GENETIC VARIATION IN ISOLATED POPULATIONS OF STONE PINE *(PINUS CEMBRA)*

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Allozyme variation at 8 enzyme loci (Lap-1, Lap-2, Got-1, Got-2, APh-1, ADh-1, Cat-1 and Est-2) was investigated by means of starch gel electrophoresis and gel isoelectric focusing in 11 populations of stone pine. Average values of expected panmictic heterozygosity (H) were relatively lower than those recorded in populations of other conifers and ranged between 0,165 and 0,397. Considerable differences were found between investigated populations with regard to the frequency of occurrence of analyzed allozymes. Values of Nei's genetic distance (D) were very high and ranged between 0,018 and 0,650. Highest values of D were found between Asiatic population Czita in comparison with the remaining populations. According to a dendrogram based on the calculated D values the investigated populations could be divided into several groups corresponding to their geographic origin.

Introduction

Most of the present biochemical studies of genetic variation in forest tree populations concern species exhibiting a wide and continuous range of distribution including numerous populations. Between such populations a considerable gene flow moderating the processes of genetic divergence is to be expected. Therefore, it would be interesting to know the amount of genetic variation in forest tree populations inhabiting relatively small and geographically isolated areas, where a block to gene flow can be expected to accelerate their genetic divergence.

Genetic variation in small and partially isolated populations of forest trees has been studied by Feret (1974) and Tigersted (1973). In the former study a remarkable genetic differentiation has been found between three small stands of *Pinus pungens*. On the other hand, the latter author concluded that small marginal populations of *Picea abies* differed only slightly from the more central populations.

In this study allozyme variation at 8 enzyme loci has been studied in 11 populations of stone pine originating from isolated parts of its natural range in Europe and Asia. In general, it is assumed, that stone pine occurring in Europe is characterized by very small variation (Bednarz 1971). Holzer (1975) suggests, that in European populations of stone pine clinal growth variation along altitudinal gradient may exist.

Materials and methods

A total of 11 populations originating from Alps, Tatra Mts., Retezat Mts. and Asinskij Khrebet have been included in this study. Georgraphic data of investigated populations are presented in Table 1. The population samples were collected from 20–25 trees per population. In the case of 3 populations namely: Czita, Retezat and Morskie Oko seed samples from individual trees were available.

Haploid female macrogametophyte (endosperm) tissue isolated from dormant seeds was used for enzyme analysis. Glutamic-oxaloacetic-transaminase (Got), (EC 2.6.1.1), leucine aminopeptidase (Lap), (EC 3.4.11.1), catalase (Cat), (EC 1.11.1.6), acid phosphatase (APh), (EC 3.1.3.2.), and alcohol dehydrogenase (ADh), (EC 1.1.1.1) isoenzymes were separated by means of starch electrophoresis. Esterases (Est), (EC 3.1.1.1, 3.1.1.2, 3.1.1.6, 3.1.1.7, 3.1.1.8) were analysed using isoelectric focusing on acrylamide slabs (0,2 mm thick) containing 2 % Ampholine pH 3,5–10 (LKB Produkter AB, Bromma, Sweden). Detailed description of separation procedures and gene identification has been presented elsewhere (Szmidt 1979; 1981; in preparation).

The following enzyme loci have been included in this study: Got-1, Got-2, Lap-1, Lap-2, Cat-1, APh-1, ADh-1 and Est-2. The genetic variation within populations has been expressed as the average heterozygosity or gene diversity (H) expected for panmictic population as proposed by Nei and Roychoundry (1974). Estimates of the genetic distance (D) between the investigated populations have been made according to Nei (1972). Using this measure a dendrogram was produced using the unweighed pair-group method of clustering (Sokal and Sneath 1963).

Table 1. Geographic data of the 11 investigated stone pine populations.

Population name and symbol	Region Country	Long	Lat.	Alt.
Salzburg (Al–1)	Salzburg Alps Austria	°00′	47°30′	1600 m
Zillertal (Al–2)	Zillertal Alps Austria	12°00′	47°00′	1750 m
Steiermark (Al–3)	Niedere Tauern Austria	14°00′	47°15′	1800 m
Chandolin (Al–4)	Berner Alps Switzerland	7°20′	46°20′	1750 m
Avers (Al–5)	Ratische Alps Switzerland	9°30′	46°30′	1800 m
Woloszyn (Ta–1)	Tatra Mts. Poland	20°10′	49°15′	1500 m
Morskie Oko (Ta–2)	Tatra Mts. Poland	20°10′	49°15′	1450 m
Bukowina (Ta–3)	Tatra Mts. Poland	20°10′	49°15′	1150 m
Bielevodska Valley (Ta–4)	Tatra Mts. Czechoslovakia	20°05′	49°10′	1240 m
Retezat (Ret.)	Retezat Mts. Rumania	22°40′	45°20′	1650 m
Czita (Czi.)	Asinskij Khreb. USSR	108°20′	49°50′	1340 m

Results and discussion

Genetic variation within populations

Frequencies of particular allozymes the 11 investigated stone pine populations are presented in Table 2. Out of 8 analysed enzyme loci, 3 loci (Got-2, APh-1, and Est 2) were polymorphic in all populations. At most examined loci, all European populations shared one and the same allozyme occurring with considerable frequency. Most striking example of the above patterns of allozyme distribution was Cat-1 locus at which all European populations were fixed for Cat-1^a allozyme.

It is assumed, that present populations of stone pine occurring in Europe arose from fragmentation of an ancestral preglacial population (Szczepanek 1971). The presence of single shared allozymes in the European stone pine populations support to some degree the above suggestion. It appears possible, that these allozymes were also present before fragmentation of the ancestral population.

It must be pointed out however, that electrophoretic identity of particular allozymes is no proof of identity of alleles coding for them and allelic identity need not be by descent. On the other hand, the occurrence of some other allozymes (Got-2^b, Lap-2^c, ADh-1^b, 1^c, Est-2^d) was restricted to only a few populations. Six allozymes (Lap-2^d, Cat-1^d, ADh-1^d, Est-2^a, Lap-1^b and Est-2^c) occurred in only one population. Most of these allozymes were found in the Asiatic population Czita.

No distinct geographic patterns of allozyme distribution were observed with the exception of Lap-2^a allozyme which was the most frequent in populations from Alps whe-

Table 2. Allozyme frequencies in 11 stone pine populations and average heterozygosity (H) calculated over 8 enzyme loci.

	Al-1	Al-2	Al-3	Al-4	Al-5	Ta-1	Ta-2	Ta-3	Ta-4	Ret.	Czi.
Got-1a	1,000	1,000	1,000	0,709	0,965	1,000	1,000	0,857	1,000	0,000	0,342
b	0,000	0,000	0,000	0,291	0,035	0,000	0,000	0,143	0,000	1,000	0,658
Got-2a	0,950	0,417	0,510	0,722	0,776	0,929	0,429	0,577	0,613	0,494	0,543
b	0,000	0,166	0.010	0,000	0,000	0,000	0,000	0,021	0,062	0,000	0,074
c	0,050	0,417	0,480	0,278	0,224	0,071	0,571	0,402	0,325	0,506	0,383
Lap-1a	1,000	1,000	1,000	0,889	1,000	1,000	1,000	1,000	0,708	0,944	0,910
b	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,060
c	0,000	0,000	0,000	0,111	0,000	0,000	0,000	0,000	0,292	0,056	0,030
Lap-2a	0,750	1,000	0,668	0,704	0,977	0,429	0,429	0,272	0,000	0,000	0,000
b	0,000	0,000	0,166	0,000	0,000	0,000	0,000	0,687	0,137	0,667	0,820
c	0,250	0,000	0,166	0,049	0,023	0,571	0,571	0,014	0,201	0,333	0,030
d	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,338	0,000	0,000
e	0,000	0,000	0,000	0,247	0,000	0,000	0,000	0,027	0,324	0,000	0,150
Cat-1a	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0.000
b	0,000	0,000	0,000	0,000	0,000	0.000	0,000	0,000	0,000	0,000	1,000
APh-1a	0,417	0,583	0,333	0,350	0,250	0,214	0,167	0,050	0,277	0,333	0,571
b	0,583	0,417	0,667	0,650	0,750	0,786	0,833	0,950	0,723	0,667	0,429
ADh-1a	0,917	1,000	0,917	1,000	1,000	0,571	0,857	1,000	0,920	0,055	0,500
b	0,000	0,000	0,000	0,000	0,000	0,429	0,000	0,000	0,080	0,389	0,000
c	0,083	0,000	0,083	0,000	0,000	0,000	0,143	0,000	0,000	0,556	0,000
d	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,500
Est-2a	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,333
b	0,583	0,583	0,500	0,550	0,583	0,643	0,740	0,583	0,818	0,556	0,333
c	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,084
d	0,000	0,000	0,050	0,000	0,000	0,143	0,000	0,000	0,046	0,000	0,000
e	0,417	0,417	0,450	0,450	0,417	0,214	0,260	0,417	0,136	0,444	0,250
H	0,199	0,200	0,269	0.300	0,165	0.246	0.236	0,224	0,313	0,316	0,397

reas its frequency was much lower in populations from Polish Tatras and it was absent in the remaining populations.

Average heterozygosity (H) values of the investigated stone pine populations were relatively lower than those calculated for populations of other conifers (Lundkvist and Rudin 1977; Mejnartowicz 1979) and ranged between 0,165 and 0,397 (Table 2). Greatest gene diversity was found in population Czita. It appears that the paucity of genetic diversity in European stone pine populations studied here can be attributed to random drift effects, that resulted from continued lack of gene flow after isolation of particular populations. On the other hand, however, it is certainly possible, that selection was also important in determining the distribution patterns of some allozymes especially those occurring with considerable frequencies in all investigated populations.

Genetic variation among populations

Populations which have been geographically isolated for a long tine are expected to be less similar than adjacent populations where a considerable gene exchange occurs. In fact remarkable genetic divergence of the investigated stone pine populations has been found.

The values of genetic distance (D) ranging between 0,018 and 0,650 (Table 3) were ge-

Table 3. Nei's genetic distance between 11 stone pine populations based upon 8 enzyme loci.

	Al-1	Al-2	Al-3	Al-4	Al-5	Ta-1	Ta-2	Ta-3	Ta-4	Ret.	Czi.
Al-1	-										
Al-2	0,050	-									
Al-3	0,036	0,027	-								
Al-4	0,034	0,048	0,092	-							
Al-5	0,018	0,033	0,045	0,024	-						
Ta-1	0,077	0,095	0,036	0,084	0,077	-					
Ta-2	0,111	0,140	0,058	0,087	0,092	0,086	-				
Ta-3	0,054	0,153	0,090	0,106	0,087	0,069	0,118	-			
Ta-4	0,119	0,167	0,102	0,105	0,136	0,079	0,094	0,109	-		
Ret.	0,481	0,555	0,414	0,387	0,509	0,395	0,315	0,395	0,454	-	
Czi.	0,603	0,631	0,548	0,531	0,627	0,624	0,428	0,650	0,591	0,431	-

nerally much higher than those calculated between populations of other conifers exhibiting continuous range of distribution (Bergmann 1974; Lundkvist and Rudin 1977; Mejnartowicz 1979).

A dendrogram based on D values for all pairs of the investigated populations of stone pine is presented in Figure 1. Most striking is that population Czita originating from the eastern border of stone pine distribution in Asia, was quite distinct from any other population examined in this study.

Romanian population Retezat originating from an isolated occurrence of stone pine in Southern Carpathians was the second most outstanding population. It differed markedly from population Czita as well as from other European populations. Much smaller genetic divergence was found among populations growing in Tatra Mts. and Alps although it is still remarkable when compared with that among populations of other conifers.

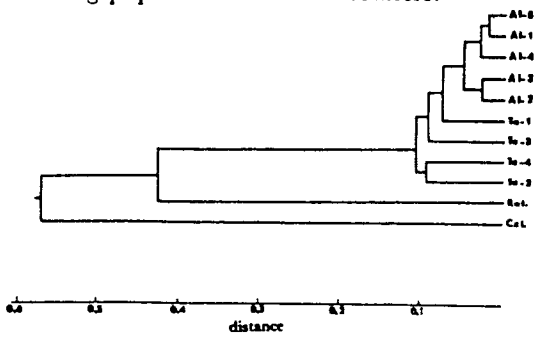

Figure 1. Dendrogram derived from Nei's genetic distance between 11 stone pine populations.

This is somewhat surprising taking into account their ecological and morphological similarity. The most homogeneous cluster embraces 5 populations from Alps. On the other hand, patterns of variation between the populations from Tatra Mts. were more complicated. Populations Ta-2 (Morskie Oko) and Ta-4 (Bielovodska Valley) are relatively distinct from other populations growing in Tatra Mts. and Alps, however they also differ markedly between each another. The two remaining populations from Tatra Mts. (Ta-1 and Ta-3) cluster with the populations from Alps.

Assuming that the investigated regions of stone pine occurrence in Europe represent fragments of the original preglacial range they derived from different and very distant parts of the original range. Furthermore the time elapsed after isolation of particular regions was presumably different. This could explain the observed remarkable genetic divergence of the investigated groups of populations. On the other hand, it still does not clarify the considerable differences between populations inhabiting one region, which was especially evident in the case of populations from Tatra Mts.

It should pointed out however, that present natural populations of stone pine in Europe occur exclusively in mountains and are separated by high summits, which can markedly reduce gene exchange between them. Furthermore, the relative closeness of these populations does not necessarily reflect their common origin. For instance, the postglacial his-

tory of stone pine forests in Tatra Mts. was rather complicated and as has been pointed out by Szafer (1966) there were at least several sources of stone pine immigration to these mountains. It is possible, that certain populations could persist in the southern (Slovakian) part of the Tatra Mts. during the last glacial period, while the populations situated in the northern parts have been destroyed by glacier. The increased genetic distance between Slovakian population Bielovodska Valley (Ta-4) and the remaining populations from Tatra Mts. can support the above suggestion. In addition, there is also some evidence, that stone pines originating from Alps and Siberia have been introduced in the XIXth century to the Polish Tatras (Paryski 1971), which could also contribute to the increased differentiation found between Polish populations of this species.

Literature

BEDNARZ, Z. 1971. Systematyka i zmiennosc. In. Limba (*Pinus cembra L.*). PWN Warszawa-Poznan.

BERGMANN, F. 1974. Genetische Abstand zwischen Populationen. II Die Bestimmung des genetischen Abstands zwischen europäisher Fichtenpopulationen *(Picea abies)* auf der basis von Isoenzym-Genhäufigkeiten. Silvae Genet 23: 28–31.

FERET, P. P. 1974. Genetic differences among three small stands of *Pinus pungens*. Theor. Appl. Genet. 44: 173–77.

HOLZER, K. 1975. Genetics of *Pinus cembra* L. Annales Forestales VI/5: 139–158.

LUNDKVIST, K. & RUDIN, D. 1977. Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. Hereditas 85: 67–74.

MEJNARTOWITCZ, L. 1979. Genetic variation in some isoenzyme loci in Scots pine (*Pinus silveatris* L.) populations. Arboretum Kórnickie 24: 91–104.

NEI, M. 1972. Genetic distance between populations. Amer. Nat. 106: 283–292.

— & ROYCHOUNDRY, A. K. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76: 379–390.

PARYSKI, W. H. 1971. Limba (*Pinus cembra* L.): Sadzenie i przesadzanie limby. PWN Warszawa-Poznan.

SOKAL, R. R. & SNEATH, P. H. A. 1963. Principles of numerical taxonomy. Freeman, San Francisco.

SZAFER, W. 1966. Dziesieć tysiecy lat historii lasu w Tatrach. PWN Krakow.

SZCXEPANEK, K. 1971. Limba (*Pinus cembra* L.): Historia limby w Polsce. PWN Warszawa-Poznan.

SZMIDT, A. E. 1979. Inheritance of catalase multiple forms in Scots pine (*Pinus silvestris* L.) endosperm. Arboretum Kórnickie 24: 105–110.

— 1981. Ultra-thin gel isoelectric focusing of Scots pine esterases Second Ann. Rep. FG-Po-326. in press.

— 1982. Enzymatyczna analiza zmienności genetycznej limby (*Pinus cembra* L.).

TIGERSTEDT, P. M. A. 1973. Studies on isozyme variation in marginal and central population of *Picea abies*. Hereditas 75: 47–60.