variation. *Geogr. Anal.* 7: 369–395 (1975). — SCHAAL, B.: Population structure and local differentiation in *Liatris cylindrica*. *Amer. Natur.* 109: 511–528 (1975). — SOKAL, R. R.: The Kluge-Kerfoot phenomenon reexamined. *Amer. Natur.* 110: 1077–1091 (1976). — SOKAL, R. R.: Population differentiation: Something new or more of the same? In *Ecological Genetics: The Interface*, P. E. BRUSSARD (ed.) Springer-Verlag,

New York, Berlin, Heidelberg. Pp. 215–239 (1978). — SOKAL, R. R. and N. L. ODEN: Spatial autocorrelation in biology: 1. Methodology. *Biol. J. Linn. Soc.* 10: 199–228 (1978a). — SOKAL, R. R. and N. L. ODEN: Spatial autocorrelation in biology: 2. Some biological implications and four applications of evolutionary and ecological interest. *Biol. J. Linn. Soc.* 10: 229–249 (1978b). — TIGERSTEDT, P. M. A.: The application of ecological genetic principles of forest tree breeding. *Silvae Genet.* 23: 62–66 (1974). — VIDAKOVIĆ, M.: Genetics of European black pine (*Pinus nigra* ARN.). *Annal. Forestales JAZU, Zagreb Yug.* 6/3: 57–86 (1974). — WORKMAN, P. L. and J. D. NISWANDER: Population studies on Southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Amer. J. Hum. Genet.* 22: 24–29 (1970). — YEH, F. and C. LAYTON: The organization of genetic variability in central and marginal populations of lodgepole pine (*Pinus contorta* ssp. *latifolia*). *Can. J. Genet. Cytol.* 21: 487–503 (1979). — YEH, F. and O'MALLEY: Enzyme variants in natural populations of Douglas-fir, *Pseudotsuga menziesii* (MIRB.) FRANCO, from British Columbia. 1. Genetic variation patterns in coastal populations. *Silvae Genet.* 29: 83–92 (1980).

Inbreeding Depression and Genetic Variances Estimated From Self- and Cross-pollinated Families of *Pinus radiata*

By M. D. WILCOX

Forest Research Institute, New Zealand Forest Service,
Private Bag, Rotorua, New Zealand

(Received 26th July 1982)

Summary

Compared with artificially cross-pollinated progenies, self-pollinated progenies of 25 *Pinus radiata* D. DON plus-trees (seed orchard clones) grew more slowly in the nursery and, up to the age of 7 years on a fertile site in Kaingaroa Forest, grew more slowly in diameter (ratio of self: cross means = 0.88), had more crooked stems (0.88), had a less desirable branching habit (0.80), had poorer needle retention, i.e., were less resistant to *Naemacyclus* and/or *Dothistroma* needle diseases (0.84), and had slightly denser wood (1.01). There was a strong interaction between parent and inbreeding depression for diameter growth, giving a very weak correlation between the relative performance of selfs and their corresponding crosses. For other traits correlations between selfs and crosses were high, and self-pollinated families could be used to measure the general combining ability of the parents.

Estimates of family variance were higher in all traits for self- than for cross-pollinated families.

Additive, dominance, and additive \times additive genetic variances were jointly estimated from separate and co-analyses of selfs and crosses. These estimates were not considered to be very reliable but nevertheless indicated that wood density, branching quality, and stem straightness were strictly under additive genetic control, diameter growth was under non-additive genetic control, whereas the genetic variance for needle retention was entirely additive and additive \times additive.

It is concluded that selfing in *P. radiata* can be detrimental to growth, stem form, branching quality, and needle cast resistance, and that selfing and other severe forms of inbreeding must be minimised in seed orchards.

Key words: *Pinus radiata*, inbreeding, genetic variances, disease resistance.

Zusammenfassung

Verglichen mit Nachkommenschaften aus kontrollierter Kreuzung wuchsen Nachkommenschaften von 25 *Pinus radiata* D. DON Plusbäumen (Samenplantagen-Klone) aus

Selbstung in der Baumschule und auf einem fruchtbaren Standort im Kaingaroa Forest bis zum Alter 7 langsamer, auch im Durchmesser (Verhältnis Selbstung: Kreuzung 0.88). Es wurden auch mehr krumme Stämme (0.88) und ein wenig wünschenswerter Zweighabitus (Aststellung (0.80) und eine schwächere Benadelung sowie geringere Resistenz gegen *Naemacyclus minor* BUTIN und/oder *Dothistroma*, jedoch eine geringfügig höhere Holzdicke (1.01) beobachtet. Im Durchmesserwachstum wurde eine Interaktion zwischen den Eltern und der Inzuchtdepression festgestellt, die eine sehr schwache Korrelation zwischen der relativen Leistung der Selbstungen und derjenigen ihrer korrespondierenden Kreuzungen erkennen ließ. Bei anderen Merkmalen waren die Korrelationen dagegen hoch, d. h. Selbstungsfamilien könnten verwendet werden, um die allgemeine Kombinationseignung von Elternbäumen zu ermitteln.

Die Varianzschätzungen für „Familien“ waren bei allen Merkmalen für die Selbstungsfamilien größer als für die Fremdkreuzungsfamilien. Mit Ausnahme des Merkmals Nadelschütte waren bei den Selbstungen die Varianzen innerhalb der Parzellen größer. Die Heritabilitäten der Familien-Mittel waren bei den Selbstungen allgemein größer, und somit die Rangfolgebildung von Familien präziser. Phänotypische Korrelationen von Familien-Mitteln zeigten für verschiedene Kombinationen von Merkmalen vergleichbare Resultate bei Selbstungen und Kreuzungen; Ausnahme war die Kombination BHD-Holzdicke, wo die negative Korrelation bei den Selbstungen (–0,65) deutlich größer war als bei den Kreuzungen (–0,32).

Additive, Dominanz- und additiv \times additive Varianzen wurden anhand separater und gemeinsamer Analyse von Selbstungen und Kreuzungen ermittelt. Obgleich diesen Schätzungen keine große Verlässlichkeit zugebilligt werden muß, deuteten sie doch an, daß Holzdicke, Aststellung und Schaftform unter ausschließlich additiver Genkontrolle stehen sowie Dickenwachstum unter nicht-additiver, währenddessen sich die genetische Varianz bei Nadelschütte gänzlich additiv und additiv \times additiv zeigte.