ACTA FORESTALIA FENNICA

Vol. 173, 1981

BIOCHEMICAL METHODS FOR DETERMINING POPULATION STRUCTURE IN *PINUS SYLVESTRIS* L.

MÄNNYN (PINUS SYLVESTRIS *L.*) *POPULAATIORAKENTEESTA BIOKEMIALLISTEN TUTKIMUSTEN VALOSSA*

Min-Sup Chung

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Min-Sup Chung

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*MÄNNYN (**PINUS SYLVESTRIS** L.) POPULAATIORAKENTEESTA BIOKEMIALLISTEN TUTKIMUSMENETELMIEN VALOSSA*

HELSINKI 1981

Arvi A. Karisto Oy:n kirjapaino
Hämeenlinna 1981

PREFACE

This present study consists of biochemical analyses of monoterpene composition and of some allozyme systems in selected Scots pine plus tree clones. The analyses of monoterpenes and allozymes respectively were carried out at the Department of Pharmacognosy and Department of Plant Breeding, University of Helsinki.

The author wishes to express his sincere appreciation to Professor Peter M. A. TIGERSTEDT for the practical guidance on allozyme analysis and on the studies on forest genetics, and to Professor Raimo HILTUNEN at the Department of Pharmacognosy for the practical guidance on monoterpene analysis. The author also wishes to extend his acknowledgement to Professor Max. HAGMAN and Dr. Veikko KOSKI at the Department of Forest Genetics, Finnish Forest Research Institute for valuable discussions and information and for giving the opportunity to work with the clone bank material.

The author is indebted to a great number of persons during his study in Finland. In this connection the author wishes to express his sincere gratitude to Professor Paavo YLI-VAKKURI, the former head of the Department of Silviculture, Professor Matti LEIKOLA, at the same Department, Professor Liisa SIMOLA and Professor Olavi LUUKKANEN at the Department of Botany, University of Helsinki.

Many thanks are due to Professor Bertil RASMUSON at the Department of Genetics, University of Umeå, Dr. Kenneth LUNDKVIST and Dr. Dag RUDIN at the Department of Forest Genetics and Plant Physiology, Swedish College of Agricultural Science for the valuable discussions and informations on biochemical genetics and isozyme techniques during the author's short visit to Umeå, Sweden.

The author wishes to express his appreciation to Professor HYUN, Sin-Kyu and the staff of the Institute of Forest Genetics, Suweon, Republic of Korea.

Many thanks are also due to Päivi RYÖPPY and Riitta LARNA for handling laboratory procurement, to Donald NEVILLE for correcting English in this thesis and Anja KORPELAINEN and Sisko SALMINEN for the typing and drawing of figures respectively. The author wishes to give thanks to his wife KIM, Jae-Il for her laboratory assistance during this study.

This work has been financed by the Department of International Development Co-operation, Ministry for Foreign Affairs of Finland in the form of scholarship since the fall of 1975.

Helsinki, march 1981

AUTHOR

CONTENTS

1. INTRODUCTION

Most of the recent studies on biochemical genetics are fundamentally based on "One Gene – One Enzyme Hypothesis" proposed by Beadle and Tatum in 1941 (AYALA and KIGER 1980, p. 342). According to the hypothesis single genes act through specific enzymes which are specific for single biochemical reactions in the metabolism of an organism. Later the hypothesis was renamed the "One Gene – One Polypeptide Hypothesis" by Ingram (AYALA and KIGER 1980, p. 350–351) who studied the chemical structure of the normal and sickle-cell hemoglobins.

There are two major ways for approaching studies on biochemical traits. One way is the direct observation of enzymatic variations through an indirect visualization of genes of which, in most cases, the primary products are the enzymes. The other way is the observation on variations in one of the final products of gene action through the primary product of enzymes, such as secondary products or substances in plants (ERDTMAN 1968).

The terpenoids, the phenols and the alkaloids constitute the three most important groups of the "secondary plant substances" (HESS 1975, p. 100), and are often used in the study of chemosystematics (ERDTMAN 1968, THIELGES 1969, 1972). Monoterpenes consist of two isoprene units and belong to one of the subgroups of terpenoids (HESS 1975, p. 99). A relatively large amount of monoterpenes are found in pine needles and cortex tissues (HILTUNEN 1976, JUVONEN 1966).

Genetic variation and inheritance of monoterpenes in various pine species have been reported (BARADAT et al. 1979, FORREST 1979, GANSEL and SQUILLANCE 1976, HANOVER 1966a, b, HILTUNEN et al. 1975a, b, HUNT and RUDLOFF 1977, TIGERSTEDT et al. 1979, TOBOLSKI and HANOVER 1971, TOWNSEND and HANOVER 1972).

With the success in separation and staining of serum proteins or enzymes in starch gel zone electrophoresis in the late 1950's (HUNTER and MARKERT 1957, SMITHIES 1955, VESELL and BEARN 1957) the isozyme technique has been widely used in biochemical and population genetics during the last two decades. In the field of forest genetics the isozyme techniques have been introduced in the late 1960's and early 1970's (cf. literature review by RUDIN 1977 b).

Recent studies on isozymes of forest trees indicate that this method can be a very useful tool for the elucidation of the following fields of studies:

(1) Inheritance (ADAMS and JOLY 1980 a, CONKLE 1971, KIM 1979, LUNDKVIST 1974, 1975, 1977, 1978, 1979 b, LUNDKVIST and RUDIN 1977, MITTON et al. 1979, NIKOLIC and BERGMANN 1974, RUDIN 1975, 1977 a, b).

(2) Mating systems (MITTON et al. 1977, MÜLLER-STARCK 1979, RUDIN 1977 b, RUDIN et al. 1974, 1977, RUDIN and LINDGREN 1977, RUDIN and EKBERG 1978, SHAW et al. in press).

(3) Population genetics (BERGMANN and GREGORIUS 1979, KRAZAKOWA and SZWEYSKOWSKI 1979, LUNDKVIST 1978, 1979 a, b, LUNDKVIST and RUDIN 1977, MUHS 1974, RUDIN and RASMUSON 1978, SAKAI et al. 1971, SAKAI and MIYAZAKI 1972, SAKAI and PARK 1971, TIGERSTEDT 1973, 1974 a, YANG et al. 1977).

(4) Chemosystematics (WELLENDORF and SIMONSEN 1979).

The term isozyme (isoenzyme) has been used to describe the multiple molecular forms of proteins which are enzymatically active and catalyzing the same reactions and occurring in the same species (MARKERT and MØLLER 1959, WILKINSON 1970, p. 2). The more specified term allozyme is used for allelic isozymes as proposed by PRAKASH et al. (1969).

In this present study glutamate-oxaloacetate-transaminase (GOT, Enzyme Commission Number 2.6.1.1.), glutamate dehydrogenase (GDH, E. C. No. 1.4.1.2.) and leucine aminopeptidase (LAP, E. C. No. 3.4.1.1.) allozymes were studied.

GOT is also known as aspartate amino-transferase or aspartate transaminase by

biochemists in medical science. It catalyzes the following reversible reactions (WILKINSON 1970, p. 15, LEHNINGER 1975, p. 562):

L-Aspartate + 2-oxoglutarate $\rightleftarrows$ oxaloacetate + L-glutamate

GOT is one of the key enzyme catalyzing transamination reactions in plants (DEVLIN 1975, p. 392) and also occurs in most human and animal tissues (DELORENZO and RUDDLE 1970, WILKINSON 1970, p. 224). Recently, the inheritance and genetic variation of GOT in forest trees was reported (LUNDKVIST 1979, RUDIN 1975).

GDH is the key enzyme of amino acid metabolism in plants through its role as the major port of entry of inorganic nitrogen into the metabolic systems (DEVLIN 1975, p. 391). GDH occurs in higher plants (DEVLIN 1975, p. 391, LUNDKVIST 1979 b, MITTON et al. 1979, see also literatures reviewed by SCANDALIOS 1974), microorganisms and mammalian tissues (WILKINSON 1970, p. 261).

LAP is also known as arylamidase (WILKINSON 1979 p. 301) and appears to catalyze protein degradation (DEVLIN 1975, p. 402–405). LAP occurs both in plants (see literatures reviewed by RUDIN 1977 b) and animals (WILKINSON 1970, p. 301–306).

The primary aim of this study was to investigate the genetic structure of *Pinus sylvestris L.* in Finland with samples of plus tree clones to determine whether there is difference between the northern, central and southern clonal groups. The secondary aim of this study was to investigate the mating probabilities in an imaginary seed orchard including self-fertilization and differential pollination between early and late flowering tree groups.

2. MATERIALS

The clone banks where the study material was collected were established with grafted Scots pine clones in 1958 (old clones) and in 1968 (young clones) at Punkaharju Tree Breeding Station (61° 48′ N, 29° 19′ E, 80 m a.s.l.), Finnish Forest Research Institute. The areas of the old and young clone banks are approximately 1.2 and 5.5 ha and the trees in the clone banks were about 6–9, and 4 m tall respectively at the time of sample collection.

Most of the clones in the young and old clone banks produced relatively abundant female strobili in the 1977 flowering year and developed cones normally except in some abnormally early flowering clones. However, only the trees in the old clone bank produced abundant pollen (usually southern clones produce more pollen) and approximately 21 % of the young clones produced male cones of which the quantity of pollen produced was definitely not enough to pollinate the female strobili within the young clone bank in 1977 flowering (The flowering in this year was observed by the author (CHUNG 1981) and by BHUMIBHAMON (1978)).

The nearest pollen sources for the young clone bank were the neighboring old clone bank and a Scots pine provenance plantation originating from various parts of Finland and one from Norway. The provenance plantation was established in 1931 on an approximately 1.2 ha land area (Fig. 1). The next nearest pollen sources would be the surrounding natural Scots pine stands that are located more than 400 m away in all directions.

Totally 146 clones were analyzed for monoterpenes and 205 for enzymes. Two-year old needles from young clones were used for monoterpene analysis. A total of 16 mature cones per clone were collected in late November 1978 for the cones developed from 1977 flowering. Seeds were extracted and bulked within a clone lot for isozyme analysis. Winter-buds were also collected at the time of cone collection for isozyme analysis.

The investigated clones were grouped into three clonal regions northern (N), central (C) and southern (S) clones according to the local temperature sums of the clonal origins (cf. CHUNG 1981). Clonal regions were represented in roughly equal numbers of clones. Genotype and gene (allele) frequencies were computed for each group. Significance test was made by t-statistics with pooled variances or Chi-square evaluation.

3. METHODS

Monoterpenes were first extracted with 10 ml of n-pentane from 3 g of fresh needles. Then the pentane was evaporated with N_2 gas. The extracts were resolved in 1.5 ml of n-hexane for the analysis by a G. L. C. The conditions for the analysis are listed in Table 1.

Isozymes (GOT, GDH and LAP) were separated from macrogametophytes and the corresponding embryos (at germinating stage) of the seeds by horizontal starch gel (12.5 %) zone electrophoresis. Eight seeds (macrogametophytes and the embryos separated) per clone and the winter-buds of the mother trees in each clone were analyzed. The probability of erroneous judgement of a heterozygote as a homozygote is $(1/2)^7$ in the allozyme analysis with eight macrogametophytes per clone. However, in most cases, the probability of erroneous judgement is close to zero because the allozyme genotypes were confirmed by the analysis of winter-buds in each clone. One may argue if the same loci function both in macrogametophytes, embryos and winter-buds. Theoritically this may often not be the case (SCANDALIOS 1974, 1979). However the selected enzyme systems control key metabolic functions and strict homology was observed in analysing mendelian segregation in the electropherograms.

Extraction of enzymes and electrode buffer systems followed the methods used by LUNDKVIST (1979 b). GDH and LAP isozymes were stained according to the methods described by SHAW and PRASAD (1970) and of GOT by SHAW and SICILIANO (1976). The conditions for electrophoresis are listed in Table 2.

In this study each allozyme locus and alleles were named alphabetically (for the loci) with subscriptions (for the alleles) of arabic numbers in the decreasing order of electrophoretic mobilities to the anode. Thus, for example, the fastest migrating region of GOT to the anode is named GOT locus A and the alleles in this region are A_1, A_2, A_3 and the second fastest migrating region is named locus B and the alleles are B_1, B_2, ---B_7 and so on.

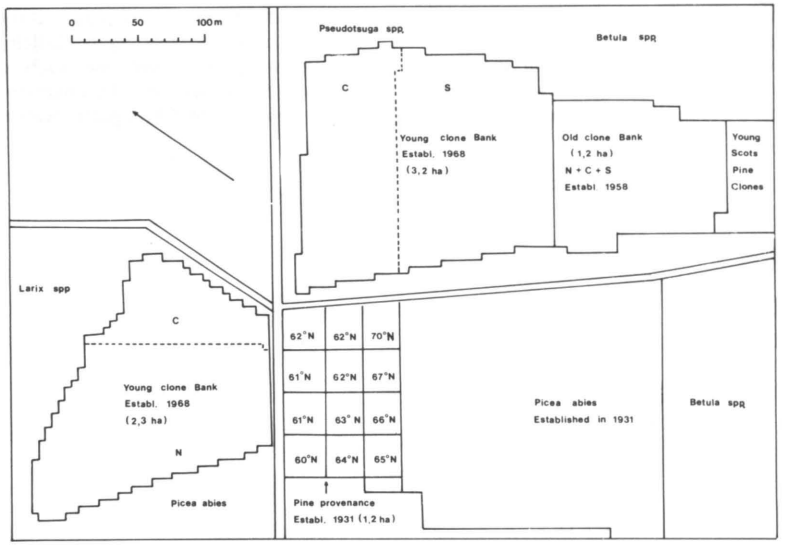


Figure 1. Distribution map of Scots pine clone banks and the surrounding tree stands at Punkaharju Tree Breeding Station. Proximal pollen sources are one Scots pine provenance plantation originating from various latitude throughout Finland

Table 1. Conditions for monoterpene analysis by a G.L.C.

Column: glass capillary 0.35 mm (dia.) x 80 m (length)
Liquid phase: FFAP
Carrier gas: N_2
Split: 1 ml/second
Injection port temperature: 200° C
Temperature program: 4° C/min. up to 240°C

Table 2. The conditions for starch gel zone electrophoresis.

Chamber temperature	: 0–4°C (air cooling)
Running voltage	: 21 V/cm.
Running time	: 6–7 hours
Migration distance (borate front)	:9–10 cm from the sample application point
Gel size	:26 cm (W) x 12 cm (L) x 7 mm (T)
No. of samples per gel	:26 samples including 2 standards

4. RESULTS

4.1. Variation in monoterpene composition between the clonal groups

The major monoterpene components in the investigated clones were α-pinene and 3-carene. On an average, the two components constitute 78 % of the total monoterpene fraction. Northern clones appear to have a larger amount of α-pinene and smaller amount of 3-carene than central and southern clones (Tab. 3). The differences of α-pinene or 3-carene contents between individual clones within a clonal group were often greater than the differences in mean values between the clonal groups.

It has been reported that the genetic control of 3-carene involves a single locus and two alleles with additional modifying genes. The allele (c) coding for "low" 3-carene is recessive and that (C) for "high" dominant (HILTUNEN et al. 1975 b, TIGERSTEDT et al. 1979, see also HANOVER 1966 b, HUNT and RUDLOFF 1977). In this study the phenotypic frequency distribution for 3-carene showed the same mode of inheritance with dominant recessive relationship as reported by the above authors (Fig. 2).

Gene frequencies (q) of the recessive allele (c) in the clonal groups were calculated according to the formula described by LI (1968, p. 14).

$$q = \sqrt{R/G}$$

where R is the number of individuals with the recessive genotype and G is the total number of individuals observed. The calculated q values are presented in Table 4.

Even though there was no statistical difference in gene frequency q between the clonal groups there was an indication of difference in gene frequency q between N and S clonal group (p = 0.0518). When the gene frequency found in northern plus tree clones was compared with that observed in northern natural populations by JUVONEN and HILTUNEN (1972) it was found to be significantly smaller ($p < 0.05$) (cf. TIGERSTEDT et al. 1979). This fact may indicate that there was a tendency for artificial plus tree selection for the "high" 3-carene type (CC or most probably Cc) in the north due to vigorous growth of such individuals.

Table 3. Monoterpene composition in different clonal groups (relative percentages from a total of 100).

Clonal group	No. of clones	Components in %	Tricyclene	α-pinene	Camphene	β-pinene	Sabinene	3-carene	Myrcene	Limonene	β-phellandrene	γ-terpinene	Unknown (No. 29)	Terpinolene	Others
N	50	mean	1.4	65.8	4.7	3.1	0.6	14.5	1.7	2.2	0.5	0.2	1.4	1.5	2.4
		s.d.	0.6	15.5	1.8	2.6	0.4	14.1	0.6	1.2	0.4	0.1	0.9	1.1	–
C	59	mean	1.7	58.8	5.1	2.9	0.8	18.2	2.6	1.4	0.8	0.3	1.4	1.8	4.2
		s.d.	0.8	15.8	2.1	1.5	0.4	13.6	1.0	0.7	0.4	0.2	1.2	1.3	–
S	37	mean	1.7	56.0	5.3	2.7	0.7	21.0	2.1	2.3	0.5	0.3	1.3	2.2	3.9
		s.d.	0.6	16.2	2.2	1.2	0.3	15.1	0.8	1.8	0.3	0.2	0.8	1.4	–
Total	146	mean	1.6	60.5	5.0	2.9	0.7	17.6	2.2	1.9	0.7	0.2	1.4	1.8	3.5
		s.d.	0.7	16.2	2.0	1.9	0.4	14.3	0.9	1.3	0.4	0.2	1.0	1.3	–
Coefficient of variation (%)*			10.3	2.2	1.8	2.2	1.5	1.6	2.4	2.3	3.0	6.8	2.7	2.5	–

* Coefficient of variation as the measure of analytical errors (the CV. was calculated from twelve consecutive analyses of a single oil extract of a clone)

Table 4. Gene frequencies (q) of recessive allele c that controls for 3-carene content in the three clonal groups.

Clonal group	n	q	Remarks
N	50	0.66	no statistical
C	59	0.55	difference
S	37	0.52	

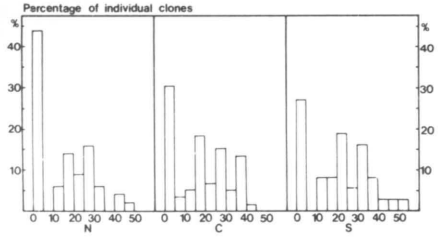


Figure 2. Frequency distribution of 3-carene content in different clonal groups. Ordinate: Percentage of individual clones. Abscissa: 3-carene contents (%).

4.2. Enzyme variarion in the clonal groups

4.2.1. Number of loci and alleles

In all cases there was allelic variation in haploid tissues of macrogametophytes. The allele pairs in the embryo, one of maternal and the other of paternal origin, could readily be recognized by comparing the zymograms of an embryo with the maternal genotype except the case when silent alleles of paternal origin were paired with functional alleles of maternal orgin at LAP A and B loci.

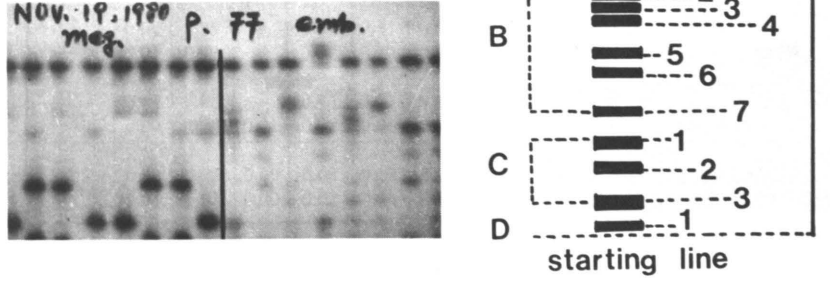


Figure 3. Allozyme pattern of GOT. Left: A photograph of GOT allozymes from seeds (eight macrogametophytes (meg.) and the corresponding embryos (emb.), from the left to the right order). Right: Total zymogram of GOT.

Isozyme patterns detected in the winter-bud of parental trees exactly coincided with the allozymes found in the macrogametophyte.

Four loci were found in the GOT isozyme system (FIg. 3). In locus D only the fastest migrating allele D_1 was found in the gel on the anodal side. Allele D_1 was linked to allele C_1. At the beginning of electrophoresis the alleles in locus D move cathodally at pH 8.1. As electrophoresis proceeds the pH of the electrode buffer on the cathodal side gradually increases from 8.1 to maximally 8.4 and the alleles in locus D move back towards the anode. Usually the allele D_1 reaches the anodal part of the gel at the end of electrophoresis.

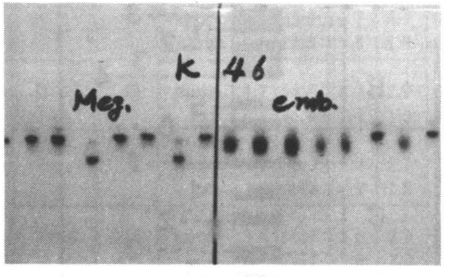
Three alleles were found at locus A, the A_2 allele having the highest frequency. Seven alleles were found at locus B. Allele B_6 is extremely rare and found only in the pollen pool. Three alleles were found in locus C. The molecular structure of GOT appears to be dimeric producing hybrid enzymes in addition to parental enzymes in heterozygotes as it was reported by RUDIN (1975).

GDH in the Scots pine clones showed a diallelic pattern at one locus (Fig. 4) just as it has been reported in *Pinus ponderosa* (MITTON et al. 1979). Homozygous individuals (winter-buds of the parental trees and the embryos of the seeds) and macrogametophytes (no matter whether its parent is homozygous or heterozygous) produce either A_1 or A_2 band while heterozygous individuals (parental trees and embryos) produce only one intermediate diffuse band A_1A_2. This fact may indicate the enzyme structure of GDH is multimeric (PRYOR 1974).

LAP was represented by four alleles at each of two loci (A and B) (Fig. 5). Silent alleles (null alleles) have been found in each locus. LAP A_2 and B_2 were the most common alleles in the investigated clones. LAP enzyme structure appears to be monomeric producing two bands in a heterozygous state.

4.2.2. Allele frequencies

Allele frequencies of the maternal (detected through macrogametophytes and winter-buds) and paternal (detected through embryos) origins in the three clonal groups are presented in Tables 5 (for GOT) and 6 (for GDH and LAP). The statistical differences in allele frequencies between the clonal groups and between male and female origins are presented in Tables 7 and 8 respectively.



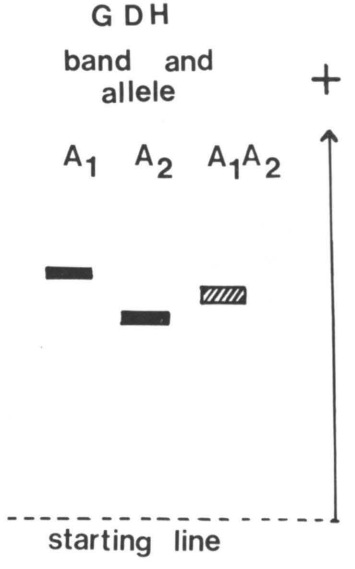
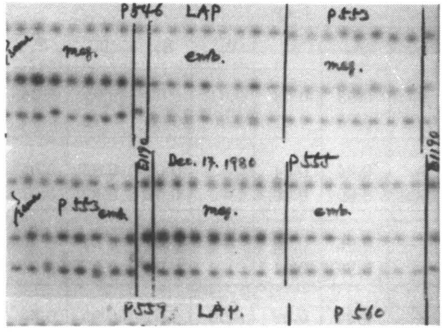


Figure 4. Allozyme pattern of GDH. Left: A photograph of GDH allozymes. Right: Zymograms of GDH. Illustration the same as in Fig. 3.



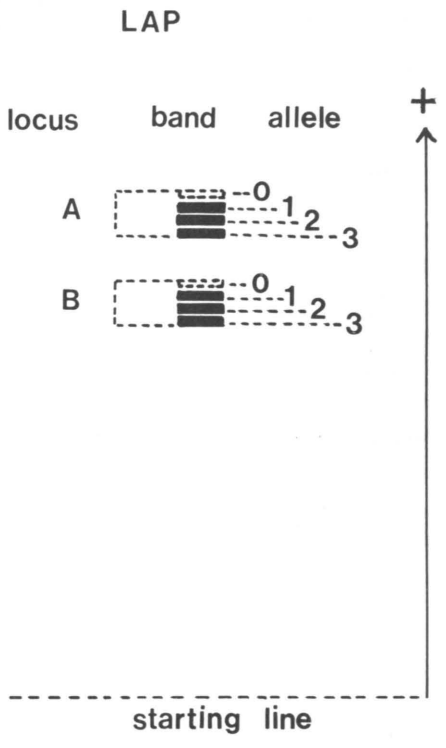


Figure 5. Allozyme pattern of LAP. Left: A photograph of LAP (two gels) from seeds (8 macrogametophytes (meg.) and the corresponding embryos (emb.), from the left to the right order) of three clones. 9th and 26th rows (vertical) are zymograms of standard samples. Right: Total zymogram of LAP. A_0 and B_0: silent alleles. Alleles in the first and second rows in each gel are identical alleles from different layers of the same gel of locus A. Third row of each gel: alleles in locus B.

Table 5. Maternal (♀) and paternal (♂) allele frequencies for GOT allozyme loci.

Clonal group	Source	GOT												
		A_1	A_2	A_3	B_1	B_2	B_3	B_4	B_5	B_6	B_7	C_1	C_2	C_3
N	♀	–	0.99	0.01	0.01	0.18	0.19	0.13	0.49	–	–	0.29	0.02	0.69
(69)	♂	0.01	0.99	–	0.03	0.08	0.21	0.10	0.56	0.00	0.02	0.29	0.02	0.69
C	♀	0.02	0.98	–	0.02	0.11	0.20	0.06	0.60	–	0.01	0.31	0.02	0.67
(61)	♂	0.01	0.99	–	0.02	0.10	0.22	0.08	0.58	–	0.00	0.29	0.01	0.70
S	♀	0.02	0.98	–	0.05	0.07	0.20	0.07	0.61	–	–	0.29	0.02	0.69
(64)	♂	0.00	0.99	0.01	0.02	0.06	0.21	0.08	0.63	–	0.00	0.33	0.01	0.66
Total	♀	0.02	0.98	0.00	0.03	0.12	0.20	0.09	0.56	–	0.00	0.29	0.02	0.69
(194)	♂	0.01	0.99	0.00	0.02	0.08	0.21	0.09	0.59	0.00	0.01	0.30	0.02	0.68

N = northern, C = central, S = southern clones
Numbers in parenthesis are the number of clones observed.
Maternal gene frequencies were computed directly from diploid clones.
Paternal gene frequencies were computed from eight embryos per clone.
– = non-occurrence, 0.00 = frequency smaller than 0.005 (rounded off).

Table 6. Maternal (♀) and paternal (♂) allele frequencies for GDH and LAP allozyme loci.

Clonal group	Source	GDH		LAP							
		A_1	A_2	A_0	A_1	A_2	A_3	B_0	B_1	B_2	B_3
N (69)	♀	0.37	0.63	0.04	–	0.93	0.03	0.01	0.01	0.92	0.06
	♂	0.36	0.64	0.00	0.01	0.98	0.01	0.00	0.03	0.92	0.05
C (61)	♀	0.31	0.69	0.05	–	0.94	0.01	–	0.02	0.93	0.05
	♂	0.36	0.64	–	0.00	1.00	0.00	–	0.04	0.89	0.07
S (64)	♀	0.36	0.64	0.02	–	0.96	0.02	–	0.02	0.92	0.06
	♂	0.38	0.62	–	–	0.99	0.01	–	0.01	0.95	0.04
Total (194)	♀	0.35	0.65	0.04	–	0.94	0.02	0.00	0.01	0.93	0.06
	♂	0.37	0.63	0.00	0.00	0.99	0.01	0.00	0.03	0.92	0.05

Designation the same as in Table 5. A_0 and B_0 are silent alleles.

Table 7. Differences in allele frequencies in the maternal (♀) and paternal (♂) gene pools between the clonal groups.

Enzymes	GOT				LAP		Remarks
Alleles	B_2	B_5	B_7	C_2	B_1	B_2	Significance at p. of
♀	N > S**	N < S*					* = 0.05, ** = 0.01,
♂	C > S**	N < S*	N > C*	C > S**	C > S**	C < S***	*** = 0.005

evaluated by 2 x 2 contingency table.

Table 8. Differences in allele frequencies between maternal (♀) and paternal (♂) origins of the clonal groups.

Enzymes	GOT			LAP		Remarks
Alleles	A_1	B_1	B_2	A_0	A_2	
N			♀>♂***	♀>♂***	♀<♂***	significance at p. of
C				♀>♂***	♀<♂***	* = 0.05
S	♀>♂**	♀>♂**		♀>♂***	♀<♂***	** = 0.01
Toltal	♀>♂*		♀>♂***	♀>♂***	♀<♂***	*** = 0.005

A_0 = silent allele
evaluated by 2 x 2 contingency table

4.3. Mating probability and population structure

4.3.1. Allele and genotype frequencies in the progeny groups

The allele frequencies of male and female gametes represent the potential mating probabilities of the parental gene pool. The expected allele frequencies of the progenies are the sum of the products of the mating combinations between the male and female gamete frequencies. The allele and genotype frequencies can be recorded directly from the progeny genotypes or can be calculated from the allele frequencies of the parental gene pool. For example, from Table 5 we get maternal allele frequencies of 0,02 and 0,98 and paternal allele frequencies of 0,01 and 0,99 for GOT A_1 and A_2 of the central clonal group respectively. The calculation method for the allele and genotype frequencies in the progeny is presented in Table 9.

Actually observed allele frequencies of the progenies are presented in Tables 10 (for GOT) and 11 (for GDH and LAP).

Table 9. An example of calculation of expected allele and genotype frequencies of progenies from the parental allele frequencies.

Parental allele frequency		♀ A_1 0.02	♀ A_2 0.98	Allele frequencies in the progeny
♂	A_1 0.01	A_1A_1 0.0002	A_1A_2 0.0098	$A_1 = 0{,}0002 + \frac{0.0098 + 0.0198}{2} = 0.0150$
	A_2 0.99	A_1A_2 0.0198	A_2A_2 0.9702	$A_2 = 0{,}9702 + \frac{0.0098 + 0.0198}{2} = 0.9850$

Table 10. Observed allele frequencies at GOT loci in the progeny groups.

Clonal groups	GOT												
	A_1	A_2	A_3	B_1	B_2	B_3	B_4	B_5	B_6	B_7	C_1	C_2	C_3
N (522)	0.00	1.00	0.00	0.02	0.13	0.20	0.11	0.53	0.00	0.01	0.29	0.03	0.68
C (488)	0.01	0.99	–	0.02	0.11	0.21	0.08	0.57	–	0.01	0.29	0.02	0.69
S (521)	0.01	0.99	0.00	0.03	0.07	0.21	0.07	0.62	–	0.00	0.32	0.01	0.67
Total (1552)	0.01	0.99	0.00	0.03	0.10	0.20	0.09	0.57	0.00	0.01	0.30	0.02	0.68

Table 11. Observed allele frequencies at GDH and LAP loci in the progeny groups.

Clonal groups	GDH		LAP							
	A_1	A_2	A_0	A_1	A_2	A_3	B_0	B_1	B_2	B_3
N (552)	0.36	0.64	0.03	0.00	0.95	0.02	0.00	0.02	0.92	0.06
C (488)	0.35	0.65	0.02	0.00	0.97	0.01	–	0.03	0.91	0.06
S (512)	0.37	0.63	0.01	–	0.98	0.01	–	0.02	0.93	0.05
Total (1552)	0.36	0.64	0.02	0.00	0.97	0.01	0.00	0.02	0.92	0.06

numbers in parenthesis are the number of embryos observed
– = non-occurrence, 0.00 = frequency smaller than 0.005 (rounded off)
A_0 and B_0 are silent alleles.

The differences in allele frequencies between the progeny groups are presented in Table 12.

Even though the overall allele frequencies were approximately the same between the maternal and paternal gene pools, except GOT B_2 and B_5 between N and S clonal groups (Tab. 7) greater differences in allele frequencies were encountered in the progeny groups (Tab. 12). The greater differences in allele frequencies among the progeny groups

Table 12. Differences in allele frequencies between the progeny groups.

Enzymes	GOT				LAP		
Alleles	B_2	B_4	B_5	C_2	A_0	A_2	Remarks
Differences between groups	N > S*** C > S***	N > C*** N > S***	N < C* N < S*** C < S*	N > S*	N > S** C > S*	N < S** N < S***	Significance at p. of * = 0.05 ** = 0.01 *** = 0.005

evaluated by 2 x 2 contingency table.

may be partly caused by the differential pollination of female gametes as shown in Table 6 and partly by the potential differences in allele frequency of maternal origin which was not accounted for in the statistical evaluation with smaller sample size.

The genotype frequencies in the parent (maternal) and progeny groups for the most heterozygous loci are presented in Tables 13 (for GOT locus B) and 14 (for GOT locus C and GDH). The differences in genotype frequencies between the clonal groups were also notably increased at GOT locus B of the progeny groups as compared to the maternal ones (Tab. 15). There was no statistical differences in genotype frequencies between the maternal groups or between the maternal and progeny pairs within clonal group except for genotype GOT B_3B_4 of which the maternal northern group had significantly (p = 0,009) larger numbers of the genotype.

4.3.2. The estimation of pollen clouds in the early and late flowering clones.

Pollen clouds were estimated in the early and late flowering maternal tree groups as to whether there is differences in allele frequencies due to differences in pollination time. The average difference in flowering times in 1977 between the early and late flowering groups was larger than 900 period units (for p.u. refer to Sarvas 1972) and approximately 3 days in time scale. The receptive intervals of female strobili between the early and late flowering clones partly overlapped during the maximum pollen shedding day in 1977.

Table 14. Genotype frequencies in the parent (M) and progeny (F) groups at GOT C and GDH A loci.

Enzyme genotype		n	GOT					GDH		
			C_1C_1	C_1C_2	C_1C_3	C_2C_3	C_3C_3	A_1A_1	A_1A_2	A_2A_2
N	M	69	0.09	–	0.41	0.04	0.46	0.14	0.45	0.41
	F	552	0.09	0.01	0.39	0.05	0.46	0.14	0.45	0.41
C	M	61	0.13	0.02	0.33	0.03	0.49	0.08	0.46	0.46
	F	488	0.08	0.01	0.42	0.02	0.47	0.13	0.45	0.42
S	M	64	0.09	0.02	0.37	0.02	0.50	0.14	0.44	0.42
	F	512	0.09	0.01	0.46	0.02	0.42	0.14	0.46	0.40
Total	M	194	0.10	0.01	0.37	0.03	0.49	0.12	0.45	0.43
	F	1552	0.09	0.01	0.42	0.03	0.45	0.13	0.45	0.42

Table 15. Differences in genotype frequencies at GOT loci B and C in the progenies.

Genotype	B_2B_2	B_5B_5	B_2B_3	B_2B_4	B_2B_5	B_3B_4	B_4B_5	C_1C_3	C_2C_3
Difference	N > S*** C > S**	N < S*** C < S**	N > S**	N > C**	N > S* C > S*	C < S*	N > S*** N > C*	N < S**	N > C* N > S*

Significance at p. of * = 0.05, ** = 0.01, *** = 0.005

evaluated by 2 x 2 contingency table.

The allele frequencies of pollen observed from the zygotes of the early and late flowering clonal groups are presented in Table 16. There was no statistical difference in allele frequencies between the two groups.

4.3.3. Rate of self-fertilization

Overall average rate of self-fertilization was calculated in a similar way as in the multilocus estimation method developed by

Table 13. Genotype frequencies in the parent (M) and progeny (F) groups at GOT locus B.

Enzyme genotype		n	Genotype frequencies at GOT locus B																		
			B_1B_1	B_2B_2	B_3B_3	B_4B_4	B_5B_5	B_1B_2	B_1B_3	B_1B_4	B_1B_5	B_2B_3	B_2B_4	B_2B_5	B_2B_7	B_3B_4	B_3B_5	B_3B_7	B_4B_5	B_5B_6	B_5B_7
N	M	69	–	0.04	0.04	0.02	0.26	–	–	–	0.03	0.03	0.06	0.19	–	0.10	0.16	–	0.07	–	–
	F	552	0.00	0.02	0.05	0.02	0.29	0.00	0.01	0.01	0.03	0.05	0.02	0.13	0.00	0.04	0.19	0.00	0.13	0.00	0.01
C	M	61	–	–	0.03	0.02	0.38	–	0.02	–	0.02	0.06	–	0.16	–	0.03	0.20	0.02	0.06	–	–
	F	488	–	0.02	0.07	0.01	0.33	–	0.00	–	0.04	0.04	0.00	0.14	–	0.03	0.22	0.00	0.09	–	0.01
S	M	64	0.02	–	0.06	0.02	0.37	–	0.03	–	0.03	0.02	0.02	0.11	–	–	0.23	–	0.09	–	–
	F	512	0.00	0.00	0.05	0.01	0.41	0.00	0.01	0.01	0.04	0.02	0.01	0.09	–	0.05	0.23	0.00	0.07	–	0.00
Total	M	194	0.00	0.01	0.05	0.01	0.34	–	0.01	–	0.03	0.04	0.03	0.15	–	0.05	0.20	0.00	0.08	–	–
	F	1552	0.00	0.02	0.06	0.01	0.34	0.00	0.01	0.00	0.03	0.04	0.01	0.12	0.00	0.04	0.21	0.00	0.10	0.00	0.01

– = non-occurrence, 0.00 = frequency smaller than 0.005 (rounded off)

Table 16. Allele frequencies of pollen observed from the zygotes of the early (E) and late (L) flowering clones.

Enzyme	GOT											GDH		LAP									n
Allele	A_1	A_2	A_3	B_1	B_2	B_3	B_4	B_5	B_6	B_7	C_1	C_2	C_3	A_1	A_2	A_0	A_1	A_2	A_3	B_0	B_1	B_2	B_3	
E (27)	0.01	0.99		0.03	0.08	0.21	0.12	0.53	–	0.03	0.30	0.03	0.67	0.33	0.67	0.01	0.01	0.97	0.01	–	0.03	0.93	0.04	216
L (28)	0.00	1.00	0.00	0.02	0.08	0.21	0.11	0.58	–	–	0.34	–	0.66	0.35	0.65	–	–	0.99	0.01	–	0.02	0.93	0.05	224

numbers in parenthesis are the number of clones observed.
n = the number of alleles of pollen detected through embryos.

SHAW et al. (1981). The estimation method used for the calculation of self-fertilization is exactly the same as that used by the above authors except that direct calculation of conditional probability of self-fertilization, instead of calculating the probability of cross-fertilization, for the non-discernible or ambiguous matings (cf. SHAW et al. 1981). The above authors estimated the proportion of crossfertilization through the estimation of mating system parameters from the maternal genotypic frequencies and the allele frequencies in the pollen pool.

In this study the matings are grouped into tow categories as those of the above authors, (1) "discernible" cross-fertilization by foreign alleles which are not present in maternal parent but present in the progenies, (2) "non-discernible" or "ambiguous" matings where uncertainty arises whether they are from selfing or cross-fertilization by pollen of other trees that carry alleles like those of the maternal parent.

The probability of self-fertilization in the discernible mating is zero. The probability of self-fertilization in the ambiguous matings was calculated as follows:

(1). Conditional probabilities of self-fertilization (CPS) were calculated for the investigated loci of an embryo based on the embryo genotype under the assumption that the progeny was produced by self-fertilization. Thus, if both the maternal parent and progeny are homozygous at a given locus the CPS for that locus was considered to be 1. If a maternal parent is heterozygous at a locus, with allele combination of A_1A_2 for example, the probabilities for the occurrence of allele combinations of A_1A_1, A_1A_2 and A_2A_2 by self-fertilization would be 1/4, 1/2 and 1/4, (these values are the CPSs of the corresponding loci) respectively provided that the alleles segregate normally.

(2) The overal CPS in an embryo is the product of the CPS of each locus over all the loci examined (cf. Tab. 17).

(3) The overall average CPS on the population as a whole is Σ Pi/N where Pi is the overall CPS of an embryo and N is the numver of embryos investigated including the embryos of cross-fertilization.

(4) Under natural conditions of open pollination, the overall CPS calculated in this way may be partly originated from self-fertilization and partly from cross-fertilization by the pollen of trees that carry alleles like those of the maternal parents for the loci in the male flowering clones.

The overall CPS calculated in this way for the non-male-flowering clones is entirely due to cross-fertilization by pollen of trees that carry alleles like those of the maternal parent. Under the assumption that the CPS calculated for the male flowering tree group consists of about the same proportion of cross-fertilization as found in the non-male-flowering tree group, the approximate rate of self-fertilization in the male flowering tree group was estimated by subtracting the CPS of non-male-flowering group from that of the male flowering group. That is:

$$S_m = \left[\Sigma P_{im} / N_m\right] - \left[\Sigma P_{if} / N_f\right]$$

where S_m is the rate of self-fertilization in male flowering tree group, p_{im} is the CPS of male flowering tree group and N_m is the number of male flowering trees under

Table 17. An example of calculation method for the conditional probability of self-fertilization (CPS) from the embryo genotypes (EG) of given maternal genotypes (MG).

MG or EG	Enzyme loci and genotype						overall CPS for the embryos
	GOT			GDH	LAP		
MG 1	A_2A_2	B_3B_5	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
EG 1	A_2A_2	B_3B_4*	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
CPS 1	1	0	1	1	1	1	0
EG 2	A_2A_2	B_3B_3	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
CPS 2	1	1/4	1	1	1	1	1/4
EG 3	A_2A_2	B_3B_5	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
CPS 3	1	1/2	1	1	1	1	1/2
MG 2	A_1A_2	B_3B_5	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
EG 4	A_2A_2	B_3B_3	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
CPS 4	1/4	1/4	1	1	1	1	1/16
EG 5	A_1A_1	B_3B_5	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
CPS 5	1/4	1/2	1	1	1	1	1/8
EG 6	A_1A_2	B_3B_5	C_3C_3	A_2A_2	A_2A_2	B_2B_2	
CPS 6	1/2	1/2	1	1	1	1	1/4

* = foreign allele

Table 18. The estimated overall average conditional probability of self-fertilization (CPS) and the rate of self-fertilization in old and young clones varying in male flowering.

Clone bank	Approx. age (yrs.)	CPS Mean ± sd.	rate of self-fert.	Male flowering
Old (22)	20	0.1410 ± 0.1515	0.0612	Abundant
Young (30)	10	0.0848 ± 0.0817	0.0050	poor
Young (92)	10	0.0798 ± 0.0698	- - -	None

Numbers in parenthesis are the number of clones observed. Eight seeds per clone were examined. Clones without either of the most common allele (GOT B_5 or C_3) were excluded.

investigation, p_{if} and N_f are the CPS and the number of non-male-flowering clones respectively. For an unbiased estimation of the rate of self-fertilization by this method the following conditions are required;
(1) no gametic selection occurs during the matings.
(2) allele frequencies in the pollen pool are uniformly distributed.
(3) maternal genotypic frequencies of the male-flowering and non-male-flowering groups are roughly equal.
(4) independent and equal segregation of the gametes over all the investigated loci.

The estimated average values of CPS and rate of self-fertilization on each tree group are presented in Table 18.

The estimated conditional probability of self-fertilization in the old clones ranged from 0.000 to 0.625. The values of conditional probability of self-fertilization in the investigated clones varied according to the presence or absence of common alleles (the alleles in question were B_5 and C_3 of GOT) at one of two investigated loci. In the absence of either of them the CPS was significantly smaller than when they were present ($t = 4.64^{***}$, $df = 131$).

Table 19. Average heterozygosity (H) and genetic diversity (V(p)) of the maternal (M) and progeny (F) groups computed over six enzyme loci.

Clonal group		N	H	V(p)
N	M	69	0.31	13.20
	F	552	0.30	11.72
C	M	61	0.29	9.54
	F	488	0.29	9.97
S	M	64	0.29	9.34
	F	512	0.28	8.47
Total	M	194	0.30	10.75
	F	1552	0.29	10.13

4.3.4. Genetic diversity

Average heterozygosity (NEI 1975, p. 128---132, NEI & ROYCHOUDHURY 1974), genetic diversity (GREGORIUS 1978), were calculated for mother clones and progeny groups and the values are presented in Table 19.

The calculations were made according to the following formulae:

$$H = \sum_{l=1}^{r} h_l/r = \text{average heterozygosity}$$

$l = l$ -th locus

r = number of loci

$h = 1-\Sigma xi^2$ = heterozygosity at a locus

xi = frequency of i-th allele

$$V(p) = \left[\frac{1}{\sum_{i_1=1}^{n_1} P1i_1^2} \cdot \cdots\cdots \cdot \frac{1}{\sum_{i_l=1}^{n_l} Pli_l^2} \right] -$$

1 = genetic diversity

i = allele ($i_j = 1 \ldots n_j$) at j-th locus ($j = 1 \ldots l$)

Pi_l = allele frequency ($Pi_j = P1i_1 \ldots P_l i_l$

Statistical evaluation for the average heterozygosity over six loci revealed that there was no differences between the maternal groups ($p = 0.2 - - - 0.4$). In other words, the maternal populations were not differentiated notably on those allozyme loci.

5. DISCUSSION

5.1. Mating system and population structure

Pines are generally known to be outcrossing species (cf. review by RUDIN 1977, SQUILLACE 1974). Although there is no mechanism of preventing self-fertilization, flowering characteristics of Scots pine favors cross-pollination (CHUNG 1981, SARVAS 1962). Such allogamous species are expected to have high levels of heterozygosity at many loci provided that allele frequencies at each locus are intermediate.

In this study three loci (GOT B, C, and GDH A) out of six appeared to maintain a high level of heterozygosity and the other loci (GOT A, LAP A and B) were highly homozygous (Tables 5 and 6). It is interesting that the GDH locus maintains a hight level of heterozygosity with a di-allelic system while GOT A and LAP (A and B) loci maintain high levels of homozyzygosity with tri- and tetra-allelic systems respectively. Therofore, homozygosity or heterozygosity at a locus does not appear to dependent on the number of alleles at the locus.

BROWN (1979) discussed what he calls the "heterozygosity paradox" and concludes that there is a trend for outbreeders to show less heterozygosity than expected, and inbreeders to show more. He explained that the situation in inbreeders depends on the tendency of geographic and microgeographic differentiation and more intense multilocus associations than in outbreeders. The reason why outbreeders tended to show less heterozygosity than expected was not clearly explained. However it is suggested that many heterozygous loci with silent alleles would not have been detected.

TIGERSTEDT (1973, 1974 a, b, 1979) examined the population structue of *Picea abies* Karst in Finland as to whether polymorphism and heterozygosity decrease towards the margins of the species range. The results indicated that the marginal populations of Norway spruce retain as much genetic variation as more central populations. In this present study on Scots pine clones the same pattern of genetic variation was found. The northern clones maintain as much genetic variation as central or southern clones even though there was an indication of differences in genotype frequencies between the clonal groups judging from the prognies. The mechanism that maintains a high level of genetic variation even in the marginal populations was interpreted as density dependent balancing selection (TIGERSTEDT 1979). However other equally fundamental causes can be suggested.

5.2. The influence of timing of female flowering on pollination and self-fertilization.

A strobilus of pine receives pollen continuously during its receptive period. Therefore the pollination of a female strobilus is a mixture from various pollen sources. Thus a mature cone pollinated in this way may contain seeds of different paternal origin. In 1977 the receptive period of female strobili in very early flowering clones began approximately 4.5 days, on an average, before maximum pollen shedding day and partially overlapped (in some clones) with the receptive period of very late flowering clones (unpublished data from 1977 flowering observation by the author). The observation on Scots pine flowering in 1977 indicated that the majority of very early flowering clones must mainly have been pollinated by different pollen sources from that of very late flowering clones.

The result in this study showed no statistical difference in paternal allele frequencies between the early and late flowering groups. This result cannot be explained properly at this moment due to the small number of observations on very early or late flowering clones. The differences in gamete frequencies between clonal groups in Table 7 indicated that the northern clonal group, to which the majority of very early flowering clones belong, was pollinated by a

significantly smaller number of pollen with the GOT B_5 allele than the southern clonal group (late flowering clones). However, it is not clear whether the difference in allele frequency is due to the difference in flowering time or to the difference in location of the two clonal groups from the pollen path and pollen source.

Synchronous flowering of male and female strobili within an individual tree may increase self-fertilization within a population. Most of the estimation of self-fertilization in forest trees were made by estimating mating probabilities by rare alleles or combined with rare and common alleles (ADAMS and JOLY 1980, MÜLLER 1976, 1977, MÜLLER-STARCK 1979, RUDIN et al. 1977). This method of estimating self-fertilization appears to be very useful in clonal seed orchards. However, it may deviate from the values of self-fertilization of trees that carry common alleles.

In this study the values of conditional probability of self-fertilization (CPS) in the trees that lack one of the most common allele was found to be much lower than that of the trees with common alleles.

The calculated average value of CPS in old clones by a multilocus estimation method was 14.1 %, 8.0 % of the CPS being caused by matings between trees that carry the same alleles to one of the maternal parent at some loci, and 6.1 % being through self-fertilization. A summary of estimated rates of self-fertilization using rare alleles or rare alleles combined with common alleles is presented in Table 20. The rates of self-fertilization estimated by other methods (mostly by chlorophyll mutant forms) than the isozyme technique is presented in Table 21.

5.3. Gene flow and natural selection

Flowering characteristics of Scots pine and the sequences of flowering time along latitudinal clines in Finland favors directional gene flow from the south to the north of Finland (CHUNG 1981, KOSKI 1970, SARVAS 1962). HILTUNEN et al. (1975 a) found that there is clinal variation of 3-carene content in Scots pine. According to the authors the frequency of recessive allelec that controls low-carene type in Scots pine is low in the south and high in the north of Finland and the variation is clinal. The authors interpreted this to be partly due to directional gene flow from the south to the north and partly due to natural selection favoring the recessive allele in the north.

If the growth character of northern trees are affected by the south-north gene flow and if the migrated alleles from the south to the north carry features for faster growth, the combined effect of gene flow and natural selection in the north would result in relatively fast growing heterozygous genotypes. Such heterozygous genotypes are apt to be selected for plus trees by artificial selection. Indeed, the result in this study on monoterpene analysis indicated that such heterozygous individuals tend to be favored in plus tree selection. The frequency of recessive allele c in the northern plus tree clones was lower than that of the corresponding Scots pine natural stands (see also TIGERSTEDT et al. 1979).

In the isozyme study, the northern clonal group has a higher frequency of allele GOT B_2 and a corresponding lower value of allele GOT B_5. In this sdudy, however, a test as to whether the variation of allele frequencies was clinal throughout Finland was not made because the number of samples are rather small and they constitute plus tree selections thus biasing criteria for random samples.

Table 20. Estimated rates of self-fertilization by isozyme technique.

Author	Year	Species	Self-fert. (%)	Remarks
MÜLLER	1976	*Picea abies*	14.7	Spruce stand (embryo)
"	1977	"	11.9	" (")
"	"	*Pinus sylvestris*	6.2	Pine stand (")
RUDIN et al.	"	"	17.0	Seed tree stand (2-year old seedlings)
"	"	"	24.0	Seed tree stand (embryo)
MÜLLER-STARCK	1979	"	12.6	Seed orchard (")
ADAMS & JOLY	1980	*Pinus taeda*	1.2	" (")
SHAW & ALLARD	in press	*Pseudotsuga menziesii*	7.0	Douglas-fir stand (")
Present study	1981	*Pinus sylvestris*	6.1	Old clone bank (")
"	"	"	0.5	Young clone bank (")

The contents in the remarks denote the parental trees and the materials analysed (in the parenthesis).

Table 21. Estimated frequency of self-fertilization by other methods (mostly by chlorophyll mutant froms) than isozyme techniques in conifer species.

Author	year	species	self-fert. (%)
EICHE	1955	*Pinus sylvestris*	9
SQUILLACE & KRAUS	1963	*Pinus elliottii*	6
FOWLER	1965a	*Pinus banksiana*	20
"	1965b	*Pinus resinosa*	12
RUDOLPH	1966	*Pinus banksiana*	17
FRANKLIN	1968	*Pinus taeda*	2
"	1971	" (upper crown)	7
		(lower crown)	34
OHBA et al.	1971	*Pinus densiflora*	5
OHBA	1972	*Pinus thunbergii*	40
KOSKI	1973	*Picea abies*	6
SORENSEN	1973	*Pseudotsuga menziesii*	7
MÜLLER	1977	*Picea abies*	5

6. SUMMARY

Studies on Finnish Scots pine plus tree clones by monoterpene and isozyme analyses have been undertaken to further investigate mating system, population structure and pollination. Six allozyme systems (3 GOT, 1 GDH and 2 LAP) were properly analysed on the basis of segregation. Monoterpenes were analysed from needle material and segregation in high and low 3-carene content was found to depend on two alleles, C and c.

Thus six allozyme systems and one monoterpene system were used as markers in this study. It was found:

- That the northern clonal group maintains as much genetic variation as the central or southern clonal groups.
- That the conditional probability of self-fertilization in about 20-year old clones estimated by the multilocus model was 14.1 %, of which 8.0 % originate from matings between trees that carry the same alleles to one of the maternal parent at some loci and 6 % through self-fertilization.
- That there was no prominent difference in allele frequences of male gametes that pollinated the very early or very late flowering clones.
- That the northern clonal group has higher and lower frequencies of alleles GOT B_2 and B_5 respectively than that of the southern clonal group.
- That artificial plus tree selection, particularly in northern Finland, appears to favor heterozygous genotypes for the alleles that control 3-carene content in Scots pine.

Seloste:

MÄNNYN (*PINUS SYLVESTRIS* L.) POPULAATIORAKENTEESTA BIOKEMIALLISTEN TUTKIMUSMENETELMIEN VALOSSA

Männyn kantapuiden klooneista on tutkittu eräiden monoterpeenien ja isotsyymien vaihtelua. Tutkimuksilla on pyritty valaisemaan männyn risteytyssysteemiä, populaatiorakennetta ja pölytystä. Tutkimukset on tehty Metsäntutkimuslaitoksen Punkaharjun männyn klooni-kokoelmissa. Kuusi allotsyymisysteemiä (3 GOT, 1 GDH ja 2 LAP) analysoitiin geneettisesti segregaation perusteella. Monoterpeenit tutkittiin neulasnäytteistä. Todettiin geneettistä segregaatiota korkean ja alhaisen 3-kareenipitoisuuden suhteen. Segregaatio johtuu kahdesta alleelista C ja c. Kaikkiaan käytettiin tutkimuksissa markereina kuutta allotsyymi- ja yhtä monoterpeenisysteemiä.

Tutkimuksissa todettiin:

- Että pohjoinen klooniryhmä on geneettisesti yhtä vaihteleva kuin keski- ja eteläsuomalainen ryhmä
- Että itsepölytyksen ehdollinen todennäköisyys noin 20 vuoden ikäisissä männyn kloonivartteissa oli 14.1 %, josta 8.0 % johtui saman alleelin aiheuttamasta vieraspölytyksestä ja 6.1 % puhtaasta itsepölytyksestä
- Että aikaisin ja myöhään kukkivien kloonien pölytyksessä ei ole havaittavissa huomattavia eroja siitepölyn geneettisessä rakenteessa
- Että pohjoinen klooniryhmä eroaa eteläisestä korkeamman GOT B_2 ja matalamman GOT B_5 alleelifrekvessin suhteen
- Että kantapuuvalinta erikoisesti Pohjois-Suomessa näyttää suosivan heterotsydotiaa 3-kareeni geenien suhteen

REFERENCES

ADAMS, W. T. & JOLY, R. J. 1980 a. Genetics of allozyme variants in loblolly pine. Jour. Heredity 71, 33–40.

– " – & – " – 1980 b. Allozyme studies in loblolly pine seed orchards: Clonal variation and frequency of progeny due to self-fertilization. Silvae Genet. 29, 1–4.

AYALA, F. J. & KIGER, J. A. Jr. 1980. Modern Genetics. The Benjamin/Cummings Publishing Co. Inc. 844 pp.

BARADAT, P. H., DAGAN, C. B. & MARPEAU, A. 1979. Variation in terpene within and between populations of maritime pine. Proc. Conf. Bioch. Genet. For Trees, Umeå, Sweden 1978, 151–169.

BEADLE, G. W. & TATUM, E. L. 1941. Genetic control of biochemical reactions in *Neurospora*. Proc. Natl. Acad. Sci. USA 27, 499–506.

BERGMANN, F. & GREGORIUS, H. R. 1979. Comparison of the genetic diversities of various populations of Norway spruce (*Picea abies*). Proc. Conf. Bioch. Genet. For. Trees, Umeå, Sweden 1978, 99–112.

BHUMIBHAMON, S. 1978. Studies on Scots pine seed orchards in Finland with special emphasis on the genetic composition of the seed. Commun. Inst. For. Fenn. 94.4, 118 pp.

BROWN, A. H. D. 1979. Enzyme polymorphism in plant populations. Theor. Popul. Biol. 15, 1–42.

CHUNG, M. S. 1981. Flowering charateristics of *Pinus sylvestris L.* with special emphasis on the reproductive adaptation to local temperature factor. In press (Acta For. Fenn. 169).

CONKLE, M. T. 1971. Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in Knobcone pine. For. Sci. 17, 190–194.

DeLORENZO, R. J. & RUDDLE, F. H. 1970. Glutamate oxalate transaminase (GOT) genetics in *Mus musculus:* Linkage, polymorphism, and phenotypes of the Got-2 and Got-1 loci. Bioch. Genet. 1970. 4, 259–273.

DEVLIN, R. M. 1975. Plant Physiology. D. van Nostrand Co. New York. 600 pp.

EICHE, V. 1955. Spontaneous chlorophyll mutations in Scots pine. Medd. Stat. Skogsforsk. 45. (13), 1–64.

ERDTMAN, H. 1968. The assessment of biochemical techniques in plant taxonomy. In Chemotaxonomy and Serotaxonomy (Ed. HAWKES, J. G.). Academic Press, London, 235–268.

FORREST, G. I. 1979. Monoterpene variation in lodgepole pine (*Pinus contorta*) and Scots pine (*Pinus sylvestris*). Proc. Conf. Bioch. Genet. For. Trees, Umeå, Sweden 1978, 136–150.

FOWLER, D. P. 1965 a. Natural self-fertilization in three jack pines and its implications in seed orchard management. For. Sci. 11, 55–58.

– " – 1965 b. Effect of inbreeding in red pine *Pinus resinosa*. Silvae Genet. 14, 36–45.

FRANKLIN, E. C. 1968. Artificial self-pollination and natural inbreeding in *Pinus taeda L.* Ph. D. Thesis, N. C. State Univ. 127 pp.

– " – 1971. Estimates of frequency of natural selfing and of indreeding coefficients in loblolly pine. Silvae Genet. 20, 194–195.

GANSEL, C. & SQUILLACE, A. E. 1976. Geographic variation of monoterpenes in cortical oleoresin of slash pine. Silvae Genet. 25, 150–154.

HANOVER, J. W. 1966 a. Genetics of terpenes I. Gene control of monoterpene levels in *Pinus monticola Dougl.* Heredity 21, 73–84.

– " – 1966b. Inheritance of 3-carene concentration in *Pinus monticola*. For. Sci. 12, 447–450.

HESS, D. 1975. Plant Physiology. Springer-Verlag, Berlin. 333 pp.

HILTUNEN, R. 1975. Variation and inheritance of some monoterpenes in Scots pine (*Pinus silvestris L.*). Planta Med. 28, 315–323.

– " – 1976. On variation, inheritance and chemical interrelationships of monoterpenes in Scots pine (*Pinus silvestris L.*). Ann. Acad. Sci. Fenn. Ser. A IV Biologica 208, Finland. 54 pp.

– " – , JUVONEN, S. & TIGERSTEDT, P. M. A. 1975 a. Geographical variation in some monoterpenes in Scots pine (*Pinus silvestris L.*) in Finland. Farm. Aikak. 84, 73–82.

– " –, TIGERSTEDT, P. M. A., JUVONEN, S. & POHJOLA, J. 1975 b. Inheritance of 3-carene quantity in *Pinus silvestris L.* Farm. Aikak. 84, 69–72.

HUNT, R. S. & von RUDLOFF, E. 1977. Leaf-oil-terpene variation in western white pine populations of the Pacific Northwest. For. Sci. 23, 507–516.

HUNTER, R. L. & MARKERT, C. L. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125, 1294–1295.

JUVONEN, S. 1966. Über die Terpenbiosynthese beeinfluessenden Faktoren in *Pinus silvestris L.* Acta Bot. Fenn. 71, 92 pp.

– " – & HILTUNEN, R. 1972. Über das Vorkommen von 3-caren-armen und -reichen Chemotypen bei *Pinus silvestris L.* in Finland. Biogenetische Studien II. Sonderdruck aus Farmaseuttinen Aikakauslehti 81, 137–145.

KIM, Z. S. 1979. Inheritance of leucine aminopeptidase and acid phosphatase isozymes in beech (*Fagus sylvatica L.*). Silvae Genet. 28, 68–71.

KOSKI, V. 1970. A study of pollen dispersal as a mechanism of gene flow in conifers. Commun. Inst. For Fenn. 70(4), 74 pp.

KOSKI, V. 1973. On self-pollination, genetic load, and subsequent inbreeding in some conifers. Commun. Inst. Forest. Fenn. 78. (10), 42 pp.

KRAZAKOWA, M. & SZWEYKOWSKI, J. 1979. Variation of 6-PGD in the populations of Polish Scots pine (*Pinus sylvestris*). Proc. Conf. Bioch. Genet. For Trees, Umeå, Sweden 1978, 86–98.

LEHNINGER, A. L. 1975. Biochemistry. Worth Publishers Inc. New York, 1104 pp.

LI, C. C. 1955. Population Genetics. The Univ. Chicago Press. Chicago & London, 366 pp.

LUNDKVIST, K. 1974. Inheritance of leucine aminopeptidase in *Picea abies. K.* Hereditas 76, 91–96.

– " – 1975. Inheritance of acid phosphatase isozymes in *Picea abies*. Hereditas 79, 221–226.

– " – 1977. Inheritance of esterases in needles and endosperms of Norway spruce (*Picea abies K.*). Hereditas 87, 27–32.

– " – 1978. Allozymes in population genetic studies of Norway spruce (*Picea abies K.*). Doctoral thesis. Univ. Umeå, Sweden, 42 pp.

– " – 1979a. Genetic differentiation within and among Swedish populations of Norway spruce (*Picea abies*). Proc. Conf. Bioch. Genet. For. Trees, Umeå, Sweden 1978, 113–117.

– " – 1979 b. Allozyme frequency distributions in four Swedish populations of Norway spruce (*Picea abies* K.) I. Estimation of genetic variation within and among populations, genetic linkage and a mating system parameter. Hereditas 90, 127–143.

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

MARKERT, C. L. & MØLLER, F. 1959. Multiple forms of enzymes; tissue, ontogenetic, and species specific patterns. Proc. Nat. Acad. Sci. 45, 753–763.

MITTON, J. B., LINHART, Y. B., HAMRICK, J. L. & BECKMAN, J. S. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado front range. Theor. Appl. Genet. 51, 5–13.

– " – , – " – , STURGEON, K. B. & HAMRICK, J. L. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. Jour. Heredity 70, 86–89.

MUHS, J. J. 1974. Distinction of Douglas-fir provenances using peroxidase-isozyme-patterns. Silvae Genet. 23, 71–76.

MÜLLER, G. 1976. A simple method of estimating rates of self-fertilization by analysing isozymes in tree seeds. Silvae Genet. 25, 15–17.

– " – 1977. Untersuchungen über die natürliche Selbstfruchtung in Beständen der Fichte (*Picea abies (L.) Karst.*) und Kiefer (*Pinus sylvestris L.*). Silvae Genet. 26, 207–217.

MÜLLER-STARCK, G. 1979. Estimates of self- and crossfertilization in a Scots pine seed orchard. Proc. Conf. Bioch. Genet. For. Trees, Umeå, Sweden, 1978, 170–181.

NEI, M. 1975. Molecular Population Genetics and Evolution. North-Holland Publishing Co. Amsterdam, 288 pp.

– " – & ROYCHOUDHURY, A. K. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76, 379–390.

NIKOLIC, D. T. & BERGMANN, F. 1974. Genetic variation of leucine aminopeptidase isozymes in seeds of *Pinus nigra Arn.* Genetika 6 (Yugoslavia), 361–365.

OHBA, K. 1972. A recessive gene producing yellow seedlings and estimation of natural self-fertilization in a Japanese black pine, *Pinus thunbergii Parl.* Jour. Jap. For. Soc. 54, 28–29.

OHBA, K., IWAKAWA, M., et. al. 1971. Estimation of the degree of the natural self-fertilization by the frequencies of chlorophyllous variants in Japanese red pines, *Pinus densiflora Sieb. et Zucc.*, and the inheritance of variants. Jour. Jap. For. Soc. 53, 327–333 (cf. For. Abs. 33, (4) 1972. PP 677–678).

PRAKASH, S., LEWONTIN, R. C. & HUBBY, J. L. 1969. A molecular approach to the study of genic heterozygosity in natural populations IV. Pattern of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics 61, 841–858.

PRYOR, A. J. 1974. Allelic glutamic dehydrogenase isozymes in maize – a simple hybrid isozyme in heterozygotes? Heredity 32, 397–401.

RUDIN, D. 1975. Inheritance of glutamate-oxalate-transaminase (GOT) from needles and endosperms of *Pinus sylvestris*. Hereditas 80, 298–300.

– " – 1977 a. Leucine-amino-peptidase (LAP) from needles and macrogametophytes of *Pinus sylvestris L.* Inheritance of allozymes. Hereditas 85, 219–226.

– " – 1977 b. The isozyme technique – a shortcut to the genes of our forest trees? Illustrations using *Pinus sylvestris L.* Doctoral Thesis, Univ. Umeå, Sweden, 53 pp.

– " – & EKBERG, I. 1978. Linkage studies in *Pinus sylvestris L.* – using macrogametophyte allozymes. Silvae Genet. 27, 1–12.

– " – , ERIKSSON, G., EKBERG, I. & RASMUSON, M. 1974. Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. Silvae Genet. 23, 10–13.

– " – , – " – , RASMUSON, M. 1977. Inbreeding in a seed tree stand of *Pinus sylvestris L.* in northern Sweden. A study by the aid of the isozyme technique. Dept. For. Genet. Royal Coll. For. Res. Notes 25, 45 pp.

– " – & LINDGREN, D. 1977. Isozyme studies in seed orchards. Stud. Forest. Suec. 139, 23 pp.

– " – & RASMUSON, B. 1973. Genetic variation in esterases from needles of *Pinus silvestris L.* Hereditas 73, 89–98.

RUDOLPH, T. D. 1966. Segregation for chlorophyll deficiencies and other phenodeviants in the X_1 and X_2 generations of irradiated jack pine. U.S.D.A. For. Serv. Res. Pa. NC-6, 18–23.

SAKAI, K. & MIYAZAKI, Y. 1972. Genetic studies in natural populations of forest trees II. Family analysis: A new method for quantitative genetic studies. Silvae Genet. 21, 149–154.

– " – , – " – & MATSUURA, T. 1971. Genetic studies in natural populations of forest trees I. Genetic variability on the enzymatic level in natural forests of *Thujopsis dolabrata*. Silvae Genet. 20, 168–173.

SAKAI, K. & PARK, Y. G. 1971. Genetic studies in natural populations of forest trees III. Genetic differentiation within a forest of *Cryptomeria japonica*. Theor. Appl. Genet. 41, 13–17.

SARVAS, R. 1962. Investigations on the flowering and seed crop of *Pinus silvestris*. Commun. Inst. For. Fenn. 53(4), 198 pp.

– " – 1972. Investigations on the annual cycle of development of forest trees; Active period. Commun. Inst. For. Fenn. 76(3), 110 pp.

SCANDALIOS, J. G. 1974. Isozymes in development and differentiation. Ann. Rev. Plant. Physiol. 25, 225–258.

SCANDALIOS, J. G. 1979. Control of gene expression and enzyme differentiation. In Physiological Genetics (SCANDALIOS, J. G. ed.) Academic Press. pp 63–107.

SHAW, C. R. & PRASAD, R. 1970. Starch gel electrophoresis of enzymes – A compilation of recipes. Bioch. Genet. 1970. 4, 279–310.

SHAW, D. V. & ALLARD, R. W. in press. Analysis of mating system parameters and population structure in Douglas-fir using single-locus and multilocus methods. Presented at the symposium: Isozymes of Forest Trees and Forest Insects.

– " – , Kahler, A. L. & ALLARD, R. W. 1981 A multilocus model for estimating mating system parameters in plant populations. Proc. Natl. Acad. Sci. 78, (2).

SICILIANO, M. J. & SHAW, C. R. 1976. Separation and

visualization of enzymes on gels. In Chromatographic and Electrophoretic Techniques 2. Zone Electrophoresis (Ed. SMITH, I), 185–209. William Hememann Medical Books Ltd. London.

SMITHIES, O. 1955. Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Bioch. J. 61, 629–641.

SORENSEN, F. C. 1973. Frequency of seedlings from natural self-fertilization in costal Douglas-fir. Silvae Genet. 22, 20–24.

SQUILLACE, A. E., KRAUS, J. F. 1963. The degree of natural selfing in slash pine as estimated from albino frequencies. Silvae Genet. 12, 46–50.

THIELGES, B. A. 1969. A chromatographic investigation of interspecific relationships in *Pinus* (subsection sylvestris). Amer. J. Bot. 56, 406–409.

– " – 1972. A chromatographic study of foliage polyphenols in pine hybrids (subsection sylvestris). Silvae Genet. 21, 109–114.

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

– " – 1974 a. Genetic structure of Picea abies populations as determined by the isozyme approach Proc. IUFRO Joint Meet. Work. Party Popul. Ecol. Genet. Breed. Theory & Progeny Testing. Stockholm, Sweden, 283–291.

– " – 1974 b. Genetical characteristics and adaptation of the tree populations in northern forests. In Ecological Problems of the Circumpolar Area (Ed. BYLUND, E., LINDERHOLM, H. & RUNE, O.) Norrbottens Museum, 65–71.

– " – 1979. Genetic adaptation of plants in the subarctic environment. Holarctic Ecology 2, Copenhagen, 264–268.

– " – , HILTUNEN, R., CHUNG, M-S. & MORÉN, E. 1979. Inheritance and genetic variation of monoterpenes in Scots pine (*Pinus sylvestris L.*). Proc. Conf. Bioch. Genet. For. Trees, Umeå, Sweden 1978, 29–38.

TOBOLSKI, J. J. & HANOVER, J. W. 1971. Genetic variation in the monoterpenes of Scots pine (*Pinus sylvestris L.*). For. Sci. 17, 293–299.

TOWNSEND, A. M. & HANOVER, J. W. 1972. Altitudinal variation in photosynthesis, growth, and monoterpene composition of western white pine (*Pinus monticola Dougl.*) seedlings. Silvae Genet. 21, 133–139.

VESELL, E. L. & BEARN, A. G. 1957. Localization of lactic acid dehydrogenase activity in serum fractions. Proc. Soci. Exp. Biol. – Medicine 94, 96–99.

WELLENDORF, H. & SIMONSEN, V. 1979. A chemotaxonomic study in *Picea* with isozymes in the seed endosperm. Proc. Conf. Bioch. Genet. For. Trees, Umeå, Sweden 1978, 182–193.

WILKINSON, J. H. 1970. Isozymes. Chapman and Hall Ltd. London, 396 pp.

YANG, J. CH., CHING, T. M. & CHING, K. K. 1977. Isozyme variation in coastal Douglas-fir I. A study of geographic variation in three enzyme systems. Silvae Genet. 26, 10–18.

1981. Biochemical Methods for Determining Population Structure in *Pinus sylvestris L.* Seloste: Männyn populaatiorakenteesta biokemiallisten tutkimusmenetelmien valossa. – ACTA FORESTALIA FENNICA Vol. 173: 1–28, Helsinki.

Studies on Finnish Scots pine plus tree clones by monoterpene and isozyme analyses have been undertaken to further investigate mating system, population structure and pollination. Six allozyme systems (3 GOT, 1 GDH and 2 LAP) were properly analysed on the basis of segregation. Monoterpenes were analysed from needle material and segregation in hight and low 3-carene content was found to depend on two alleles, C and c.
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Author's present address: Department of Plant Breeding, University of Helsinki, Viikki, 00710 Helsinki 71.
Permanent address: Institute of Forest Genetics, Suweon, Kyunggi-Do, Republic of Korea.

CHUNG, MIN-SUP O.D.C. 161.6 + 165.3 *Pinus sylvestris*

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TEOLLISUUDEN PUUYHDISTYS
OY TAMPELLA AB
JOUTSENO-PULP OSAKEYHTIÖ
KAJAANI OY
KEMI OY
MAATALOUSTUOTTAJAIN KESKUSLIITTO
VAKUUTUSOSAKEYHTIÖ POHJOLA
VEITSILUOTO OSAKEYHTIÖ
OSUUSPANKKIEN KESKUSPANKKI OY
SUOMEN SAHANOMISTAJAYHDISTYS
OY HACKMAN AB
YHTYNEET PAPERITEHTAAT OSAKEYHTIÖ
RAUMA-REPOLA OY
OY NOKIA AB, PUUNJALOSTUS
JAAKKO PÖYRY CONSULTING OY
KANSALLIS-OSAKE-PANKKI
SOTKA OY
THOMESTO OY
ASKO-UPO OY
SAASTAMOINEN YHTYMÄ OY
OULU OY
OY KESKUSLABORATORIO
METSÄNJALOSTUSSÄÄTIÖ
SUOMEN METSÄNHOITAJALIITTO RY
OY KYRO AB
SUOMEN 4H-LIITTO
SUOMEN PUULEVYTEOLLISUUSLIITTO RY
OY W. ROSENLEW AB

Arvi A. Karisto Oy:n kirjapaino
Hämeenlinna 1981

ISBN 951-651-048-5