Allozyme Variation in Scots Pine (Pinus sylvestris L.) in Sweden

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Abstract

Nine native stands of Scots pine from five Swedish locations between the latitudes 59° and 65° have been described by eleven polymorphic allozyme loci. Three of the locations have diverse site conditions and here we have described two or three adjacent stands representing different sites.

We found significant allele frequency differences between locations and between one set of adjacent stands, but the subdivision was very low. More than 98 per cent of gene diversity was within populations and no obvious distributional pattern was found. No stand, except one, showed a significant deviation from Hardy-Weinberg equilibrium. The fixation index did not deviate from zero nor could we find any deviations from random distribution on the two loci level.

It was concluded that our allozyme data did not support a division into subspecies in the area under study. Further the data from this and other studies on conifers by allozymes suggests that in areas permanently covered by ice during the glacial periods conifers have a lower differentiation than outside these areas.

Key words: Allozyme variation, Scots pine, Sweden

Zusammenfassung

Es wurden neun autochthone Bestände von *Pinus sylvestris* L. auf 5 schwedischen Standorten zwischen dem 59. und 65. Breitengrad durch 11 polymorphe Allozym-Loci beschrieben. Drei der Standorte haben verschiedene Standortsbedingungen, zu denen wir zusätzlich 2—3 angrenzende Bestände beschrieben haben, die unterschiedliche Standorte repräsentieren.

Es wurden signifikante Allel-Häufigkeitsunterschiede zwischen Standorten und zwischen einer Reihe von angrenzenden Beständen gefunden, wobei die Unterteilung sehr gering war. Mehr als 98% der genetischen Mannigfaltigkeit lag innerhalb der Populationen; dabei wurde kein deutliches Verteilungsmuster gefunden. Kein Bestand, bis auf einen, zeigte eine signifikante Abweichung vom Hardy-Weinberg Gleichgewicht. Der Bestimmungsindex wich weder von 0 ab, noch konnten irgendwelche Abweichungen von der randomisierten Verteilung auf dem zwei Loci-Niveau gefunden werden.

Es wird daraus geschlossen, daß unsere Allozym-Daten ersatzweise eine Einteilung in Unterarten in der Region nicht zulassen. Weiterhin weisen die Daten dieser und auch anderer Untersuchungen der Allozyme bei Koniferen darauf hin, daß in Gebieten, die während der Eiszeiten vollkommen mit Eis bedeckt waren, Koniferen eine geringere Differenzierung zeigen als außerhalb dieser Gebiete.

Introduction

The morphological data and studies on mating system of conifers indicate that most of them have a small amount of population differentiation with few examples of discrete races or abrupt changes over short distances (Stern and Roche, 1974). This picture of low differentiation and clinal variation is also supported by allozyme studies indicating that several forest trees have less differentiation between populations than most other plants (Brown, 1979; Wheeler and Guries, 1982). Continuous spatial distribution, predominantly outcrossing mating system and the potential for long distance gene flow and seed dispersal may contribute to this situation (Hamrick *et al.*, 1981).

There is evidence, however, that even coniferous species may have a high amount of local adaptation (Eriksson, 1982) as well as a high genetic differentiation and spatially abrupt changes in their genetic composition. Thus several species have been subdivided into subspecies, and some of these subdivisions are reflected in allozyme studies, e.g. for lodgepole pine (*Pinus contorta* Dougl.) (Wheeler and Guries, 1982). Szmidt (1982) has also observed an exceptionally high population subdivision in stone pine (*Pinus cembra* L.) for allozymes, possibly as an effect of a long period of a patchy spatial disribution. Ledig and Fryer (1972) have found a drastic change for cone serotiny in pitch pine (*Pinus rigida* Mill.) a character supposedly controlled by a single gene. Furthermore Bergmann (1978) and Mitton *et al.* (1977, 1980) have observed population differences for allozymes between adjacent stands of Norway spruce (*Picea abies* (L.) Karst.) and ponderosa pine (*Pinus ponderosa* Laws.), respectively, and they interpret them as a result of adaptation to environmental differences. Ledig and Conkle (1983) and Millar (1983) have also observed drastic changes in allozymes over short distances for Torrey pine (*Pinus torreyana* Parry ex Carr) and bishop pine (*Pinus murricata* D. Don) but they do not explain their findings with adaptions to the environments in question. Our present information on the population structure of Scots pine indicates that the pattern agrees with that observed on coniferous species generally, but it is to some extent contradictory and inconclusive. Scots pine is taxonomically subdivided into several subspecies, but systematists describe different numbers of taxonomic units (for review see Bialobok, 1967). In one of the more recent and exhaustive systematic studies Pravdin (1964) divides the species into five subspecies, i.e. *P. s. lapponica* Fries which is found in Europa north of 62° latitude. *P. s. sibirica* Ledebour north of 52° latitude in Asia, *P. s. kulundensis* Sukachev south of 52° latitude in Asia, and *P. s. hamata* Fomin. in Crimea and Caucasus. The fifth subspecies *P. s. sylvestris* L. covers the rest of the distribution area, i.e. Europe south of 62° latitude but excluding the south-eastern part of the European continent. There has been no attempt to examine whether this differentiation into subspecies is in agreement with allozyme data. The evidence from other studies, however, does not fully support the division into *P. s. sylvestris* L. and *P. s. lapponica* Fries in Sweden: the genetic variation for survival and height is continuous and clinal (Eriksson, 1982), and does not show any abrupt change in the border between these subspecies.

The allozyme studies, allthough not related to the taxonomic treatments, still give some indications that the picture of low genetic differentiation and gradual change could be wrong. Thus Mejnartowitcz (1979) has found a

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relatively high subdivision of Scots pine in central and south-eastern Europe. Gullberg *et al.* (1982) also observed allozyme differences between adjacent stands in Sweden. In contrast our calculations on the data published by Rudin et al. (1974) (see *Table 6)* indicate a low differentiation in Sweden as a whole.

The limited and contradictory information on the population structure of Scots pine in Sweden led us to take a closer look at the subdivision of allozyme variation within and between Swedish populations. In the present study we present the results of an electrophoretic analysis of eleven loci for five Swedish locations from a wide latitudinal altitudinal range. Within three of these locations we have also studied adjacent stands representing different local conditions.

Materials and Methods

Seeds were collected from nine native stands at five locations distributed over a large parts of Sweden *(Figure 1, Table 1).* To get as complete a description as possible, we have included materials collected during several years and also used data published in other contexts (Gullberg *et al.*, 1982; Yazdani *et al.*, 1984a, b). The locations differ considerably in length of the growing season. Within locations adjacent stands are separated from each other by 200 to 2000 meters; and they are located in different site types. A stand comprises the trees on a relatively homogenous site, in this study ranging from one to ten hectares with 150 to 300 trees. Gullberg *et al.* (1982) showed that there is a difference in flowering time between the stands within the locations D and E. Zackrisson (1977) studied stands close to location B and found similar differences between north and south facing slopes as those observed at D and E. By analysing fire scars he also found that the occurrence of fires is much more frequent on the south facing slope suggesting differences in rotation time on the two slopes, since natural regeneration occurs after fires.

Only trees with seeds could be selected for study, which means that every second to every fifth tree in a stand has been analysed. The electrophoretic analysis was made on macrogametophytic tissue in the Umeå laboratory, and the genotypes of each parental tree were inferred from seven seeds. Sample sizes and the loci and alleles scored are listed in *Table 2.* Only polymorphic loci are included in the study, so the estimates of the absolute amount of electrophoretically detectable genetic variability are not unbiased ones. The low number of trees scored for ADH-A at location D results from insufficient electrophoretic separation due to small seeds, and the different number of trees in stand B2 is due to incomplete analysis during one of the sampling years. GDH and GOT-C were not analysed in

Figure 1. — Distribution of sample population.

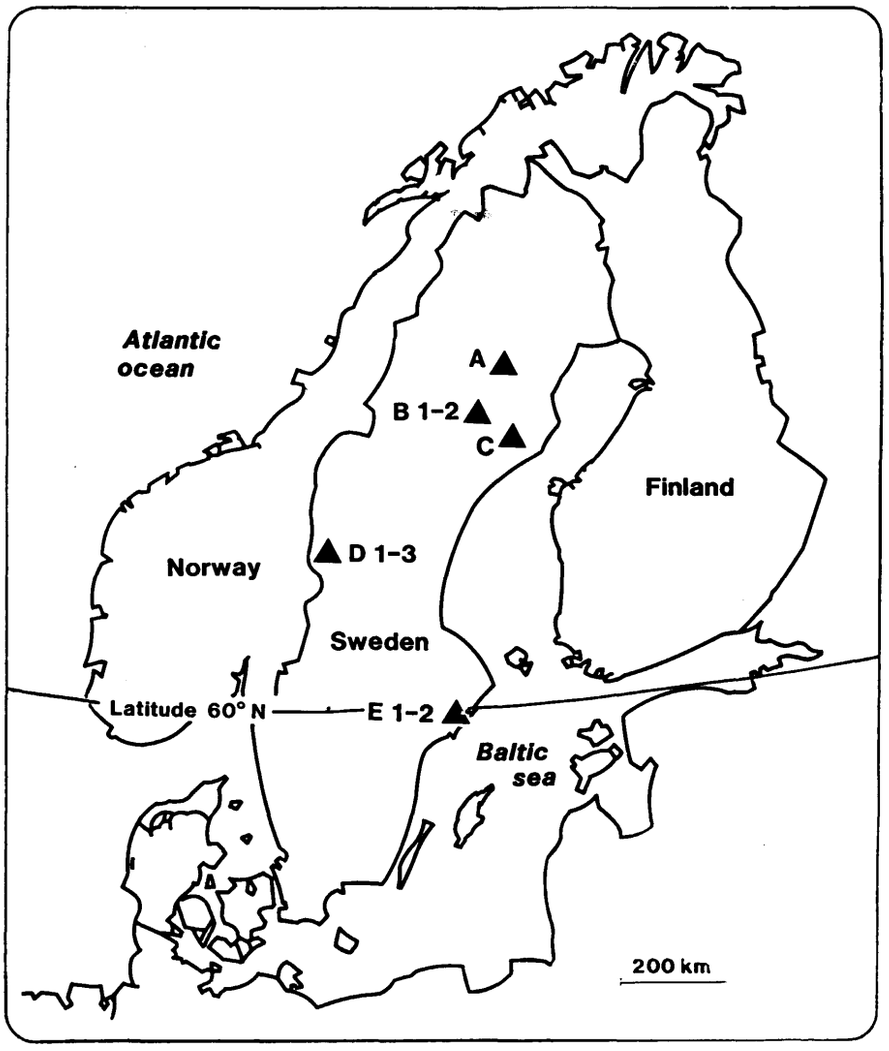

Table 1. — Stand data.

Locality	Map code	Latitude N	Longitude E	Altitude above sea level (meter)	Age of trees (year)	Year of collection
Gårdsjärn	A	65°29'	19°17'	400	80-100	1981
Remjaurliden, north facing slope	B1	64°40'	18°08'	420	60-120	1981
Remjaurliden, south facing slope	B2	64°40'	18°08'	420	60-120	1981
Svartberget	C	64°14'	19°47'	200	100	1980
Idre, in a valley	D1	61°51'	12°45'	675	120-150	1978-81
Idre, at the tree limit	D2	61°51'	12°45'	860	200-	1978-81
Idre, north facing slope	D3	61°51'	12°45'	750	120-150	1978-81
Älta, on a hill	E1	59°16'	18°11'	50	200-	1980
Älta, on a bog	E2	59°16'	18°11'	30	80-150	1980

all samples due to technical reasons. The electrophoretic techniques are described elsewhere, i.e. preparation of gels in Gullberg *et al.* (1982) and sample preparations in Lundkvist (1974), Rudin (1975, 1977), Rudin and Ekberg (1978), Adams and Joly (1980) and Yazdani and Rudin (1982). The electrophoretic variability patterns and their interpretation have been described by Yazdani *et al.* (1984a, b). The nomenclature for designation of loci is that used in the Umeå laboratory (Rudin, 1975; Yazdani *et al.*, 1984a). Studies indicating a Mendelian mode of inheritance have previously been performed for at least some of the alleles at the following loci: F-EST, GOT-A, GOT-B, LAP-A, LAP-B, MDH-A and MDH-B (Rudin, 1975; Yazdani *et al.*, 1984a). We have also, for comparison, calculated the gene diversity in earlier studies on Scots pine (Rudin *et al.*, 1974; Mejnartowitcz, 1979), in Norway spruce (*Picea abies* (L.) Karst.) (Lundkvist and Rudin 1977; Lundkvist, 1979), and stone pine (Szmidt, 1982) studies.

Results

There are highly significant allele frequency differences among locations (*Tables 2* and *3*). The stands at location D also differ significantly, whereas no heterogeneity was observed among the stands at locations B and E.

Although statistically significant, the magnitude of the absolute allele frequency differences are generally very small. The values of genetic distance (Nei, 1975) based on the nine loci scored in all samples (GDH and GOT-C excluded) are small ranging between .003 and .027. The lack of substantial differentiation is further reflected by the generally good fit to Hardy-Weinberg expectations observed in the total material (*Table 4*), and a significant deficiency of heterozygotes is found at a single locus only (ADH-A). Finally, there are no indications of gametic phase (linkage) disequilibrium as may be expected in a subdivided and genetically differentiated population; contigency chisquare

Table 2. — Allele frequencies and number of trees analysed.

Locus	Allele	Locality								
		A	B1	B2	C	D1	D2	D3	E1	E2
ADH-A	1	.239	.344	.363	.288	.101	.300	.234	.344	.442
	2	.761	.656	.638	.680	.900	.700	.766	.656	.558
	1-2	0	0	0	.032	0	0	0	0	0
	n	88	48	40	111	15	35	32	61	60
ADH-B	1	.106	.094	.112	.066	.026	.167	.112	.090	.083
	2	.756	.781	.775	.781	.882	.700	.724	.689	.692
	3	.139	.125	.112	.145	.092	.133	.163	.221	.225
	0	0	0	0	.009	0	0	0	0	0
	n	90	48	40	114	38	45	49	61	60
F-EST	1	.706	.625	.705	.682	.816	.713	.745	.754	.758
	2	.089	.179	.143	.120	.053	.128	.128	.139	.058
	3	.206	.161	.143	.190	.132	.148	.128	.107	.167
	01	0	.036	0	.008	0	.011	0	0	.017
	28	0	0	.009	0	0	0	0	0	0
	n	90	56	56	121	38	47	47	61	60
GOT-A	1	.017	.009	0	.004	.026	0	0	0	0
	2	.956	.991	1	.996	.974	1	1	1	1
	3	.028	0	0	0	0	0	0	0	0
	n	90	56	59	115	39	47	49	61	60
GOT-B	1	.072	.030	.050	.018	0	.021	.011	.008	.058
	18	.139	.180	.101	.080	.079	.128	.170	.148	.101
	2	.144	.230	.170	.345	.224	.213	.319	.189	.208
	22	.167	.110	.130	.066	.211	.096	.117	.139	.075
	3	.472	.450	.550	.487	.487	.543	.372	.516	.558
	0	0	0	0	.004	0	0	0	0	0
	*	.006	0	0	0	0	0	.011	0	0
	n	90	50	50	113	38	47	47	61	60
LAP-A	1	.033	.027	.017	.004	0	0	0	.033	0
	2	.911	.955	.898	.934	.974	.967	.980	.918	.925
	3	.044	.009	.042	.048	.013	.033	.020	.025	.067
	0	.011	.009	.042	.013	.013	0	0	.025	.008
	n	90	56	59	114	39	45	49	61	60
LAP-B	1	.011	.036	.026	.004	.026	0	.020	.017	0
	2	.933	.866	.914	.957	.949	.944	.929	.908	.908
	3	.056	.089	.052	.035	.013	.044	.041	.075	.083
	4	0	0	0	0	0	0	.010	0	0
	01	0	.009	0	.004	0	.011	0	0	.008
	0	0	0	.009	0	.013	0	0	0	0
	n	90	56	58	115	39	45	49	60	60
MDH-A	1	.072	.071	.019	.031	.101	.117	.094	.057	.025
	2	.922	.929	.981	.969	.900	.883	.906	.943	.975
	3	.006	0	0	0	0	0	0	0	0
	n	90	49	54	114	40	47	48	61	60
MDH-B	1-3	.706	.713	.704	.691	.675	.704	.638	.656	.763
	2-4	.278	.287	.296	.305	.300	.296	.351	.303	.237
	0	.006	0	0	0	.012	0	0	.041	0
	1-0	.011	0	0	0	0	0	0	0	0
	*	0	0	0	.005	.012	0	.011	0	0
	n	90	47	54	110	40	49	47	61	57
GDH	1	.361	.400	.314		.419	.383	.357	.369	.358
	2	.639	.600	.686		.581	.596	.643	.631	.633
	22	0	0	0		0	.011	0	0	.008
	*	0	0	0		0	.011	0	0	0
	n	90	55	59		37	47	49	61	60
GOT-C	1-3	.311	.306	.300					.402	.367
	2	.689	.684	.700					.590	.633
	1-0	0	.010	0					0	0
	0-3	0	0	0					.008	0
	n	90	49	40					61	60

n = number of trees analysed
* = rare allele, not yet designated

Table 3. — G-statistics (Sneath and Sokal, 1973) and degree of freedom (below) for allele frequency homogeneity. (In a few cases alleles had to be lumped to provide reasonable expectations.)

	BETWEEN				
Locus	B_1,B_2	D_1,D_2,D_3	E_1,E_2	A,B,C,D,E	All stands
ADH-A	0.067	5.197	2.411	17.401**	25.076**
	1	2	1	4	8
ADH-B	0.209	12.939*	0.037	12.906	26.091
	2	4	2	8	16
F-EST	1.863	4.482	3.987	16.185*	26.517*
	2	4	2	8	16
GOT-A	1.444	5.004	0.000	18.614**	25.062**
	1	2	1	4	8
GOT-B	3.077	13.688*	2.692	41.760***	61.217***
	3	6	3	12	24
LAP-A	2.818	0.306	0.041	10.840*	14.005
	1	2	1	4	8
LAP-B	1.336	0.357	0.000	9.313	11.006
	1	2	1	4	8
MDH-A	3.600	0.290	1.646	15.448**	20.984**
	1	2	1	4	8
MDH-B	0.020	0.945	3.309	1.075	5.349
	1	2	1	4	8
Total	14.432	43.208*	14.124	143.541***	215.305***
	13	26	13	52	104

* : $P < 0.05$
** : $P < 0.01$
*** : $P < 0.001$

tests on the total material for all pairwise combinations of loci revealed no case of significant departure from random association.

Within samples the fit of genotypic distributions to Hardy-Weinberg expectations was tested by the exact method of Vithayasai (1973). For loci with more than two alleles we applied an extended version of this test (Leimar, in preparation). Given the observed counts of the different alleles the exact hypergeometric probability of obtaining the observed genotypic distribution is calculated. More extreme cases are defined as those with lower probabilities than that of the case observed, and the total probability used for accepting or rejecting the null hypothesis is obtained by summing the probabilities of all cases being equally extreme or more than the one observed. In a few cases alleles had to be lumped to avoid unreasonably time-consuming computer operations. Out of the 94 tests performed within samples only four indicated a significant departure from the expected genotypic distribution *(Table 4)*. Two of the significances are at the same location (C), and all but one (GOT-C in stand E1) reflect a deficiency of heterozygotes.

The dendrogram (Upgma; Sneath and Sokal, 1973) constructed from genetic distance values does not reveal any obvious tendency for geographically proximate locations to cluster together *(Figure 2)*. In contrast, the major branching separates the D1 stand from the two neighbouring stands at the same (D) location. It should be noted, however, that the D1 sample size is one of the smaller ones, and particularly the allele frequency estimate at ADH-A is based on a fairly small number of trees *(Table 2)*.

As estimated from a hierarchical analysis of gene diversity (Nei, 1973, 1975; Chakraborty *et al.*, 1982) the major part of the total gene diversity (98.3 per cent) is found within stands *(Table 5)*, whereas the diversities attributable to differences among locations (1.0 per cent) and between stands within locations (0.7 per cent) are fairly small and similar in magnitude. The relation between the G-values for different sources of variation *(Table 3)* indicates that the diversity component among locations reflects real differences which are not explained from the variation between stands within locations.

Discussion

The present study has shown that there are significant allele frequency differences between distant and proximate stands. The absolute amount of genetic differentiation is small, however, and we could not find any pattern related to environmental conditions or to geographic distance. A generally good fit to Hardy-Weinberg expectations at both the one and two loci-level was also found for the total material and for each population indicating minor deviations from random distribution of allozymes for the whole area considered.

In contrast to taxonomists, who have based their conclusions on characters with an unknown genetic background, we have no evidence for a genetic division of Scots pine in Sweden into two subspecies. Thus, our electrophoretic data give a picture that is different from that suggested by Prus-Glowacki and Rudin (1981) who based their conclusions on the variation of a limited number of antigenic proteins.

The good agreement with Hardy-Weinberg equilibrium is notable considering the excess of homozygotes observed at the seed stage in other studies on Scots pine. Both Rudin *et al.* (1974) and Yazdani *et al.* (1984b) reported such deviations. It should be noted, however, that the only population significantly deviating from equilibrium in this study is the one observed by Yazdani *et al.* (1984b) which may indicate that their data are from a non-typical population.

A comparison of the gene diversity observed in this study with other studies of *Pinaceae* suggests that areas totally

Figure 2. — Dendrogram based on genetic distance.

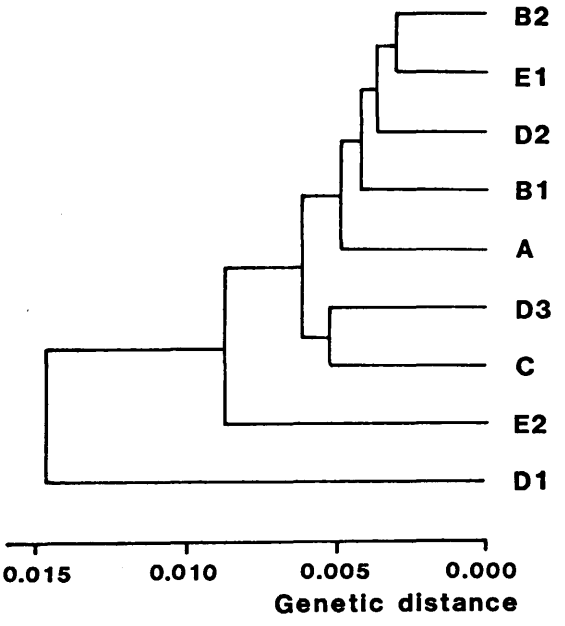

Table 4. — Summary of tests for correspondence with HARDY-WEINBERG expectations.

		A	B1	B2	C	D1	D2	D3	E1	E2	Total
ADH-A	P	0.139	0.356	0.732	0.002**	1.000	1.000	0.652	1.000	1.000	0.0001***
	F	+	-	+	+	-	-	-	-	-	0.064
ADH-B	P	0.623	0.775	0.319	0.679	0.414	0.647	0.218	0.316	0.059	0.464
	F	+	-	+	-	+	+	+	+	+	0.057
F-EST	P	0.090	0.683	0.344	0.818	0.769	0.013*	0.868	0.695	0.456	0.269
	F	+	+	-	-	-	+	-	+	-	0.042
GOT-A	P	1.000	1.000	mono	1.000	1.000	mono	mono	mono	mono	1.000
	F	-	0		+	-					-0.050
GOT-B	P	0.885	0.410	0.203	0.007**	1.000	0.927	1.000	1.000	0.922	0.141
	F	-	+	+	+	+	-		+	+	0.056
LAP-A	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F	-	0	-	-	0	-	0	-	-	-0.049
LAP-B	P	0.132	1.000	0.479	1.000	1.000	1.000	1.000	0.077	1.000	0.156
	F	+	-	-	-	-	-	+	+	-	0.000
MDH-A	P	1.000	1.000	1.000	1.000	0.320	1.000	1.000	0.166	1.000	1.000
	F	-	-	0	-	+	-	-	+	0	0.026
MDH-B	P	0.121	1.000	0.753	0.376	1.000	0.505	0.313	0.925	1.000	0.315
	F	+	+	-	-	+	-	+	-	-	0.002
GDH	P	0.822	0.164	0.134		0.742	0.123	0.348	0.277	0.860	0.056
	F	-	-	-		+	-	+	-	-	-0.090
GOT-C	P	0.810	0.539	0.279					0.003**	0.403	0.036*
	F	-	-	+					-	-	-0.123

* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$

P = The probability of getting the observed or a genotypic distribution even more deviating from the expected, assuming HARDY-WEINBERG equilibrium.

F = $1-H_{obs}/H_{exp}$. The values are given for the total material but is only indicated by sign within the different samples.

mono = monomorphic

covered by ice during the glacial periods have a much lower subdivision than those outside this area. *(Table 6).* The exceptions from this pattern are bristlecone pine *(Pinus longaeva* BAILY) and virginia pine *(Pinus virginiana* MILL.), which, according to HIEBERT and HAMRICK (1983) and WHEELER *et al.* (1983), could have low differentiation due to glacial events. The species of *Pinaceae* have, as a maximum, spent 100 generations in these areas after the latest invasion, and one obvious explanation for smaller differentiation could be that this period has been to short to permit substantial differentiation. As a consequence, one would expect that the species in question are in a differentiation process in he glacial areas and this has to be considered in their management.

Table 5. — Gene diversity statistics (NEI, 1975).

	Absolute values of H_T	Relative values (%) Within stands	Between stands within B,C,D	Between A,B,C, D,E
ADH-A	0.421	96.0	1.4	2.7
ADH-B	0.401	98.4	0.9	0.7
F-EST	0.441	98.8	0.5	0.7
GOT-A	0.018	98.0	0.6	1.4
GOT-B	0.674	98.4	0.8	0.7
LAP-A	0.115	98.7	0.5	0.8
LAP-B	0.145	99.0	0.3	0.7
MDH-A	0.123	98.2	0.4	1.4
MDH-B	0.431	99.5	0.3	0.2
Mean	0.307	98.3	0.7	1.0
S.E.	0.072	0.4	0.1	0.3

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Table 6. — Gene diversity statistics (Nei, 1975) for *Pinaceae*. $G_{ST} = \frac{D_{ST}}{H_T}$ where $H_T = H_S + D_{ST}$, H_T being the total gene diversity, H_S the average gene diversity within populations.

Geografic area	Species	Number of pop	Number of loci	G_{ST}	Data from
Eurasia covered by landice[1]	Pinus sylvestris	3	3	0.03	RUDIN et al.(1974)[2]
	Pinus sylvestris	9	9	0.02	This study
	Picea abies	8	4	0.04	BERGMANN (1974)[3]
	Picea abies	8	4	0.02	LUNDKVIST & RUDIN (1977)[2]
	Picea abies	4	10	0.03	LUNDKVIST (1979)[2]
	Picea abies	10	6	0.05	TIGERSTEDT (1974)[3]
Eurasia not covered by ice					
	Pinus sylvestris	19	3	0.16	MEJNARTOWITCZ (1979)[2]
	Pinus cembra	11	8	0.32	SZMIDT (1982)[2]
	Pinus nigra clus.	7	3	0.12	BONNET-MASIMBERT et al (1978)[4]
	Pinus nigra lari	12	4	0.07	" "
	Pinus nigra nigr.	11	3	0.11	" "
	Pinus nigra pall	10	4	0.07	" "
	Pinus nigra	28	4	0.14	NIKOLIC & TUCIC (1983)
Eastern North American covered by landice					
	Pinus resinosa	5	7	0.00[5]	FOWLER & MORRIS (1977)
	Pinus rigida	11	21	0.02	GURIES & LEDIG (1982)
Eastern North America not covered by ice					
	Pinus virginiana	4	2	0.03	WITTER & FERET (1984)[4]

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Tab. 6. — Continued

Western North American covered by landice					
	Pinus banksiana	3	21	0.02	DANICK & YEH (1983)
	Pinus contorta lat.	5	21	0.02	" "
	Pinus contorta	32	42	0.06	WHEELER & GURIES (1982)
	Pinus contorta lat.	9	25	0.04	YEH & LAYTON (1979)
	Picea sitchensis	10	24	0.08	YEH & EL-KASSABY (1980)
	Pseudotsuga menziesii	6	2	0.03	MEJNARTOWITCZ (1976<9
	P. menziesii COAST	9	4	0.03	YANG et al (1977
	P. menziesii COAST	11	21	0.03	YEH & O'MALLEY (1980)
	P. menziesii INTER	11	21	0.04	YEH, F.C.(1981)
Western North American not covered by ice					
	Pinus longaeva	5	14	0.04	HIEBERT & HAMRICK (1983)
	Pinus monticola	28	12	0.15	STEINHOFF et al.(1983)
	Pinus ponderosa	10	23	0.12	O'MALLEY et al.(1983)
	Pinus radiata	5	19	0.13	BROWN & MORAN (1981)

[1]) The area studied has been totally covered by the landice during one or several glaciations during the Quaternary period.
[2]) Calculated in this study.
[3]) Calculated by BROWN (1979).
[4]) Calculated by BROWN and MORAN (1981).
[5]) Even H_T was equal to zero in this study.

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Geographical variation in the relative proportion of monoterpenes in cortical oleoresin of Pinus sylvestris in Sweden

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Summary

Quantities of several monoterpenes were determined by gas-chromatographic method in individuals belonging to 26 populations of *Pinus sylvestris* planted in Sävar, Sweden, latitude 63° 54', longitude 20°33', altitude 10 m. Pines originating from various parts of Sweden appear to have different monoterpene compositions. Southern populations contain more individuals with low limonene, low β-pinene and high $\triangle$ 3-carene than northern ones. The number of individuals with a high quantity of limonene gradually increases with latitude. The means of the six coefficients of variation for six major monoterpene variables demontrate that southern populations, when transferred from south to north, contain lower genetic variation than those from the central and northern Sweden.

Since there is a cline for relative proportion of limonene in Swedish *Pinus sylvestris* populations, it is proposed that such a pattern has evolutionary significance and is caused by the process of long term natural selection. Therefore, population descriptions based on monoterpene composition can be helpful in breeding work and gene resource conservation programs.

Key words: monoterpenes, *Pinus sylvestris*, population

Zusammenfassung

Nach gaschromatographischer Untersuchung haben quantitative Analysen verschiedener Monoterpene an Einzelbäumen von 26 Populationen der Kiefer *(Pinus sylvestris* L.) in einem Herkunftsversuch in Nordschweden (Sävar 64° 54') folgende Ergebnisse gezeigt:

Kiefern verschiedener schwedischer Herkünfte haben verschiedene Monoterpenmuster. In südlichen Populationen (56—60°) treten häufiger Individuen mit niedrigem Limonen- und β-Pinen- sowie hohem 3-Caren-Gehalt auf als in nördlichen Populationen (64—68°).

Mit zunehmender nördlicher Breite der Herkunft steigt die Anzahl der Individuen mit hohem Limonen-Gehalt.

Mittelwerte der 6 Variationskoeffizienten für 6 Hauptmonoterpen-Variable zeigen, daß südliche Herkünfte, die im Norden angebaut wurden, eine durchschnittlich geringere genetische Variation aufweisen als mittelschwedische (60—64°) und nordschwedische Herkünfte. Dieser Verlust genetischer Variation könnte die Folge einer Selektion am Anbauort sein.

Der gefundene Süd-Nord-Klin für Limonen in schwedischen Kiefern-Populationen hat als Folge langanhaltender natürlicher Selektion evolutionäre Signifikanz. Eine Herkunftsbeschreibung mit Hilfe von Monoterpenmustern kann daher wertvolle Hinweise für die Züchtung und Erhaltung von Genressourcen geben.

Introduction

Conifers produce the greatest amount of terpenes among all plant families. In *P. sylvestris* the heart wood contains 8% resin consisting of 25% monoterpenes (PARHAM 1976).

Monoterpene composition has been shown to be very informative in investigations of infrastructure and population variation for a wide variety of species. For the most part, monoterpene variation is found to be genetically determined and little influenced by environmental factors, and each component is identifiable with high accuracy (SQUILLACE 1976). Studies of monoterpene composition are used for characterization of populations and provenances and determination of seed sources of *Pinus elliotti* (GANSEL and SQUILLACE 1976; SQUILLACE 1977; SQUILLACE *et al.* 1980), *Pinus contorta* (FORREST 1977) and *Pinus maritima* (BARADAT *et al.* 1978). In *Picea abies* monoterpenes have been used as markers in clone identification (ESTEBAN *et al.* 1976). Monoterpenes of the needles and oleoresin of *Pinus sylvestris* are under strong genetic control (HILTUNEN 1975; YAZDANI *et al.* 1982), and little influenced by the environment or the physiological condition of the trees (YAZDANI unpublished data). Some monoterpene fractions show clinal variation among populations of *Pinus sylvestris* in Finland (HILTUNEN *et al.* 1975).

Monoterpene composition seems to play an important role in the defense mechanism against pathogenic fungi (RISHBETH 1972; SCHUCK 1980), and has been found to be related to resistance against insect attacks (HANOVER 1975).

Since monoterpene quantity in *P. sylvestris* is under strong genetic control, it can be used as a multigene marker for studying population structure. This study deals with a comparison of the geographical pattern of monoterpene composition among 26 populations of *P. sylvestris* in Sweden.

Materials and Methods

The reference material consisted of 26 representative and well-documented natural populations of *P. sylvestris* from different latitudes. Further details of the origins are presented in *Table 1* and *Fig. 1*. Seed was sown in 1971 and planted during autumn 1972 at Sävar outside, Umeå, Sweden. (Latitude 63° 54' N, longitude 20° 33 E and altitude 10 m) by the Institute for Forest Improvement.

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