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EDITOR: SEPPO OJA

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Silva Fennica

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SEPPO OJA

TOIMITUSKUNTA:

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Silva Fennica, joka vuosina 1926–66 ilmestyi sarjajulkaisuna (niteet 1–120), on vuoden 1967 alusta lähtien neljännesvuosittain ilmestyvä aikakauskirja. Suomen Metsätieteellinen Seura julkaisee myös *Acta Forestalia Fennica*-sarjaa vuodesta 1913 (nide 1) lähtien.

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POPULATION GENETICS OF FOREST TREES

Proceedings of symposium held in Helsinki 1981

METSÄPUIDEN POPULAATIOGENETIIKKA

Helsingissä 1981 pidetyn sympoosion tutkimusraportit

CONTENTS

PREFACE

Population genetics of forest trees is a most important field of research today. It is important partly due to its contributions to population genetics of plants generally and partly due to its applications in forest tree breeding.

We may dissect the general subject in a hierarchal way and thus place genecology at the top of the classification and then proceede down to the level of analysing differences between populations and finally look at the genetic variation within populations, between individuals. We conclude that forest tree breeding is basically applied population genetics and may be looked upon analogously. At the top we have questions of seed transfer, provenance research if you will, next comes racial differences between seed stands of a climatic zone, selection of qualitatively superior seed sources if you will, and thirdly the genetic variation of growth and quality characters in populations, selection in conjunction with thinnings or selection of plus trees if you will.

Forest tree breeding must of course be based upon population genetics of forest trees. Without such a sound base breeding is more or less a training in futility. We can never expect to achieve maximum benefits from breeding trees if the genetic structures of tree populations are unknown to us. Many of the reports in this volume have a direct effect on tree breeding using the seed orchard method. Also the management of cultivated trees, which is the first step in domestication, must be based on a thorough understanding of the genetic structure of trees. We are actually just now in many parts of the world wittnessing a process of domestication in the forest. We may greatly improve the forest crop by selecting proper seed but we may also embark upon an ecologically unsound path if we do not consider population genetics of trees and forest ecosystems.

Today we have good tools in our hands. We have the isozyme analysing technique whereby trees can be genetically identified and marked. We also have other chemical analyses of e.g. monoterpenes and enzyme activity. Monoterpenes can now be used to identify chemotypes of trees simply by 'sniffing' the air surrounding the needles of the tree. Measurements of enzyme activity can now be done on a population level and we can expect much new information from both of last mentioned techniques.

We have also learned many new theoretical methods from scientists working on herbaceous plant species, mostly wild grasses and cultivated crop plants. Specialists in those fields of plant population genetics have opened a new way of thinking for forest tree breeders and geneticists. The concept of mating-system is now a normal term for the tree breeder. We are actually wittnessing a process of new knowledge entering into forest management principles. We would like to stress that sound management of forest ecosystems, either man made or natural, can not live without steady inputs from basic research on the genetics of natural forest populations. Also the reverse is true, sound population genetic research of trees should be well aware of forest management practise, particulary of the management of new man made forests.

This meeting has been a continuation of one held in Umeå, Sweden in 1978. We think that different aspects of research on the population biology of trees is now moving so quickly that a 3-year period is a proper time interval for meetings of this kind. Only by gathering our knowledge in this way can we meet the challenge of the new cultivated and domesticated forest.

The University of Helsinki has provided us with meeting facilities and has also given us direct economic support. We extend our warm appreciation and thanks to the Finnish Academy and to the Central Association of Finnish Forest Industries for their support in financing these proceedings. Finally the Finnish Society of Forest Research has provided us with an unusually large share of their publication thus making the proceedings available to a larger group of specialists.

Helsinki, April 1982

P. M. A. Tigerstedt

ALKUSANAT

Elämme aikakautta jolloin metsien puista on tulossa viljelykasveja. Metsäpuiden populaatiogenetiikasta muodostuu tällöin metsäntutkimuksen keskeinen tutkimusala. Metsäpuiden populaatiorakenteen tunteminen vaikuttaa suoraan metsänjalostuksen ja metsänhoidon perusteisiin ja menetelmiin. Lisäksi alan tutkimus tuo uutta perustietoa kasvien populaatiogeneettisestä rakenteesta ja edistää näin geneettistä tutkimusta. Metsän puu on sikäli edullinen tutkimuskohde, että se pysyy paikallaan vuosikymmeniä ja tutkija voi välillä palata saman puun juurelle hakemaan lisää tutkimusmateriaalia.

Tutkijoita ja soveltajia kiinnostaa tietää kuinka paljon eri ilmastoista peräisin olevat metsäpopulaatiot eroavat toisistaan. Geneetikko mittaa näitä eroja geneettisen etäisyyden tai diversiteetin avulla, soveltaja provenienssien välisinä eroina jotka ovat todettavissa kasvurytmissä ja heijastuvat suorina kasvueroina.

Toisaalta kaikkia osapuolia kiinnostaa myös tiedot siitä, miten saman ilmastovyöhykkeen sisällä olevat eri populaatiot poikkeavat toisistaan. Geneetikko käyttää jälleen geneettisen etäisyyden tai diversiteetin mittaa ja vertaa saatuja tuloksia metsiköiden välisiin edafisiin kasvupaikkaeroihin. Soveltajat haluavat tietoja metsiköiden välisistä perinnöllisistä laatu- ja/tai kasvueroista sekä vastauksia kysymykseen eroavatko lähellä toisiaan mutta eri kasvualustoilla kasvavat metsiköt geneettisesti toisistaan.

Kun siirrytään metsikön tai populaation sisään geneetikkoa kiinnostaa yksilöiden väliset perinnölliset erot. Hän voi tietojen perusteella tehdä laskelmia ja arviointeja puulajien risteytymisjärjestelmistä sekä yleensä ominaisuuksien välisistä riippuvaisuuksista, kytkennöistä. Soveltajat haluavat tietää miten kasvun ja laadun eri osa-ominaisuudet kytkeytyvät ja periytyvät.

Kirjassa esitetyt tutkimukset ja tarkastelut edustavat nopeasti kehittyvän metsägeneettisen tutkimusalan uusimpia tietoja. Suomessa 1981 pidetty kansainvälinen symposio on jatkoa Ruotsissa 1978 pidetylle vastaavalle kokoukselle. Kolmessa vuodessa on tietomme metsän geneettisestä rakenteesta lisääntynyt valtavasti. Tuloksista voi päätellä, että ongelmiin pureudutaan nyt niin perusteellisesti että 3 vuoden väliajoin tapahtuva tutkijoiden ja soveltajain tapaaminen on välttämätöntä jotta siirtyminen viljelymetsien aikakauteen tapahtuisi biologisesti järkevimmällä mahdollisella tavalla.

Helsingin yliopisto on tällä kertaa toiminut kokouksen isäntänä ja osittain myös rahoittajana. Kiitämme Suomen Akatemiaa sekä Suomen Metsäteollisuuden Keskusliittoa saamastamme julkaisuavusta. Esitetyn tutkimusaineiston julkaiseminen on ollut mahdollista Suomen Metsätieteellisen Seuran tuella ja myötävaikutuksella. Seura on antanut meille poikkeuksellisen suuren tilan julkaisusarjassaan.

Helsinki, huhtikuussa 1982

P. M. A. Tigerstedt

LIST OF PARTICIPANTS

Dormling, Ingegerd Dept. of Forest Genetics, Swedish University of Agricultural Sciences, Building 4, Roslagsvägen 113, S-104 05 Stockholm, Sweden

Elo, Kirsi Forest Research Institute, Dept. of Forest Genetics, Unionink. 40 A, 00170 Helsinki 17, Finland

Eriksson, Gösta Swedish University of Agricultural Sciences, Dept. of Forest Genetics, Box 7027, S-750 07, Uppsala, Sweden

Friis, Anders Swedish University of Agricultural Sciences. Inst. of Forest Genetics and Physiology 90183 Umeå, Sweden

Gullberg, Urban Swedish University of Agricultural Sciences. Dept. of Forest Genetics. S-750 07 Uppsala, Sweden

Hagman, Max. Forest Research Institute, Dept. of Forest Genetics, Unionink. 40 A, 00170 Helsinki 17, Finland

Hiltunen, Raimo University of Helsinki, Dept. of Pharmacognosy, Fabianinkatu 35, 00170 Helsinki 17, Finland

Kamra, Krishan Swedish University of Agricultural Sciences, Dept. of Forest Genetics and Plant Physiology, 90183 Umeå, Sweden

Korpelainen, Heikki Foundation for Forest Tree Breeding, Alkutie 69, 00660 Helsinki 66, Finland

Koski, Veikko Forest Research Institute, Dept. of Forest Genetics, Unionink. 40 A, 00170 Helsinki 17, Finland

Krutzsch, Peter National Board of Forestry, Vallgatan 6, S-55183 Jönköping, Sweden

Krzakowa, Maria Dept. of Genetics, Adam Mickiewicz University, Dabrowskiego Str., 165, Poznan, Poland

Kukkonen, Hannu Foundation for Forest Tree Breeding, Alkutie 69, 00660 Helsinki 66, Finland

Kärki, Lauri Foundation for Forest Tree Breeding, Alkutie 69, 00660 Helsinki 66, Finland

Lepistö, Martti Foundation for Forest Tree Breeding, Alkutie 69, 00660 Helsinki 66, Finland

Lindgren, Dag Swedish University of Agricultural Sciences, Inst. of Forest Genetics and Physiology 90183 Umeå, Sweden

Lundkvist, Kenneth Swedish University of Agricultural Sciences, Inst. of Forest Genetics, S-750 07 Uppsala, Sweden

McMullan, Eleanor Pacific Forest Recearch Center, 506 West Burnside Rd. Victoria, B.C. V8Z IM5 Canada

Mikola, Jouni Forest Research Institute, Dept. of Forest Genetics, Unionink. 40 A, 00170 Helsinki 17, Finland

Muona, Outi University of Oulu, Dept. of Genetics, Linnanmaa, 90570 Oulu 57, Finland

Müller-Starck, Gerhard Institut für Forstgenetik und Forstpflanzenzüchtung, Büsgenweg 2, D-3400 Göttingen-Weende, FRG

Nieminen, Pia University of Helsinki, Dept. of Plant Breeding, Viikki D-house, 00710 Helsinki 71, Finland

Niiranen, Juhani Tapio Central Board of Forestry, Salomonkatu 13, 00100 Helsinki 10, Finland

Prus-Glowacki, Wieslaw Dept. of Genetics, Adam Mickiewicz University, Dabrowskiego Str., 165, Poznan, Poland

Pöykkö, Tapani Forest Research Institute, Dept. of Forest Genetics, Unionink. 40 A, 00170 Helsinki 17, Finland

Reinikainen, Jukka University of Helsinki, Dept. of Silviculture, Unionink. 40 A, 00170 Helsinki 17, Finland

Rudin, Dag Swedish University of Agricultural Sciences. Inst. of Forest Genetics and Physiology 90183 Umeå, Sweden

Ryynänen, Martti Forest Research Institute, 95900 Kolari, Finland

Ryöppy-Ekbom, Päivi University of Helsinki, Dept. of Plant Breeding, Viikki D-house, 00710 Helsinki 71, Finland

Shaw, Doug International Forest Seed Co. Box 76008, Birmingham, Alabama 35253, U.S.A.

Skrøppa, Tore Norwegian Forest Research Institute, Box 61 1432 Ås-NLH, Norway

Szmidt, Alfred Institute of Dendrology, Polish Academy of Science 62-035 Kornik, Poland

Tammisola, Jussi University of Helsinki, Dept. of Plant Breeding, Viikki D-house, 00710 Helsinki 71, Finland

Tigerstedt, P. M. A. University of Helsinki, Dept. of Plant Breeding, Viikki D-house, 00710 Helsinki 71, Finland

Vidgren, Jukka Forest Tree Breeding Station, 01590 Maisala, Finland

Velling, Pirkko Forest Research Institute. Dept. of Forest Genetics, Unionink. 40 A, 00170 Helsinki 17, Finland

Yazdani, Reza Swedish University of Agricultural Sciences, Inst. of Forest Genetics and Physiology, 901 83 Umeå, Sweden

Ziehe, Martin Institut für Forstgenetik und Forstflanzenzüchtung, Büsgenweg 2, D-3400 Göttingen-Weende FRG

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PART I

ESTIMATION OF MATING SYSTEMS OF FOREST TREES

HOW TO STUDY THE RATE OF INBREEDING IN POPULATIONS OF PINUS SYLVESTRIS AND PICEA ABIES

VEIKKO KOSKI

There are several terms connected with inbreeding: self-pollination, self-fertilization, selfing, and proportion of offspring originating form self-fertilization, which are not synonyms. It is necessary to apply the proper term in each connection, especially when numeric values are given. None of the above mentioned phenomena has precise and constant values, but a range within which the actual values vary due to many factors. The rate of inbreeding in the offspring after a partial self-pollination depends essentially on the number of embryonic lethals, less on the abundance of pollination. Reliable estimates can be calculated only based on the results of controlled self-pollinations. Then it is possible to calculate ratios between self-pollination and individuals originating from self-fertilization. Some examples are given.

Inbreeding is a fundamental factor of the population biology at any species. The genetic structure of natural populations and genotype frequencies of autogamous species are essentially different from those of allogamous species. Consequently the choice of the breeding strategy depends on the image of the population structure and mating pattern of the species in question. The estimates of the proportions of self-pollination and inbreeding at *Pinus sylvestris*, *Picea abies*, as well at several other conifers published in earlier reports, are, because of their diversity, quite confusing. For stands of *Pinus sylvestris* Sarvas (1962 p. 123–142) calculated that, on an average, 26 per cent of the pollination of the ovules is *self-pollination*, but due to the embryonic lethals only 12 % of full seed have an embryo from *self-fertilization*. Further, provided that inbreeding embryos are competitively weaker during seed development their proportion drops to 7 %. Sarvas points out that the proportions depend on the abundance of pollination. Stern (1972) reports that *self-pollination* of *Pinus sylvestris* is 0...82 %. The present author (Koski 1973) has concluded that, on average, the proportion of *self-fertilizations* is around 10 % both for Pinus sylvestris and Picea abies, and the proportion of full seeds originating from self-fertilization is only 1 %. Wright (1976 p. 39) mentions that the share of natural *selfing* of some conifer species is 7 % or less. Recently, isoenzyme analyses have yielded several estimates of inbreeding ratios both in ordinary stands and seed orchards.

E.g. Müller (1977) obtained self-fertilization estimates of 7,3...17,7 % for *Picea abies*

and 2,9...9,6 % for *Pinus sylvestris*. Shen et al. (1981) report 2,9 %, 9,4 % and 8,5 % selfing for the top, middle and bottom part of the crown in three results of one clone of *Pinus sylvestris*.

These few examples show that several concepts have been used; self-pollination, self-fertilization, selfing, and proportion of self-fertilized embryos within germinable seeds. These terms are by no means equal and values given in one meaning cannot be converted to another with any universal coefficient. Good definitions are given by Stern and Roche (1974, pp 74–77), for example. In some papers it even remains uncertain which stage is dealt with. In all reports where data from individual trees have been given the great differences between trees can be noticed. Hardly any results of the same trees from different years have been published. Obviously the influence of own pollen depends on the abundance of male flowering in the tree itself and in its surroundings and on the meteorological conditions during the flowering period. Thus observation from one single year may deviate considerably from the general case.

From the practical point of view the proportion of seedlings originating from self-fertilization is most interesting. Before the isoenzyme methods, the proportion could be estimated only within offspring of such mother trees that happen to carry a marker gene eg. the albino-factor. Efforts have been made to estimate the rate of inbreeding from the empty seed percentages. As the percentage of empty seed is easy to asses from X-ray pictures it would ideally be desirable to calculate, for example, the proportion of self-pollination and rate of inbreeding in the progenies.

The reproductive system of spruce and pine is, however, of such kind that empty seed percentage alone does not give all the necessary information. Polyembryony connected with embryonic lethals makes up a rather complex system where interrelations are not linear. This system has been throughly discussed in connection of complete (=controlled) self-pollination (Koski 1971, 1973, Bramlett and Popham 1971, Lindgren 1975, Bishir and Pepper 1977). The applied formula has given surprisingly high numbers of lethals per genotype, but also shows a considerable variation between individuals. By means of controlled self-pollinations and X-ray radiography the number of embryonic lethals have been estimated for 135 *Pinus sylvestris* and 87 *Picea abies* individuals from Finnish populations. The distributions of trees according to the number of embryonic lethals are as follows.

Pinus sylvestris

	Number of lethals per genotype							
Unit	1–3	4–6	7–9	10–12	13–15	16–18	>18	Total
Trees	6	32	46	30	11	9	1	135
Per cent	4	24	34	22	8	7	1	100

Picea abies

	Number of lethals per genotype							
Unit	1–3	4–6	7–9	10–12	13–14	16–18	>18	Total
Trees	2	20	21	27	8	7	2	87
Per cent	2	23	25	31	9	8	2	100

The estimated number of embryonic lethals is obtained with the aid of the empty seed percentage after controlled self-pollination. It is worth noticing that this estimated value must not be taken as a precise value. Due to the rather small number of seeds obtained, the confidence limits of the per cent value are rather broad. Empty seed formation is certainly caused by other factors as well. Reduced pollen viability, or an environmental stress (draught, cold) during the primary embryo stage, probably results in increased empty seed frequencies. In order to illustrate this kind of error variation a comparison of empty seed percentages from the very same clones of *Pinus sylvestris* after a controlled self-pollination in years 1968–69 and 1979 respectively are given in the following scatter diagram. (Fig. 1).

One immediately detects 3 major deviations from the main series. The high values may be caused by inferior pollen. The exceptionally low empty seed percentage in 1979 may even originate from cross-pollination. In a large scale programme some mistakes occur; the tags of the ramets may be mixed, the

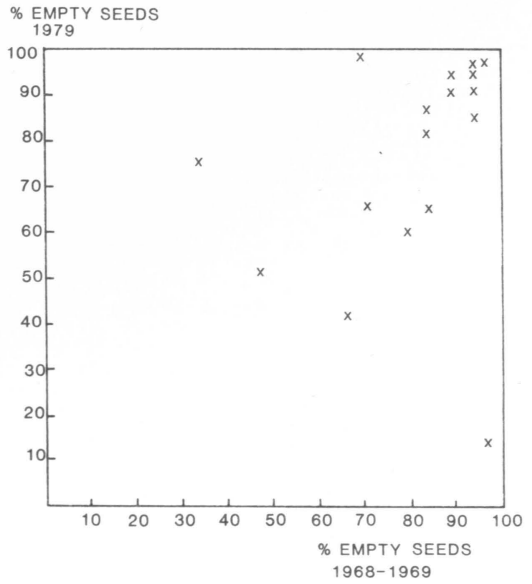

Figure 1. *Pinus sylvestris*. Empty seed percentages after controlled self- pollination in the same clones.

isolated female strobili may be pollinated with a wrong pollen syringe etc. One has to be quite cautious regarding the unexpected extremes.

Several efforts have been made to calculate the proportions of self-pollination, self-fertilization, and number of embryonic lethals, etc, merely on the seed material from open pollination. As long as the number of embryonic lethals is unknown all the calculations are quite uncertain. In other words, controlled pollinations, including complete self-pollination, are necessary for reliable calculations. The number of embryonic lethals, or degree of self-sterility, influences the percentage of empty seeds as well as the proportion of viable individuals originating from self-pollination. By means of extending the formula of the expected proportion of empty seed after self-pollination (Koski 1971, Bramlett & Popham 1971) it is possible to calculate expected values for empty seed percentages and proportions of inbred seeds in sound seed for any proportion of self-pollination. Lindgren (1975) has calculated expected values in a case where a complete self-pollination yields 80 per cent empty seed. He studied different alternatives for a partial self-pollination, providing 1 or 2 fertilizations per ovule and different probabilities of embryo abortion after cross-fertilization.

The modifying factor is that embryo abortions also take place after cross fertilization. Sarvas (1962 p. 137) reports 10,1 per cent empty seed in the stands. The present author (Koski 1971) reported 12,7 per cent in young grafts after out-crossing. If one assumes 2 fertilizations per ovule (k = 2) one can approximate that the probability of embryo abortion after a cross fertilization is as high as 0,3.

Using the following symbols and approximations

S = proportion of self-pollination
C = proportion of cross-pollination
E = expected proportion of empty seeds
I = expected proportion of germinable seed originating from self-fertilization
n = number of embryonic lethals
k = number of fertilizations per ovule
$P_n(k)$ = probability of all self-fertilized zygotes' abortion.

For instance, if n = 4 and k = 2, when $P_4(2) = 0.55$ and $P_4(1) = 0.69$,

we can write

$$E = 0{,}550\ S^2 + 0{,}414\ SC + 0{,}09\ C^2$$

$$I = \frac{0{,}45\ S^2 + 0{,}39\ SC}{(1 - E)}$$

The numerical values of E and I are given in the compilation using per cent units corresponding values for

1) n = 4 k = 2

S, %	E, %	I, %
0	9	0
10	12	4,0
20	15	9,4
30	18	14,9
40	22	21,2
50	26	28,4
60	31	37,0
70	36	47,2
80	42	60,4
90	48	76,8
100	55	100,0

2)

n = 8, k = 2	S, %	E, %	I, %
	0	9	0
	10	14	1,6
	20	19	3,5
	30	23	5,8
	40	29	8,7
	50	36	12,5
	60	44	17,8
	70	52	25,1
	80	61	36,6
	90	71	53,1
	100	81	100,0

3)

n = 8, k = 1	S, %	E, %	I, %
	0	30	0
	10	36	1,6
	20	42	3,4
	30	48	5,8
	40	54	8,7
	50	60	12,5
	60	66	17,6
	70	72	25,0
	80	78	36,4
	90	84	56,2
	100	90	100,0

4)

n = 12, k = 2	S, %	E, %	I, %
	0	9	0
	10	13,5	0,5
	20	18,8	1,1
	30	25,1	1,8
	40	32,2	2,8
	50	40,3	4,1
	60	49,2	6,1
	70	59,1	9,2
	80	69,8	14,8
	90	81,5	28,2
	100	94,0	100,0

The same examples have also been drawn as diagrams in order to make the comparison easier (Fig. 2). Figure 2 should convince anybody that the estimation of the proportion of self-pollination or proportion of inbred germinable seeds based only on empty seed percentage is not very reasonable.

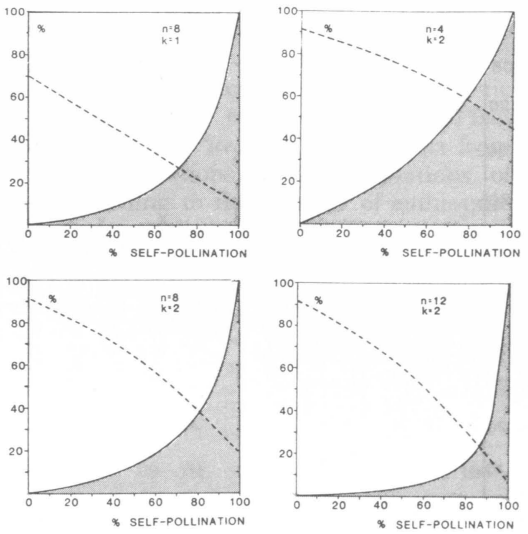

Figure 2. Some examples of the expected proportions of filled seeds and germinable seeds originating from selfing with different numbers of embryonic lethals (n) and fertilizations per ovule (k). It is assumed that the probability of embryo abortion after crossfertilization is 0.3. The dashed line indicates percentage of full seeds, the solid line and shaded area indicate the proportion of germinable seeds originating from self-fertilization.

The mechanism of embryonic lethals seems to be an effective measure against inbreeding for up to some 50 per cent of self-pollination in an average case of *Pinus sylvestris* and *Picea abies* ($n > 8$). At more self-fertile individuals or species ($n < 4$) the rate of inbreeding is essentially higher if the proportion of self-pollination is 50 per cent. Surprisingly, the effect of k on the rate of inbreeding is negligible relative to the effect of n, when comparing $n = 8$, $k = 2$ to $n = 8$, $k = 1$. Lindgren's (1975) example is almost equal to the case $n = 8$ given in this paper. The numerical results agree, even though Lindgren applied more sophisticated calculations. However, the differences between Lindgren's alternative curves are so small that it is doubtful whether they can empirically confirmed. As long as the detailed biological knowledge concerning the embryonic lethals is lacking, rather simple mathematical models are appropriate. The proportion of self-pollination (S) varies between 0 and 1. Even though it is rather simple to asses the abundance of male flowering, e.g. at seed orchard clones, we do not know yet how these figures are actually reflected on the composition of the pollination. When pollen measurements, controlled pollinations with different proportions of own pollen, and progeny analyses with genetic markers are plotted against the above mentioned calculations we should gradually have a better knowledge of self-pollination and related phenomena.

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GENETIC STRUCTURE OF OPEN-POLLINATED PROGENIES FROM A SEED ORCHARD OF PINUS SYLVESTRIS

DAG RUDIN and INGER EKBERG

Five clones carrying isozyme marker alleles in the glutamate-oxalatetransaminase and leucine-amino-peptidase loci were chosen in a seed orchard of *Pinus sylvestris* in central Sweden. In three of them, cones were collected from two vertical sections and in the two others from three sections, one top section two meters above ground, one inner section and one peripherial section below the two meter level. Cones from the closest four neighbouring grafts at a distance of five meters as well as from four grafts at a distance of 14 meters from the marker grafts were also included in the collection program. The frequencies of marker alleles found in the surrounding grafts reached one to three per cent which agrees with the expected, following panmixis. Based on seven different estimates from heterozygous marker loci the mean selfing frequency amounted to 16 per cent. The lower inner sectors of the crowns have a selfing frequency of seven per cent. These selfing frequencies are surprisingly high. The relevance and significance of these results are discussed.

Introduction

Seed orchards for forest trees are aimed at producing superior seeds for plant production. The seed orchards are often located on good sites which promote regular flowering and a good seed maturation. This generally results in well-developed seeds which give the plants a good start in nurseries. Eriksson and Lindgren (1975), however, pointed out that an inbreeding depression will not always become evident under nursery conditions. The

possibility of culling selfed plants under good forest nursery conditions is therefore limited if only plant height in one- or two-year-old plants is examined. With these facts in mind, the importance of information concerning the pattern of pollination within seed orchards becomes obvious. It would be worth-while to have a test system wich leads to a certification of the different years crops of forest seeds. This certification should, if possible, be based on data from individual clones in order to present an opportunity for optimizing the genetic gain which might be obtained from a seed orchard. If a clone-wise field test of the seeds is performed together with the parameters mentioned below, even better seed mixtures for reforestation areas could be made.

Based on individual clones the following parameters could be included in a seed certification system:

1. Weight of 1000 seeds.
2. Germination power.
3. Average heterozygosity.
4. Structure of the father population.
5. Level of contamination from outside the seed orchard.
6. Selfing frequency.
7. Relative amount of seeds from the different clones in a seed orchard.

In Sweden points 1 and 2 are regularly checked on a seed orchard basis. For the Långtora seed orchard, points 4, 5 and 7 were studied by Jonsson et al. (1976). Points 3–6 are suitable for studies using biochemical marker techniques. Points 4 and 6 will be illustrated and discussed in the present paper. The investigation was performed in the seed orchard No. 48 at Långtora (lat. 59°43', long. 17°08', alt. 15 m).

Material and methods

By the time of cone collection, the Långtora seed orchard was 10–12-year-old and the grafts were at a height of 3.5–5.5 m. The grafts were originally planted in a 5×5 m spacing. The seed orchard is comprised of 36 clones.Seeds were collected from all clones in the seed orchard and the macrogametophytes were isozyme-analysed for gene markers. One year later, cones were collected from three sectors in two marker ramets(F 2029 and W 1038). The sectors were the following: one top section over 2 m above ground, one inner and one peripheral section below this level (see Figure 1). For three marker ramets (C 5006, E 4006 and F 1008) only two sections, cutting the trees in two halves from the top to the bottom, were analysed by a simultaneous embryo/macrogametophyte analysis.

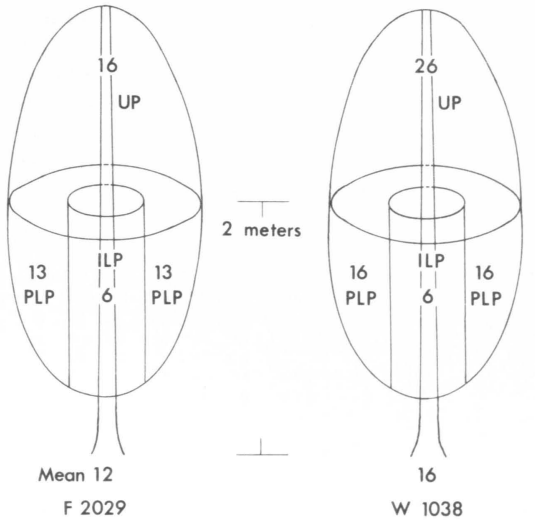

Figure 1. Mean selfing frequencies in per cent in different sectors of the two main marker ramets. UP = upper part, ILP = inner lower part and PLP = peripheral lower part.

Around each marker ramet, the four closest neighbours and four more at a distance of 14 meters were studied. These eight surrounding trees were each divided into two sectors from wich cones were collected – a northwest and a southwest sector (see Figures 2a–2d). About 100 embryo/macrogametophyte analyses were made for each sector of each tree.

For the separation of the LAP and GOT isozymes, embryos and macrogametophytes were homogenized separately and starch gel electrophoresis in a modified discontinuous buffer system was performed (Ashton and Braden 1961). Patterns of inheritance were checked by Rudin (1975, 1977) and 1:1 segregation of alleles in endosperms was checked in this study.

The LAP-A locus contains the alleles A_1 and A_2, the LAP-B locus the alleles B_1, B_2 and B_3, the GOT-A locus, the alleles A_1 and A_2 and, as we have discovered, the GOT-B locus the alleles B_{18}, B_2, B_{22} and B_3. It was rather difficult to distinguish between the band positions for B_{18}, B_2 and B_{22} because they were very close to one another. In this situation, rather than running the risk of getting unreliable results and even though we thereby lost some information, the B_{18}, B_2 and B_{22} bands were pooled into one group called B_2.

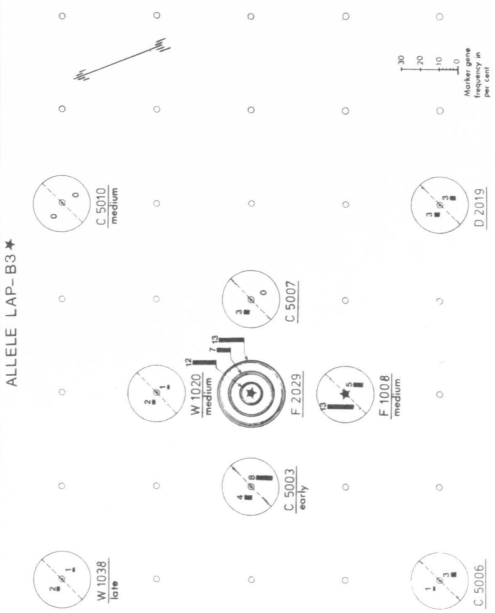

Figure 2b. Spatial dispersal of gene marker LAP-B_3 from clone F 2029 and F 1008 to themselves and to some surrounding ramets.

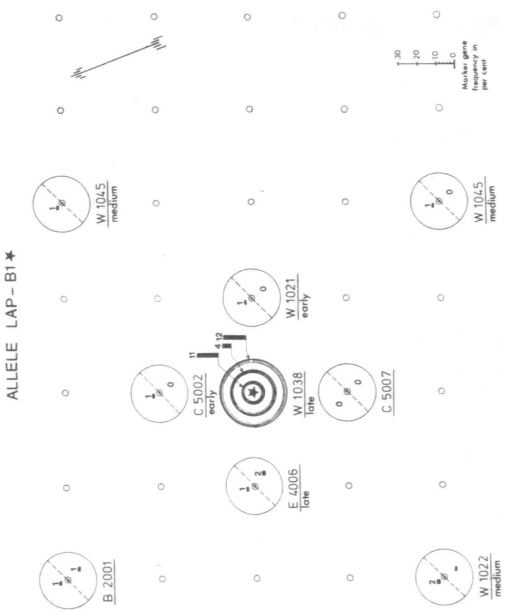

Figure 2d. Spatial dispersal of gene marker LAP-B_1 from clone W 1038 to itself and to some surrounding ramets.

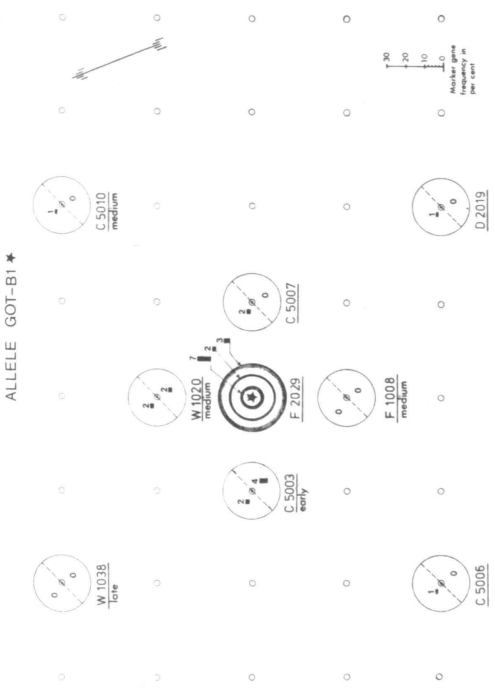

Figure 2a. Spatial dispersal of gene marker GOT- B_1 from clone F 2029 to itself and to some surrounding ramets. The frequencies of the marker allele (%) are shown; small circles (o) denote ramets not analysed. Late, medium and early indicate relative flowering time.

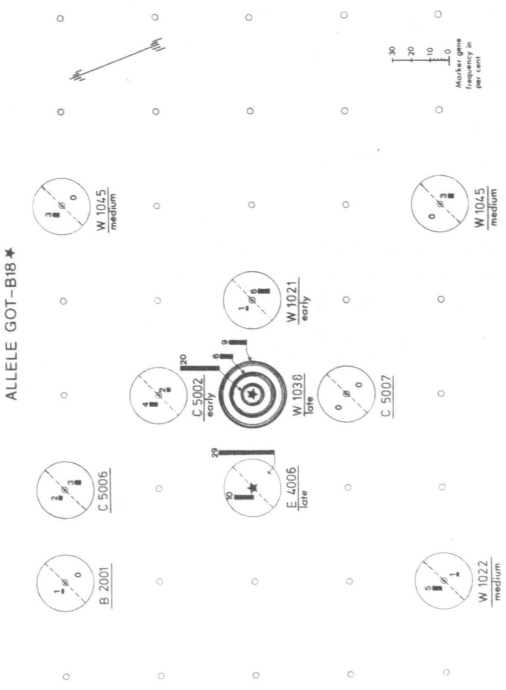

Figure 2c. Spatial dispersal of gene marker GOT-B_{18} from clone W 1038 and E 4006 to themselves and to some surrounding ramets.

Results and discussion

The father population

The father population was studied by means of the dispersal of marker alleles to the eight ramets surrounding the marker clones. The dispersal of pollen from the two clones F 1008 and W 1038 was studied. The dispersal of pollen in the marker clone itself is discussed further below, together with the analysis of the selfing frequencies. The ratio between the general mean gene frequencies for the closest four ramets and the peripheral four is 2:1. This ratio varies between different marker alleles ranging from 1.0: 1.0 to 3.9: 1.0. If the peripheral four ramets reach the same level as the closer four, this certainly is due to an impact from other marker ramets carrying the same gene marker. This pattern of distribution of markers to surrounding ramets is quite logical.

The result from the only unique seed orchard allele, GOT-B_1, shows the greatest difference between the two four-ramet groups. The mean impact of the marker allele on the four closest neighbouring ramets varies between 0.8 and 3.0 per cent. The corresponding figures for the peripherial four are 0.4 and 1.6 per cent. The gene frequencies for the inner four show a tendency toward higher gene frequencies in the directions north and west. This result might be caused by the prevailing winds during the pollen dispersal season. The sectors of the inner four which are facing the direction of the marker ramet also show higher marker gene frequencies than the others with one exception only (GOT-B_{18} around W 1038). The expected impact of each clone on the others is 3 per cent. Jonsson et al. (1976) reported mean impact for three consecutive years to be 1.3 per cent for F 2029 and 5.1 per cent for W 1038. This difference does not very well coincide with our results. However, different years could show great variation.

The recovery of marker genes in the marker ramets themselves

The results from the three-sector ramets show a significantly lower mean allele frequency in the inner lower part of the crowns (4.2 per cent), than in the upper part (12.5 per cent) and the lower peripheral part (9.2 per cent) (see Figure 1 and Table 1). This might be caused by a shading effect on the inner lower part of the crown. The deeper shade might delay the development of female flowering in such a way that self-pollination occurs less frequently here. In any case, it is a somewhat surprising result.

The total recovery in the two-sector trees is of the same magnitude as that of the three-sector trees. However, it is evident that the gene frequency of the sector facing another marker ramet is about three times higher than of the section opposite. The total mean level of recovery in each sector of the two-sector trees differs by a factor of two (20 per cent and 9 per cent). The individual mean value is considerably higher in the direction west of the main marker ramet (see Figure 2a, 2c). This corresponds well with the common pattern of pollen dispersal.

The level of selfing

If the studied gene marker is not unique to the seed orchard, it is necessary to reduce the above-mentioned frequencies of recovered gene markers through pollen dispersal in order to calculate the actual level of selfing in a proper way. The only marker which is unique to the seed orchard studied is GOT-B_1. The markers GOT-B_{18}, LAP-B_1 and LAP-B_3 are rare alleles. The corrections applied are reviewed in Table 1 and vary between two and three per cent. The correction is obtained by calculation of an average impact of the marker ramet on the surrounding ramets already described above and shown in Figure 2. Because the gene markers appear in a heterozygous genotype, the selfing frequency is calculated by reducing the original gene marker recovery frequencies with an estimated per cent of pollen coming from another clone with the same marker and finally by multiplying the reduced figure by two, assuming that the frequency of the non-detectable selfings are of the same magnitude as those which are detectable. Segregation data, however, do not show any significant deviation from 1:1 segregation.

In this way, calculated selfing frequencies show a mean level of 16 per cent. The mean value for each tree studied varies between 12 and 30 per cent (Table 2). The highest value is presented for one ramet of E 4006 (marker GOT-B_{18}) which deviates markedly from the other mean values. The reason for deviation might be that the impact from the neighbouring marker tree is higher than that allowed for by the 3 per cent correction.

Results presented by Shen et al. (1981), indicate that the impact from a neighbouring tree could be as high as 31.4 per cent if wind directions and flowering times are favourable. Another possible explanation is that the GOT-B_{18} bands in the gels could be somewhat difficult to profile in relation to the other close-lying bands. The mean selfing values for the marker trees, F 2029, W 1038, C 5006 and F 1008, vary between 12 and 16 per cent which is a remarkably narrow variation. The total mean selfing value for all marker trees is 16 per cent. This figure might be somewhat high due to underestimated correction factors.

All those in Table 3 reported estimations of selfing frequencies are based on isozyme gene

Table 1. Selfing in different sectors of Scots pine grafts in the Långtora seed orchard

Clone	Marker allele	Sector in crown (see Figure 1)	Marker alleles from fathers	Embryos analysed	Marker allele freq. (%)	Correction for other contributing fathers	Corrected selfing freq. (%) in sector	Mean
F 2029	LAP-B2/**B3**	UP	8	69	12	– 3	18	
		ILP	4	60	7	– 3	8	15
		PLP	9	69	13	– 3	20	
	GOT-**B1**/B2	UP	5	69	7	0	14	
		ILP	1	60	2	0	3	8
		PLP	2	69	3	0	6	
MEAN								12
W 1038	LAP-**B1**/B2	UP	7	65	11	– 2	18	
		ILP	3	68	4	– 2	5	14
		PLP	9	76	12	– 2	20	
	GOT **B18**/B3	UP	13	65	20	– 3	34	
		ILP	4	68	6	– 3	6	17
		PLP	7	76	9	– 3	12	
Mean								16

Table 2. Selfing in different clones in the Långtora seed orchard.

Clone	Marker allele	Corrected freq. %	mean selfing
F 2029	LAP-B3	15	
	GOT-B1	8	12
W 1038	LAP-B1	14	
	GOT-B18	17	16
C 5006	LAP-B1		14*
E 4006	GOT-B18		30 (blurry bands)
F 1008	LAP-B3		12
		Mean	16

* not shown in Figure 2
Mean if E 4006 is omitted: 13

Mean values (%) from F 2029 and W 1038	
Upper part	22.0
Inner lower part	6.5
Peripheral lower part	15.5

Table 3. Selfing frequencies estimated by isozyme gene markers

Species	Selfing frequency in per cent	Number of clones	Remarks	Author
Pinus sylvestris	6	51	High mortality	Shen et al., 1981
–"–	12–14	36		Müller-Starck, 1979
–"–	2–5	21–25		Rudin & Lindgren, 1977
Pinus taeda	1.2	50		Adams & Joly, 1980
Pinus radiata	14	30	One clone	Moran & et al., 1980
–"–	7–10	30		–"–

markers comprising two senses of selfing – within the single ramet and between ramets of the same clone. A lower number of clones and high mortality should both lead to high selfing frequencies based on panmixi statistics and wind velocity factors.

Moran et al. (1980) calculated an out-crossing frequency in a *Pinus radiata* seed orchard based on a comparison between isozyme types of parents and progenies by maximum likelihood statistics. They found an out-crossing rate varying between 0.90 and 0.93 for three different years.

Somewhat older studies of selfing frequencies based on morphological characteristics and chlorophyll deficiencies often present lower levels of selfing than the studies referred to above. For example, Franklin (1968) reported an average estimate of selfing of 1.75 per cent in a natural stand of *Pinus taeda*. The frequencies for single trees varied between 0 and 13 per cent. Johnsson (1972) reported less than one per cent in a *Pinus sylvestris* seed orchard.

The following factors might increase the degree of selfing in a seed orchard.

1. A low genetic load (of recessive lethals).
2. Male and female flowers closer to each other.
3. A closer synchronization of male and female flowering time within a clone.
4. A lower proportion of cross-fertilizing pollen in the pollen cloud due to:
   4.1 A younger seed orchard.
   4.2 A lower number of abundant flowering clones (♂).
   4.3 A wider spacing.
5. A lower wind velocity.

With knowledge from Jonsson et al. (1976) and other unpublished information about this studied seed orchard, some of the above-mentioned points can be discussed.

1. The genetic load can be estimated if controlled selfing is performed and the proportion of empty seeds from each clone serves as a base for calculations of recessive lethals (Koski 1973). Results from controlled selfing are available for the seed orchard studied. The results indicate that those studied marker trees which have been artificially selfed are representing an average empty seed frequency.
2. In many ramets, the male and female flowers are situated close to each other, which promotes selfing.
3. With evenly spread ramets in a seed orchard on level ground, the possibility of synchronization between different ramets of the same clone is good. This is the case in the present seed orchard. According to Jonsson et al. (1976) there was a considerable overlap between receptivity and pollen dispersal within each clone. With a high self fertility this would promote selfing.
4. If the clones involved are very early and late flowering clones, respectively, the proportion of cross-fertilizing pollen in the pollen-cloud in the seed orchard will be lower. Clone W 1038 is a late female flowering clone. But the others are not conspicuous in this respect.

   This seed orchard is young. Therefore, the flowering of a number of clones has been scanty. The percentage of male strobili turned out to be as follows for the clones studied (Jonsson et al. 1976; the expected mean value at equal frequency of male flowering is 2.7 per cent):

C 5006 – 0.4 per cent
E 4006 – 7.8 "
F 1008 – 6.4 "
F 2029 – 1.3 "
W 1038 – 5.1 "

This variation in flowering frequencies might give rise to variation in selfing frequencies as well. But this was not borne out in this particular study.

5. The wind-velocity in the seed orchard during the flowering season seems to be moderate.

The frequencies of selfing are higher than expected based on the study of frequency of flowering and flowering phenology by Jonsson et al. (1976). One reason for this could be a substantial within-ramet pollination, thus biassing panmixis.

Acknowledgements

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SEXUALLY ASYMMETRIC FERTILITY SELECTION AND PARTIAL SELF-FERTILIZATION

1. Population genetic impacts on the zygotic genotypic structure

MARTIN ZIEHE

Two frequently observed reproductive components for monoecious plant populations are theoretically considered: genotypically differential ovule and pollen production (called sexually asymmetrical fertility selection) and mixed random mating and selfing (partial self-fertilization). Sexually asymmetrical fertilities controlled by a gene locus may be suggested if in the zygotic genotypic structure the corresponding contributions via ovules differ from those via pollen. Asymmetrical fertility selection alone leads to a multiplicative genotypic structure in the offspring generation, which generally presents a homozygotic disadvantage relative to corresponding Hardy-Weinberg proportions. Partial self-fertilization as an additionally acting reproductive factor is able to counterbalance or even obscure this effect. The resulting offspring genotypic structures under both reproductive components (under consideration) are discussed. Some results are applied to the interpretation of the genotypic structure in a Scots pine seed orchard offspring in part 2 (Mueller-Starck 1982).

Introduction

The intensified use of biochemical gene markers often leads to a comparison between the genotypic structures of an adult population and their offspring generation. When the influence of subsequently acting viability selection is reduced, this comparison allows a more detailed study of those selective components which are involved in the reproductive system. Nevertheless, number and complexity of these components and their interactions are too extensive to allow a sufficiently global analytic description incorporating all reproductive system effects. Fig. 1 lists (certainly incompletely) well-known examples of breeding system components.

In the subsequent analytical considerations, we confine ourselves to the following factors which are frequently observed and accepted as main components of the reproductive system in monoecious populations: Differential ovule and pollen production, generally described as asymmetric fertility selection, and partial self-fertilization as a cumulative effect of several mating system components. Zygotic genotypic structures at a gene locus controlling these effects shall be characterized in this paper. These characterizations may be used to confirm hypotheses about the main mechanisms involved in the reproductive system of plant and, in particular, tree populations (Müller-Starck 1982).

Some basic characterizations of each breeding system component

Sexually asymmetric fertility selection

Sexually asymmetric fertility selection (gamete production) may directly be observed by estimating the production rate of ovules and pollen grains for genotypes in an adult population. Though sexually differential flowering behavior for genotypes in forest tree populations is not unusual, concrete data for gametic production rates are seldom available. Nevertheless, the electrophoretic determination of embryo and endosperm genetic information in conifers offers the possibility to infer the existence of sexual specific patterns within the reproductive system: The mentioned technique allows a comparison between the adult allelic contributions via female and male gametes to offspring (Müller 1976). An observed nonrandom difference between female and male contributions rules

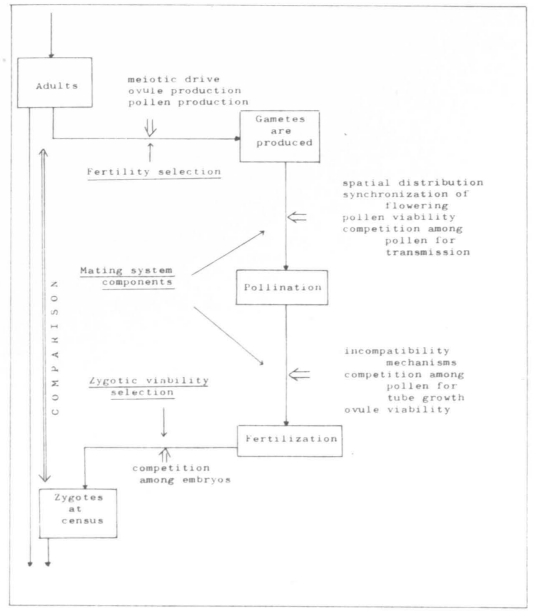

Figure 1. Examples for reproductive components in different phases of reproduction.

out a population genetic interpretation in terms of the classical (symmetrical) fertility theory alone. One of the more realistic hypotheses to check in this case is the effect of genotypically differential ovule and pollen production.

Assuming that gametes fuse to zygotes at random, the resulting zygotic structure exhibits a characteristic feature. Let P'_{kl} (primes indicate the offspring generation) denote the relative frequency of zygotes which received the allele A_k from a female and A_l from a male parental gamete. *The influence of sexual asymmetrical selective effects must be accepted if at least one P'_{kl} is "significantly" different from P'_{lk}.* If $p_k^{\varphi\prime}$ is the proportion of the allele A_k among the female successful gametes (incorporated in zygotes) and $p_l^{\sigma\prime}$ is the proportion of the allele A_l among successful male gametes, then *random gametic fusion leads to the* following *multiplicative structure:*

$$P'_{kl} = p_k^{\varphi\prime}\, p_l^{\sigma\prime} \qquad \text{for all k, l.}$$

Several authors already compared this multiplicative structure with those Hardy-Weinberg proportions (HWP) based on the corresponding allelic frequencies p_k',

$$p_k' = \frac{1}{2}\,(p_k^{\varphi\prime} + p_k^{\sigma\prime}).$$

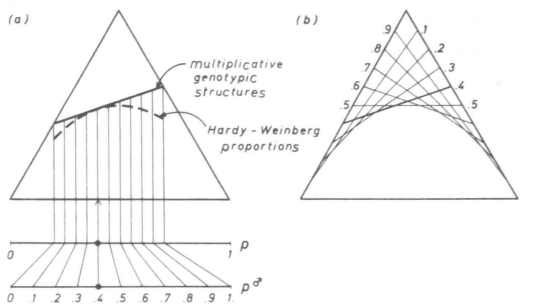

Figure 2. (a) Multiplicative genotypic structures for fixed $p_f^{\varphi} = 0.4$ and varying p_f^{σ} relative to the corresponding HWP. (b) Multiplicative genotypic structures for fixed p_f^{φ} and varying p_f^{σ}.

Robertson (1965) proved a heterozygotic excess and a homozygotic deficiency relative to HWP in the diallelic case. [It is easy to verify

$$p_k^{\varphi\prime}\, p_l^{\sigma\prime} + p_l^{\varphi\prime}\, p_k^{\sigma\prime} = 2p_k'p_l' - \frac{1}{2}(p_k^{\varphi\prime} - p_k^{\sigma\prime})\,(p_l^{\varphi\prime} - p_l^{\sigma\prime}).$$

Thus the deviation from Hardy-Weinberg proportions is proportional to the differences between female and male allelic contributions.] Purser (1966) applied this result to differential fertility selection. Ziehe and Gregorius (1981) recently analyzed in more detail the amount of these deviations as a function of newly defined asymmetric fertility coefficients.

The concrete locations of multiplicative genotype structures with fixed $p_f^{\varphi} = 0.4$ in the diallelic case are illustrated in the de Finetti diagrams of Fig. 2.

Equilibrium structures and the stability properties for special diallelic cases are investigated by Bodmer (1965), the multiallelic equilibrium deviations from HWP by Ziehe and Gregorius (1981).

Partial self-fertilization

For many of the self-compatible monoecious tree species, a self-fertilization rate is suggested which is much higher than expected under random mating models. In this context we will use a self-fertilization parameter σ as follows: a proportion σ of all those ovules which are produced by an individual and will be fertilized during the reproductive

phase shall be reserved to be fertilized by pollen from the same individual. The remaining part of the ovules is assumed to merge at random with pollen from the pollen cloud. Assuming further that total ovule and pollen production as well as the contribution to the pollen cloud are the same for all individuals and considering one multiallelic gene locus in an effectively infinite population reproducing in separated generations, the allelic frequencies remain constant over the generations ($p'_k = p_k$) while the genotypic frequencies change in the following manner:

$$P'_{kk} = p_k^2 + \frac{\sigma}{2}(p_k + P_{kk} - 2p_k^2) \quad \text{and}$$

$$P'_{kl} = P'_{lk} = p_k p_l - \frac{\sigma}{2}[4p_k p_l - (P_{kl} + P_{lk})] \quad \text{for } k \neq l.$$

Here $(p_k + P_{kk} - 2p_k^2) \geqslant O$ for any genotypic structure. [If $O \leqslant p_k \leqslant \frac{1}{2}$, we already have $p_k \geqslant 2p_k^2$. $\frac{1}{2} \leqslant p_k \leqslant 1$ together with the inequality $P_{kk} + 1 \geqslant 2P_{kk} + \sum_{j \neq k} (P_{kj} + P_{jk}) = 2p_k$ leads to $2p_k^2 - p_k = p_k(2p_k - 1) \leqslant 2p_k - 1 \leqslant P_{kk}$. Thus the assertion holds for all $O \leqslant p_k \leqslant 1$.]

Therefore offspring homozygotic frequencies exhibit a homozygotic excess relative to HWP (or in special cases exactly HWP), and the sum of heterozygotic frequencies shows a corresponding deficiency. Nevertheless, a single offspring heterozygotic frequency in the multiallelic case may be higher than HWP.

[For example: $P_{13} + P_{31} = p_2 = \frac{1}{2}$ implies $P'_{13} - p'_1 p'_3 = P'_{31} - p'_3 p'_1 = \frac{\sigma}{16}$, which is positive for $\sigma > O$.]

It should be noted that the deviations from the corresponding HWP therefore have sign opposite to that of the deviations resulting from sexually asymmetric fertility selection alone.

Investigating the long-term effect, we obtain equilibrium frequencies

$$P^*_{kk} = p_k^2 + \frac{\sigma}{2-\sigma} p_k(1-p_k) \text{ and}$$

$$P^*_{kl} = P^*_{lk} = p_k p_l - \frac{\sigma}{2-\sigma} p_k p_l \quad \text{for } k \neq l,$$

where p_k, p_l are the initial allelic frequencies. The equilibrium is approached in the following geometric manner (assuming $P_{kl} = P_{lk}$, which is valid after one generation at the latest):

$$(P'_{kl} - P^*_{kl}) = \frac{\sigma}{2}(P_{kl} - P^*_{kl})$$

Resulting offspring structures

Summarizing both preceding sections the following genotypic offspring structures occur under models of either partial self-fertilization or sexually asymmetric fertility selection:

i) $P'_{kl} = p_k p_l$ under panmictic reproduction,
ii) $P'_{kl} = p'_k p'_l$ (Hardy-Weinberg proportions) under symmetrical fertilities and $\sigma = O$,
iii) $P'_{kk} = p'^2_k + \frac{\sigma}{2}(p_k + P_{kk} - 2p_k^2)$ and
$P'_{kl} = p'_k p'_l - \frac{\sigma}{2}[4p_k p_l - (P_{kl} + P_{lk})]$ for $k \neq l$
under $\sigma \geq O$ and no fertility selection,
iv) $P'_{kk} = p'^2_k + \frac{\sigma}{2-\sigma} p'_k(1-p'_k)$ and
$P'_{kl} = p'_k p'_l - \frac{\sigma}{2-\sigma} p'_k p'_l$ for $k \neq l$
(equilibrium inbreeding structure) in equilibrium under $\sigma \geq O$ and no fertility selection,
v) $P'_{kl} = p'^{\varphi}_k p'^{\sigma}_l$ (multiplicative genotypic structure) under sexually asymmetric fertility selection and $\sigma = O$.

The combined influence of fertility selection and partial self-fertilization on offspring and equilibrium genotypic structures

Sexually symmetric fertility selection and partial self-fertilization

Several papers deal with solutions for equilibria in models incorporating mixed random mating and self-fertilization and sexually symmetrical viability or fertility selection. As noted by Bodmer (1965), both last mentioned selection models are analytically equivalent if census is made of zygotes in each of the separated generations. Nevertheless all presented equilibrium solutions are difficult to survey with respect to characterizations of the genotypic structure: Hayman (1953) assumed the diallelic case with overdominance in viability coefficients. Workman and Jain (1966) simplified his equilibrium solutions and estimated selection coefficients based on observations in some predominantly self-fertilizing plant populations. Weir (1970) extended the complex rules for computation of equilibria to the multiallelic case.

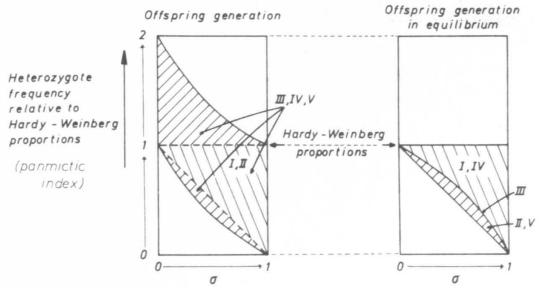

Figure 3. Upper and lower limits for the panmictic index under several diallelic selection models (following Ziehe, 1982). The models are: I. Symmetrical viability and fertility selection with heterozygote advantage. II. Symmetrical viability and fertility selection with heterozygote disadvantage. III. Selection in pollen production. IV. Selection in ovule production with heterozygote advantage. V. Selection in ovule production with heterozygote disadvantage.

Fertility selection with respect to one gametic sex only and partial self-fertilization

Fertility selection with respect to one gametic sex only is already a case of sexual asymmetry. Analytical considerations are generally restricted to the diallelic case. Workman and Jain (1966) gave equilibrium solutions for varied maternal fertility effects and showed that these equilibria are the same as in a model with these genotypical fertility coefficients in the sexually symmetrical fertility case. Though the same equilibrium structures result, genotypic trajectories do not develop in the same way. Ziehe (1982) determined upper and lower limits for deviations from HWP in the offspring generation and in equilibrium. These deviations are measured in terms of the panmictic index I_p as the heterozygotic frequency relative to the corresponding HWP [$I_p = (P_{12} + P_{21})/2p_1p_2$] or the fixation index F (introduced by Wright), $F = 1 - I_p$. Figure 3 illustrates some of the results.

The general case

Let Φ_{kl} denote the number of ovules (all to be fertilized) which are produced by an individual with genotype A_kA_l or A_lA_k, such that the ordering of the alleles has no influence on ovule and pollen production ($\Phi_{kl} = \Phi_{lk}$). σ is taken as the proportion of ovule self-fertilization as defined on p. 95–96. The remaining part $(1-\sigma)\Phi_{kl}$ of the genotypical amount of fertilized ovules is fertilized at random by pollen from the pollen cloud which consists of all the pollen not needed for self-fertilization.

Then the following offspring deviations from the corresponding multiplicative genotypic structure hold:

$$P'_{kk} - p^{\varphi}_k p^{\sigma}_k = \frac{\sigma}{2}\left(p^{\varphi}_k + P_{kk}\frac{\Phi_{kk}}{\bar{\Phi}} - 2p^{\varphi}_k p^{\sigma}_k\right) \quad \text{and}$$

$$P'_{kl} - p^{\varphi}_k p^{\sigma}_l = -\frac{\sigma}{4}\left[4p^{\varphi}_k p^{\sigma}_l - (P_{kl} + P_{lk})\frac{\Phi_{kl}}{\bar{\Phi}}\right] \quad \text{for } k \neq l,$$

where $\bar{\Phi}$ is the mean ovule production: $\bar{\Phi} = \sum_{k,l} P_{kl}\Phi_{kl}$.

Some consequences:

i) The deviations from the corresponding multiplicative genotypic structure in the offspring are proportional to σ. If in particular $\sigma = O$, the zygotic genotypic structure represents a multiplicative structure. Thus the deviations from the multiplicative structure reflect the additional self-fertilization effect.
ii) The deviations from the corresponding multiplicative genotypic structure are independent from any genotypical contribution to the pollen cloud. Differential pollen cloud contributions are already contained in the allelic structure of male successful gametes (p^{σ}_k, $k = 1, 2\ldots$) and thus involved in the multiplicative genotypic offspring structure.
iii) The heterozygotic offspring deviations are symmetrical with respect to their female and male gametic representations:

$$P'_{kl} - p^{\varphi}_k p^{\sigma}_l = P'_{lk} - p^{\varphi}_l p^{\sigma}_k.$$

A significant difference between these deviations for at least two offspring heterozygotes A_kA_l and A_lA_k requires the consideration of further or entirely other breeding system components.

iv) If ovule production in adults does not vary ($\Phi_{kl} = \Phi$ for all k, l), the sign and amount of deviations of the zygotic genotypic frequencies are the same as those from HWP under partial self-fertilization alone (compare section 2b):

$$P'_{kk} - p^{\varphi}_k p^{\sigma}_k = \frac{\sigma}{2}(p_k + P_{kk} - 2p_k^2) \text{ and}$$

$$P'_{kl} - p^{\varphi}_k p^{\sigma}_l = -\frac{\sigma}{4}\left[4p_k p_l - (P_{kl} + P_{lk})\right] \text{ for } k \neq l.$$

v) Comparing the zygotic genotypic structure with the corresponding HWP, we get

$$P'_{kk} - p'^2_k = -\frac{1}{4}(p^{\varphi}_k - p^{\sigma}_k)^2 + \frac{\sigma}{2}\left(p^{\varphi}_k + P_{kk}\frac{\Phi_{kk}}{\bar{\Phi}} - 2p^{\varphi}_k p^{\sigma}_k\right)$$

and $[P'_{kl} + P'_{lk}] - 2p'_k p'_l = \frac{1}{2}(p^{\varphi}_k - p^{\sigma}_k)(p^{\sigma}_l - p^{\varphi}_l) - \frac{\sigma}{2}\left[4p^{\varphi}_k p^{\sigma}_l - (P_{kl} + P_{lk})\frac{\Phi_{kl}}{\bar{\Phi}}\right]$

for $k \neq l$. Here the difference between pollen and ovule allelic contributions to zygotes already include a self-fertilization effect.

Concluding remarks

Sexually symmetrical selection in reproduction is a dubious assumption if *sexual specific selective effects* are involved in fertilities and mating system components. These sexually asymmetric effects may be supposed if ovule and pollen (allelic or gametic) contributions to the offspring generation differ significantly. For an example compare Müller-Starck (1982).

Assuming differential ovule and pollen production and random gametic fusion, a multiplicative genotypic structure in zygotes results. Multiplicative genotypic structures and their relationship to Hardy-Weinberg proportions are characterized in 2nd section. If we assume mixed random gametic fusion and self-fertilization in addition to differential gametic production, deviations of the zygotic genotypic structure from the corresponding multiplicative genotypic structure occur. These deviations are investigated in section 3c. This may serve to interpret genotypic offspring structures with respect to special hypotheses about the influence of reproductive components. Some of the theoretical considerations given here are applied to a *Scots pine* seed orchard in part 2 of this series (Müller-Starck, 1982).

Acknowledgement

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SEXUALLY ASYMMETRIC FERTILITY SELECTION AND PARTIAL SELF-FERTILIZATION

2. Clonal gametic contributions to the offspring of a Scots pine seed orchard

GERHARD MÜLLER-STARCK

Enzyme gene markers were applied to identify the gametic contributions of single clones among the open pollinated offspring from a Scots pine (*Pinus sylvestris L.*) seed orchard. Seeds from two flowering periods were studied. The genetic control of the enzyme system GOT was verified by analysis of seeds from controlled crossings. Three clones carried unique alleles and functioned as marker clones. Their gametic contributions were monitored in all-orchard seed probes.

The obtained deviations between the female and male contribution of each clone indicate differential ovule and pollen production. Such occurrence of sexually asymmetric fertility selection was also reflected in the genotypic structure of each offspring population by applying the "multiplicative structure". Individual and clonal self-fertilization were indicated. The obtained results stress the importance of taking into account the effects of sexually asymmetric fertility selection in order to avoid misleading interpretations of genotypic structures among the offspring.

Introduction

The genotypic structures of parental and offspring populations can be expected to be identical in the case of a population in panmictic equilibrium. Required conditions for this are for instance that the fertilities and viabilities of each individual of any given genotype in a population and its gametes are the same and stay the same for all subsequent generations. Substantial deviations from this assumption are evident in forest tree populations, including seed orchards, as a consequence of the realized reproductive system which comprises both the fertilities and the mating system. Due to the first, differential ovule and pollen production occurs in Scots pine seed orchards (e.g. Jonsson et al. 1976, Bhumibhanon 1978), and due to the latter, non-random fusion of gametes occurs (e.g. Shen et al. 1981,Müller-Starck 1979 and 1981). A survey of phenomena which may play a role in the relationship between parental and offspring genotypic structures is given in part 1 by Ziehe (1982).

In the present paper the gametic contribution of particular clones to the open pollinated crop of a total seed orchard ("all-orchard offspring") is studied for two flowering periods. The gametic contribution of a clone is described in terms of the frequency of seeds which descend from this clone by containing either its fermale or male contribution or both. The last-named are necessarily descendants from clonal self-fertilization and may originate from individual self-fertilization or from cross-fertilization between genotypically identical individuals from the same clone. The clones which are included in this study are designated as "marker clones" due to the fact that they carry unique alleles.

All results refer to viable seeds and thus to the successful gametes which are incorporated in them. This implies that impacts of gametic or zygotic viability selection and possibly mating specific effects are included in the results. Therefore, the detected gametic contributions of the marker clones can refer only approximately to the actual ovule and pollen production.

It is the aim of this study to determine the extent to which single parental clones are represented gametically among the all-orchard offspring and to use the obtained results for a testing of hypotheses on predominant components of the reproductive system of

a Scots pine seed orchard: genotypically differential ovule and pollen production ("sexually asymmetrical fertility selection") and individual self-fertilization.

Material and methods

The Scots pine clonal seed orchard was established in 1959 by the Hessische Forstliche Versuchsanstalt, Hann. Münden. The actual number of clones is 36, the average number of individuals per clone is 25, and the population size is 900. The seed orchard can be expected to be isolated sufficiently from external pine pollen (for further details see Müller–Starck 1981). The seed probes originate from open pollination during the flowering periods 1975 and 1976 and are part of the harvests of cones from the total orchard in the winters of 1976/77 and 1977/78, respectively. The seeds were supplied by the Hessische Forstliche Versuchsanstalt.

The parental clones andf their offspring were identified by enzyme gene markers, applying the system of leucine aminopeptidase (LAP, EC 3.4.1.1) and of glutamate oxalate transaminase (GOT, EC 2.6.1.1). Endosperm and embryo tissues were homogenized with 0.13 M tris hydrochloric acid buffer pH 7.3 and the enzymes separated by means of starch gel zone-electrophoresis in a discontinuous buffer system based on Poulik (1957), modefied by Bergmann (1973): 0.07 M tris citrate gelbuffer pH 8.7, and 0.3 M sodium hydroxide borate electrodebuffer pH 8.2. Gel concentration 11 %, voltage distribution 15 V/cm, bridge distance 10 cm, water cooling temperature 8°C. Staining solution for LAP see Bergmann (1973), for GOT see Rudin (1975). Both enzyme systems were studied simultaneously by slicing the starch gels horizontally.

Needle probes were harvested outside of the vegetation period and pre-treated as follows: Crushing together with insoluble polyvinylpyrrolidone in fluid nitrogen at –196°C with an Ultra-Turrax equipment (Jahnke and Kunkel, T 18/10, 18 N) and permanent storage of the pulverized material at –60°C. Homogenization of small portions of the cooled material with 50–100 µl tris hydrochloric acid buffer pH 7.3, the following substances being added per 100 ml buffer: 4 g soluble polyvinylpyrrolidone 10, 0.15 g EDTA II, 0.1 g diethyldithiocarbamic acid, 1 ml 2-mercaptoethanol, 0.01 g cysteine, 0.5 g sodium bisulfite 1.0 g sodium ascorbate (selected agents from Rhodes, 1977).

To verify the genetic control of the GOT-system, parents and the offspring from controlled crossings were analyzed. For related studies which verify the LAP-B gene locus and for further references see Müller–Starck (1981). For characterization of the LAP-system by molecular properties see Müller-Starck and Hüttermann (1981).

Based on these results, the genotypes of the parental seed orchard clones were identified by endosperm analysis, using at least nine seeds per clone. In addition, clonal needle probes and the available pollen probes were analyzed. The all-orchard offspring was studied by analysis of endosperm and corresponding embryo of each individual seed. By means of this, the female and the male gametes can be identified always as ordered pairs (Müller 1976). The gametic contribution of each marker clone to the all-orchard offspring was derived by identifying those seeds which carried the unique allele of the respective marker clone in their female or male contribution or in both.

The genotypic structure among the all-orchard offspring at one enzyme gene locus was used as an example to test hypotheses on components of the reproductive system. Some of the methods presented by Ziehe (1982) were applied.

Results and discussion

Identification of genotypes

Under the given conditions, the GOT-polymorphism was apparent in the zymograms within three zones, designated according to their decreasing relative mobility as A, B, and C. An overlapping of the zones A and B was noticeable. In the case of haploid tissue, a single isoenzyme band was obtained in each zone, while in the case of diploid tissue either a single band or a combination of two single bands with an additional intermediate hybrid band was observed. The latter fact indicates dimeric enzyme structures. Individuals of the same clone always had identical GOT-phenotypes. For any given clone, these phenotypes coincided, independent of whether needle tissue, endosperm mixtures, or pollen probes were used for identification. Some results of analyses of endosperm and corresponding embryo of seeds which originate from controlled crossings are presented in table 1.

The obtained phenotypic segregations among the offspring do not deviate significantly from the proportions expected under the assumption of a simple Mendelian mode of inheritance. Isoenzyme bands contributed by the female and male parent are apparent in the embryo in the form of direct combinations with an additional hybrid band. In the case of uniform parental phenotypes, a segregation among the offspring is not evident. As an example, results from a controlled self-pollination are illustrated in figure 1. The parental phenotype is represented by both single endosperms and needles.

The parental clones which were involved in the controlled crossings were uniform with respect to GOT-A. Additional studies of open pollinated seeds from other clones which all carry two distinct GOT-A-phenotypes resulted in the following: For each clone a 1:1 segregation was obtained among the endosperms, and in the embryos all possible combinations of isoenzyme bands were present independently from GOT-B and GOT-C.

Table 1. Results of analyses of seeds from controlled crossings. The given chi²-values refer to the expected Mendelian proportions among the GOT-phenotypes

Crossing of clones	Parental clones GOT-phenotypes ♀♀ × ♂♂	Number of seeds	Offspring Detected segregation of GOT-phenotypes		$\chi^{2(1)}$
CH10×CH10	$A_2A_2 \times A_2A_2$	19	A_2A_2 uniform	= 19	
	$B_3B_5 \times B_3B_5$		B_3B_3:B_5B_3:B_3B_5:B_5B_5	= 4:5:7:3	1.84 n.s.(2)
	$C_1C_3 \times C_1C_3$		C_1C_1:C_3C_1:C_1C_3:C_3C_3	= 2:5:9:3	6.05 n.s.
CH10×CH2	$A_2A_2 \times A_2A_2$	46	A_2A_2 uniform	= 46	
	$B_3B_5 \times B_2B_5$		B_3B_2:B_5B_2:B_3B_5:B_5B_5	= 9:7:14:16	4.61 n.s.
	$C_1C_3 \times C_2C_3$		C_1C_2:C_3C_2:C_1C_3:C_3C_3	= 16:7:8:15	5.65 n.s.
CH9×CH2	$A_2A_2 \times A_2A_2$	40	A_2A_2 uniform	= 40	
	$B_1B_3 \times B_2B_5$		B_1B_2:B_3B_2:B_1B_5:B_3B_5	= 10:10:6:14	3.20 n.s
	$C_3C_3 \times C_2C_3$		C_3C_2:C_3C_3	= 17:23	0.90 n.s.
CH9×CH3	$A_2A_2 \times A_2A_2$	50	A_2A_2 uniform	= 50	
	$B_1B_3 \times B_5B_5$		B_1B_5:B_3B_5	= 26:24	0.08 n.s.
	$C_3C_3 \times C_1C_3$		C_3C_1:C_3C_3	= 25:25	0.00 n.s.

(1) Chi²-test "Goodness of fit"
(2) At 5 % level

It can be concluded for the present that the GOT-polymorphism in needle tissue, endosperm, embryo, and pollen is under the genetic control of at least three gene loci GOT-A, GOT-B, and GOT-C, each of which codes for several codominant alleles. These alleles are represented phenotypically by single isoenzyme bands. Classifying the offspring from each controlled crossing according to the particular genotypes at one GOT-locus, it is then possible to study within these classes the segregation at the remaining two GOT-loci. For the available material it can be stated for the present that in each classified group of offspring all possible genotypes at the respective other gene loci were represented.

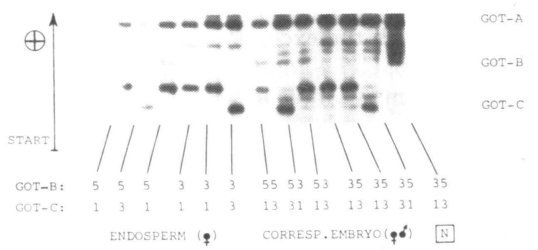

Figure 1. GOT phenotypes of the offspring from a controlled self-pollination (endosperm ♀ and corresponding embryo ♀♂ of 6 seeds) and the parental clone CH 10 (N=needle tissue). The designation refer to GOT-B and GOT-C; GOT-A is uniform.

The results of our studies in Scots pine seed orchards and stands indicate that the gene locus GOT-A codes for three alleles, GOT-B for seven alleles and GOT-C for at least three alleles. Some of the alleles were represented by faintly stained isoenzyme bands, but a lack of enzyme activity ("null-allele") was never obtained. Scaled between 0 and 100, the relative mobilities are (synonymous designation of alleles and corresponding isoenzyme bands):

GOT-A: $A_1 = 72$, $A_2 = 66$, $A_3 = 59$
GOT-B: $B_1 = 61$, $B_2 = 57$, $B_3 = 55$, $B_4 = 52$, $B_5 = 44$, $B_6 = 38$, $B_7 = 33$
GOT-C: $C_1 = 27$, $C_2 = 24$, $C_3 = 18$, $C_4^? = 5$.

The designation $C_4^?$ is preliminary because the available material did not allow unequivocal exclusion of the possibility that this band might be expressed by an additional gene locus.

The obtained results correspond to other studies in Scots pine as follows: The gene loci GOT-A and GOT-B are identical to the two loci detected by Rudin (1975) in needle tis-

sues and endosperms and by Shen et al. (1981) also in the embryos. All three loci correspond to the loci I, II, and III which were described by Krzakowa et al. (1977) for endosperms. In a recent study Chung (1981) describes the same loci GOT-A, GOT-B, and GOT-C and an additional locus GOT-D for winter buds, endosperms and embryos. The only isozyme band at the latter locus may correspond to the band C_4 in the present study.

In the actual seed orchard the alleles A_3, B_4, B_6, and C_4^2 are not represented. Three clones were proven to carry unique alleles in the heterozygous state: The two marker clones CH 4 and CH 14 have the allele GOT-A_1 and GOT-B_7, respectively, while the third marker clone, CH 2, carries two unique alleles, namely LAP-B_3 and GOT-B_2.

Clonal gametic contributions

For each of the flowering periods 1975 and 1976 a sample of 640 all-orchard seeds was studied. Because the marker clones carry the unique alleles in the heterozygous state, the gametic contributions can be identified only with respect to one out of two alleles. Therefore, the detected number of seeds which descend from one of the marker clones was multiplied by the factor 2 if these seeds contained the female or the male contribution. In the case of descendants from self-fertilization, only those seeds can be positively identified which carry the respective unique allele in the homozygous state. This implies a multiplication by the factor 4. All subsequently given values were estimated according to these methods.

In table 2 the estimated numbers of seeds descending from a particular marker clone are presented separately according to whether they contain the female or male contribution or both. Each of these offspring groups is contrasted with the respective number of seeds which can be expected according to the panmixia hypothesis. The gametic contribution of the clone CH 2 was estimated by means of each of the two unique alleles separately. One of them, LAP-B_3, was also applied in a recent study (Müller-Starck 1981) which referred to the same clone in the flowering period 1976 but was based on another seed probe from the same all-orchard crop. The resulting genotypic structures at the LAP-B gene locus of both seed probes did not deviate significantly.

The female and male gametic contribution of particular clones to the all-orchard offspring can vary substantially: In the case of the clone CH 4 in the flowering period 1975, the frequency of seeds with its female contribution exceeds the frequency of those with the male contribution by a factor of 5.3. The opposite extreme is realized by the clone CH 14 in the period 1976: Seeds with its male contribution can be expected 6 times as frequently as those with its female contribution. Under the assumption of random fusion of gametes, these facts clearly indicate differential ovule and pollen production and thus the occurrence of sexually asymmetric fertility selection. The deviation between the female and male gametic contribution was tested for each clone and flowering period separately: Both frequencies and the corresponding remainder to the sample size of 640 were contrasted in a 2 × 2 contingency table, and the chi^2-values were calculated. The results are given in table 3.

In most cases significant deviations were obtained within single clones between the frequencies of seeds containing the female and those containing the male gametic contribution. These two estimations for the clone CH 2 (LAP-B and GOT-B) result in deviating chi^2-values, especially in 1975. As can be seen in table 2, this is due to the different values of the male contribution of CH 2. Nevertheless, the two estimations for the frequencies of the seeds which descend from CH 2 cannot be proven to deviate significantly.

The deviations between the flowering periods with respect to either the female or the male gametic contribution are not significant, with the exception of the male contributions of CH 4 and CH 14. In both cases, a more intensive male flowering in the period 1976 is indicated. A testing with respect to the offspring from clonal self-fertilization is not useful, since the identified frequencies vary only between 1 and 3.

The total gametic contribution of a clone to the all-orchard offspring (see table 2) was derived by adding up the expected frequency of seeds from clonal self-fertilization and the averaged frequency of the seeds with the

Table 2. Estimated number of seeds with the gametic contributions of the clones CH 4, CH 14 and CH 2 in all-orchard seed probes from two flowering periods in contrast to the expected panmixia values.

						Offspring from self-fertilization.			
			♀ or ♂			♀ and ♂	♀ and ♂	Total contribution	
Marker clone	Flower period	Number of seeds	exp. under panmixia (1)	♀ contribution estimated (2)	♂ contribution estimated (2)	exp, under panmixia (3)	contribution estimated (4)	exp. under panmixia	estimated
CH 4	1975	640	17,3	32	6	0,5	0	17,8	19
	1976	640		32	26		4		33
CH 14	1975	640		10	28		0		19
	1976	640		8	48		4		32
CH 2	1975(5)	640		26	52		4		43
	1976(5)	640		34	54		8		52
	1975(6)	640		26	42		4		38
	1976(6)	640		32	48		12		52

(1) $(N-1)/N^2 \times 640$, N = Number of clones
(2) identified genotypes × 2
(3) $I/N^2 \times 640$
(4) identified genotypes × 4
(5) LAP-B
(6) GOT-B

Table 3. Comparison in pairs between the frequencies of seeds with the male and those with the female gametic contribution for each of three clones in two flowering periods. The given chi^2-values result from 2×2 contingency tables. Satistical significance at levels of 5% (*), 1% (**), 0.1% (***).

	Clone CH 4		Clone CH 14		Clone CH 2			
	1975♂	1976♂	1975♂	1976♂	1975♂(1)	1976♂(1)	1975♂(2)	1976♂(2)
1975♀	18.33***		8.79**		9.23**		3.98*	
1976♀		0.65 n.s.		29.88***		4.88*		3.41 n.s.

(1) LAP-B (2) GOT-B

Table 4. Comparison in pairs between the total gametic contribution of each of three clones and the expected value under panmixia. For further explanation see table 3.

	Clone CH 4		Clone CH 14		Clone CH 2			
	1975	1976	1975	1976	1975(1)	1976(1)	1975(2)	1976(2)
Panmixia	0.04 n.s.	4.74*	0.04 n.s.	4.31*	10.97***	17.72***	7.65**	17.72***

(1) LAP-B (2) GOT-B

female or male contribution from the same clone. The obtained frequencies were compared statistically (chi^2-test "goodness of fit") with the expected values according to the panmixia hypothesis. As can be seen in table 4, the deviations between both are in most cases significant. These deviations result from the overrepresentation of each clone as compared with the expected panmixia values (see table 2). The total gametic contribution of the marker clones is on the average 1.8 times more frequent than expected. Specific effects of the flowering periods are noticeable: The panmixia value is exceeded by the factor 1.5 in 1975 and by the factor 2.2 in 1976. The maximum deviation is valid for the clone CH 2 in 1976: The estimations by means of the loci LAP-B and GOT-B both confirm a total gametic contribution which exceeds that expected by the factor 2.9.

Possibly also due to its rareness, the estimates for self-fertilization of the marker

Table 5. Genotypic structures at the GOT-C gene locus of parental clones and all-orchard offspring in contrast to the expected frequencies under panmixia and under random combination of the successful gametes ("multiplicative structure") for two flowering periods.

Genotype ♀♂		Frequency parental clones	Expected frequencies offspring under panmixia	under random combinat. of successful gametes 1975	1976	Detected frequencies offspring 1975	1976
C_1C_1		4	49.4	42.8	64.4	42	59
C_2C_2		0	0.5	0.7	0.9	2	3
C_3C_3		19	308.6	316.5	265.8	322	266
C_1C_2	}	1	4.9	5.5	10.0	9	14
C_2C_1			4.9	5.4	5.6	7	5
C_1C_3	}	11	123,5	93.6	131.6	91	133
C_3C_1			123.5	144.7	130.0	144	136
C_2C_3	}	1	12.4	11.9	11.5	9	10
C_3C_2			12.4	18.7	20.1	14	14
		36	640.1	639.8	639.9	640	640

clones are on the average 7.3 times more frequent than expected. The contribution of the marker clones as female parent exceeds the expected panmixia value on the average by the factor 1.4, as male parent by the factor 2.

The substantial overrepresentation of the marker clones among the all-orchard offspring necessarily implies a simultaneous underrepresentation of parental clones other than the studied ones.

Testing hypotheses on fertility selection and self-fertilization

As an example, the genotypic structure of the open pollinated all-orchard offspring is studied at the GOT-C gene locus. A larger number of different genotypes is realized at this locus than at GOT-A, and the particular genotype classes are better represented and thus more suitable for statistical comparisons than at the GOT-B or LAP-B locus. In table 5 the genotypic structures of the offspring populations from 1975 and 1976 are given and contrasted with the expected values under the conditions of panmixia and of random combination of the successful gametes.

The two offspring populations can be proven to deviate significantly (k × 2 table ch^2-test, e.g. Sachs 1974, topic 6.1.1). With the same test, the deviations between the female and male allelic frequencies among each offspring population are significant in 1975, bot not in 1976. Comparing each of the two female and male gamete pools with the panmixia expectation (chi^2-test "goodness of fit"), all except the males in 1975 can be proven to deviate significantly.

Highly significant deviations are obtained between each of the detected offspring structures and the panmixia expectation. The homozygote C_1C_1 is underrepresented in 1975 and overrepresented in 1976, While the opposite holds for the homozygote C_3C_3. Of these four values, only C_3C_3 in 1976 shows a significant deviation from the panmixia value (2 × 2 contingency table). More important is the fact that significant deviations are obtained between the frequencies of the reciprocal heterozygotes: This is the case for C_1C_3 and C_3C_1 in 1975 and for C_1C_2 and C_2C_1 in 1976. This asymmetry cannot be explained by means of the panmixia standard, since this standard is based on the assumption of absence of differences in the gamete production and in the successful gametes in the offspring.

The Hardy-Weinberg proportions are also not suitable in this case: The necessary averaging of the female and male gametic frequencies would mask the detected asymmetries. This is not valid for a standard which is based on the assumption of random combination of successful gametes ("multiplicative structure" according to Ziehe 1982). As can be seen in table 5, the application of this standard results in a much better approximation to the detected offspring stuctures than was possible by the panmixia standard: The deviations between the multiplicative structure and each offspring structure are no longer significant. This result shows that the occurrence of sexually asymmetric fertility selection is clearly indicated. Nevertheless, the involvement of other phenomena of the reproductive system cannot be excluded.

The fact that the frequencies of some reciprocal heterozygotes cannot be approximated to a similar extent by the multiplicative structure suggests the existence of individual self-fertilization (Ziehe 1982, topic 3.c). This is more valid for 1976 than for 1975 and may explain the greater frequency of offspring from self-fertilization in this period (see table 2). In particular, self-fertilization is indicated by the slight overrepresentation of the homozygote C_3C_3 in 1975 and C_1C_1 in 1976. The small extent of this deviation and the fact that other homozygotes are underrepresented may be interpreted for the present as being a consequence of the counterbalance by sexually asymmetric fertility selection, which was proven by Ziehe and Gregorius (1981) to reduce the proportion of homozygotes.

Concluding remarks

The parental clones in a forest seed orchard are usually assumed to contribute to the subsequent generation to approximately the same extent. This aspect was studied by identifying all-orchard seeds which descend from single specified clones. Due to the effective isolation of the seed orchard, interference by external pollen can be expected to be at a minimum. A multi-locus study on quantifying such contamination is in progress.

The results of this study make it clear that gametic contributions to the all-orchard offspring can vary substantially between clones and between flowering periods and can also deviate significantly from sexual symmetry. The total gametic contribution of the studied marker clones was on the average 1.8 times as frequent as expected under panmixia. This overrepresentation was more pronounced with respect to the male contribution than to the female. Maximum deviations appeared in relation to descendants from clonal self-fertilization and also between the female and male gametic contributions of a particular clone in a particular flowering period. In addition, evidence for differential ovule and pollen production, and thus for the occurrence of sexually asymmetric fertility selection, was obtained from the studied genotypic structure among the offspring populations. A testing of this hypothesis on the basis of classical fertility models was not possible. It could be done only by applying the "multiplicative structure". The same was true for hypotheses on individual self-fertilization.

Interpretations of genotypic offspring structures should in general not be restricted to analysis of the detected proportions of homozygotes, since this parameter reflects the effects of two major, counterbalanced phenomena: Self-fertilization tends to induce an increase in the frequency of homozygotes, whereas sexually asymmetric fertility selection tends to have the opposite effect. Misleading interpretations can only be avoided if hypotheses are tested by means of standards which can reveal each phenomenon separately. In this sense, the application of the "multiplicative structure" is essential.

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PART II

POPULATION STRUCTURE OF FOREST TREES

POTENTIAL CAUSES FOR MULTILOCUS STRUCTURE IN PREDOMINANTLY OUTCROSSING POPULATIONS

OUTI MUONA

Multilocus theory and results are reviewed especially with reference to forest trees. Two aspects are discussed: gametic disequilibrium and zygotic disequilibrium, i.e. correlations between loci in heterozygosity. There are sevaral different ways of quantifying disequilibrium. Gametic disequilibrium consists of two components, between and within individual disequilibrium. Both components of disequilibrium can be measured in conifers, which is a rare, as yet unused opportunity. Several different causes, drift, population subdivision or migration and different patterns of selection can give rise to disequilibrium. It is often difficult to distinguish between these non-selective and selective causes. Even when selection is implied, it may be difficult to pinpoint the loci it is acting at. There are relatively few studies on disequilibrium in plant populations, and very few on trees. Significant disequilibria have been found only rarely, but the results are not yet conclusive. The rarity of disequilibria may be due to the fact that random pairs of loci have been studied. Zygotic disequilibrium may arise due to partial selfing alone, or as a result of a combination of initial disequilibrium and selection at only one locus, or due to epistatic selection. When zygotic disequilibrium exists, general heterozygosity may be predicted on the basis of few marker loci, otherwise usually not.

Since disequilibria are rare, and may be due to spurious causes giving rise to unstable and variable correlations, it seems doubtful that such correlations could generally be used for breeding purposes. Correlations due to pleiotropy would of course be an exception. However, correlations between marker loci and polygenic, economically important traits may be rare.

Introduction

Much of classical population genetics has dealt with genetic change at single loci. However, natural selection acts on whole organisms, not on single loci. Many evolutionists have emphasized the role of of interactions between loci, the integrity of the genotype, and coadapted gene complexes (e.g. Mayr 1970, Lewontin, 1974). Recently, Wright (1980) has discussed the importance of organismic as opposed to genic selection.

Brown (1978) has reviewed the manifold ways in which different authors use the term coadaptation. According to Mayr (1970), alleles coexisting in the same population at different loci are coadapted by definition. Interactions between loci are not a prerequisite for coadaptation. Dobzhansky (1970) used coadaptation to describe the phenomenon that heterokaryotypes of locally occurring chromosomes are fitter than those between chromosomes of disparate origin. Allard et al. (1972) discussed gametic phase disequilib-

rium as evidence of coadaptation between allele combinations.

This paper deals with multilocus structure in populations where the mating system is one of partial selfing and random mating. Most of the discussion will be limited to two-locus systems, of which two aspects will be considered: gametic disequilibrium and zygotic disequilibrium. There are several reviews on both the theoretical and experimental findings on evolution in multilocus systems, e.g. Allard et al. (1968), Karlin (1975) Hedrick et al. (1978) and Barker (1979). Brown (1979) has reviewd experimental results on plant populations. I will briefly deal with findings on the effects of partial selfing, consider conifers in particular, and also discuss some implications of the results for practical applications.

Gametic disequilibrium

Definitions and measurement

Let us consider a two locus system with alleles A and a at locus A and alleles B and b at locus B. The frequencies of the alleles are p_A (and 1-p_A) and p_B (and 1-p_B). A genotype formed by the union of gametes AB and ab has frequency P^{AB}_{ab}. The gametic frequency of AB is $P^{AB}_{..}$ This notation is according to Weir and Hill (1980). Weir (1979) has described the system with several alleles at a locus, but for simplicity, only two alleles per locus will be onsidered here. The conventional measure of gametic disequilibrium is $D = P^{AB}_{..} - p_A p_B$. This measure can be used for either gametic or genotypic data. When gametic data are available, estimation is straighforward. If genotypic data are used, random mating has to be assumed to estimate the proportion of coupling and repulsion heterozygotes (Weir 1979).

Gametic disequilibrium in non-random mating populations can be divided into two components (Cockerham and Weir 1973, 1977, Weir and Hill 1980). These are Within individual disequilibrium, D_w, and between individual disequilibrium, D_b. The latter measures departures from random mating.

$D_w = P^{AB}_{..} - P^{A}_{.B}$ $\quad D_b = P^{A}_{.B} - p_A p_B$ $\quad D = D_b + D_w$

If it is not possible to distinguish coupling and repulsion heterozygotes, these components cannot be estimated independently. In fact, when mating is not at random and only genotypic data are available, Weir (1979) recommends estimating a composite measure of disequilibrium, Δ, first suggested by Burrows (Cockerham and Weir 1977)

$$\Delta = P^{AB}_{..} + P^{A}_{.B} - 2p_A p_B \quad (= D_w + 2D_B).$$

The use of this measure does not require any assumptions about the mating system. Furthermore, it is easily computed and unbiased. It has been used e.g. by Laurie-Ahlberg and Weir (1979) and Muona (1982). The statistical significance of either D or Δ can be tested by using χ^2-tests or Fisher's transformation of the correlation coefficient. Despite the non-normality of the data, such tests perform quite well (Weir 1979).

In any studies on gametic disequilibrium it is important to use large sample sizes. Brown (1975) has shown that the power of tests remains small if allelic frequencies are skewed and disequilibria are small, unless very large samples are available.

There have been only few studies on gametic equilibrium in conifers. However, they would offer a rare methodological advantage. Usually distinguishing between coupling and repulsion heterozygotes requires special techniques, such as progeny testing in *Drosophila*. Even then, only tightly linked locus pairs can usually be studied. (Admittedly, these are likely to be most interesting for this kind of studies.) In conifers, the same haploid cell gives rise to both the haploid endosperm and the egg cell. Electrophoretic techniques have proven suitable for identifying the genotypes of both the endosperm and the embryo. The maternal contribution to the embryo can be obtained from the endosperm, and the paternal contribution can then be deduced from the genotype of the embryo (Shaw and Allard 1981). This system has many advantage for studying different problems about population structure, one of which is that coupling and repulsion heterozygotes can be easily distinguished. As mentioned above, this allows estimation of components of disequilibrium. As it is known that many confers are partial selfers, there is a potential for finding both components of disequilibrium in conifer populations. No such studied have been made yet.

Causes of disequilibrium

Measurement of gametic disequilibrium involves two sampling processes: first the sampling of gametes in the population when successive generations of zygotes are formed, and second, the sampling of gametes for estimating disequilibrium (Weir and Hill 1980). I will ignore the second sampling and discuss the different mechanisms that give rise to disequilibrium between loci: genetic drift, migration and population subdivision, selection and hitchhiking (the effect of a selected locus on neutral loci). The general effect of partial selfing is that disequilibrium is generated and maintained under a wider range of conditions than in random mating populations.

Hill and Robertson (1966) showed that in small populations and for tightly linked loci, drift may cause substantial disequilibrium between neutral loci. This disequilibrium will be transient: in small populations the loci will become monomorphic and there will be no more potential for disequilibrium. Golding and Strobeck (1980) studied the joint effects of mutation, partial selfing and drift. Permanent disequilibrium is possible under tight linkage and high degrees of selfing, in populations with large enough effective sizes to maintain variability. In conifers, the levels of selfing are so low that disequilibria due to drift, at least between unlinked loci, are not likely to remain for many generations, aside from those due to very severe population bottlenecks. However, new disequilibria may be generated each year.

Population subdivision may give rise to excess homozygosity at the single locus level. This is known as the Wahlund effect (see e.g. Shaw and Allard 1982). There is an analogous result at the two locus level. If allelic frequencies at two loci differ in subpopulations, there will be gametic disequilibrium (e.g. Feldman and Christiansen 1973) Although the differences in allelic frequency could be due of differing selection in the two subpopulations, *epistatic* selection is not needed in this case. Migration from populations differing in allele frequencies would have a similar consequence. Thus it is important to study the populations in detail, so that data are not inadvertently pooled to generate "artificial" disequilibria. It is also important to distinguish between correlations among loci due to within and between population phenomena.

Epistatic selection has been the focus of numerous theoretical investigations on disequilibrium (e.g. Karlin 1975). Roughgarden (1979) has summarized some of the main results: even with simple models there exists a plethora of stable and unstable equilibria, the details depending on the exact constellations of recombination and selection parameters. For tightly linked loci, many selection models give rise to permanent gametic disequilibrium. Jain and Allard (1965, 1966) were the first to study the effects of partial selfing in combination with selection. Their numerical results showed that with high degrees of selfing, disequilibria can be maintained under a wide range of selection models, even between unlinked loci. In fact, the effect of partial selfing was more pronounced for loosely than closely linked loci. There were complex interactions between parameters, but in general, the potential for disequilibrium was a function of the degree of selfing. Holden (1979) obtained the first analytical results on the effects of selfing with selection. He studied a special case, the symmetric viability matrix. With selection, the effects of selfing and linkage were not equivalent in their effect on the maintenance of disequilibrium.

Most investigations have dealt with models where only two loci are selected, and the rest of the genome is assumed to be neutral. This is of course an unrealistic simplification: the loci are imbedded among other loci under varying selection pressures. Thomson (1977) has reviewed studies on hitchhiking, the effect of selected loci on neutral loci. The allelic frequencies at the neutral locus can change if there is some initial disequilibrium. Hitchhiking can also give rise to disequilibrium between neutral loci. Hedrick (1980) has compared the effects of partial selfing and linkage. High degrees of partial selfing can cause more important effects than linkage: hitchhiking occurs more rapidly and can cause changes in the sign disequilibrium.

It is evident that there exists a variety of causes for disequilibrium. In a large popula-

tion, only disequilibrium maintained by epistatic selection will remain at equilibrium. It is of importance to consider the dynamics of disequilibrium: how fast is equilibrium attained. Weir and Cockerham (1973) have shown that in a neutral situation disequilibrium changes at the rate

$$\frac{1}{2}\left\{\left(\frac{1+\lambda+s}{2}\right) + \left[\left(\frac{1+\lambda+s}{2}\right)^2 - 2\lambda s\right]^{1/2}\right\}$$

, where λ (= 1–2c) is a recombination parameter (c is the map distance between the loci), and s is the proportion of selfing. This shows that linkage and selfing are equivalent in their effects of maintaining disequilibrium in a neutral situation. Selfing can slow down the decay of disequilibrium considerably. Avery and Hill (1979) showed that disequilibria generated by extreme bottlenecks can remain in the population for long times, even if the population size is large after the bottle neck. Epistatic selection can of course retard the decay, or even maintain permanent disequilibria. On the other hand, some patterns of selection can increase the rate of decay. Clegg (1978) showed this in his computer simulations, and found similar results in experimental populations. The reason for rapid decay was heterotic selection, which generated excess double heterozygotes. The most extensive studies on disequilibrium in plant populations are those of Allard and coworkers on Composite Cross populations of barley. E.g. in Composite Cross II, there are very rapid changes in the magnitude of disequilibrium over a time span of five to ten generations, even between very closely linked loci (see e.g. Allard 1975, Muona 1982). Such changes cannot be due to the neutral decline of initial disequilibrium, but must be affected by selection. Note that selection can either retard or speed up the decay of association. The important point is that the changes over time are highly unpredictable, because sufficient information will probably never be available for considering all selective and other factors. As environmental conditions change, it may take extremely long times to reach equilibria in nature, if indeed they are ever reached.

Findings in natural populations

Epistatic selection is expected to be fairly common in nature. Interactions on fitness scale are generated e.g. by stabilizing selection, which is a well documented phenomenon in natural populations. Given the above theoretical results, disequilibria could be expected to occur frequently between loci. However, the general result from random mating populations is that disequilibria between marker loci are found only rarely. Most studies to date have dealt with animal populations. The most thorough studies on plant populations are on predominant inbreeders (see Allard 1975, Brown et al. 1980) The few studies on predominantly outcrossing plant populations are presented in Table 1. No disequilibria were found in either *Zea mays* or *Silene maritima*. The significant associations found between loci *Oenothera biennis* are expected because of the permanent heterozygosity and restriction of recombination. Five species of trees have been studied. In general, the number of loci has been small, no more than seven in any study. No associations were found between loci in the two *Eucalyptus* species studied. Rasmuson (1978) gave results on four populations of *Picea abies* and seven populations of *Pinus sylvestris*. There were several significant associations in Norway spruce, but only one of 21 possible tests was significant in Scots pine. Mitton et al. (1980) found several significant associations in *Pinus ponderosa*. The causes of all these disequilibria are unknown, they could be due to either selective or non-selective factors. In fact, it is quite difficult to exclude factors such as bottle necks or drift and then attribute the assiciations to selective causes. Such exclusion would require information about the history of population, which in most cases will not be available. The barley Composite Crosses are again an important exception, the initial conditions and population sizes over time are known. Changes in disequilibria are due to selection, but even then it is not known what loci have been selected (Clegg et al. 1978).

Multilocus studies in natural populations were not feasible before electrophoretic technique became available. At first, it was thought that multilocus studies could resolve to what extent the loci studied are selected.

Table 1. Findings on disequilibrium in predominantly outcrossing plant populations

Species	Number of loci	Result	Reference
Zea mays	9	–	Brown & Allard 1971
Oenothera biennis	6	+	Levy & Winterheimer 1977
Silene maritima	4	–	Baker et al. 1975
Eucalyptus obliqua	3	–	Brown et al. 1975
Eucalyptus pauciflora	7	–	Brown and Philips 1976
Picea abies (4)	7	9/84	Rasmuson 1978
Pinus sylvestris (7)	3	1/21	Rasmuson 1978
Pinus ponderosa (3)	5	5/30	Mitton et. al. 1980

However, later theoretical work has shown that this remains a difficult problem (Avery and Hill 1979).

Another question is the reason for lack of disequilibrium in most populations. Why do we not find disequilibria due to factors such as drift or migration? And why does epistatic selection not give rise to frequent associations? Even if few plant populations have been studied, the results on animal populations are quite clear.

Disequilibria have usually been studied between random pairs of loci. If these are not closely linked, chance effects do not give rise to associations in large populations. Epistatic selection should be effective between functionally related loci, not between random pairs. In fact, many of the cases where disequilibria or fitness interactions have been found have involved such functionally related pairs, e.g. leucine amino peptidase and amino peptidase in mussels (Mitton and Koen 1973), or 6PGD and G6PD in *Drosophila melanogaster* (Bijlsma 1978). Another possible explanation has been forwarded by Clark and Feldman (1981). The selection models that have been studied have only contained the viability component of selection. In single locus models, viability and fertility selection often have opposite effects, and similar results could hold for two locus models. It is certainly true that fertility selection is an important component in plant populations (Clegg and Allard 1973, Clegg et al. 1978).

More studies are clearly needed on plant populations. The results on animal populations, many of them on different species of *Dorosophila*, cannot be directly generalized to, say, forest tree populations, because of the important differences in so many aspects of their biology.

Zygotic associations

The other aspect of multilocus structure to be discussed is zygotic associations, i.e. correlations between genotypes at different loci. Haldane (1949) pointed out that inbreeding in human populations would give rise to excess multiple heterozygosity and multiple homozygosity. Bennet and Binet (1956) studied the effects of partial self-fertilization. For neutral loci, there will be no gametic associations at equilibrium, but partial selfing will give rise to an excess of multiple homozygosity and multiple heterozygosity. The biological explanation for this phenemenon is that outcrossing events make zygotes heterozygous at multiple loci simultaneously. Allard et al. (1968) gave values for excess heterozygosity for different degrees of selfing and linkage. Harding and Allard (1969) studied a population of the predominantly self-fertilizing *Phaseolus lunatus*, and showed that the Bennett and Binet effect was quite important.

Correlations between loci in levels of heterozygosity and allelic frequency change can also arise without any inbreeding, if there is some disequilibrium between the loci. Many authors have studied such associative overdominance. When there is some initial disequilibrium between a neutral locus and and a locus under heterotic selection, the neutral locus will behave as if it also displayed heterozygote advantage. Another consequence will be that double heterozygotes will be more frequent than expected under independence (Sved 1968, Cockerham and Rawlings 1967, Ohta and Cockerham 1974, Strobeck 1979). Correlations between heterozygosity at different loci can also be due to heterotic selection, e.g. with fitness matrices where double heterozygotes are

more fit than other genotypes (see Allard et al. 1968).

If there are zygotic associations between loci, one can predict levels of individual heterozygosity on the basis of few loci. When such correlations do not exist, heterozygosity at a few loci should be a poor predictor of overall heterozygosity. Mitton and Pierce (1980) made a simulation study with 100 independent loci. When twelve loci were sampled, the correlation between the sample heterozygosity at the total 100 loci was 0.35. As the loci were independent, there would have been no correlation between the 12 loci and the remaining 88 loci. As the number of loci is very large in any genome, such small samples do not give a picture of overall heterozygosity. Mitton et al. (1981) have found a relationship between heterozygosity at a few marker loci and mean growth rate in aspen, and a positive correlation between heterozygosity and growth variability in aspen and ponderosa pine, a negative correlation in lodgepole. The biological reasons for such correlations remain poorly understood.

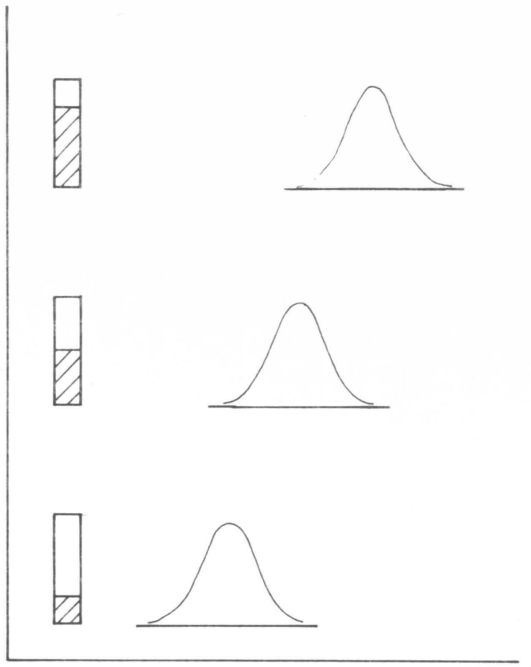

Figure 1. Parallel clines in allelic frequency and quantitative character give rise to a between population correlation. The bars give allelic frequencies in different populations. The abscissa gives measures for some quantitative trait, of which the curves give the frequency distribution. See text for explanation.

Implications for breeding

Karlin (1977) has suggested that disequilibria between loci could be used for breeding purposes. If there were a consistent correlation between loci controlling quantitative characters and marker loci, the easily studied marker loci could be used for selecting for economically important quantitative traits. One example of such correlations are those found in *Avena barbata* by Hamrick and Allard (1975). In this case, two different ecotypes, "xeric" and "mesic", are characterized by different sets of quantitative characters and alleles at electrophoretic loci.

In this connection it is important to distinguish within and between population correlations. This has been illustrated in Fig. 1. There is a cline in a quantitative character and a parallel cline in allelic frequency. If the data are pooled, there will be an overall correlation between the enzyme locus and the quantitative character. However, it does not necessarily follow that there would be a significant *within* population correlation between the two characters. Such a correlation would be needed for selecting within populations, and for selection to be successful, the correlation would have to be high.

An example of changing within population correlation is given in Table 2. The population studied was Composite Cross II, an experimental barley population, which has been grown annually without conscious selection in Davis, California. Seed has been stored from many generations. I studied enzyme genotypes and quantitative characters in families from four different generations (Muona 1980). Different enzyme genotypes were compared with respect to quantitative characters. The esterase loci Est-1 and Est-3 and heading date have been chosen as examples. In F_8 genotypes at the Est-3 locus differed significantly with respect to their heading dates. However, this correlation had disappeared by generation F_{13}. On the other hand, there was no correlation between genotypes at the Est-1 locus and heading date in the earlier generations, but a correlation

Table 2. Relation between esterase loci and heading date in different generations of barley Composite Cross II. The mean heading dates (after March 23rd) are given for different genotypes at the loci Est-1 and Est-3. Further details in Muona (1980).

	Est-1			Est-3			
	22	33		11	22	33	
F_8	15.74	16.15	NS	18.13	13.01	16.03	++
	37	41		22	18	39	
F_{13}	15.40	15.07	NS	15.73	15.36	15.10	NS
	52	26		30	8	42	
F_{23}	16.59	16.06	NS	15.71	12.97	16.97	+
	41	33		30	15	34	
F_{45}	17.62	22.29	+++	19.12	–	17.80	NS
	75	4		3		77	

appeared by F_{45}. This illustrates that even in this highly self-fertilizing population, correlations change rapidly over time. Obviously such correlations would not be of much use to a barley breeder, who would do better selecting directly for heading date. Transient correlations, such as these, would also probably differ from population to population due to differences in population history. In specific crossing schemes however, correlations between enzyme genotypes and quantitative characters are expected, and can be used for breeding purposes (see e.g. Tanksley et al. 1981).

It is of course possible to find useful associations in populations, but these would probably have to be of relatively simple nature, e.g. a pleitropic association between an enzyme locus and disease resistance. Such cases are likely to be rare. Despite the results of Mitton et al. (1981) discussed previously, it does not seem likely that very useful and consistent correlations would be found between a few enzyme loci and such polygenic traits as growth characteristics in forest tree populations.

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ISOZYME HETEROZYGOSITY IN ADULT AND OPEN-POLLINATED EMBRYO SAMPLES OF DOUGLAS-FIR

D. V. SHAW and R. W. ALLARD

Eleven electrophoretically detectable loci were used as markers to examine patterns of heterozygosity in both adults and open-pollinated progenies of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Heterozygotes were less frequent in samples of embryos than expected under panmixia--an observation that is consistant with previously estimated outcrossing rates. Conversely, a slight excess of heterozygotes was found in adults relative to expected panmictic proportions. Most of the differences between the adult and the offspring genotypic distributions probably result from viability selection favoring outcrosses i.e., by selective removal of selfed offspring prior to reproductive maturity. However, viability difference between selfs and outcrosses cannot be responsible for the excesses of heterozygotes observed in the adult samples. Several mechanisms that might account for the excesses are discussed.

Introduction

The genotypic arrays of reproducing adults in populations of perennial plants and the open-pollinated progenies produced by these adults represent two distinct phases of the life cycle. The distribution of embryo genotypes depends primarily on pollen movement, on the mating system, and on fertility components of selection. The array of adult genotypes may differ from the array of genotypes in embryos as a result of forces acting throughout the diploid phase of the life cycle. In this paper we examine heterozygosity at 11 isozymes--in both adults and their open-pollinated progenies--from eight natural stands and a plustree seed orchard of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*).

In most forest tree species the majority of fertilizations result from outcrossing. However, many species produce some selfed offspring upon open-pollination, and thus have mixed mating systems. Squillace (1974) reviewed estimates of the proportion of selfed offspring for 12 coniferous tree species and reported values ranging from 0 to 20 percent. Previous analyses of the natural stand and seed orchard samples used in our study indicated that the embryos were formed with a mixed mating system featuring 90 percent outcrossing, t, and 10 percent selffertilization, $s = 1\text{-}t$ (Shaw and Allard 1982).

Another common characteristic of forest tree species is that they express moderate to severe inbreeding depression for characters of commercial importance, such as height and volume growth (reviewed by Franklin 1970). The studies of Sorensen and Miles (1974) have shown that selfed Douglas-fir seedlings can suffer an 18 percent reduction in first year height growth relative to cross-fertilized seedlings; Rehfeldt (1978) demonstrated that this reduction in yield can be as large as 30 percent in subsequent years. Because characters such as height and volume are presumed to be important to adaptive and competitive ability, most selfed embryos are expected to be at a selective disadvantage. Brown et al. (1975) and Phillips and Brown (1977) have reported measurable deviations from panmictic genotypic proportions, due to self-fertilization, in embryo or seedling samples, but found no evidence for accumulated inbreeding in adult populations. Such results are consistent with the hypothesis that selection removes most inbred offspring from the eventual adult populations.

We use the mating system parameters estimated for our experimental populations (from Shaw and Allard 1982) and the patterns of heterozygosity observed for embryos and adults to evaluate the long-term significance of self-fertilization to populations of Douglas-fir. We also assess the likely contribution of other forces to the observed patterns of heterozygosity.

Table 1: Location, sample size, age and height data for the eight natural stands sampled for this study, and sample size data for the Jefferson seed orchard.

		Location		Sample size			
		Latitude	Longitude	open-pollinated families	total seeds assayed	Stand age (approx.)	Stand height (m)
Springfield	1	44° 18'	122° 32'	24	274	35	14
	3	44° 5'	122° 43'	25	299	25	16
	4	44° 5'	122° 40'	23	232	100+	30
	5	44° 17'	122° 34'	25	288	40	18
Longview	1	46° 9'	122° 32'	18	167	60	26
	3	46° 12'	122° 38'	25	320	35	12
	4	46° 13'	122° 46'	25	289	70	26
	5	46° 11'	122° 33'	20	244	60	24
Jefferson				42*	727		

*The Jefferson seed orchard contains several ramets of each clonal genotype. Seventy-six ramets were sampled representing 42 distinct genotypes.

Materials and Methods

Collections and electrophoretic assay

Open-pollinated progenies were collected from eight stands of naturally regenerated trees and also from a clonal plus-tree seed orchard. The location of stands, the age and size of trees within stands, the number of families sampled, and the number of seeds assayed by electrophoresis, are given in Table 1. The eight natural stands are located on sites considered ecologically central to the distribution of coastal Douglas-fir. A variety of environments and topographies are represented; all sites are located between 400 and 600 meters elevation. Both diploid embryos and haploid maternal gametophytes were analyzed by starch-gel electrophoresis for each seed assayed; 11 loci were revolved and scored. Adult allele and genotypic frequencies were inferred from the haploid genotypes of not fewer than seven gametophytes per family, using methods given by Morris and Spieth (1977). Embryo allele and genotypic frequencies were calculated directly from the observed codominant isozyme phenotypes. Descriptions of the experimental populations and electrophoretic procedures are given by Shaw and Allard (1982).

Statistical methods

Expected diploid genotypic frequencies for a neutral marker locus can be predicted with Wrights's equilibrium formulae (Wright 1931) as

$$\begin{aligned} f(A_iA_i) &= p_i^2 + F\,p_i(1-p_i) \\ f(A_iA_j) &= 2p_ip_j(1-F), \end{aligned} \qquad (1)$$

in which $f(A_iA_i)$ and $f(A_iA_j)$ are the frequencies of the A_iA_i homozygote and the A_iA_j heterozygote at a locus, p_i and p_j are the frequencies of the i^{th} and j^{th} alleles at this locus, and F is the inbreeding coefficient (correlation between uniting gametes, Wright, 1931; also the probability that the alleles of an individual are identical by virtue of descent from a common ancestor, Malécot 1948). Wright (1951) derived the fixation index, $\hat{F}$, as an estimator of the inbreeding coefficient. $\hat{F}$ is calculated as

$$\hat{F} = 1 - \frac{\sum_{ij} h_{ij}}{\sum_{ij} 2\,p_ip_j} \ldots i < j. \qquad (2)$$

In (2) h_{ij} is the observed frequency of the heterozygote bearing the i^{th} and j^{th} allele at a locus and p_i and p_j are the frequencies ot the i^{th} and j^{th} alleles respectively. The denominator of the fraction in (2) is often referred to as the *expected panmictic heterozygosity* (H_e) because it reflects the proportion of heterozygous genotypes expected under random union of gametes. H_e has also been referred to as the *polymorphic index* (Marshall and Allard 1970), the *average heterozygosity* (Nei 1975), and the *genic diversity* (Brown *et al.* 1980); H_e is a useful measure of allelic variability. Estimates of $\hat{F}$ greater than zero indicate an excess of homozygous genotypes and estimates of $\hat{F}$ less than zero indicate an excess of heterozygous genotypes relative to expected panmictic proportions. Several factors affect the precision and accuracy of the fixation index as an estimate of the inbreeding coefficient in predominantly outcrossing species:

1) The sampling variance of $\hat{F}$ depends on the actual inbreeding coefficient, and on the allelic variability at the marker locus used for estimation (Rasmusson 1964). As noted by Brown (1979), it is unfortunate that the sampling variance of $\hat{F}$ is maximum in predominantly outcrossed species for marker loci with intermediate allele frequencies because such loci are the most useful markers for estimation of many other genetic parameters, as well as in making between-population comparisons. Because the precision of $\hat{F}$ depends on the allelic frequencies at each marker locus we calculated average fixation indices by weighting the estimate of $\hat{F}$ obtained for each locus by its approximate maximum likelihood variance (Brown 1970).

2) $\hat{F}$ is biased downward when estimates are made from small samples (Levine 1949). Fixation indices can be corrected for this bias by multiplying the expected panmictic heterozygosity in (2) by $[1 + 1/(2N - 1)]$, in which N is the sample size. This bias is large only with very small samples and thus we have corrected only adult fixation indices in this report.

3) Although the fixation index was originally developed as an estimator of the inbreeding coefficient it provides, in simplest form, a comparisons of observed levels of heterozygosity with levels expected under panmixia. Brown (1979) lists more than a dozen factors that can affect $\hat{F}$; several of these will be discussed later with reference to empirical data.

An additional feature of the Douglas-fir data is that the esterase marker locus *(Est)* yielded estimates of selfing that were lower than the estimates obtained with other marker loci (Shaw and Allard 1982). Because the level of heterozygosity observed for this marker did not fit the general pattern, individual fixation indices estimated with the esterase marker were excluded when calculating the average $\hat{F}$ for each population.

Results

Allelic variability

A summary of expected panmictic heterozygosities is given in Table 2. These values range from zero (for locally fixed loci) to 0.70. The average allelic variability as measured by H_e was similar among all sample populations. Further, Spearman rank correlation coefficients (Siegel 1956) between H_e and $\hat{F}$ within each population are all non-significant, indicating no association between the degree of allelic variability and the estimated fixation index.

Individual-locus fixation indices

Table 3 gives individual markerlocus fixation indices for both embryos and adults from the Springfield 1 and Springfield 3 natural stands. The results for these two stands are

Table 2: Average and range of expected panmictic heterozygosities (H_e) for adult embryo samples from eight natural stands and a plus-tree seed orchard of Douglas-fir

		Embryo samples		Adult samples	
		$\bar{H}_e$	Range	$\bar{H}_e$	Range
Springfield	1	.200	(0.02–0.63)	.215	(0–0.67)
	3	.202	(0–0.63)	.205	(0–0.62)
	4	.200	(0–0.63)	.210	(0–0.65)
	5	.219	(0.02–0.68)	.230	(0.04–0.64)
Longview	1	.216	(0–0.66)	.243	(0–0.66)
	3	.212	(0–0.61)	.235	(0–0.59)
	4	.201	(0–0.70)	.207	(0–0.70)
	5	.214	(0–0.63)	.250	(0–0.65)
Natural stand average		.209		.230	
Pooled natural stand data		.219	(0.01–0.63)	.225	(0.01–0.65)
Jefferson		.228	(0–0.63)	.237	(0.02–0.64)

Table 3: Fixation indices ($\hat{F}$)for 11 isozymes in the Springfield 1 and Springfield 3 natural stands.

	Springfield 1 $\hat{F}$*		Springsfield 3 $\hat{F}$*	
Locus	Adults	Progeny	Adults	Progeny
Got 1	0	.136	...	...
Got 3	0	0	−.020	0
G6pd	...	0	−.065	.12
Gdh	0	0	...	...
To	−.085	−.067	.020	.091
Est	.013	.276	−.075	.121
Lap 1	−.118	.038	−.065	.051
Lap 2	−.118	.373	−.162	.091
Pgm 1	.210	.039	.243	.091
Pgm 2	−.080	.181	−.021	−.052
Pgi	0	.091	.021	−.110

*Fixation indices can be calculated only for variable loci.

Table 4: Fixation indices ($\hat{F}$) estimated for embryo and adult samples from 8 natural stands and a plus-tree seed orchard of Douglas-fir. (Sample standard errors for mean $\hat{F}$ in parentheses.).

		Embryo $\hat{F}$*	Adult $\hat{F}$*
Springfield	1	.067 (.040)	−.020 (.034)
	3	.040 (.029)	−.030 (.041)
	4	.025 (.046)	−.005 (.052)
	5	.041 (.029)	0 (.052)
Longview	1	.090 (.027)	−.017 (.024)
	3	.027 (.027)	−.058 (.024)
	4	.042 (.022)	−.047 (.030)
	5	.015 (.024)	−.038 (.042)
Natural stand average		.050	−.027
Pooled natural stand data		.236 (.069)	.024 (.063)
Jefferson		.029 (.017)	.030 (.066)

*Avarage fixation indices were calculated excluding estimates based on the esterase locus due to their obvious and systematic deviations.

typical of those for all sample populations. A conspicuous feature of the results is that the estimates of $\hat{F}$ obtained with the different marker loci differ strikingly from each other within the two populations. Fixation indices are given only for marker loci with expected panmictic heterozygosities greater than zero; allelic variability at a marker locus is necessary for its use in estimating $\hat{F}$.

Average fixation indices

Despite the large between-locus variability in $\hat{F}$ for embryo samples, only one estimate in ten is negative when all samples are considered. Average fixation indices calculated for embryo samples in all experimental populations are positive (Table 4), indicating an excess of homozygous genotypes over the proportions expected under panmixia. The grand mean for all embryo fixation indices is $\bar{\hat{F}} = 0.050$. Values of t and s for these populations are 0.90 and 0.10 (Shaw and Allard 1982). The inbreeding coefficient expected to result from a single generation of mating with 10 percent selfing (Hayman 1953) is F = 0.05, which is exactly the observed value. The observed value is also very close to that expected with neutral inbreeding equilibrium, $F_e = 1 - t/\ 1 + t = 0.053$ (Fyfe and Bailey 1951). The comparison of observed $\hat{F}$ with expected F is not very informative in this case because, when t is near unity, inbreeding equilibrium is approached rapidly, and the expected inbreeding coefficients for single-generation and equilibrium inbreeding are nearly the same. The sample standard errors for mean estimates of $\hat{F}$ given in Table 4 were calculated using unweighted individual-locus fixation indices. These standard errors are intended only as an indication of dispersion, as it is unlikely that the criteria necessary for valid formation of confidence intervals from sample standard errors will be met (e.g. homogeneity of variance for all observations). Note that the average fixation index for the pooled natural stand data is considerably more positive than individual natural stand estimates (Table 4), a point that will be discussed later.

Substantial between-locus variability in $\hat{F}$ was also found for adults. However, unlike the pattern observed for embryos, most adult fixation indices are negative. Average adult fixation indices are slightly less than zero for seven of eight natural stands and zero for the eighth (Table 4), indicating an excess of heterozygotes over expectations based on panmixia. The grand mean for all adult natural stand fixation indices is $\bar{\hat{F}} = -0.027$. The average $\hat{F}$ for the pooled adult natural stand data and for the Jefferson seed orchard adults are both positive. Standard errors are again included in Table 4 as indicators of within-population variation of $\hat{F}$.

Discussion

Caution is required in making inferences about the dynamics of population change from comparisons of adult and progeny genotypic distributions: the two samples do not necessarily represent a continuum of genetic event, i.e., the embryo pool from which the current generation of adults was drawn may have been formed from a gene pool (the grandparental gene pool), and with a mating system, that differed from those presently observed. However, the consistency of estimated mating system parameters and the general similarity of gene pools among the eight natural stands sampled suggests that the assumption of reasonable continuity for these factors, over at least a single generation, may be valid for the populations of the present study.

Comparison of embryo and adult genotypic distributions indicates that considerable genetic change occurs between the two life cycle phases. Most of the decrease in homozygosity between embryo to adult phases can be explained by the selective removal of selfs from the populations or, stated alternatively, by "heterosis for outcrosses" (Brown 1979). If all selfed offspring are removed from adult genotypic distributions then the *effective outcrossing rate* (Workman and Allard 1962) for the adult population is $t^* = 1.0$. The hypothesis of an effective outcrossing rate of $t^* = 1.0$ does not, however, account for two observations: 1) adult fixation indices for the pooled natural stand data and the Jefferson seed orchard data are positive, which indicates an excess of homozygous genotypes over expectations based on $t^* = 1.0$; 2) adult natural stand fixation indices are usually negative, which indicates an excess of heterozygous genotypes over expectations based on panmixia.

Causes for excess homozygosity

Homozygosity in excess of that predicted by the mating system, in this case $t^* = 1.0$, can result from a variety of causes (Brown 1979). Perhaps the most likely cause for excess homozygosity found in the pooled adult natural stand sample and the adult Jefferson seed orchard sample is the Wahlund effect (Wahlund 1928), or variance in allele frequency among the subpopulations from which the diploid genotypes were drawn. The apparent excess of homozygotes in the pooled population can be quantified for a diallelic locus as

$$F_{ST} = \sigma^2_{\bar{p}_i}/2\bar{p}_i(1 - \bar{p}_i), \qquad (3)$$

in which $\bar{p}$ and $\sigma^2_{\bar{p}_i}$ are the mean and variance of the i^{th} allele frequency over subpopulations and F_{ST} is Wright's measure of population subdivision (Wright 1951). F_{ST} can be substituted for F in equation (1) to obtain expected genotypic frequencies when subdivision and variance in allele frequency occur. Note that the Wahlund effect can also explain the observation that $\hat{F}$ is larger for the pooled embryo natural stand data than for individual natural stand samples (Table 4).

Causes of excess heterozygosity

Several mechanisms can produce an excess of heterozygous genotypes over expectations based on the mating system (Brown 1979); however, only a few of these mechanisms can lead to significant excesses of heterozygotes when the effective outcrossing rate, t^*, approaches unity.

The two most commonly cited selective mechanisms for generating heterozygote excess (with $t^* \simeq 1.0$) are: 1) heterozygote advantage for chromosomal segments including the marker locus and; 2) associative overdominance. The first mechanism implies either selection favoring heterozygotes at the marker locus itself, or heterozygous advantage for a block of genes of which a particular marker locus is an integral component. Associative overdominance implies that the marker locus itself is neutral and that the observed excess of heterozygotes results from

association between the marker and selected loci. Mixed mating systems can lead to non-random distributions of heterozygous loci among diploid individuals (Bennett and Binet 1956) and thus to associations between marked and unmarked loci. Gametic disequilibrium, resulting from various modes of selection (Allard et al., 1968; Hendrick et al., 1979; Feldman et al., 1977) as well as from non-selective causes (Hill and Robertson 1968), can also result in such associations.

No consistent patterns of heterozygote excess associated with specific marker loci were found in our study. There was no significant heterogeneity among the average fixation indices estimated for each marker locus (averaged over populations) and there was no correlation between populations for the rankings of individual-locus fixation indices ($\bar{r}_s$ = 0.04; Siegel 1956), i.e. the marker showing the largest heterozygote excess in one population did not necessarily show the largest excess in other populations. These results exclude neither of the hypotheses involving selection because they are consistent with both associative overdominance (with varying degrees of between-locus association) and also with heterozygote advantage in which selection intensities vary among populations.

Heterozygote excess can also occur when there are differences between male and female gametic allele frequencies (Robertson 1965). This potential cause for heterozygote excess is often ignored in monoecious plants because each individual is expected to contribute to both the pollen and ovule pool. However, allelic frequencies may differ in the male and female gametic pools of monoecious species because each parent contributes unequally to the two pools (Horovitz and Harding 1972). Migration of pollen between populations differing in allele frequency (Prout 1981) can also lead to such inequalities. The change in the fixation index resulting from differing male and female allele frequencies can be calculated as

$$\Delta F = 1/2(t)^2 \sum_i (p_{im} - p_{if})^2. \quad (4)$$

In (4) p_{im} and p_{if} are the frequencies of the i^{th} allele at a locus for male and female gametes respectively and t is the outcrossing rate (Brown 1979). The outcrossing rate can in general be replaced by the migration rate (m) if differences within populations are negligible and the outcrossing rate is $t = 1.0$. A more complete solution involving both within and between population differences is not yet available. Note that the excess heterozygosity generated by the above process is not the result of selective differences among diploid individuals. Note also that, if some inbreeding occurs, the resulting negative bias in $\hat{F}$ may be masked in embryo samples; however samples of adults drawn at random from the outbred portion of the embryo population will exhibit heterozygosity in excess of expectations based on panmixia. The average within-stand difference between male and female gametic allele frequencies in our study was 0.05 (range 0.00 to 0.15). These differences in gametic allelic frequencies are too small to account for the negative fixation indices observed in the adult samples. However, the average within-stand difference may underestimate the actual differences in allele frequency between uniting gametes because each natural stand sample may contain progenies from several breeding cohorts. It is interesting to note that variation in allele frequency among subpopulations causes an excess of heterozygotes when migration occurs during the haploid phase of the life cycle (pollen migration) and an excess of homozygotes when migration occurs during the diploid life cycle phase (seed migration).

Conclusions

The limitations of our data set, and the unknowns of population history, do not allow discernment among the several selective and/or nonselective force that may be responsible for the patterns of heterozygosity observed in this study of Douglas-fir. However, the results suggest that the dynamics of genotypic distributions in Douglas-fir are complex and that they are affected by forces whose effects tend to counterbalance, e.g. significant amounts of self-fertilization occurred during the formation of our sampled progenies, but excesses of homozygotes resulting from inbreeding in previous generations had disappeared in the observed adult populations. Thus selfed offspring appear to be of little importance in the long-term biology of the Douglas-fir populations studied.

The variability in $\hat{F}$ was substantial and it is interesting that this variation, as measured by sample standard errors, was of the same order of magnitude for the individual natural stand samples, the larger pooled natural stand samples, and the Jefferson seed orchard sample. This may indicate that variability in $\hat{F}$ is not due to inefficiency of estimation or sampling error, but rather to invalidity of various assumptions made in formulating the mixed mating model. It follows that experiments to test specific hypotheses concerning the mixed mating model may provide information useful in understanding the population structure of Douglas-fir.

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COMPETITION AND NEIGHBOURING EFFECT IN A NATURALLY REGENERATING POPULATION OF SCOTS PINE

P. M. A. TIGERSTEDT, D. RUDIN, T. NIEMELÄ and J. TAMMISOLA

A natural pine stand, 300–400 years old, and a progeny generation underneath, about 100 years old, were analysed for population structure by means of isozyme analysis. The genetic structure of old growth conformed to Hardy-Weinberg equilibrium and neighbouring trees appeared on the average to be slightly related. The genetic structure of regeneration departed significantly from Hardy-Weinberg equilibrium and appeared more inbred with high proportions of homozygotes. Regeneration groups around old growth trees did not appear to be particularly related to the tree above. It is suggested that seeds in natural stands move in cohorts and land in cohorts thus mixing up the population structure. It is also suggested, that selection against high proportions of inbreds in seedling populations is important to avoid inbreeding depression in natural populations.

Introduction

A tree is sedentary. It is confined to live its entire life on the location where it was born.

Seeds from a tree are mainly dispersed around its source, very few seeds find their way far from the mother. The dispersal is leptocurtic and covers only a restricted area with a radius of at the most a few tree heights.

Trees are predominantly wind-pollinated, anemophilous. Pollen from a particular tree can find its way far from the source of release, but just as in seeds, the bulk of pollen is distributed leptocurtically around the father. These basic ideas result in a conception about the genetic structure of tree populations, and plant populations generally. We visualize populations as consisting of groups of relatives, suffering from inbreeding depression.

Natural forest tree populations often regenerate through bursts of highly dense and relatively even-aged seedling formation. Such bursts can be caused by ecological disruption, such as a forest fire, or a storm-felling, or sometimes just because of favourable conditions for flowering, seed set and seed germination. Thus the regenerated population may have a density of magnitudes higher than a final mature population. A selection intensity of $10^{-3}-10^{-6}$ is here normally involved. In the intervening years selection, either stochastic or naturally determined, must cause drastic reduction in population density.

Genetically plant populations must thus depart considerably from 'mendelian' or random-mating populations. In plants, random mating seems unrealistic because individuals are sedentary. Plants experience heterogenous environments differently, they can not actively choose their habitat. Assortative mating is unlikely, possibly with the exception of temporal synchrony in flowering of certain phenotypes. A forest genecologist is aware of the often strong clinal variation in fitness characters, such as growth initation or cessation, flowering rhythm or vegetative and generative dormancy.

Many of these caracters are of imminent interest to the tree breeder looking for higher yields or better adaptation.

But the forest population geneticist has a vague idea about the structure of his population. We say this being fully aware of recent studies of tree populations using isozymes as markers. There are still many open questions and in this study we have deliberately chosen a new approach to this problem. In our opinion the spatial structure of tree populations needs more careful scrutiny so that the findings of genecologists can be extended to the local population. We also believe that the structure of natural tree populations is important for the selection of individuals in breeding work.

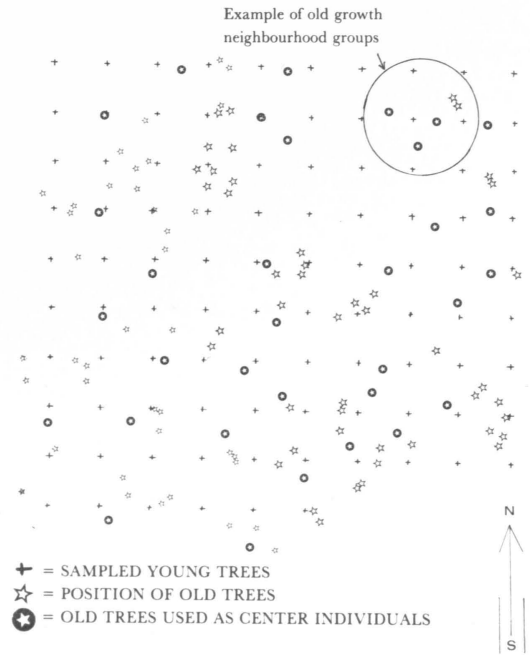

Figure 1. Map of the Scots pine stand at Oulanka.

Material

The desirable features of the selected pine stand should be as follows.

1. It must be natural so that influence by man can be proven unimportant.
2. It must be ecologically as uniform as possible.
3. It should be in the process of regeneration so that two different generations, a parental and a juvenile could be analysed simultaneously.
4. It should be dense enough so that sampling of groups around parental trees and random samples along grid-lines could be taken.

Such a stand was found near the Oulanka biological station in east-central Finland. It appeared to have been untouched by man for 300–400 years with a forest fire burning the ground some 100 years ago, after which regeneration had taken place.

The research area is a square of about 100×100 meters (1 ha).

This area has a relatively uniform distribution of old growth of which about 100 individuals (nearly all) were genetically analysed (Fig. 1).

The area also has a 'juvenile' generation, about 80 years old, partly very dense and grouped around parental old growth trees. There are at least 50.000 individuals on the research area of this generation.

The whole stand grows very slowly because it covers an unfertile sandy soil of *Cladonia*-type.

Three different kinds of samples were taken from the stand.

First twigs of virtually every parental tree were taken. The trees were tagged and the twigs deepfrozen for later needle analysis of 4 enzyme loci: 2 leucin-aminopeptidase and 2 glutamate-oxalate-transaminase. The same enzyme systems were analysed in all subsequent samples. However, only the GOT-B system was used for complete population analysis. Rare alleles in other systems were used to estimate the spatial distribution of alleles within the stand.

Second transects were run through the stand in roughly south-north direction and with 10 meters spacing between lines. Along the line, twigs were taken from the regeneration at 10 meter intervals, thus the whole stand was covered by a 10×10 meter grid, in all about 100 small trees were sampled.

Third groups of about 30 small trees were sampled under 37 parental trees dispersed more or less regularly over the area. The 30 closest trees were taken for the sample. Thus circles with radii of 2–3 meters marked the sampling of groups. The pine stand had a very regular structure such that most of the old growth individuals had clearly distinguished groups of plants or young trees arranged more or less circularly around them (Fig. 2). Thus one of the obvious research propositions was to analyse old and young trees for kinship.

Methods

Electrophoresis was mainly run on needle material, in some cases on buds. Standard gel-electrophoretic procedures were used (Rudin 1975, 1977). All 4 loci analysed had anodal bands. Most loci includeed multiple alleles but only one system, GOT-B, appeared to carry alleles at fairly intermediate frequencies, thus most of the analyses were done on the 5 alleles included in the system.

Rare alleles of some systems were used to estimate the spatial distribution of alleles within the stand.

Statistics

Non-parametric chi-square statistics was used comparing genotype distributions to Hardy-Weinberg expectations. Observed classes with less than 5 trees were generally lumped with the next smallest to make up artificial classes of at least 5 trees. A 2×C contingency test was applied to compare the population of old trees to that of regeneration. Again, observed classes smaller than 5 were lumped.

The genetic distribution of plants under old trees was determined as follows. Expected genotype frequencies were first calculated on the basis of a random old growth gene pool. As the genotypes of the maternal trees were known, an expected plant genotype distribution could thus be calculated and compared to observed numbers. In addition a heterogeneity chi-square over all 37 groups was computed in accordance with Sokal & Rohlf 1969 p. 623.

To further verify the genetic structure of plant groups under old trees a chi-square test of gene frequency heterogeneity was done (Snedecor & Irwin 1933):

$$\chi^2 = 2N \left(\frac{\Sigma\sigma^2_{pi}}{\bar{p}_i}\right)$$

where N is the total number of individuals, σ^2_{pi} is the variance in allele frequency and $\bar{p}_i$ is the weighted mean frequency. To complete subgroup comparison, Wright's $\hat{F}_{IS}$ statics was computed (Wright 1965, Kirby 1975):

$$\hat{F}_{IS} = (\sum_i w_i q_i p_i F_{ISi})/(\sum_i w_i q_i p_i)$$

where $w_i = \frac{N_i}{N}$ and F_{ISi} is Wright's fixation index corrected for finite population size (Kirby 1975). A negative $\hat{F}_{IS}$ value represents an excess of heterozygotes, a positive value indicates homozygote excess.

We define a local F-value as the probability that a random allele from a center tree is identical by decent with a randomly taken allele from 4–8 neighbouring trees.

For the purpose of running such tests the adult and the regeneration population was grouped around a center tree taking 4–8 surrounding trees for the analysis. In case of the adult population, groups were formed with the help of a detailed map of the stand where each analysed tree was positioned to the nearest meter in the coordinate (Fig. 1). On this map a flexible rubberband representing a diameter of 30 meters was placed so that it encompassed an approximate center tree and its 4–6 neighbours. In this way totally 33 groups were formed as indicated on the map. Cases where two or more old trees were growing close together, thus having the same neighbours, were excluded. A second criterion for determining center trees and groups was that they had to be fairly evenly distributed over the whole stand. It must be stressed, however, that neighbour groups of this kind are quite irregular, in some cases a number of trees in the neighbourhood of a center tree may grow close together, in other cases they may be widely and irregularly dispersed. Thus the local F_A-value (A = adult) of old trees and on the other hand of regularly spaced regeneration (10 or 14 meters from the center and symmetrically arranged) may not be properly compared. This fact will be discussed later.

In the regenerating young population sampling was done in 3 different ways in order to compare local F_R-values (R = regeneration). As the whole stand had been sampled in form of a 10×10 meter grid the composition of groups was done on this basis. First, 4-tree groups were formed taking the four trees within 10 meters from the center tree and running this system of sampling down the cruising lines taking every second tree in the row or column as a center. Thus the neighbourhood area was a square of approximately 14×14 meters with a neighbour at each corner and a tree in the center. Group size was then increased to 8 neighbours using squares of approximately 20×20 meters. The distance of neighbours to the center was now 10 or 14 meters. Local F_R was then computed using either every or every second tree in the row or column as a center.

The model behind the computation of local F can be described as follows: Let the center tree have the genotype A_1A_1 or A_1A_2. Among the 4–8 neighbour trees we have found a_1 number of alleles of A_1, a_2 alleles of A_2 and so on. Let the allele frequencies of the whole population of the old parental tree population be p_1, p_2 – – etc. and let the alleles be ordered according to decreasing frequency, such that $p_1 > p_2 > - - - > p_n$.

If the neighbouring trees were totally random samples from the population and thus virtually unrelated to the center tree, then the expected value $a_1/2N = p_1$, $a_2/2N = p_2$ – – – $a_n/2N = p_n$, N being the number of neighbour trees.

However, if the center tree is more closely related to the neigbours than to the population at large, then the expected values for the frequencies of A_1, A_2 etc. are $(1-F)p_1+F$, $(1-F)p_2$, – – – $(1-F)p_n$ when the center tree is homozygote A_1A_1 and $(1-F)p_1+½F$, $(1-F)p_2+½F$, $(1-F)p_3$, – – –, $(1-F)p_n$ when the center tree is heterozygote A_1A_2 and so on. Thus F is the probability that a random allele from the center tree is identical by descent with a randomly taken allele from the proximal trees. A theoretical paper on the local F will be published elsewhere. By using the numbers of alleles found in the neighbours F can be computed by a maximum likelihood method.

A list of F-values has been computed for each center tree and its 4–8 neighbours. F-values generally vary from about −0.7 to +1.0. For each analysis, an average $\bar{F}$-value has been counted. If the value of $\bar{F}$ is greater or smaller than zero its significance can be tested by a t-test which is then a crude measure of relatedness of neighbours. The computation of F by a maximum likelihood method is briefly as follows:

The investigated tree is homozygous A_1A_1

$$F = \frac{a_1 - 2Np_1}{2N(1-p_1)}$$

The investigated tree is heterozygous A_1A_2

case 1. $p_1 < ½$: $F = -\frac{B}{2A} + \sqrt{\frac{B^2 - 4AC}{4A^2}}$

case 2. $p_1 > ½$: $F = -\frac{B}{2A} - \sqrt{\frac{B^2 - 4AC}{4A^2}}$

$A = 2N(½-p_1)(½-p_2)$, $B = ((2Np_1-½a_1)(½-p_2) + (2Np_2-½a_2)(½-p_1))$,
$C = ½(p_2(2Np_1-a_1) + p_1(2Np_2-a_2))$, $p_1 > p_2$
p_i = gene frequency in the whole population
a_i = number of alleles i in neighbouring trees

Results

The genetic structure of old growth

The total number of old trees was 118 and they were fairly evenly distributed over the 1 hectare area (Fig 1). Of these 109 were analysed for 4 enzyme loci (LAP-A, LAP-B, GOT-A and GOT-B). Only GOT-B with 5 alleles showed adequate variation for further population genetic computations. The other loci had gene frequencies close to fixation and thus they were mainly used for studying the spatial distribution of rare alleles. In fact, all further computations have been done exclusively on the locus GOT-B.

After pooling observed genotype classes smaller than 5 observations it was found that there was no significant departure from an expected Hardy-Weinberg equilibrium in the 109 old trees of 300–400 years age (χ^2=11,27, $p < .20$). It should be stressed, that this old growth stand represents the end of a long life cycle which was most probably started with a population of 50.000–100.000 individuals.

To investigate possible kinships of proximal neighbour groups the 'rubberband' method was applied. Groups of size 4–6 around the center trees were formed as indicated on the map (Fig. 1). The genotype of the center tree was recorded and gene counts were made on the neighbours. Local F-values were computed for each numbered center tree and are given here as an example. The tree number is given first and the F_A value in brackets.

2 (1.00), 3 (0.33), 4 (1.00), 7 (1.00), 10 (0.02), 11 (0.54), 12 (0.00), 14 (–0.28), 23 (0.53), 24 (0.00), 25 (0.43), 30 (–0.03), 31 (–0.16), 32 (0.13), 33 (0.03), 37 (–0.40), 40 (–0.43), 42 (0.05),46 (0.51), 53 (0.49), 56 (–0.02), 64b (–0.16), 72 (–0.72), 74 (0.31), 80 (–0.26), 85 (0.16), 88 (0.14), 93 (0.13), 96 (–0.16), 104 (0.00), 109 (0.16), 113 (0.44), 115 (1.00), 118 (–0.17).

A closer inspection of the individual values shows that only 11 negative values appear among 34. The mean $\bar{F}_A$- value 0.165, is significantly positive in the T-test ($t_{(33)} = 2.269$, $p < .025$).

The genetic structure of regeneration

From the 10×10 meter grid of young trees a population of N=86 was taken, excluding only a few cases where the enzyme analysis appeared uncertain. For GOT-B chi-square analysis showed a highly significant departure from Hardy-Weinberg equilibrium

($\chi^2_{(6)} = 29.08$, $p < .001$).

This is due to a much higher than expected observation on homozygotes in two genotype classes (B_2B_2 and $B_{22}B_{22}$) and a much lower than expected observation on heterozygotes in one class (B_2B_{22}).

Local F_R-values and average local F_R for regeneration were computed as for old growth. Individual local F_R-values are not given here but 31 negative values appeared among 51 in the most complete set of data.

Local F_R-values and average local $\bar{F}_R$ for regeneration were computed with 3 different sets of material as mentioned earlier. This was done in order to observe wether the size and spread of neighbouring groups has an influence on the F-values. Results are given in tables 1,2 and 3.

In all cases a few groups were abandoned due to failing of some enzyme analysis. However, in all 3 cases the groups were well spread over the whole stand. As can be seen from the tables the average $\bar{F}_R$-values did not depart significantly from zero in any of the cases, indicating that there was no greater than expected relatedness of neighbouring trees, a somewhat unexpected result that will be discussed later.

Table 1. The genetic structure of regeneration. t-test for neighbour groups of 4 (every second tree used as center).

$\bar{F}_R = -0.075$
Hypothesis 0 : Expected $\bar{F}_R = 0$
Hypothesis 1 : Expected $\bar{F}_R > 0$
$t(24) = -1.01$
$0.85>P>0.8$ *not significant*

Table 2. The genetic structure of regeneration. t-test for neighbour groups of 8 (every second tree used as center).

$\bar{F}_R = -0.055$
Hypothesis 0 : Expected $\bar{F}_R = 0$
Hypothesis 1 : Expected $\bar{F}_R > 0$
$t(23) = -0.734$
$0.8>P>0.75$ *not significant*

Table 3. The genetic structure of regeneration. t-test for neighbour groups of 8 (every tree used as center).

$\bar{F}_R = -0.044$
Hypothesis 0 : Expected $\bar{F}_R = 0$
Hypothesis 1 : Expected $\bar{F}_R > 0$
$t(50) = -0.834$
$0.8>P>0.75$ *not significant*

Comparison of old growth and regeneration populations

It is apparent that local F_A-values of adults are significantly larger than local F_R-values of

Table 4. Comparison of the genetic structure of old growth and regeneration. Mann–Whitney U-test between F-values (most complete set of data).

Hypothesis 0 : Probability ($F_A > F_R$) = 0.5
Hypothesis 1 : Probability ($F_A > F_R$) > 0.5
Z = 2.36**
P<0.009 *significant*

regeneration (Table 4). In other words, old growth groups are more closely related within the group than are regenerations, regardless of how regeneration groups were formed. This is a most interesting finding. It may be due to the fact the average distance of the old growth center individuals to their 4–6 neighbours is not as regular as in the regeneration where they are spaced at either 10 or 14 meter distance. But it may also depend on the fact, that old growth neighbours are often unevenly dispersed around center trees and often grow in groups of 2–3 within a few meters from each other. A final plausible reason may be the fact, that a 10–14 meter radius from a young center tree is too large to represent related neigbour individuals considering the fact that the area under investigation totally holds 118 adult trees and an estimated 50.000 young trees, slightly grouped around old trees (Fig. 2).

A 2×k chi-square test was done between 109 well identified adults and 86 regenerations that represented the 10×10 meter grid sample ($\chi^2_{(9)} = 30.43$, $p < .001$). The two populations differ significantly in the distribution of genotypes. The reason for this difference may be manyfold. First the regeneration appears to be more homozygotic than expected under random mating, and second there seem to exist decisive differences in gene frequencies between adult and regeneration as can be seen from table 5.

The genetic structure of regeneration groups

Around 37 old trees, groups of 30 young individuals were analysed as described above. Observed genotype frequencies were

Figure 2. Typical structure of the stand under investigation. Observe the grouping of regeneration under old trees.

Table 5. Gene frequencies in the adult and the regeneration population.

	GOT-B_1	GOT-B_2	GOT-B_{22}	GOT-B_3	Population size N
adult	0.11	0.40	0.07	0.42	109
regeneration	0.02	0.33	0.22	0.43	86

tested against those expected. Expected frequencies were counted on the bases of the genotype of the old center tree and the gene (pollen) pool of the old growth. Significant departure from expected genotype frequencies was observed in 28 groups of the 37, and departures generally seemed to be due to a higher than expected homozygosity in the young groups. Combining the probabilities of the 37 chi-square tests (Sokal & Rohlf 1969 p 621) indicates a highly significant departure from expectation (Table 6). This suggests that plants under old trees are significantly more homozygous than expected under panmixia.

A test of gene frequency heterogenity in the progeny groups was then carried out using the technique of Snedecor and Irwin (1933). Gene frequencies were significantly different between groups in all cases (Table 7). The structure of the subdivided population was in

Table 6. The genetic distribution of regeneration under old trees (expected genotype distribution calculated on the basis of a random old growth paternal gene pool).

$\chi^2_{(90)} = -2\sum_i \ln P_i$ (Sokal & Rohlf 1969)

$= 343.27$***
P<0.001 *highly significant*

Table 7. Gene frequency heterogeneity in subgroups under old trees.

All 4 alleles: χ^2 (36) (3) = 414.9***
B_1+B_{22} pooled: χ^2 (36) (2) = 260.0***
Two classes: χ^2 (36) (1) = 74.7***

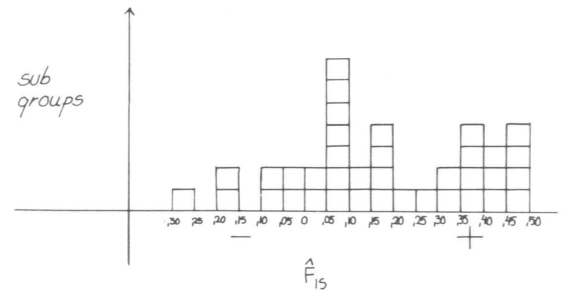

Figure 3. Plotted distribution of F_{IS}-values of regeneration groups.

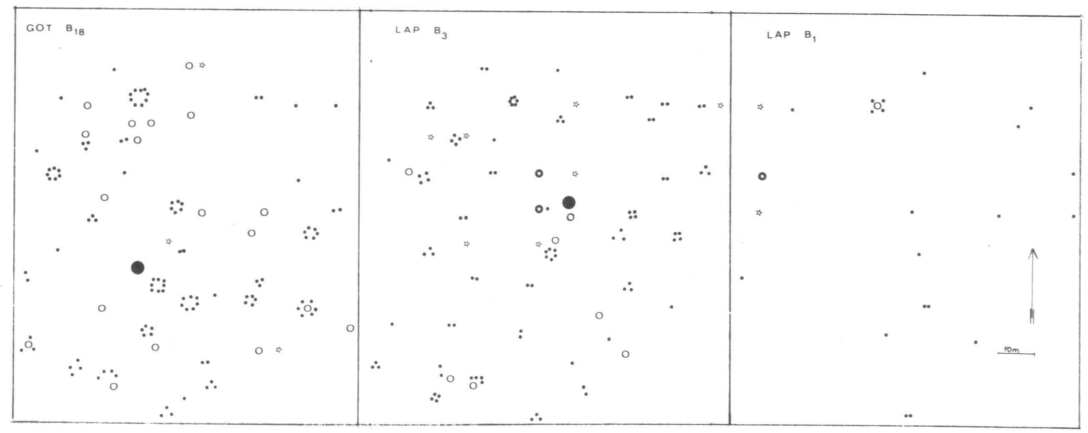

Figure 4. Spatial distribution of 3 different rare alleles. Observe the tendency of allele groups under particular mother trees.

○ ● heterozygote and homozygote old tree
☆ ✪ heterozygote and homozygote young tree on 10×10 m grid
● number of rare alleles in subgroups under old trees

addition analysed by Wright's F-statistics. Individual $\hat{F}_{IS}$-values have been plotted in Figure 3. Generally, the values appear to be on the positive side indicating an exess of homozygotes (or too few heterozygotes). Of 37 subgroups only 7 deviate in the direction of excess heterozygotes. This is yet another proof of high homozygosity in the regeneration. Most groups have a high positive $\hat{F}_{IS}$-value and the distribution is very skewed.

Finally some observations on the spatial distribution of rare alleles were made. It appears as if there would be little or no large scale clustering of rare alleles within the hectare stand under investigation (Figure 4). However, there is one peculiarity to be mentioned. Particulary the rare allele GOT-B_{18} seems to be clustered in subgroups under old trees and what more, often independent of whether the old tree above has the same allele. One is tempted to conclude from this that seeds sail in cohorts and land in cohorts, but not necessarily under their common mother. Pollination may also come in cohorts from adjacent trees.

Discussion

Allogamous anemophilous plant populations, like pine stands, have a population structure that is influenced by (1) wind and temperature at the time of pollination, (2) chance at the time of seedling establishment and (3) competition during stand development. The outcome of such effects may vary from case to case, populations may e.g. exhibit varying degrees of inbreeding or selfing, also varying degrees of migration from adjacent or distant populations. Above all, long-living trees are subject to the effects of natural selection during scores of vegetative cycles, often attaining maturity-age of several hundred years.

We have set out to clarify at least some questions related to population structure by making detailed observations on the spatial (geographical) distribution of genotypes within a stand of pine trees.

We conclude that large differences in the genetic structure of two successive pine generations may occur. There must be a number of reasons for the difference in genotype distribution of the 300–400 years old population and the 80 year old regeneration. As a most important factor we would like to suggest competition between genotypes in the regeneration which, as time goes on, decimates an initial population of say 100.000 individuals per hectare to one that involves about 100. It appears from the analysis that a regeneration population may for a long time carry inbred individuals, which gradually die off as the population approaches maturity. It also appears that two tree generations occupying the same area may be very different genetically. The reason may be a gradual decline of inbred individuals, but it may also be due to real differences in gene frequencies in the successive populations, as can be seen from Table 5. Several explanations to this problem could be offered, but all explanations are inconclusive. One could conclude, however, that the genetic structure of the regenerating population is interesting from an evolutionary point of view as it has not yet reached the stage of reproduction. If development continues naturally within the stand, it would probably take another 200 years before the regeneration population would be ready to shed seed and reproduce again. By then, we believe, excess inbreeding would have been taken care of by competition and natural selection. However it is permissible to conclude, that inbreeding depression may become problematic under conditions of artificial regeneration through sowing or particulary planting of spaced individuals. We feel that much more attention should be paid to the question of inbreeding in cultivated forests where competition and natural selection is almost eliminated.

Tests of neighbour kinship in subgrouped populations have been proposed by S. Wright (1965 and earlier). We have partly used Wright's F-statistics for studying population subdivision in groups of 30 young trees under particular old center trees. We have also developed a local F-concept to find out if neighbours are more related than the population at large.

We found that such subgroups are extremely heterogeneous, deviating from expected genotype composition, showing gene frequency heterogeneity, showing generally a higher than expected degree of homozygosity and, most perplexing, showing no particular extra kinship to the seed producing generative old mother trees above them. It is as if nature seeks all possible means of scrambling up a population that could otherwise, as time goes by, succumb due to heavy inbreeding and subsequent depression. We visualize heavy plant competition as a decisive factor in keeping the population well adapted and free of genetic decay. We are forced to believe, that large and unpredictable changes, possibly to the worse may take place in cultivated tree populations if competition is excluded. Equally we would like to emphasize the importance of artificial selection in natural stands of trees for improving yield and quality. However, natural and artificial selection are not mutually exclusive but should rather be combined in modern forestry practice.

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GENETIC VARIATION IN QUALITY CHARACTERISTICS OF SCOTS PINE

PIRKKO VELLING

Three progeny tests of Scots pine *Pinus sylvestris* L. were evaluated for a number of quality or growth habitus characters. Sib-families, either half- or full-sib, differed significantly from each other for most characters indicating generally a high heritability of quality traits interesting for breeding of better stem quality.

Introduction

Quality characters have been of great importance as selection criteria since the first phase of forest tree breeding work, the selection of plus trees. The importance of the characters affecting the quality of the stem and wood was strongly revealed again about a few years ago when the Forestry Seed and Seedling Board made an inguiry concerning the improvement of timber quality through forest tree breeding activities by questioning the different interested parties in the fields of forestry and forest tree breeding, (the National Board of Forestry, the Central Forestry Boards Tapio and Skogskultur, the Finnish Forest Research Institute, the Foundation for Forest Tree Breeding, the Central Association of Finnish Forest Industries and the Finnish Pulp and Paper Research Institute).

On the basis of the replies, proposals were made also for Scots pine, and these are summarized below.

Improvement of quality

- Most important of all is *the fine-branchiness;* in addition to the small size of the branches, *perpendicularity, small number* and *good natural pruning* of the branches are the characters to strive for
- Other important characters are the *minimum tapering* of the stem and *high wood density*

Maintaining quality at the present level

- *Stem straightness,* at least the present day level of the pine forest naturally regenerated; presupposes retaining damage resistance

Breeding for fast growth – breeding for quality

- Breeding for increased growth must under no circumstances be done at the expense of quality. On the contrary:
- Breeding for fast growth can be reduced if this is necessary when maintaining or improving quality, however
- There is little advantage in reducing the present day *rate* of growth, in general.
- To be able to meet any new requirements in the future, breeding should be kept as broadly-based as possible so that it can respond to any rapid changes.

Thus quality problems have attained greater relevance because of the increasing concern being expressed by the various parties about the bad external stem quality in future, artifically regenerated forests. At the same time, the oldest pine seed orchards are approaching the stage at which thinning will have to be carried out and second generation seed orchards will have to be established.

Material and methods

The results presented in this report are based on measurements made in three field tests of Scots pine. The two older tests, no. 159 in Kuorevesi (latitude 62°02′ N and longitude 24°45′ E, altitude 120 m) and no. 160 in Savitaipale (61°10′ N and 27°31′ E, 92 m), established in 1960, consist of open-pollination progenies (half-sib families). The third test, no. 396/2 in Padasjoki (61°22′ N and 25°06′ E, 125 m), is established in 1972 of control-pollination progenies (full-sib families). In all 52 selected trees, 49 of which real plus trees and 3 peatland pines are represented in these three tests. Some of the trees are represented in more than one test examined, for example the very peculiar plus tree no. E 1101, the so called "Kanerva pine" is in the progenies of all three tests. Normal stand progenies are used for comparison in the tests studied.

The three tests measured are not altogether suited to the study but for the present the best ones we have. There are for example only 5 progenies for comparison in test no. 160. Further the site within the tests varies, especially in test no. 159; there are incomplete blocks (no. 159 and 396/2) and so on. Test orchards, the management of which is more intensive and the site more homogenous than in the field tests, will possibly in future years offer better objects of study, even if all the characters present in the long-term field tests will not be present.

There is one additional aspect to be taken into account when examing the wood quality characters inside the stem, namely the more or less destructive sampling making it difficult to get suitable material for the study.

In the present study the tests no. 159 and 160 were measured in the autumn 1979, no. 396/2 in the spring 1981. In the test no. 160, sample discs, for the determination of wood density, were also collected. In test no. 159 the density was determined earlier and the results published in Folia Forestalia 188.

The same characters were measured in the same way in both of the older trials. In principle the measurements carried out in all the three tests were similar. In addition, in spring 1981, evaluation of quality by visual classification was made before the measurements.

The following quality (and growth) characters were measured:

Character	Test No. 159	Test No. 160	Test No. 396/2
Breast height diameter	x	x	x
Diameter at 6,0 (3,5) to evaluate the tapering	x	x	
Tree height	x	x	x
Length increment of the leader shoots, over the last 3 years			x
Crown limit; the lowest green branch	x	x	
Crown width, at the widest point of the crown			x
Branch thickness, the thickest branch of the whorl, 7.–11. whorl from the top	x	x	x, 3. and 5. whorl
Branch angle of the same branches as for thickness	x	x	x
Number of branches/ whorl	x	x	x
Wood basic density	(x) earlier	x	

Both expected and unexpected factors disturbing the development of the tree, and thus influencing the results obtained, were noted during the evaluation of all the three tests, as well as the crookness and sweep of the stem in the tests no. 159 and 160. The characters, visually classified into five classes beforehand in test no. 396/2, were relative crown width, branch angle, average branchiness and stem straightness. At the same time, every tree evaluated was given a score from 1 (the best possible) to 10 (the worst possible), taking into account all the visible quality and growth characters and weighing the quality.

Every second three/plot more than 2 m high and normally developed was measured in test no. 396/2. In all, 10 trees/plot were measured. There were 6 blocks, 3 of which were complete and included into the calculations. In the older tests, dominant trees (that means, the probable future sawtimber trees) were measured (in test no. 159 5 trees/plot from 4 blocks, and in test no. 160 10 trees/plot, from 5 blocks). The number of sample trees was thus quite small so that the variation within the plot probably was insufficiently represented in the study.

About 2 cm thick discs, at two meter intervals, starting from the stump level, were taken in test no. 160 from 20 trees/ plot and all the 5 blocks. The wood basic density was then determined using the V^D -method described by Olesen (1971), as was earlier determined from the sample discs (5 trees/plot, 4 blocks) in test no. 159.

The results obtained from the visual classification in test no. 396/2 and their comparison with the results obtained by measuring are not discussed in this paper.

Results

The tests means, and coefficients of variations of family means of the characters measured and the results from the two-way analysis of variance are presented in tables 1 and 2. It can be seen that in test no. 160, the half-sib families significantly differ from each other with respect to nearly all the characters examined. The differences between blocks are small. In test no. 159 the differences between blocks are, on the contrary, mostly significant, thereby preventing the real variation between different families to appear. All characters measured in test no. 396/2 generally vary very significantly between the full-sib families concerned, between blocks the variation is less significant.

In the three tests examined (and especially in the test no. 160 including only 5 progenies, and in the test no. 396/2, where more than 1/3 of all the trees are the progeny of the tree mentioned below) the variation between families is noticeably influenced by the presence of the progenies of the plus tree no. E 1101, the so called "Kanerva pine". About one half of the trees of its progenies has some features very typical to the original tree: extremely narrow crown, fine branches at nearly right angles to the stem, minimum tapering – and as disadventages, the greater number of branches/whorl, very steep sinus-angle, and most important, more or less serious crookness and/or sweep of the stem. With all its peculiarities, the "Kanerva pine" and its numerous progenies offer an interesting object for study.

In general, the plus tree progenies were better than normal stand progenies, both in quality as well as in height growth in all the three tests studied (table 3). The only exception to this rule was the larger number of branches/whorl in the plus tree progenies.

The phenotypical correlations between the characters measured were also calculated from the three tests. The main results of these calculations made on the individual level were in quite good agreement which each other (tables 4 and 5):

- the correlations between the *growth characters* (breast height diameter, diameter at 6,0 m tree height and leader shoots increment, crown limit and width) were, as expected, positive and significant
- the correlations between the *growth and branch characters* (branch thickness, angle and number/whorl) were positive and significant, or there was no correlation. Exceptions were as follows. In test No. 160: between the diameter at 6,0 m and branch thickness, a very significant negative correlation caused by the influence of the "Kanerva effect" (i.e. fine branches together with minimum tapering) and in test No. 396/2, a very significant negative correlation between the crown width and branch angle (i.e. narrow crown together with right angle branches).
- the correlations between *the branch characters* in all three tests were in good agreement – between branch thickness and angle significantly negative, thickness and number/whorl negative or with no correlation and angle and number/whorl generally positive
- the correlations between the *same characters* for example branch thickness measured from the different branch whorls (from the top 7.–11.) in test No. 159 and 160 were nearly always positive and very signifacant. So in

test No. 396/2 it was considered to be sufficient to measure the characters from only two whorls, the 3rd and 5th from the top, which were also in good agreement.

The upper whorl was considered to represent that part of the crown which still can develop free from the influence of the neighbouring trees, the lower whorl represents that part which already is susceptible to competition.

It seems that in general it would be enough to measure the characters from two branch whorls, different in their relative position with regard to the influence of the neighbouring trees. In the older tests, like no. 159 and 160 in this case, the measuring of the upper whorl would, however, be difficult and slow.

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Table 1. Mean values of the characters measured in tests no. 159 and 160, coefficients of variation of the family means and results from the two-way analysis of variance.

Character measured	Mean values		CV, %		Analysis of variance, F-values: Families		Blocks	
	159	160	159	160	159	160	159	160
Diameter (d.b.h.), cm	11,9	13,0	4,7	1,5	1,51	0,73	8,25***	1,63
Upper " (6.0 m), cm	6,6	7,5	5,2	5,9	0,78	2,20	9,74***	3,74
Tree height, m	8,2	10,0	5,1	4,1	1,73*	4,71*	8,51***	2,90
Crown limit, m	2,7	3,9	10,9	5,5	1,99**	1,71	14,93***	3,32**
Volume, dm^3	54,9	65,7	8,8	5,4	1,54*	1,27	10,29***	3,27*
Branch thickness, 7. whorl f.t., cm	2,5	2,4	5,8	6,3	1,79*	4,09*	1,95	1,39
" " 8. " " "	2,6	2,5	5,4	7,6	1,64*	9,07***	6,45***	0,76
" " 9. " " "	2,6	2,6	4,4	5,8	0,99	6,10**	5,26**	1,20
" " 10. " " "	2,4	2,5	5,5	7,3	1,42	7,32**	5,34**	1,54
" " 11. " " "	2,2	2,4	5,4	7,2	1,14	7,20**	4,60**	0,84
" " $\bar{x}$ " " "	2,5	2,5	4,8	6,8	1,51	7,33**	5,73**	1,21
Branch angle,° 7. whorl f.t.,	48,5	51,8	5,2	7,6	1,73*	7,55**	3,17*	1,03
" " 8. " "	49,5	52,1	3,9	5,3	1.17	4,36*	3,24*	1,02
" " 9. " "	50,2	54,2	4,1	5,6	1,44	2,75	6,90***	0,30
" " 10. " "	51,9	55,9	4,5	3,8	1,61*	2,76	2,54	0,43
" " 11. " "	54,3	57,0	4,4	4,4	1,69*	2,75	2,72*	0,58
" " $\bar{x}$ " "	50,8	54,2	3,7	5,1	1,72**	4.73*	5,18**	0,55
Numb. of branches/whorl 7. wh. f.t.	5,7	5,8	8,1	6,6	2,24***	10,22***	1,11	1,77
" " " 8. " "	5,7	6,3	6,4	8,4	1,44	16,58***	0,05	3,22*
" " " 9. " "	5,7	6,2	5,9	5,7	1,72*	5,12**	1,37	1,85
" " " 10. " "	6,0	6,4	5,2	10,7	1,14	21,67***	3,49*	3,59*
" " " 11. " "	5,6	6,1	4,6	4,6	1,28	3,44*	7,50	3,34*
" " " $\bar{x}$	5,7	6,2	4,6	6,8	1,76*	17,84***	2,51	4,33
Wood basic density, kg/m^3	355	377	7,7	1,2	3,18*		3,37*	

Table 2. Mean values of the characters measured in test no. 396/2 coefficients of variation of the family means and results from the two-way analysis of variance.

Character measured	Mean values	CV, %	Analysis of variance F-values: Families	Blocks
Diameter (d.b.h), cm	3,8	9,9	2,40**	4,70**
Tree height, m	2,7	6,7	2,89***	3,89*
Length of the leader shoot,[1] m	1,2	9,4	4,02***	7,07***
Crown width, m	1,6	7,1	2,65**	3,97*
Branch thickness, 3. whorl f.t., cm	1,4	6,6	2,38**	16,13***
" " 5. " "	1,5	11,5	7,30***	5,90**
Branch angle, 3. whorl f.t., cm	60,0	7,9	6,03***	2,17
" " 5. " " "	73,1	6,8	5,17***	5,28**
Number of branches/ 3. whorl f.t.	5,4	8,5	3,57***	2,14
" whorls 5. " "	5,3	11,2	2,93***	1,83

[1] Sum the last 3 years

Table 3. Comparison of normal stand progeny and plus trees progenies in test No. 160

Character measured	Mean values: Normal stand progeny	Plus tree progenies	Family: E 42	E 637 D	E 645 D	E 1101
Diameter, cm	12,8	13,1	13,1	13,3	12,9	12,9
Tree height, m	9,52	10,17	9,68	10,28	10,28	10,45
Crown limit, m/Tree height, m	0,38	0,40	0,39	0,40	0,39	0,40
Upper diameter (6,0 m), cm/Diameter, d.b.h., cm	0,55	0,59	0,54	0,59	0,60	0,62
Volume, dm^3	59,6	67,3	66,1	68,5	66,4	68,0
Branch thickness, 7. whorl f.t., cm/$D_{1,3}$, cm	0,20	0,18	0,19	0,18	0,19	0,17
" " 11. " "	0,20	0,18	0,19	0,19	0,18	0,16
" " $\bar{x}_{7.-11.}$ "	0,21	0,19	0,20	0,19	0,19	0,17
Branch angle,° 7. whorl f.t.	49,8	52,3	50,0	49,9	50,6	58,8
" " 11. "	54,7	57,6	56,8	56,1	56,0	61,3
" " $\bar{x}_{7.-11.}$ "	52,7	54,6	53,4	52,1	53,9	59,0
Number of branches/whorl, 7. whorl f.t.	5,5	5,9	5,5	6,0	5,8	6,4
" 11. "	5,7	6,3	6,2	6,2	6,1	6,5
" $\bar{x}_{7.-11.}$	5,8	6,3	5,9	6,4	6,0	6,8
Wood basic density, kg/m^3	370	379	382	379	376	380
Number of stems crook or sweep, %	6	14*)	0	8	4	44*)

Table 4. Correlations between the characters measured in tests no. 159* and 160.**

Character / Corr. coefficient	D1.3. 1	D6.0 2	H 3	CL 4	7. BT 11. 5	6	7. BA 11. 7	8	7. NB 11. 9	10
1 D1,3 Breast height diam.	1.00									
2 D6,0 upper diameter	+++* +++**	1.00								
3 H Tree height	+++ +++	⊕ ⊕	1.00							
4 CL Crown limit, lowest green branch	+++ +++	⊕ +++	+++ +++	1.00						
5 BT_7. Branch thickness, 7. whorl from top	+++ ⊕	+++ ⊕	+++ +++	++ +++	1.00					
6 BT_{11}. Branch thickness, 11. whorl from top	+++ ⊖	+++ −−−	+++ +++	⊖ ⊕	+++ +++	1.00				
7 BA_7. Branch angle, 7. whorl from top	⊕ +++	⊕ ++	++ ⊖	+++ +	−−− −−−	⊖ −−−	1.00			
8 BA_{11}. Branch angle, 11. whorl from top	⊖ +++	⊕ ⊕	⊕ ⊖	+ ⊕	⊖ −−−	−−− −−−	+++ +++	1.00		
9 NB_7. Number of branches, 7. whorl from top	0 +++	⊕ +++	⊕ ⊕	⊕ +++	−− −−−	− −−−	+ ⊕	+++ +++	1.00	
10 NB_{11}. Number of branches, 11. whorl from top	+ +++	⊕ +++	+ +	⊕ +++	⊕ −	⊖ ⊖	+ +	+++ +	+++ +++	1.00

+++ = significant positive correlation, 0,1 %
++ = significant positive correlation, 1 %
+ = significant positive correlation, 5 %
⊕ = not significant positive correlation,

−−− = significant negative correlation, 0,1 %
−− = significant negative correlation, 1 %
− = significant negative correlation, 5 %
⊖ = not significant negative correlation,

Table 5. Correlations between the characters measured in test no. 396/2 (symbols same as in table 4).

Character / Corr. coefficient	DI.3 1	H 2	LS 3	CW 4	3. BT 5. 5	6	3. BA 5. 7	8	3. NB 5. 9	10
1 Diameter, b.h.	1.00									
2 Tree height	+++	1.00								
3 Lenght incr. of the leader shoot	+++	+++	1.00							
4 Crown width	+++	+++	+++	1.00						
5 Branch thickness 3. whorl f.t.	+++	+++	+++	+++	1.00					
6 Branch thickness 5. whorl f.t.	+++	+++	+	+++	+++	1.00				
7 Branch angle, 3. whorl f.t.	⊕	⊕	+	−−−	−−−	−−−	1.00			
8 Branch angle, 5. whorl f.t.	⊖	⊖	+	−−−	−−−	−−−	+++	1.00		
9 Number of branches, 3. whorl f.t.	+++	+++	+++	+	+++	⊕	+++	+++	1.00	
10 Number of branches, 5. whorl f.t.	+++	+++	+++	⊕	⊕	⊕	++	⊕	+++	1.00

GENETIC VARIATION IN QUALITY CHARACTERS OF SCOTS PINE

An evaluation by means of the heritability concept

TAPANI PÖYKKÖ

Three progeny tests of Scots pine were evaluated. A number of different quality and growth characters were used for computing heritability values. It was found, that open pollinated half-sib analysis is very unreliable for estimates. Also controlled mating designs give variable results depending on the structure of the analysed population of progenies. However results indicate, that quality characters generally have heritability values of 40–50 % while growth characters have values of less than 30 %.

Introduction

Heritability can be estimated in either of two ways. The most direct estimates are derived from the relation between parents and offspring obtained by measuring the parents, growing the offspring and measuring the offspring. The other way is to establish a half-sib or full-sib progeny test, calculate the mean squares and variances, and compute heritability as a function of the variances.

When forest trees are concerned, the estimation of heritability with a parent – progeny regression is seldom possible. Sufficiently old progenies are not available and characters measured from young progenies are not always same as those measured from parent trees. Thus to estimate heritability one has to use sib-progeny tests and the analysis of variances.

In this report I have used two methods based on 2-way analysis of variances and one method based on the half-sib groups of controlled crossings to estimate the values of heritabilities of different quality and growth characters. The progeny tests needed for these methods are open-pollinated offsprings or sib-progenies of F families replicated (for blocks) B times at one site using N-tree plots.

Material

The study material is drawn from three different field tests of the department of Forest tree breeding. Detailed introduction of the field tests and measurements carried out in them is given in the earlier paper by Velling. Field test 159/1 and 160/1 are open-pollinated progeny tests of Scots pine. Field test No. 396/2 is established with full-sib-progenies of Scots pine. In these field tests there are also 1–2 controls.

For the measurement, ten dominant trees per a plot were selected in the field tests No. 160/1 and 396/2 and five dominant trees per a plot in the field test No. 159/1. Before calculation, the basic study material was restricted to fullfill the requirements of the heritability analysis. In field test 159/1 all those families with an empty plot in any of four blocks measured were rejected. After the rejection there were 34 families left with four blocks and five trees per plot in field test 159/1. In field test No. 160/1 no rejection was needed. In this field test there were 5 families in five blocks with ten trees per plot.

Field test 396/2 consisted of 21 full-sib progenies and 2 open pollinated families as controls. Before analysis these controls were rejected and the study material was then 21 full-sib families with three blocks and ten trees per plot.

As the mating design shows it was also possible to select half-sib groups according to the father trees. The first grouping was four fathers with three mothers. The second grouping was three fathers with five mothers. The heritability values were calculated for every character measured, and also for the means of three different branch angles, branch diameters and numbers of branches per a whorl. (Table 2).

Methods

In all three field tests the phenotypic variance is divided into variance components. The division is carried out by means of the classical 2-way analysis of variances (Table 1). When there are unequal number of trees per plot the analysis of variances is usually carried out in two stages. First the 2-way analysis is carried out by levels of plot means. Then the analysis is made between plots and within plot to calculate within plot variance. Because of the orthogonality ot the study material there was no need to divide the analysis into two stages. It was carried out in the normal way.

Table 1. Half-sib family.

Analysis of variances

Source	DF	MS	Expected MS
Blocks	(r–1)	R	$\delta_e^2 + p\,\delta_R^2$
Families	(p–1)	A	$\delta_e^2 + r\,\delta_F^2$
Family × Block	(r–1) (p–1)	B	δ_e^2
Trees within a plot	rp (n–1)	C	$\delta_E^2 + 3\,\delta_F^2 + \delta_D^2$

Estimation of heritability

	Johnsson (1978)	Kempthorne (Tigerstedt 1969)	
COV_{HS}	(A–B/r	(A–B)/r	¼ V_A
δ_e^2	no calcul.	$B–n_hC$	
$\delta_f^2 + \delta_g^2$	(A–B)/r+C	(A–B)/r+C	
h^2	$\frac{4\,COV_{HS}}{\delta_f + \delta_g}$	$\frac{4\,COV_{HS}}{\delta_f^2 + \delta_e^2 + \delta_g^2}$	

Table 2. Mating design of experiment 396/2.

	Fathers / Mothers	1 E 144	2 E 1101	3 E 360	4 E 635
1	E 108	×			
2	E 48	×			
3	E 106	×			
4	E 1002	×		×	
5	E 635C	×	×	×	
6	E 112		×		
7	E 147		×		
8	E 127		×		
9	E 1007		×		
10	E 622		×		
11	E 719D		×		
12	E 1001		×	×	×
13	E 138			×	
14	E 104			×	×
15	K 44				×

The heritability is evaluated by two different methods (Table 1); Johnsson 1979 and Tigerstedt 1969. In table 1 the parameters needed and their estimation is introduced separately to each of the two methods. The difference between methods in question lies in the evaluation of the environmental variances. The enviromental variance can be considered to consist of two components 1) an individual factor (f) and a common factor (e) (Tigerstedt 1969). The common factor, which is the same for all individuals of the same plot, is calculated and included in the phenotypic variance in Tigerstedt's method but not in Johnssons's. Due to this, the heritability values calculate by the former methods are smaller than those calculated by the latter method.

The evaluation of the genetic covariances is the same in both methods. Table 1 shows the situation in field tests 159/1 and 160/2. The family variation signifies the covariances of half-sibs equal to 1/4 of the additive genetic variation. In field test 396/2 of full-sib progenies, the family variance signifies covariance of the full sibs equal to half of the additive variance and 1/4 of the dominance variance.

Besides the two methods in question, in field test 396/2 halfsib groups were also formed, and by means of higher ordered analysis of variances the variance components of father trees and mother trees were derived separately. The heritability was calculated as a function of variances due to different parent groups (Becker 1967). In this case, the variance of father trees is equal to the covariance of the half-sibs and the variance of mother trees within fathers is the covariances of full sibs minus the covariance of half-sibs. Thus the variation between fathers is 1/4 of the additive genetic variation and the variation between mothers is 1/4 of the additive variation plus 1/4 of the dominance variation. When both of parents are involved in the heritability, their variance is half of additive variation plus 1/4 of the dominance variation (Becker 1967).

Results

Open pollinated progenies

The evaluation of the heritability in open-pollinated progenies does not give reliable results. Values of heritabilities of characters calculated in field tests 159/1 and 160/1 do not agree with each others. There are also negative values and values over one, which are not in accordance with the theory of heritability. Values of heritability of the same character measured at different heights on a tree varies considerably and without any noticeable control. Calculated values of heritability are in such contradiction that meaningful conclusions are difficult to draw.

However, one may find some possible explanations for this confusion. First, there is heterogenity in the growing conditions within the field test and micro site. Also, the topography of a test field varies. These factors cause unpredictable variations which are difficult to separate by means of test planning and analysis of variances. Due to this variation, the genetic variation disappears or, in some cases, becomes overestimated. For instance, when results of Johnssons's and Tigerstedt's methods are compared it will be noticed that results differ remarkably for some characters. This is due to the interaction between family and block, which is taken in consideration in Tigerstedt's method but not Johnssons's.

Secondly, the sample of progenies in the field test do not always represent the true variation of a Pine stand. This is the case in field test no. 160. There are only five families in this test. These families are gathered from a very large area and the variation between them is great. For these reasons, when the variation between the groups is large and biased and the variation due to environment is reduces, the value of heritability will be high and in some cases it will be over one. Further, the ratio of full-sibs and half-sibs in open pollinated progenies is unknown. According to theory, the open-pollinated progeny consists of half-sibs. This means that variation between families is 1/4 of the additive genetic variation. According to mating analysis, it is probable that there are also full sibs among half sibs and the family variation is less than 1/4 of the additive variation. If this is taken into consideration, the covariation of half sibs, which is equal to the family variation, has to multiple with an number

Table 3. Values of the heritability of the characters measured in field tests 159/1 and 160/1 (J = Johnsson, T = Tigerstedt)

Character	Field test 159 J	159 T	160 J	160 T
Height	.57	.32	over 1.00	0.95
Crown limit	.77	.49	.31	.21
D 1.3	.43	.25	neg.	neg.
D 6.0	neg.	neg.	.50	.37
Branch angle 7	.20	.19	.68	.54
” ” 8	.06	.05	.25	.25
” ” 9	.12	.12	.29	.26
” ” 10	.17	.16	.15	.15
” ” 11	.13	.13	.19	.18
Diameter of branch 7	.31	.26	.77	.62
” ” 8	.26	.22	over 1.00	over 1.00
” ” 9	.01	.01	.74	.68
” ” 10	.15	.13	.83	.77
” ” 11	.08	.07	.74	.70
Number of branches per a whorl 7	.33	.31	.36	.38
” ” 8	.09	.09	.83	.84
” ” 9	.16	.15	.31	.31
” ” 10	neg.	neg	over 1.00	over 1.00
” ” 11	.07	.07	.22	.22
Mean of the branch angle	0.14	0.12	0.47	0.44
Mean of the diameter of the branch	0.26	0.20	over 1.00	.97
Mean of the number of the branches per a whorl	0.24	0.22	0.84	0.84

Table 4. Values of the heritability of the characters measured in the field test 396/2.

Character	COV_{HS}	B	Expectations C	$COV_{HS}+C$	$COV_{HS}+B-\frac{1}{n_n}C+C$	h^2 Johnsson	h^2 Tigerstedt
Height	2,23	4,70	12,73	14,96	18,39	0,30	0,24
Growth of the terminal shoot	0,97	1,30	3,41	4,38	5,34	0,44	0,36
Diameter of the crown	0,60	1,80	6,33	6,93	8,10	0,17	0,15
$D_{1,3}$	8,18	24,40	64,22	72,40	90,38	0,23	0,18
Branch angle, upper whorl	18,87	14,80	78,61	97,48	104,42	0,39	0,36
" " lower whorl	20,12	19,16	106,88	127,00	135,47	0,32	0,30
Diameter of branch, upper whorl	0,58	1,43	4,69	5,27	6,23	0,22	0,19
" " lower whorl	2,89	1,69	10,00	12,89	13,58	0,45	0,43
Number of branches per a whorl, upper whorl	0,09	0,23	1,04	1,13	1,26	0,16	0,14
Number of branches per a whorl, lower whorl	0,24	0,48	1,73	1,97	2,28	0,24	0,21
Mean of branch angle	18,70	14,80	68,12	86,82	94,81	0,43	0,39
Mean of diameter of branch	1,44	1,48	5,20	6,64	7,58	0,43	0,38
Mean of number of branches per a whorl	0,17	0,18	0,77	0,94	1,05	0,37	0,33
Diameter of the crown/height	3055,73	443,88	6186,47	9242,20	9624,23	0,66	0,64
$D_{1,3}$/height	33,45	82,49	258,13	291,13	348,26	0,23	0,19
Diameter of branch/$D_{1,3}$	3774,48	26,47	6478,14	10252,62	–	0,74	0,71

less than four but greater than 2 in order to obtain a more precise estimate for additive genetic variation.

Full-sib progenies

The values of heritability of different characters was calculated by tree different method in field test 396/2 (control-pollinated progenies). Table 4 shows the values evaluated by both Johnsson's and Tigerstedt's methods. The values of heritability of growth characters are lower than those of quality character. The additive variation of height is 20–30 % of the total variation. The heritability of the terminal shoot growth is a little higher. The heritability of the crown diameter and that of the Bole diameter at breast height is less than 20 %. Of the quality characters the heritability of branch angle and branch diameter is equal and almost 40 %. The heritability of number of branches per whorl is less than branch angle but higher than those of growth characters. Also the heritabilities of the ratio of the crown diameter and height and the ratio of the branch diameter and bole diameter are high.

Table 5 shows four different heritability values of height and the branch angle. The first three values are calculated from half-sib groups and the fourth is based on full sib analysis.

In the first column of the table is the case number which shows the mating design included in the analysis. There are 6 different cases. Cases A to A 4 are the four father trees mated with three mother trees. In the first case, all four fathers are included in the analysis. In the second case father group 1 is rejected, in the next case father group 2 and so on. In the case B there are three father groups, each father mated with five mother trees.

The heritability values of both character studied are very similar in cases A and B. In these cases the number of families is greatest. In case A the number is 4×3=12 and in case B 3×5=15. The heritability of height, calculated from father groups, is about 20 % and that of the branch angle about 70 %. When the heritabilities are calculted from mothers, grouped within father trees, the values are about 70 % and 40 %, respectively. Mothers

Table 5. Test No. 396/2. Heritabilities. Becker (1967), Johnsson (1978).

	Sources of variation / cases	Between father-groups	Between mothers within fathers	Between families	Trees within a plot	h^2 Fathers	h^2 Mothers	h^2 Fathers + Mother	h^2 Johnsson full-sibs
Height	A	0,56	2,71	3,17	11,71	0,14	0,72	0,43	0,43
	A1	0,80	3,18	3,78	11,15	0,20	0,83	0,52	0,51
	A2	– 1,23	2,77	1,85	11,06	– 0,34	0,77	0,21	0,29
	A3	1,74	1,16	2,46	12,77	0,44	0,30	0,37	0,32
	A4	0,97	3,07	4,41	11,88	0,24	0,77	0,51	0,54
	B	0,80	2,79	3,50	12,04	0,20	0,71	0,46	0,45
									0,30*
Branch angle	A	20,27	11,00	27,58	80,64	0,73	0,39	0,56	0,51
	A1	30,80	8,54	31,65	91,89	0,94	0,26	0,60	0,51
	A2	13,04	10,12	19,90	67,31	0,58	0,45	0,52	0,46
	A3	4,93	14,86	18,55	88,08	0,18	0,55	0,37	0,35
	A4	32,29	10,47	34,69	75,27	>1,00	0,35	0,72	0,63
	B	18,10	12,63	25,56	71,29	0,71	0,50	0,60	0,53
									0,39*

* = total material

within fathers are mean values of those values calculated separately from father groups or mothers within fathers and they are equal to those calculated from full-sibs using Johnsson's method.

The structure of the population has a strong effect on heritability values. In cases A 1 to A 4 one of the father groups is rejected from each case. Due to this rejection values of heritability varies from negative value to 0,50 in the height character and from 0,20 to over one in the branch angle. The fathergroups 2 and 3 deviate most from mean of the population but in opposite directions. When both of these are present in the data, with one of the other father groups, the value of heritability of the branch angle calculated from fathers is very high but that calculated from mothers is low, when compared with cases A and B. When height is concerned the situation is reversed. When one of these groups is absent, the value of heritability of the branch angle drops drastically.

With the height character, the absence of father group 2 gives negative heritability and absence of father group 3 gives a very high heritability.

Discussion

The evaluation of the genetic variation of forest trees by the concept of heritability has many restrictions. The concept of heritability seems to be sensitive to disturbances caused by the structure of progenies, restriction of the sample population and environmental factors within the test areas. Particularly, values of the heritability calculated from open-pollinated progenies are, in many cases, unreliable. They are also mostly very high overestimations of the genetic variation.

However, on the basis of results of the three experiments studied it is possible to say that the genetic variation of the quality character seems to be higher than that of the growth character. The amount of the additive genetic variation of the quality character is approximately 40–50 % of the total phenotypic variation. In some cases it may rise to 70 %. When the growth characters are concerned, the amount of the additive genetic variation is mostly less than 30 %. In some character and in some cases it can be lower than 10 %.

Due to these contradictions two questions can be raised. These are:

1) Are postulates of quantitative genetics concerning the concept of the heritability valid in empirical investigations of forest tree breeding particularly in open-pollinated progenies?
2) Does heritability signify the amount of the additive genetic variation and in broad sense the total amount of genetic variation?

If the answers are "no", what method is to replace the concept of heritability in the evaluation of the genetic variation of forest trees?

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PART III

PATTERNS OF ADAPTATION IN FOREST TREES

GENETIC STRUCTURE IN NATURAL AND CULTIVATED FOREST TREE POPULATIONS

KENNETH LUNDKVIST

Little is known today about the changes in genetic structure and genetic variation brought about by forest tree breeding programs as compared to natural populations. Modern breeding methods, will increase the possibilities to control and manipulate the genetic composition of cultivated forests. Clonal forestry, with its absence of sexual recombination, signifies the most extreme case of genetic control. Such a control of genetic structure and variation emphasizes the necessity to determine the importance of genetic variation on population and individual level for the adaptability of forest trees.

In the breeding work, the potential of future genetic change will be contained in the breeding populations rather than in the cultivated forests. However, a certain amount of genetic variability must be retained in the planted forests to offer a buffering capacity for unexpected fluctuations in the environment. It is, though, conceivable that a phenotypically homeostatic population needs less genetic diversity to meet with environmental heterogeneity in time and space. One of the central questions in this context is whether such homeostasis is correlated with individual heterozygosity.

Introduction

The adaptation of a plant population to habitat is mainly a genetic process in which its mean fitness is continually increased by natural selection. In cross-breeding species, the optimal adaptation is likely to be an act of balance between specialization to the prevailing conditions and maintenance of the genetic flexibility necessary to meet with environmental change in future generations.

Natural populations of forest trees therefore have a genetic homeostasis in its gene pool as well as genotypes specialized to thei· environment. A genetic diversity among individuals is, however, necessary to secure a buffering capacity of the population against temporal fluctuations in the environment. Finally, the individual's own ability to withstand environmental fluctuations, i.e. its developmental homeostasis, plays a decisive role in the process of adaptation. The genetic structure of natural populations will thus to a large extent be influenced by the species' adaptive strategy.

In contrast to the situation in natural populations, domestication of forest trees by breeding will upset the genetic system of the species and its gene pool will we sub-divided to allow high genetic homeostasis in the breeding populations, while the level of genetic variability in the production populations

may be reduced to allow specialization, and high fitness under the prevailing environmental conditions. It is thus reasonable to anticipate a different optimum genetic structure in cultivated populations than in natural ones, and improvement of the plant material will to a higher extent be based upon the interaction between genetic structure and the specific requirements of the planting site.

Advanced breeding methods will enhance the breeder's control of the genetic structure in cultivated forests and it is imperative that the relationship between genetic structure and adaptation to heterogenous environments is better understood. Research should therefore be concentrated to the optimum genetic structure within the limits of the species' genetic system in nature as well as to the changes in genetic structure induced by the breeding work and its effects on yield and stability.

Adaptive strategy and genetic structure

In forest tree species, as for most organisms, the degree of genetic variability is controlled by their genetic system, i.e. their reproductive, breeding and chromosomal systems, all of which are under genetic control. Being present in the populations, either in a free or a potential state, the variability is a prerequisite for their evolutionary response to natural selection. The optimum strategy of adaptation, i.e. evolutionary response, in a heterogenous environment will be to optimize the mean fitness of the population, and its genetic structure will be determined by the species' genetic system as well as the pattern of environmental variation.

A method for determining the optimal genetic structure of a species was proposed by Levins (1962) in which the fitness in different environments is graphically represented by fitness-sets. For small niche differences the fitness set will be convex, while a concave set suggests large niche differences.

The different optimum population structures predicted by Levins' model are summarized in table 1. We observe that when niche differences are small in comparison with individual homeostasis, i.e.a convex fitness set, the optimum strategy will be a monomorphism for an intermediate phenotype. In case of large niche differences, i.e.a concave fitness set, the optimal genetic structure for fine-grained environmental variation will be a monomorphism for specialists to the most common type of environment, while a coarse-grained variation will favour a polymorphism with specialists to each type of environment.

Table 1. The resulting optimal genetic structures for the different adaptive strategies manifested in figure 2.

	Arithmetic mean fitness Fine-grained variation	Geometric mean fitness Coarse-grained variation
Convex $\alpha(X)$ similar environments	Monomorphic for intermediate phenotype "jack-of-all-trade"	Monomorphic for intermediate phenotype "jack-of-all-trade"
Concave $\alpha(X)$ dissimilar environments	Monomorphic for specialists to most common type of environment	Polymorphic with specialists to each type of environment

(From Roughgarden 1979, slightly modified).

With reference to the expectations derived from this model, Levins (1963) predicted the optimum phenotype, the optimum population structure and variation patterns along geographic gradients for natural plant populations (Table 2). Interpretation of tables 1 and 2 indicate that, in case of large niche differences, heterogeneity *in space* will be experienced as fine-grained variation, while heterogeneity *in time* is experienced as coarse-grained by a plant population.

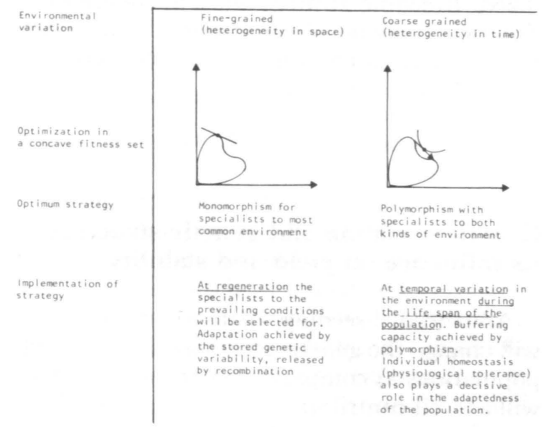
With respect to the patchiness of the environment, it is considered that coarse-grained species can exist in two or more niches, but individuals spend most of, or all, their lifetime in only one of these. Fine-grained species, on the other hand, are such that the individuals can explore and exploit all, or nearly all, the patches in the environmental mosaic (c.f. Soulé 1976 for references).

It is then conceivable that a forest tree population will experience the environment as coarse-grained when exposed to between-year fluctuations on the site conditions, while hetetrogeneity in space, i.e. the fine-grained situation, is manifested to the population at the time of regeneration when a large number of zygotes explore and exploit the various niches in a heterogenous environment (Table 3).

Table 2. Optimum phenotype, optimum population structure and variation pattern along geographic gradients for natural plant populations. (From Levins 1963)

	Difference small between niche optima as compared to individual homeostasis	Difference large between niche optima as compared to individual homeostasis	
		Environment heterogenous in space	Environment heterogenous in time
Optimum phenotype	Intermediate between optima in the two niches	Optimun phenotype for more frequent niche (specialized to one niche)	Specialized to one or the other niche
Optimun population structure	Monomorphic of moderate fitness in each niche	Monomorphic, specialized	Polymorphic mixture of specialized types
Pattern along geographic gradient in niche frequency	Continuous cline in phenotype	Discrete races separated at some critical value of niche frequency	Cline in proportions of same polymorphic types

Table 3. Optimal strategies of adaptation for a plant population subjected to spatial and temporal environmental fluctuations. Niche differences large in comparison with individual homeostasis.



In the latter case, the best strategy to meet a spatially heterogenous environment will be to have a sufficient genetic homeostasis for production of a wide array of genotypes, among which natural selection will favour those which are best adapted, i.e.specialized, to the conditions of the patch where the seed happened to fall. In the former case, heterogeneity in time, the optimal strategy will be a polymorphism where the phenotypes have different fitness optima. The population will thus be buffered against environmental fluctuations between years. It is likely, as well, that the individual's physiological tolerance plays a decisive role in the adaptedness of a population to both temporally and spatially heterogenous environments.

Changes in genetic structure induced by breeding – implications for breeding strategy

The changes in genetic structure brought about by the domestication of a forest tree species is mainly due to the following factors: disruption of the species' genetic system and a subdivision of its gene pool into breeding populations and production populations.

The species' breeding system will be upset by introducing vegetative propagation into breeding work, its mating system upset by bringing plus trees together in seed orchards and applying flowering stimulation and selective pollination control. In advanced breeding generations it is conceivable that the genetic load of the species may be reduced to such an extent that self-fertility is increased without any adverse effects on the vitality of the progeny. Such a process will require a large number of generations of mass-selection, but experiments with selfing of redwood

(Libby et al. 1981) have shown that some progenies from selfing perform as well as, or better than, their outcrossed relatives. Whatever the reason, these results suggest that increased homozygosity is sometimes tolerated in forest trees.

Furthermore, if optimization of fitness in different environments should involve accumulation of different sets of favourable genes, these gene combinations would be disrupted in the second generation following hybridization, shifting the genetic structure of cultivated stands still further from that of natural ones. Intercrossing of selected plus trees from different regions or from different provenances might have such an effect on population structure.

Finally, the genetic control of recombination frequencies in forest trees is virtually unexplored, but working with a narrow genetic material, e g in sub-lines of a breeding population, may well have effects on recombination frequencies, and thus on the genetic structure of the progeny.

However, the major effects on forest tree population structure caused by breeding work will be due to the sub-division of the species' gene pool into breeding and production populations, respectively. The evolutionary success of a population is very much dependent on its genetic homeostasis, substantiated by its stored genetic variability. However, the ability to evolve constitutes at the same time a genetic load (c.f. Wallace 1970, p. 35), manifested in the multitude of recessive deleterious genes which to some extent will be expressed in homozygous progenies. In cultivated stands, this genetic load is no longer necessary, since the ability to evolve will be consigned to the breeding populations. Optimum genetic structure, no longer involving genetic homeostasis, will thus differ from that of natural stands in this context as well.

With respect to forest tree breeding, Tigerstedt (1974) stated that . . . "breeding for . . (ecological tolerance). . means a deliberate effort to preserve population heterogeneity . . . in the future it may also involve active breeding efforts whereby diversifying selection is applied". This statement may be further elaborated on by taking into account the conclusions from Levins' theory presented above: For Scandinavian forest trees, the environmental variation is such that niche differences must be considered to be large with respect to individual homeostasis, as well for spatial as for temporal variation (c.f. Eriksson 1982). It is thus the genetic structure connected with concave fitness sets that is of the highest interest for forest tree breeding (c.f. Table 3). The conclusions relevant to breeding strategy may then be summarized as follows:

For the heterogeneity in space, the breeder must apply diversifying selection to meet the specific requirements of the different planting sites. The genetic diversity necessary for this "evolution" is contained in the breeding populations, while a reduction in the genetic variation will take place in the production populations due to "specialization". This effect would arise e.g. from a further delineation of present breeding zones (c.f. fine-grained variation in table 3), aiming at discrete varieties of planting material.

However, population diversity must not be reduced to such an extent that yield is adversely affected or the buffering capacity against environmental fluctuations jeopardized. Breeding strategy must therefore take genetic polymorphism and individual homeostasis into account as well (c.f. coarse-grained variation in table 3).

Genetic variation and genetic structure – its influence on yield and stability

Advanced breeding methods in forestry will change the genetic structure of cultivated populations as compared to natural ones, but will as well contribute to a better *control* of the genetic structure of the stands. Since optimum structure presumedly will differ from natural ones, one major objective of the breeder must be to gain information about the causal relationship between the level of genetic variation as well as genetic structure and the stability and yield of the populations.

Genetic variation at the individual level

Since plant are stationary and cannot avoid temporary unfavourable conditions by moving to another niche, individual homeostasis is of importance mainly when temporal variation is encountered, i.e. in coarse-grained environments. To some extent, the individual stability may also be significant in adaptation to fine-grained variation, e.g. for the ability to colonize niches differing from that of the parental population.

It is generally considered that there is a close relationship between the individual genetic variation, i.e. heterozygosity, and homeostasis. The observation that polyploids demonstrate a higher degree of phenotypic stability than their diploid ancestors, e.g. as invaders of disturbed habitats, may serve as an example to illustrate this point of view. The increased stability may be ascribed to the balanced hybridity of polyploids, i.e. the preservation of a high level of heterozygosity due to a reduced genetic recombination (Tal 1980, p. 61–62. 67).

Several examples of circumstantial evidence for increased homeostasis in heterozygotes have been presented for agricultural species as well (c.f. Simmonds 1977, p. 118). With respect to forest trees, the promising results with species hybrids of larch, pines and poplars are believed to be due to heterotic effects (Stern and Roche 1974).

Genetic variation at the population level

Experience from the performance of agricultural species, mainly physical mixtures of pure lines of inbreeders, suggest that as well yield as stability is positively correlated with the genetic heterogeneity and heterozygosity of a population. Additional support for this view is found in the experiments by Allard and Adams (1969) showing that bulk propagated lines of barley over several generations evolved a mutual adaptation, by Harper (1964) called" ecological combining ability". The yield of such a mixture does exceed that of randomly combined lines.

The exact nature of the interactions between phenotypes promoting yield and stability in heterogenous populations is not yet identified. It is, however, conceivable that the different constituents of a mixture may exploit the resources more efficiently, thus giving relative total yield higher than unity (c.f. Spitters 1980 for references).

Suggested research

The considerations on genetic structure of populations, and implications for breeding strategy which have been treated, are of course applicable to forest tree breeding in general. However, the problems to be solved are emphasized by the restraints on genetic structure imposed by clonal forestry.

Furthermore, vegetative propagation of selected phenotypes offers a means to focus the experiments on specific phenomena related to genetic structure, eliminating errors due to within-family variation, etc. Consequently, suggestions on problems to be studied and suitable experiments to be carried out, will be made primarily with respect to clonal forestry.

In this context, the critical matters at issue appear to be:

- to what extent can genetic variability in production populations be reduced without jeopardizing stability?
- what is the optimum genetic structure in cultivated populations with respect to yield and survival?
- methods for early selection of clones with high adaptability and production.

Determination of individual homeostasis

Transferring the genetic homeostasis of the species to the breeding populations means that the genetic variability of the production populations may be reduced, the lower limit of population heterogeneity mainly being determined by the homeostasis of the individual phenotype and genetic polymorphism (c.f. the optimum strategy for heterogeneity in time, table 3).

Several methods for measuring individual stability which have been used for agricultural and herbaceous species are applicable to forest trees. In the following sections are cited a number of analytical methods which generally apply to field experiments. It is, however, important in this context to observe the possibility, in some cases even the necessity, to make model experiments in nurseries or climate chambers (c.f. Eriksson 1980, 1981) before large field experiments are made.

The main advantages offered by experiments in climate chambers, as pointed out by Dormling (1980), are that the growth conditions can be reproduced with a high precision and several environmental parameters can be kept constant while one or two parameters are varied in different treatments. The prospects of assessing the degree of stability for individual clones are indeed promising if the opportunities offered by climate chambers are utilized.

Measuring adaptation by linear regression

Finlay and Wilkinson (1963) and Eberhart and Russell (1966) have suggested methods for measuring variety adaptation by using linear regression of yield on the mean yield of all varieties for each site and the deviation from regression. These are statistical measures designed to demonstrate and select superior varieties or clones, with respect to low genotype × environment interaction.

The critical issue appears to be the description of the environmental parameters of the site: . . . "a good estimate of the regression coefficients can be obtained from a few environments if they cover the range of expected responses" (Eberhart and Russell 1966). This method is suitable for estimating genotype × environment interaction of forest tree clones on field trials, but climate chamber experiments should be used as well, since the opportunities are then better to study only one or a few environmental parameters, the range of which can be determined before the experiment. This is of some importance since experience from field crops have shown that seasonal variability is a decisive factor of the environment, i.e. the same site and varieties will produce different mean yields in different years. Genotype × environment interaction will then give information about the stability of the vareties, or clones, but little is known about *what* environmental factors the homeostasis is connected with.

Stability measure developed from statistical inference

To clarify the concept of individual stability associated with low genotype × environment interaction, Hanson (1970) has suggested a stability measure to be constructed by using the deviations of expected yield of a a genotype from its yield if totally stable. The measures integrates the information from regressions and deviations from regressions which were discussed above, and should be valuable in attempts to quantify phenotypic stability of clones in field trials.

Quantification of individual stability

Levins' model of fitness may well be used in experiments to study individual as well as population fitness in different environments. There is, however, one difference to be considered, namely the quantification of fitness in experiments. The Darwinian fitness used in Levins' work, often defined as "the average contribution which the carriers of a genotype, or a class of genotypes, make to the gene pool of the following generation, *relative to* the contributions of other genotypes", is a relative measure, and there is a need to find a measure which permits quantification of the adaptedness of a genotype or a population to its environment.

In this context, Ayala (1969) suggests "the ability of the population to transform the available materials and energy into living matter of its own kind", i.e. dry matter production per unit time and area, as a measure to quantify adaptedness to a given environment.

McNaughton (1970) used this method to study the adaptedness of different species and populations of Typha. The results indicated that *Typha* species with an adaptive strategy of generalization were situated within the fitness sets, while species specialized to the environmental parameters that were studied defined the limits of the fitness sets.

Similar experiments should be made with forest tree clones in a series of different environments, generated in climate chambers. Analysis of single clones in two similar environments would presumedly yield convex fitness sets, while increasing environmental differences is expected to create increasing concavity, provided the genotypes are adapted to the environmental parameters that are studied. In such cases, where the environmental differences create concave fitness sets, it is decidedly of interest to compare the adaptedness of monoclone cultures and different clonal mixtures.

The long generation time of forest trees obstructs conclusive experiments on the postulated relationship between heterozygosity and individual stability. However, clones originating from the Åkersberga trial outside Stockholm (c.f. Eriksson et al. 1973, Andersson et al. 1974) constitutes a suitable material for experiments aiming at the quantification of stability for clones with different degrees of heterozygosity. Crossings between a number of parental trees have been made for two successive generations, generating progenies with inbreeding coefficients ranging from 0 to0.75, the latter being the second generation progeny by selfing. Presumedly, such progenies are more homozygous than those from open pollination, and should provide opportunities to study the alleged connection between heterozygosity and stability.

Stress experiments

In connection with comparisons between the adaptedness of different clones, information can be obtained about the range of tolerance for various environmental parameters. If a correlation between tolerance-range and genotype × environment interaction is demonstrated, stress experiments would be an efficient method for early selection of clones with high stability for different sites and between year variation. These experiments should include such parameters as water- and nutrition supply, light and temperature, all of which can be kept under control in climate chambers.

For the purpose of early selection, it is, furthermore, of high interest to extend the stress experiments to include physiological differences between clones demonstrating high and low genotype × environment interaction, respectively. The phenotypes studied in experiments are the products of the genotype and its reaction with its environment, with physiological processes being an intermediary step (Figure 1). The buffering capacity of the individual, expressed as homeostasis, is most certainly mediated by those processes in the plant cells. Any detectable physiological difference would therefore serve as an efficient criterion for early selection of homeostatic clones. This is one further

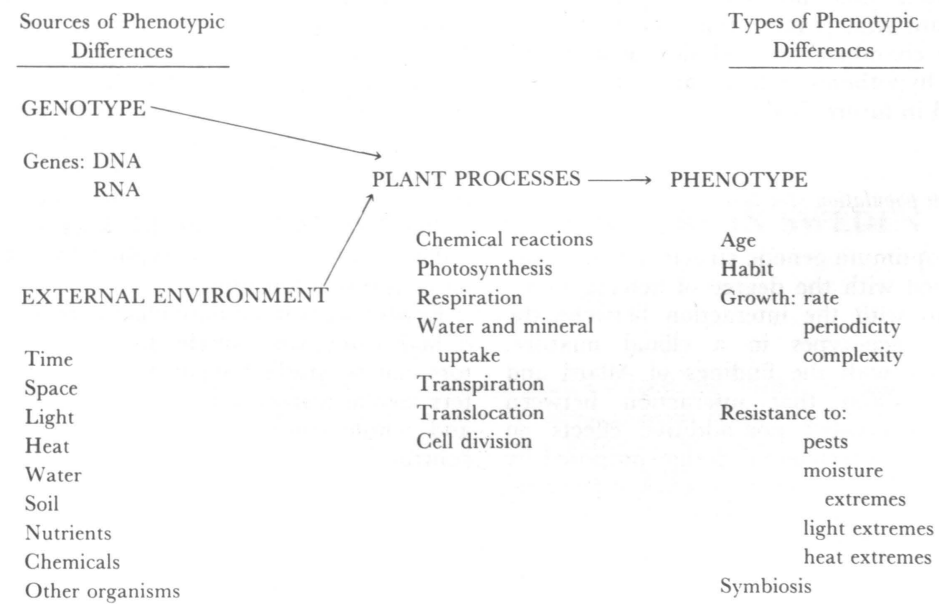


Figure 1. Relationship of an individual's phenotype to its genotype and environment. Phenotypic differences of the individual become apparent as physiological processes occur in the internal environment of plant cells. (From Spurr and Barnes 1980).

incitement for climate chamber experiments on genotype × environment interaction of forest tree clones.

Determination of population homeostasis and optimal population structure

Apart from the individual homeostasis, population heterogeneity will be the main factor promoting adaptedness in clonal forests (c.f. table 3). Another main issue for research must therefore be to determine the lower limit of heterogeneity in clonal mixtures and the optimal genetic structure with respect to yield and stability.

Population homeostasis

Concerning homeostasis, the experiments outlined for single clones in climate chambers are applicable to clonal mixtures as well. In addition, long term field trials must be established in which yield and stability of different clonal mixtures are compared with monocultures and seed plants. Such experiments may be established with a general *à priori* hypothesis that heterogenous populations are more stable than monocultures, but it is also important that results from experiments in climate chambers are included in more detailed hypotheses which are further developed in future field trials.

Optimum population structure

The optimum genetic structure is not only connected with the degree of heterogeneity, but also with the interaction between the different genotypes in a clonal mixture, analogous with the findings of Allard and Adams (1969) that interaction between genotypes created non-additive effects on yield. The experimental design proposed by Spitters (1980), in which a series of mixtures is produced by replacing plants in a monoculture with different proportions of another clone (or variety) until the other monoculture is obtained, seems to be suitable for forest tree clone experiments.

If the yield of each clone is plotted against the proportion it occupies in a mixture, a straight line relationship indicates that the clones have equal competitive abilities for the shared resources. Deviations from linearity, on the other hand, would mean that the clones occupy in part different niches and the mixture will thus exploit the resources more efficiently than either monoculture. Measuring the yield as biomass production per unit time, such experiments are suitable on a short term basis, e.g. in climate chambers.

Long-term experiments, with an explicit *à priori* hypothesis, intended to study optimum population structure and yield, are difficult to design and should be preceded by short-term experiments on competition and interaction among genotypes.

Conclusions

Changes in the genetic structure, imposed by domestication of forest tree species, will carry significance for the breeding strategy. The breeding populations will carry the genetic variability necessary for adaptation to heterogeneity in space, thus permitting a reduction of the genetic variability of the production populations.

The lower limit of population heterogeneity, being determined by individual homeostasis and genetic variation among individuals, is of particular interest for clonal forestry. Optimum population structure must be studied in experiments, primarily with respect to individual homeostasis, population heterogeneity and interaction among genotypes. Field experiments are necessasy, but should be preceded by model experiments in order to formulate more explicit hypotheses on causal relationships.

Experiments in climate chambers are then of high value, since single environmental factors can be studied separately and parameters such as water- and nutrition supply, light and temperature can be kept under strict control.

Acknowledgement

I am indebted to professors Gösta Eriksson and Peter Tigerstedt for valuable comments and critical reading of the manuscript.

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ECOLOGICAL GENETICS OF CONIFERS IN SWEDEN

GÖSTA ERIKSSON

In this paper the results from studies of the ecological genetics of conifers in Sweden are compared with the hypothesis presented by Levins (1963). This supposes that the taxonomic species responds to heterogeneity in time by developing clinal variation along ecological gradients and polymorphisms within populations. All results supported this expectation. More research is needed to test the existence of a heterogeneity in space.

The results were obtained both from field trials and experiments in climate chambers.

Introduction

Gene ecological research with forest trees is now a prominent part of Swedish forest genetics. The whole field will not be reviewed in this paper, which concentrates on work carried out over the past decade.

Mostly the research has aimed at setting

guidelines for efficient forest tree breeding. This means that the formulation of new concepts and theories in gene ecology has not been attempted. Some of the projects making use of isozyme genes constitute exceptions to this, but these investigations will not be treated in this paper.

The rules for transfer of seeds have contributed greatly to the economy of Swedish forestry. A utilization of the recently suggested method to test plus tree candidates with respect to frost hardiness is expected to promote rapid progress in Swedish forestry (Jonsson 1980).

Levins (1963) presented a table of the expected intra- and interpopulation structure for large and small differences in niche optimum as compared to individual homeostasis. By individual homeostasis is understood the ability of an individual to tolerate transitory fluctuations in the environment.

Climatic adaptation

Population level

Extensive provenance research was carried out for all species of major interest in Swedish forest tree breeding. These are *Picea abies, Pinus contorta* and *P. sylvestris*. For *Pinus sylvestris,* the investigation by Langlet (1936) on dry matter content in different provenances soon became a classic. During the late forties Eiche (1966) established a nation-wide series of field trials with the purpose of elucidating the transfer effects. Whenever possible, the populations included at each experimental plantation originated from northern as well as southern localities. Transfers upwards and downwards in altitude were also made. Plant damage and survival were recorded every year up to an age of 20 years.

Eriksson et al. (1980) reported on the results obtained from the northern trials as well as on four trials established by the Institute for Forest Improvement. The effect of transfer on plant survival can easily be seen in Fig. 1. The survival increased with increasing latitude of origin. Similarly, the lower the altitude of origin the lower the survival. The strong latitudinal and altitudinal influence on the survival called for a stepwisen regression analysis with the change in latitude and altitude as well as their squares and cross product as independent variables, plant mortality being the dependent variable. The results of this analysis are illustrated in Fig. 2. The latitudinal transfer exerts the greatest influence on survival. For this character there was a good agreement of the results from all the trials in northerly Sweden. Therefore, a parallel regression analysis was carried out. The following equation was obtained:

$$\text{Plant mortality} = k + 10.8\triangle\text{lat} + 3.0\triangle\text{alt} \quad (R^2 = 0.98)$$

This means that the transfers in latitudinal and altitudinal directions can be used to obtain a particular degree of survival. This also means that latitude and altitude well describe the climatic conditions of a particular site. An extrapolation of this conclusion to other parts of Scandinavia not covered by the trials is obviously not permissible. This was well reflected by some of the Norwegian populations with an origin west of the Scandinavian mountain chain. Their survival corresponds to the survival of Swedish populations originating 3–5 degrees of latitude further south.

The results obtained suggest a clinal variation in *Pinus sylvestris* with respect to plant mortality. A clinal variation for plant height was also noted by Remröd (1976). Similar results were previously reported by Johnsson (1971). He estimated the growth reduction at 0.8 per cent per degree of latitude southward transfer. In some south and central Swedish trials some of the German populations showed a promising growth up to the age of 20 years when they became severely damaged by wet snow.

Transfers over the same latitudinal distance in northerly Sweden (>60°) affect plant mortality more dramatically than tree height in *Pinus sylvestris*.

The implication of this for the breeding of *Pinus sylvestris* is that many climate zones must be recognized. If this is not done an intolerable variation of survival within a zone would be the result. In each zone, breeding for survival must be given the highest priority. Therefore a transfer southward must take place. This will cause a minor loss of growth at the individual tree level. This loss of yield is more than compensated by the increase in yield due to higher number of surviving trees.

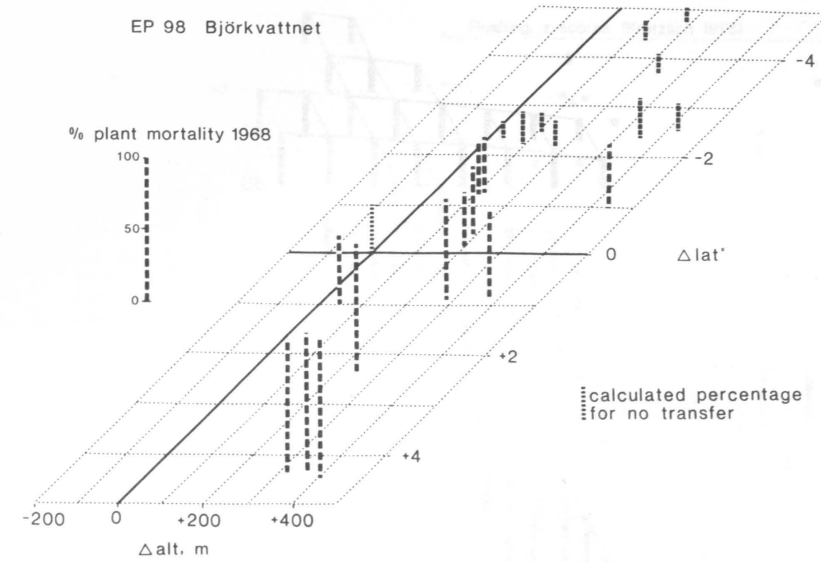

Figure 1. The percentage of dead plants in a provenance trial of *Pinus sylvestris* at Björkvattnet lat 63.43, alt 460 m. The locations of the columns in the grid indicate the altitudinal and latitudinal transfers. Thus, the columns below the horizontal line indicate populations that were moved in a northward direction. Columns to the right of the diagonal axis show provenances that were moved to a higher altitude. Two types of screen are used in the columns, one for the provenances showing a lower percentage plant mortality than that calculated for no transfer, and the other for the remaining provenances.

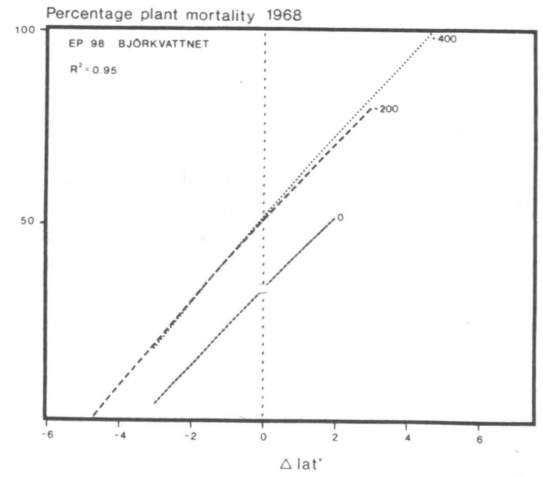

Figure 2. The effect of latitudinal transfer at constant altitudes on the percentage of dead plants at Björkvattnet. Plus signs indicate a transfer upwards or in a northward direction. The R^2 =square of the multiple correlation coefficient.

The variation in critical night length for bud set in *Picea abies* depended almost exclusively on the latitude and altitude of the populations according to Dormling (1979). Her study comprised a latitudinal range of 47–68°. The same type of regression analysis was performed by her, the R^2-value amounting to 0.93 (Fig. 3). Also in this case a seemingly smooth cline existed.

Similar results were also obtained for frost hardiness of *Pinus contorta* studied under field conditions (Hagner and Fahlroth 1974, Lindgren et al. 1980) as well as in climate chambers (Jonsson et al. 1981). One example from the latter study is illustrated in Fig 4.

To test the existence of a latitudinal influence on bud flushing in the provenances of *Picea abies* at Bornsjön studied by Krutzsch (1975), Fig. 5 was drawn. Krutzsch calculated what he called z-scores to make possible

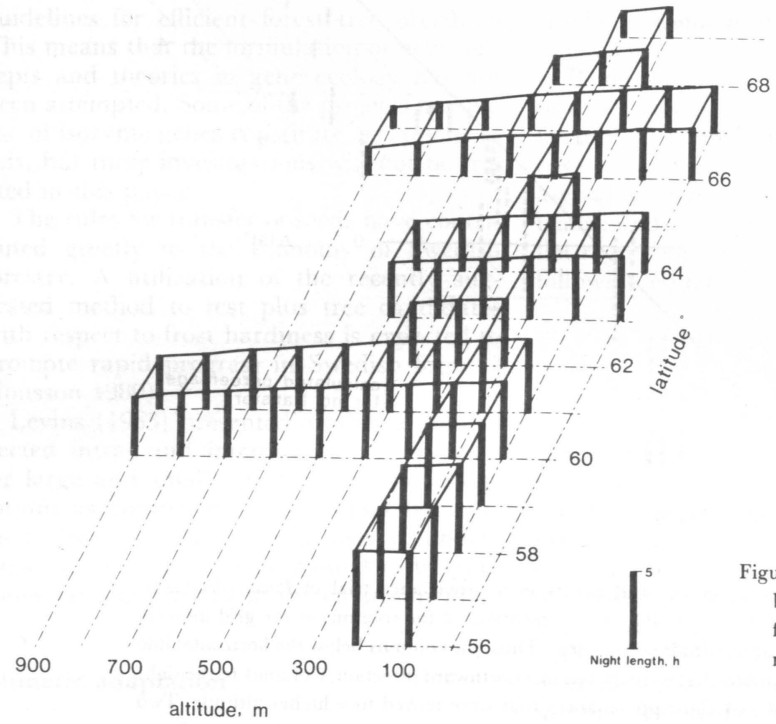

Figure 3. Critical night length for bud-set in *Picea abies* seedlings from different origins in Scandinavia (after Dormling 1979).

a comparison of the data from two trials, in which

$$Z = 500 + \frac{X_i - \bar{X}}{S},$$

X_i is the value of the provenance according to the classification system for bud flushing of Krutzsch (1973). In Fig. 5 a clinal variation with latitude can be excluded. For the provenances in the range of latitude 46–50° the z-scores were plotted against the longitude. As seen from Fig. 6 a fairly good relationship was obtained. The rest of the material did not vary much with respect to longitude.

It is easy to understand that a northern – southern cline as well as a western – eastern cline may be obtained since the climate changes in these two directions. Especially within Sweden the northern – southern climatic gradient is pronounced (Liljeqvist 1970). This is probably due to the absence of an eastern – western mountain chain in Sweden or any features of the landscape creating conditions, like high or low temperature valleys. The occurrence of such valleys seems to be the reason for the low fitness to the regressions with geographical variables observed in the Nordic provenance trials of *Picea abies* reported by Dietrichson et al. (1977). Similar results were obtained by Persson (personal communication) for the 1 100 provenances of *Picea abies* studied in three field trials in Sweden. In both reports tree height was the variable studied.

Family level

In the previously discussed Eiche series of *Pinus sylvestris* each population was represented by 20 open pollinated families. Results from one of the trials – Nordanås lat 64° 19' alt 400 m – were reported by Eriksson et al. (1976). At this trial 16 populations were represented by 20 open pollinated families each planted in three replications of which each consisted of a 15-tree row plot. Data on survival and tree height were presented. In spite of the low number of replications, significant differences in plant mortality between the families were observed in four populations. In populations with low or high percentages of plant mortality a significant within-population difference cannot be expected.

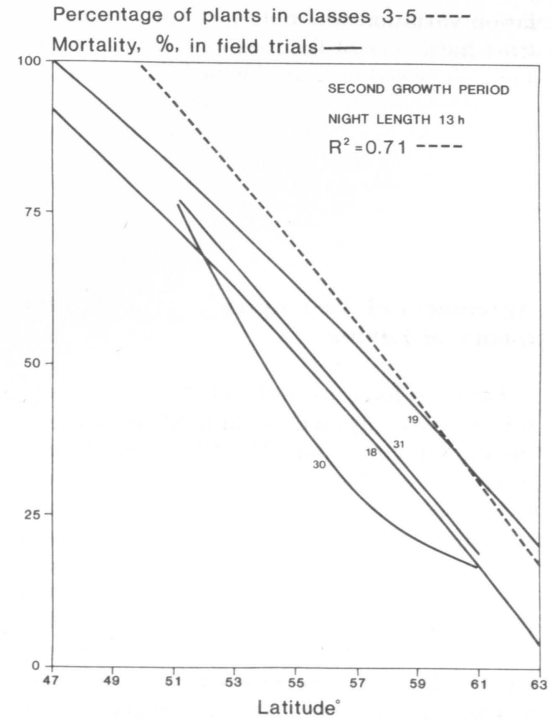

Figure 4. Regression of percentages of plants in classes 3–5 on latitude. Freeze testing at –10°C was performed after treatment with 13 h night in second growth period. This regression obtained in a phytotron is compared to regressions of percentage mortality on latitude for field trials 18 and 19 (based on results obtained by Lindgren et al. 1976) as well as trials 30 and 31 (based on results placed at our disposal by Dr. S. Hagner, Swedish Cellulose Company). The curves are drawn for an altitude of 800 m. Trials: No 18-Kompo, Nattavaara, lat 66°44'N, alt 375 m; No 19-Lappeasuando, Svappavaara, lat 67°30'N, alt 390 m; No 30-Lappträskberget, lat 65°55'N, alt 225 m; No 31-Volgsele, lat 64°46'N, alt 435 m.

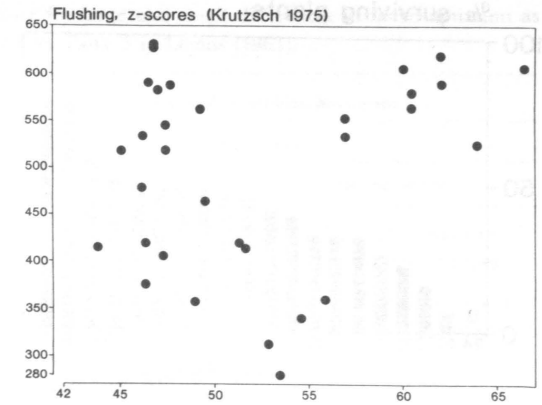

Figure 5. The relationship between flushing of *Picea abies* provenances and their latitudinal origin. The study was carried out at Bornsjön lat 59.23, long 17.77, alt 15 m by Krutzsch (1975).

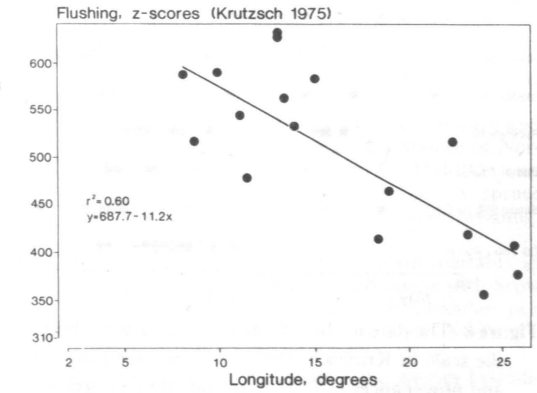

Figure 6. The relationship between flushing of *Picea abies* provenances from latitudes 46–50° and their longitudinal origin. The study was carried out at Bornsjön lat 59.23, long 17.77, alt 15 m by Krutzsch (1975).

The within-population difference in tree height was analyzed in 13 populations. Of these, five showed a significant within-population difference.

An extreme example of the variation in plant mortality is shown in Fig. 7 based on data from a trial at lat 68° 20' alt 440 m. In this case each family was replicated only twice.

In open pollinated families of *Picea abies*, too, great variations were noted in this case with respect to flushing (Fig. 8). For one population from Benus, the arrival at stage No 3 according to the classification system introducted by Krutzsch (1973) showed an amplitude of three weeks. This occurred with high repeatability from year to year and trial to trial.

Characters of high adaptive value, such as bud flushing and bud set in *Picea abies*, show polygenic inheritance (Eriksson et al. 1978) which favours the development of clines with the individual populations polymorphic.

In *Pinus contorta* a significant within-popu-

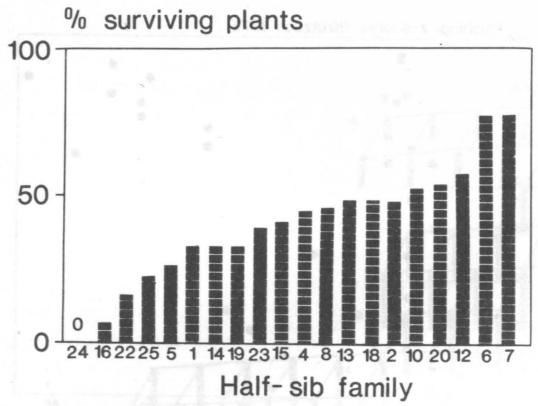

Figure 7. The percentage plant survival of single tree progenies of a *Pinus sylvestris* population originating from Korpilombolo lat 66.88, alt 175 m studied at Kåbdalis lat 66.27, alt 440 m.

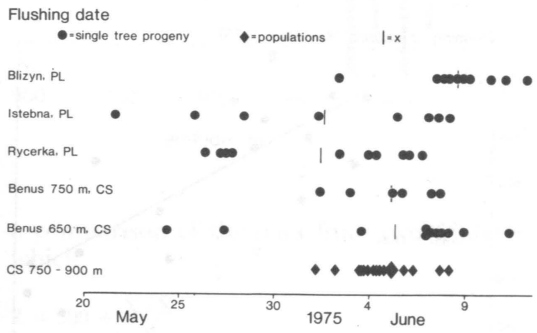

Figure 8. The date for bud flushing (stage 3 according to the scale in Krutzsch, 1973) of single tree progenies and provenances of *Picea abies* studied in a nursery at Bogesund 59.40, alt 15 m. PL = Poland, CS = Czechoslovakia.

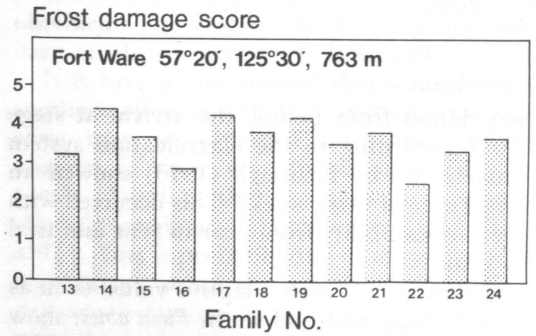

Figure 9. Frost damage score after freezing to –10°C of single tree progenies of the *Pinus contorta* population, Fort Ware. The scoring of frost damage comprised 6 classes: 0 = no damage, 5 = dead apical bud.

lation variation was obtained with respect to frost hardiness of families exposed to –10°C. Fig. 9 is based on unpublished data by Jonsson et al. Similar results were obtained for *Pinus sylvestris* cultivated in green-house and artificially freeze tested (Andersson and Rosvall, personal communication).

Agreement of the empirical data with the theory of Levins

First we have to find out if the niche optimum as compared to individual homeostasis is large or small. With the wide area of distribution of Norway spruce, Scots pine and lodgepole pine, no single genotype can possess such individual homeostasis that it will survive under all environmental conditions occupied by the species. Heterogeneity in time is especially pronounced. The weather conditions certainly vary over the life time of a tree. Moreover, the weather conditions vary at the phase of regeneration, a phase of crucial importance for the survival of a species. Thus, for our forest tree species under study, the difference in niche optimum as compared to individual homeostasis must be regarded as large.

The gene ecological investigations carried out so far fully agree with the expectation of the intra- and interpopulation structure with a heterogeneity in time as presented in Table 1. All investigations in which variation along an ecological gradient was studied have disclosed a clinal variation. Whenever tested a polymorphic intrapopulation structure was obtained in the investigations carried out.

Polymorphism may be regarded as an insurance of the population against changes of the environment. Thus, there will always be some individuals which have high fitness values.

No studies have so far been conducted to investigate the existence of discrete races along an ecological gradient. Since our conifers cover a wide range of site conditions there should be possibilities for the development of races adapted to particular site conditions.

The migration caused by pollen and seed dispersal over considerable distances may counteract the development of populations genetically adapted to particular site condi-

Table 1. The expected intra- and interpopulation structure for large and small differences in niche optimum as compared to individual homeostasis. The table is based on Table 3 in Levins (1963).

	Difference in niche optimum as compared to individual homeostasis		
		large=concave	
	Small = convex	heterogeneous in space	heterogeneous in time
Optimum population structure	Monomorphic of moderate fitness	Monomorphic specialized	Polymorphic mixture of specialized types
Pattern along ecological gradient in niche frequency	Continous cline in phenotype	Discrete races separated at some critical value of niche frequency	Cline in proportions of same polymorphic types

tions. However, if the interpollination between adjacent populations is largely prevented by differences in time of receptivity and pollen dispersal of the two populations, the evolution of discrete races may be promoted (cf. Gullberg 1982).

An adaptation of populations to different site conditions is not a purely academic question but has bearing on the strategy to be pursued by the breeders. If such an adaptation has taken place, the number of breeding zones must probably be increased or clones be actively selected that show little interaction with site conditions.

Investigations of a possible adaptation in space (= site conditions) are urgently needed.

On the family level it has been shown that genotype × site interaction exists (Jenkinson 1974, Johnstone et al. 1978). This does not mean that an adaptation to site had taken place but rather that the populations are polymorphic with respect to the characters tested.

Acknowledgement

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FRACTIONATION OF SEED ORCHARD SEEDS BY WEIGHT DOES HAVE GENETIC IMPLICATIONS

DAG LINDGREN

Seeds from a Scots pine clonal seed orchard were weighed one by one. A partition of causes of variation gave 43 per cent between clones, 12 per cent between ramets within clones, and 45 per cent between seeds within a ramet. In a heavy or light seed fraction the representation of clonal progenies will be highly unequal. The genetic diversity of a seed lot will be reduced by fractionation. It is recommended to avoid unnecessary seed fractionation of seed orchard seeds.

Introduction

Seed fractionation is a common practice in Sweden today. It is known that seeds from individual trees in stands may differ considerable, and therefore seed grading of stand seeds by weight may have genetic implications and reduce the genetic base (Hellum 1976; Silen and Osterhaus 1979). Use of seeds from clonal seed orchards become more and more common, and the possibility of a reduced genetic base is more severe in seed orchard seeds, where the limited number of clones constitute a severe restriction.

The aim of the present investigation was to find out to what degree genetic composition may change following weight fractionation of seeds from a clonal seed orchard.

Material and methods

The investigation deals with seeds collected in a Scots pine (*Pinus sylvestris* L.) seed orchard at Långtora (lat. 59°43', long. 17°08', alt. 15 m), approximately 30 km west south west of Uppsala. The seed orchard comprised 36 clones, (9 with a Finnish origin). The flowering was studied intensively in 1973–1975. The results (as well as more details of the seed orchard) were published by Jonsson et al. 1976. Seeds from open pollination were collected in 1974. For each clone two ramets were harvested.

Seeds from two ramets were not available. The corresponding two clones are omitted from the ANOVA. Empty seeds were removed by blowing. Twenty seeds from each ramet were measured individually on a balance with a precision of approximately ± 0.1 mg. Seed weights are expressed in mg.

For the statistical analyses the following model was used:

$y_{ijk} = \mu + c_i + r_j + s_k$

y_{ijk} = Seed weight
c_i = Contribution of clone i
r_j = Contribution of ramet j (within clone i)
s_k = Contribution of seed k (within ramet j and clone i) including error

c_i, r_j and s_k are regarded as random variables with variances σ_c^2, σ_r^2 and σ_s^2. It is an hierarchic design (nested design).

The effect of a (hypothetical) weight fractionation of a seed population consisting of the 20 seeds weighed from the 68 ramets were studied. The probability of two seeds taken at random from a fraction (with replacement) originating from the same mother clone was calculated. The inverse of this value may be denoted "effective clone number". If all clones contribute equally the probability of two seeds having the same mother is the inverse of the clone number. The "effective clone number" divided by the actual clone number (34) is denoted "relative effective clone number" (N_r). Also, the probability that one seed taken from a light fraction has the same mother as one from a heavy fraction was calculated. A "shared proportion" was calculated as a measure of to what extent the same mother gave contribution to both the light and heavy fraction. Expressed in another way, the smallest of the contributions to the heavy and the light fraction were added for all clones.

It must be pointed out that the measures used to quantify the fractionation effect do not take the random variation of sampling into consideration, and thus interference with the real population introduces an error.

Results

Some of the seed weight distributions obtained are presented in Table 1. An analysis of variance gave the results shown in Tables 2 and 3.

There are evidently considerable differences between clones, and there is almost no overlap between seeds from the clone giving the lightest and the one giving the heaviest seeds. It was noted in the field work that clone W 1037 (giving the lightest seeds) had an exceptional number of female strobili per shoot.

A considerable part of the variation is caused by clonal differences. Heritability (in broad sense) was calculated as:

$H^2 = \sigma_c^2 / (\sigma_c^2 + \sigma_r^2) = 0.77$

Table 1. Distribution of seed weights

Fraction (mg)	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	Σ
Per mille of seeds.	4	56	217	313	250	125	35	1 000
W 1037, the clone giving the lightest seeds, per cent of seeds (40 Measured).		75	22	3				100
C 5002, the clone giving the heaviest seeds.				5	35	35	25	100
No of clones with average seed weight in this fraction (average of two ramets).		1	6	13	11	3		34

Table 2. Analysis of variance.

	Square sum	Degrees of freedom	Mean square	Estimates of mean square
Between clones	984.51	33	29.83	$\sigma_s^2 + 20\ \sigma_r^2 + 2 \times 20\ \sigma_c^2$
Within clones between ramets	149.10	34	4.39	$\sigma_s^2 + 20\ \sigma_r^2$
Within ramets between seeds	877.10	1 292	0.679	σ_s^2
Total	2 010.71	1 359	1.48	

$F = 4.39/0.679 = 6.5$ $p>0.999$

Thus, there is a highly significant difference between ramets of the same clone.

$F = 29.83/4.39 = 6.8$ $p>0.999$

Thus, there is a highly significant difference between clones.

Table 3. Components of variance.

		%	σ
Between clones,	$\sigma_c^2 = 0.636$	43	0.431
Between ramets,	$\sigma_r^2 = 0.186$	12	0.431
Between seeds,	$\sigma_s^2 = 0.679$	45	0.824

Thus, the seed weight is a factor under strong genetic control.

A fractionation of the population of 1 360 seeds studied into 1 mg fractions would give effects presented in Table 4.

If all clones contributed equally to all seed weight fractions the effective clone number would always be 34. Large deviations from this ideal situation, in which all clones give an equal contribution, occur following fractionation. The genetic diversity will be reduced to a considerable extent in the extreme fractions.

Two hypothetical fractions, were studied more in detail, a light fraction with seeds below 5 mg and a heavy with seeds above 7 mg (see Table 5).

Fifteen of the 34 clones gave rise to seeds in only one of the fractions. The probability that a seed drawn at random from the heavy fraction originated from the same mother clones as one drawn from the light fraction was calculated to be 0.0091. If the clones contributed equally the probability (P) expected would be 1/34 = 0.0294. The probability of getting two seeds from the same mother in the light fraction was 0.0563 and in the heavy 0.0670. Thus there was more than a factor of 6 difference in probabilities "within" and "between" fractions. The "shared proportion", calculated as described above, was 0.2175.

It is concluded that, to a large extent, the seed of the heavy and the light fractions have different mother clones.

Discussion

Seed fractionation has considerable genetic effects. The "effective population number" in the extreme fractions will be considerably decreased. The effects add to other factors decreasing the effective population number in seed orchards (differences in gene contribution caused by differences in pollen and seed production, differences in earliness etc.). The clone number in seed orchards ought to be increased if fractionation is intended. The different fractions will be genetically different. When the seed harvest is tested, the test will be unreliable for fractionated seeds.

Fractioning may take place unintentionally in seed handling. It is wellknown that empty seeds often are unequally distributed in seed containers, and similar process may influence weight distribution.

Unintentional seed fractionation may be common in many stages; seed extraction, seed cleaning or moving seed bottles (especially in seeding).

It is probable that the genetic composition in different compartments of the same seed lot may differ significantly. Therefore, if

Table 4. Effective population size.

Fraction (mg)	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9
(P=) Probability that two seeds taken at random from the fraction (with replacement) will have the same clone as mother.	0.222	0.203	0.054	0.038	0.041	0.063	0.114
Effective clone number (=1/P)	4.5	4.9	18.5	26.3	24.4	15.9	8.8
Relative effective clone number (N_r=1/ (34×P)	0.132	0.145	0.544	0.765	0.720	0.470	0.259

Table 5. Comparison of light and heavy fraction.

Fraction	Below 5 mg	Above or equal to 7 mg
P	0.0563	0.0670
N_r	0.522	0.439
Per mille of total	377	217

plants from the same seed lot are taken from different parts of a nursery, they may constitute different populations.

There might exist considerable differences in seed weight of Scots pine between ramets of the same clone. Simak (1954) found considerable differences (up to 2.5 mg). The investigation was done at an early stage with small grafts, probably not representative of a producing seed orchard. Shen and Lindgren (1981) got a difference of 1,5 mg. This is compatible with the clones in this study showing the greatest between ramet variation. Thus, earlier studies are not contradictory to the present, and gives no firm support to the possibility that within clone variation is greater in other orchards.

The practice of using pollen donators as "common testers" may be criticized. The mothers not only differ genetically, but also produce seed of different weights, and seed weight is known to influence early growth. If common testers are used, it is preferable to use common mothers. Maternal effects and clonal seed characters will then not influence calculated values of general combining ability for fathers.

As there are seed weight differences between ramets, it seems advisable to replicate the same cross on several mothers. To study clonal effects on seeds, collection of seeds from several ramets per clone is recommended.

This investigation deals with seed fractionation for weight, but seed weight is probably closely correlated with e.g. size (such a close correlation was found for Douglas fir by Silen et al. 1979). Thus other types of seed grading probably cause similar effects.

As pointed out by e.g. Righter (1945) selection within progenies according to seed characteristics would probably have no adverse genetic consequencies. Thus, some of the genetic effects of seed fractionation would be avoided if fractionation was done for seeds harvested from different clones separately and then mixed. Another way to avoid genetic consequencies would be to mix plants from different fractions before planting.

If selfing occurs, there is a possibility that seeds produced are lighter than out-crossed seeds. The effects of selfing may be proportionately greater in light seed fractions.

This investigation deals with seeds collected in one seed orchard a single year. The situation may change from year to year and from seed orchard to seed orchard. There might be a danger in making too far reaching generalizations.

Concluding remarks

There are often good reasons to fractionate seeds. The removal of immature or damaged seeds increases germinability and decreases the probability of empty pots. Plant development becomes more even if fractions of different weight are sown separate. But as the

procedure has significant effects, especially on seed orchard seeds, my recommendation is that seed fractionation should be done when there is a good reason and not become a routine for seed orchard seeds. This is particularly important if there is only a low number of clones in the seed orchard.

Acknowledgements

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GENETIC VARIATION IN GROWTH RHYTHM CHARACTERISTICS WITHIN AND BETWEEN NATURAL POPULATIONS OF NORWAY SPRUCE
A preliminary report

TORE SKRØPPA

The elongation of the leading shoots was measured during the growth seasons of 1978 and 1980 in a diallel experiment comprising three complete Norway spruce diallels, each performed within a natural stand, and in a provenance experiment comprising 36 provenances. A considerable genetic variation was found in shoot elongation parameters, both between different provenances and between full-sib families within the same stand. The central and eastern European provenances generally had a longer growth season than the Nordic ones, and this was the main reason for their better growth. Within two of the diallel stands the best-growing families had a much higher rate of elongation per day than the poor-growing ones, while the length of the growth period was not much different. Within the third stand the length of the growth period was the main cause of variation.

Introduction

Good information about the genetic variation at different levels for a large number of characters is of major importance in any breeding program. It is also important to estimate the correlations between characters and study the inheritance patterns of the different traits. In 1973, a project was started in Norway to fulfil some of these requirements by means of diallel crosses. In this preliminary rapport some results from the project will be presented. No complete statistical analyses of the diallels are attempted here.

Materials

In the spring of 1973, a number of controlled crosses were performed in each of three natural stands of Norway spruce. In each stand ten trees standing more than 50 m apart were randomly chosen among the trees having both female and male flowers. The ten trees were crossed in all possible combinations including reciprocal crosses and selfings, thus resulting in three complete diallels. Due to sterile pollen the dimension of one of the diallels was reduced to 9×9, the others being 10×10.

The seed was sown in multipots in the spring of 1974, and the seedlings were planted out two years later. All families were planted in a short term experiment at 60 cm spacing at our experimental farm at Ås. Each half-diallel (no reciprocal crosses) was also planted in a long term experiment at a forest site. The parental clones were grafted, and the grafts were planted at the same site as the nursery experiment.

The three stands are all from the south-eastern part of Norway, less than 60 km apart, and from elevations 270 m, 300 m, and 500 m. The stands are shown on Fig. 1 together with the site of the short term experiment.

The sowings in 1974 also included a provenance experiment comprising 36 different provenances mostly from Latvia and Poland (Fig. 2). The seedlings were given approximately the same treatments as the diallel plants and were planted in 1976 in a seperate experiment adjecent to the short term diallel experiment. Each provenance represents a stand and is in most cases based upon seed from 20 to 25 parents.

Methods

The purpose of the diallel experiment is to study the genetic variation within natural stands of Norway spruce and estimate the inheritance patterns of important characters. Consequently, a large number of measurements have been made. Most measurements have been done simultaneously both in the diallel and provenance experiments, making it possible to compare the within-stand genetic variation with the variation between stands and provenance regions.

As the growth rhythm is an important factor in determining both adaptability and growth capacity, it was decided to put effort into the study of growth rhythm characters. So, in the summer of 1979, the elongation of the annual shoot was measured seven times during the growth season. In 1980 similar measurements were done nine times. All plants in six replicates were measured in the diallel and provenance experiments at the experimental farm. The same measurements were also done on the grafts of the parental clones. In two of the field experiments the shoot elongation was measured three times during the growth season in 1980 and 1981. Other growth rhythm characters such as flushing time, lammas shoot formation, and lignification have also been studied. In this paper, however, preliminary results from only the shoot elongation measurements will be presented.

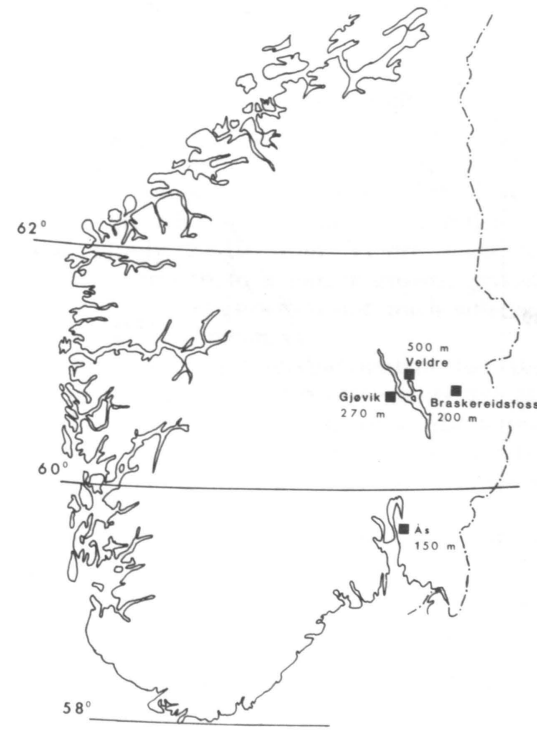

Fig. 1. Location of the three diallel stands and the short term experiment.

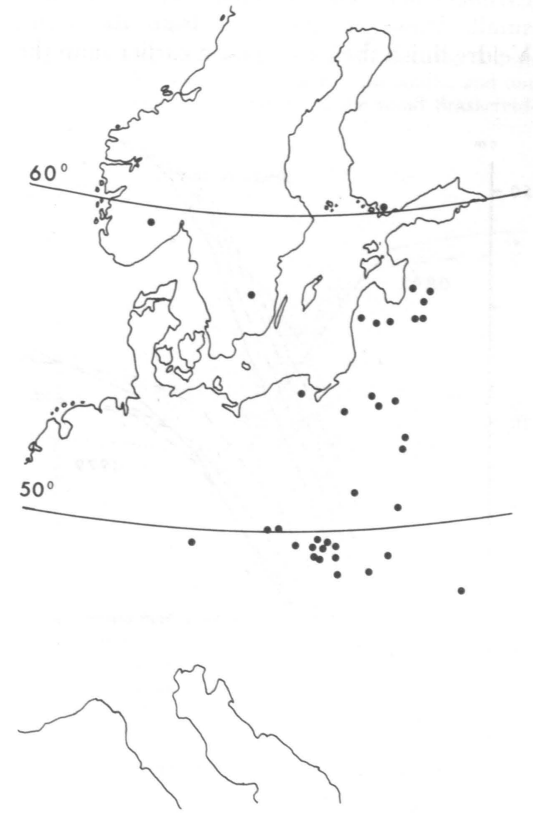

Fig. 2. Seed sources included in the provenance experiment.

Results

The elongation curves for 1979 and 1980 are shown in Fig. 3 for five groups of provenances. The Nordic provenances flush much earlier than the more southern ones, but also terminate the growth much earlier. By July 1 1980 the trees from these provenances had finished 85 % of their total growth, compared to less than 60 % for the provenances from south-eastern Poland, which started elongation very late. The Nordic sources grew considerably less than the Latvian and Polish ones, particularly in 1980. There is some variation between the stands within each provenance group, but as will be shown later, this variation is not substantial. The Finnish provenance flushes very early in the spring and also has a much earlier growth cessation than the Norwegian and Swedish sources.

Fig. 4 shows the elongation curves for the means of all diallel progenies from each of the three Norwegian stands. The differences in earliness between the stand means are quite small. However, the trees from the stand Veldre finish their elongation earlier than the trees from the other two stands. The elevation of this stand is 200 m higher than the other two, and the climate is much more severe.

Next, the elongation curves for each of the two years 1979 and 1980 in Figs. 5–7 show some of the within-stand variation. The three curves represent the best-growing full-sib family, the poorest growing full-sib family, and one intermediate full-sib family within each stand. The correspondence between the two years is very good. It seems that the best-growing families start their growth relatively early and continue for some more days than the poor families. The latter ones are slow growers already from the start of the growth season. One exception is family 19 in the stand Veldre. This family is relatively late in spring, but has a steep elongation curve for a longer period of time. It is interesting to compare the families 19 and 9 in this stand, which have the same pollen parent.

The growth curves can be described by several parameters. Table 1 shows 1980 data for total elongation, number of days between 20 % and 80 % of total elongation, and elongation per day for the stand mean, for the

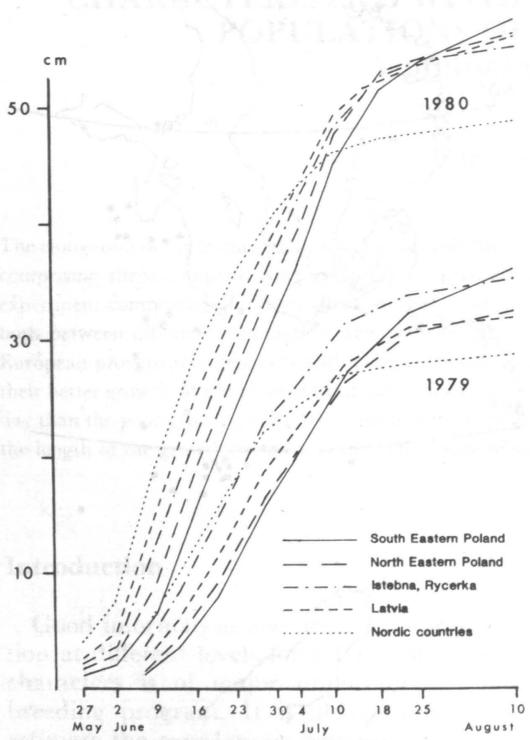

Fig. 3. Elongation curves for five groups of provenances in the provenance experiment.

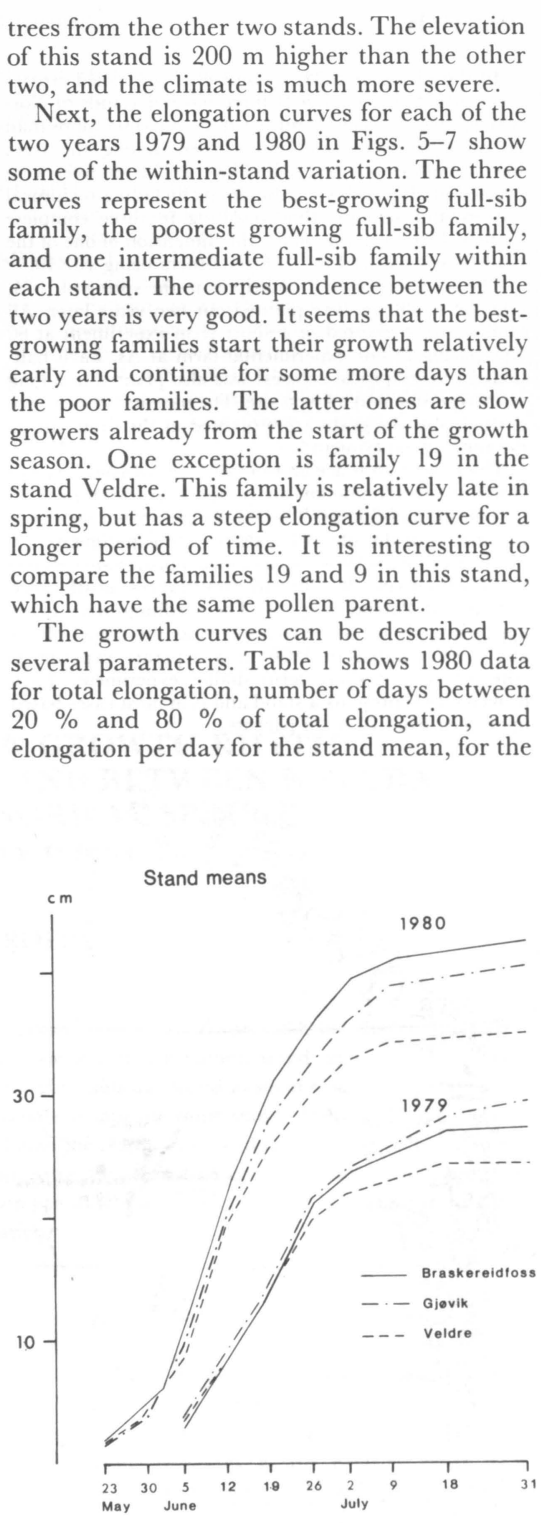

Fig. 4. Elongation curves for the means of the three diallel stands.

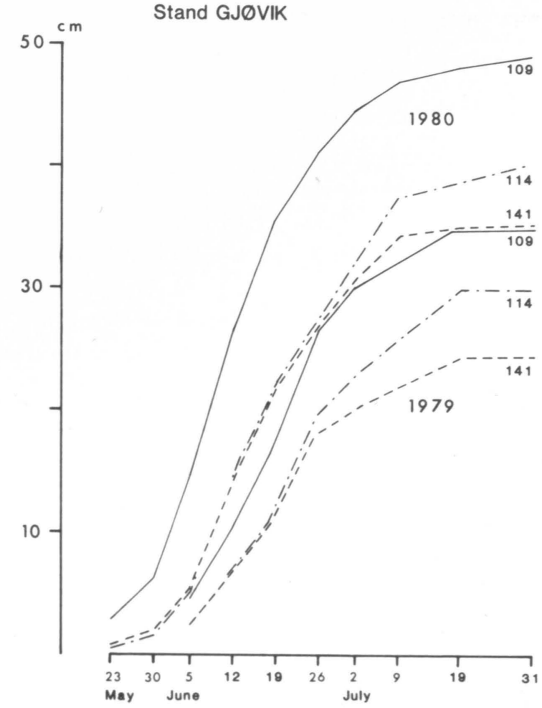

Fig. 5. Elongation curves for the best-growing full-sib family, the poorest-growing full-sib family, and one intermediate full-sib family in the stand Gjøvik.

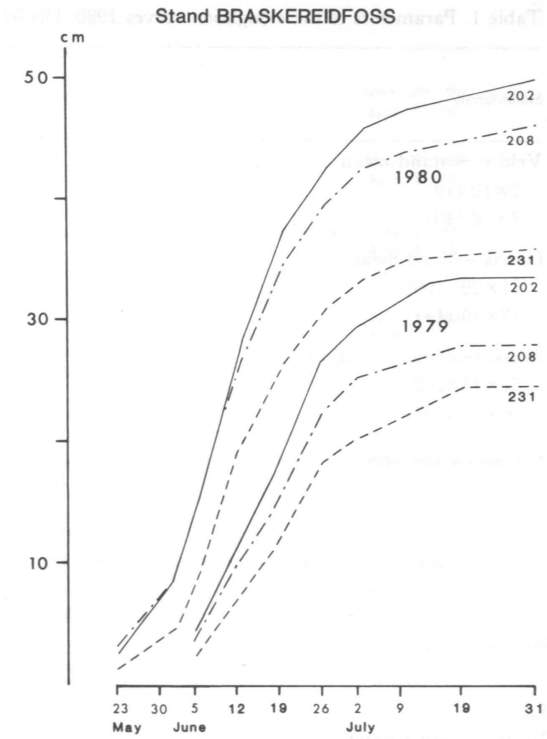

Fig. 6. Elongation curves for the best-growing full-sib family, the poorest-growing full-sib family, and one intermediate full-sib family in the stand Braskereidfoss.

best-growing, and for the poorest-growing full-sib family within each stand. It can be seen that in two of the stands the best families have a much higher rate of elongation per day than the poor-growing ones, while the length of the growth period is not much different. The family 2×10 from Veldre owes its superior growth to a longer growth period, while the rate of growth is not much different from the stand mean.

The corresponding parameters for the elongation curves of the provenance groups (Table 2) show that the more southern and eastern provenances have a much longer period of elongation, and that this is the main reason for their better growth.

Analyses of variance between the full-sib families within each stand and between the provenances all show strongly significant values both for measured elongation values and for relative elongation, defined as the percentage of elongation attained at different dates of measurements. The differences between the stand means within the provenance groups, are, however, small compared to the

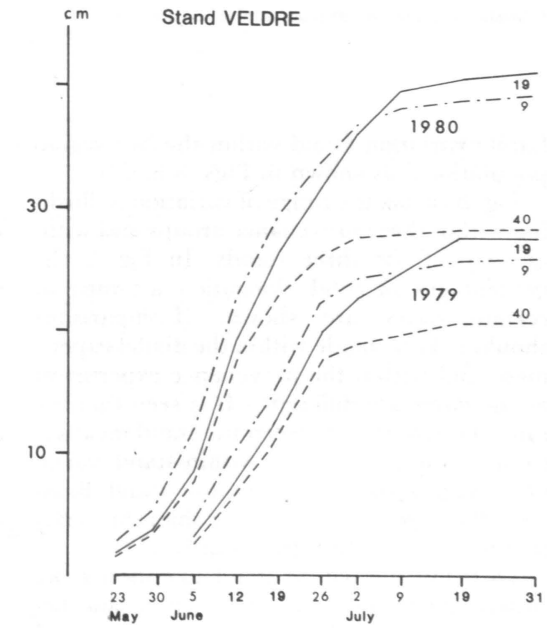

Fig. 7. Elongation curves for the best-growing full-sib family, the poorest-growing full-sib family, and one intermediate full-sib family in the stand Veldre.

Table 1. Parameters of the elongation curves 1980. Diallel experiment.

Stand/family	Total elongation cm	Elongation period*) days	Elongation per day mm
Veldre – stand mean	35.1	20	10.6
2×10 (19)	41.5	23.5	10.8
7× 8 (40)	28.3	16.5	10.3
Gjøvik – stand mean	40.8	21.5	11.4
11×20 (109)	49.5	22.5	13.2
17×19 (141)	35.5	21.5	9.7
Braskereidforss – stand mean	42.7	20	12.8
21×23 (202)	49.8	21	14.2
26×27 (231)	36.2	20	10.9

*) Number of days between 20 % and 80 % of total elongation.

Table 2. Parameters of the elongation curves 1980. Provenance experiment.

Provenance group	Total elongation cm	Elongation period*) days	Elongation per day mm
Latvia	55.3	28	12.0
North-eastern Poland	56.5	27.5	12.3
Istebna, Rycerka Poland	56.2	28.5	11.8
South-eastern Poland	57.2	27.5	12.5
Nordic countries	49.1	23	12.8

*) Number of days between 20 % and 80 % of total elongation.

family variation found within the Norwegian populations, as shown in Figs. 8 and 9.

Fig. 8 shows the range of variation in flushing within three provenance groups and within each of the three stands. In Fig. 9 the percentages of total elongation attained at certain dates are shown. (Comparisons should only be made within the diallel experiment and within the provenance experiment as the dates are different.) It is seen that the range of variation between the stand means is small compared to the within-stand variation. The variation within the stand Braskereidfoss seems to be smaller than the variation within the other two stands.

To study the within-stand variation more closely the range of variation within and between the ten half-sib families in the stand Gjøvik is shown in Fig. 10 for the character relative elongation attained on June 26 1980. There is a large variation between the full-sib families within each half-sib family. Family 20 is the best-growing half-sib family with an early flush and also terminates the elongation early. This family has a steep growth curve and is desireable in a tree breeding program. It has high general combining ability (GCA) effects both for height growth, early flushing, and early cessation of shoot growth as shown in Table 3, where the GCA and SCA effects are calculated for the data in Fig. 10. Both types of effects are highly significant.

Shoot elongation measurements in the field experiments showed that the elongation period is much shorter under field conditions. However, significant correlation coefficients were found between full-sib family means for corresponding characters with estimated correlation coefficients between 0.6 and 0.8.

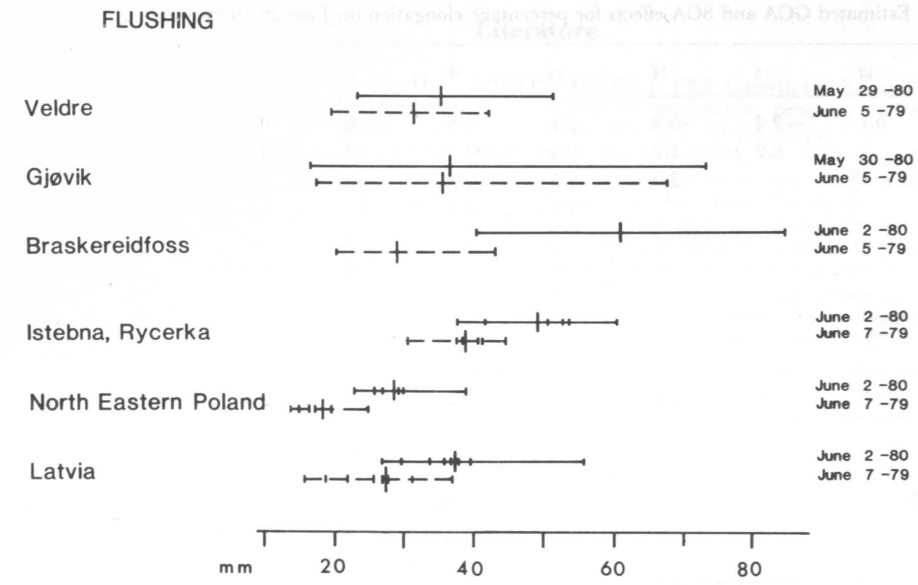

Figure 8. Range of variation in flushing within the three diallel stands and within three groups of provenances.

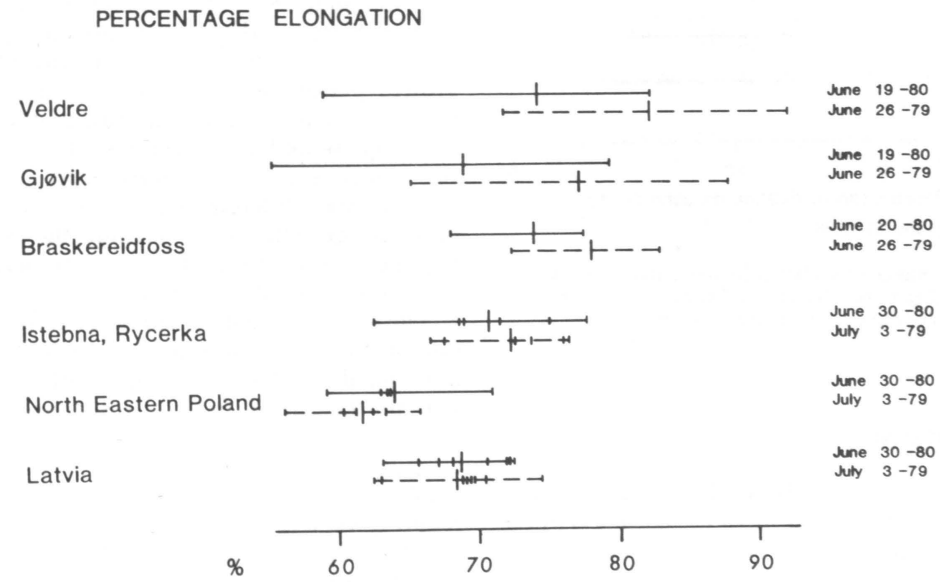

Fig. 9. Range of variation in relative elongation at certain dates within the three diallel stands and within three groups of provenances.

Table 3. Estimated GCA and SCA effects for percentage elongation on June 26 1980. Stand Gjøvik.

Family	12	13	14	15	16	17	18	19	20	GCA
11	0.1	—2.2	–0.9	2.1	0.8	–3.6	0.1	0.7	0.9	–1.5
12		0.2	1.7	0.3	1.3	–5.8	–4.0	1.7	2.0	–2.1
13			–2.0	–2.5	1.3	4.0	1.2	–0.5	–0.6	–0.4
14				–1.4	–0.1	2.6	–1.4	3.3	1.2	2.4
15					3.7	1.1	1.8	1.2	–3.4	3.0
16						–1.0	–1.9	–5.8	2.7	1.0
17							0.4	–0.8	–1.3	–4.7
18								0.1	1.5	–2.1
19									0.4	0.7
GCA	–2.1	–0.4	2.4	3.0	1.0	–4.7	–2.1	0.7	4.1	

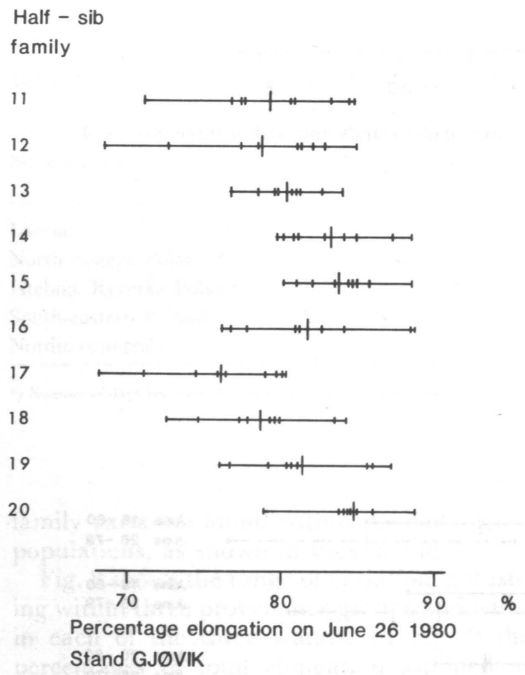

Fig. 10. Range of variation between and within half-sib families in the diallel stand Gjøvik for the character relative elongation on June 26 1980.

Discussion

These results show that there is much genetic variation in shoot elongation parameters within natural stands of Norway spruce and that this variation can be as large or larger than the variation between populations in the same or adjacent provenance regions. Similar amount of genetic variation has previously been demonstrated by Dietrichson (1969) and Ståhl (1977). Eriksson and Gagov (1975) found variation in plant heights within full-sib families partly caused by variation in the duration of the growth period.

Genetic variation is found for several parameters of the shoot elongation curve, such as the date of initiation of the growth, the duration of growth period, the date of growth cessation, and the rate of growth. Different patterns of variation were found between populations and within populations, and the latter showed different expressions in different populations. The differences in total growth between the provenance groups are mostly caused by varying length of the growth period, while intensity of growth is nearly constant. Within the two populations from Gjøvik and Braskereidfoss the length of the elongation period varies little, and the large growth differences are associated with different growth rates. Within the Veldre population the length of the growth period is the main cause of variation.

The shoot elongation curve describes only one part of the total annual growth rhythm, and the date of cessation of height growth may not be critical for frost resistance. More important are the physiological processes associated with hardening off and cessation of cambial activity. Nevertheless, Ståhl (1979) found a very good correspondence between the duration of the growth period at a southern site and the number of frost damaged terminal buds at a site 4.5 degrees latitude further north.

Acknowledgements

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FROST RESISTANCE DURING BUD FLUSHING AND SHOOT ELONGATION IN PICEA ABIES

INGEGERD DORMLING

Norway spruce (*Picea abies* (L.) Karst.) seedlings of 10 populations (47–67° N) were grown in climate chambers. Conditions known to promote different degrees of total hardiness were applied during hardening. A low temperature treatment to break dormancy was given to most plants but was in some cases excluded. Bud flushing was studied at different temperatures and photoperiods. Plants were freeze tested, mostly to –7 °C, at different stages of shoot development.

The developing shoots had little resistance from the appearance of new needles until completed shoot elongation. The susceptibility was the same in plants of all origins if they were freeze tested at the same flushing stage and were pretreated in a way giving the same degree of basic hardiness. Shoots on plants with a high degree of hardiness survived better than other. The whole plant was dehardened during bud flushing and was susceptible to frost if the basic hardiness was insufficient. Well hardened plants flushed earlier than less hardy ones.

Spruce populations moved to the north are never as hardy as the autochtonous ones. This fact might be of importance after an autumn with unfavourable conditions.

Introduction

The late bud flushing of Norway spruce (*Picea abies* (L.) Karst.) from Romania, Poland and White Russia is well known to forest owners in the parts of Scandinavia where provenances from these more southern latitudes are recommended for use in reforestation. In spite of its obvious advantages there are still doubts about using this introduced material. One common question is: when at the same stage of bud bursting and development of new shoots, is the introduced spruce not more susceptible to frost than the autochtonous one? To answer that question was the aim of the experiments reported here.

Material and methods

Three experimental series, here named A, B and C, have been grown from seeds under controlled conditions in the Stockholm phytotron.

Origin of seeds

A. Vitebsk 55°10′ N
Brunskog 59°42′ N
B. The same as A plus
Kalix 66° N
C. Ten populations (1–10) within the range 47° to 67° N, see Table 1

Table 1. Origin of the seed lots in series C.

Nr	Locality	Latitude	Longitude	Altitude, m
1	Nattavaara	66°45'	20°55'	300
2	Burträsk	64°30'	20°30'	200
3	Bälinge	59°59'	17°26'	40
4	Brunsberg	59°42'	12°59'	134
5	Ulvshult	56°25'	14°27'	135
6	Vitebsk	55°10'	30°10'	200
7	Minsk	54°10'	27°30'	200
8	Spisska Nova	48°51'	20°30'	650
9	Frasin II	47°33'	25°48'	750–1000
10	Toplita	46°50'	25°25'	1000

Growing conditions

The seeds were sown in a vermiculite-sand mixture at 20 °C under continuous light. Fourteen days later the seedlings were planted in pots with mineral wool. The pots were watered once or twice a day with a complete nutrient solution at low concentration, 2L–6513, 100 mg N/l (Ingestad 1979). The number of plants in each treatment or test is given in the figure texts. In each of the growing conditions of series B the total number of plants per population cultivated was 128, of series C 192 in V and 96 in VI, VI a, VII and VII a. The following treatments were used:

a. *Treatments in the first growth period*
A. 11 weeks (w) of continuous light, 22 000 lux, temperature 20 °C, followed by 8 w with 16 hour (h) night.
B. 8 w of continuous light, 20 °C, followed by
I night prolonged by 1 h/w to 10 h night followed by 16 h night during 6 w (Kalix, Brunskog, Vitebsk)
II as I up to 13 h night followed by 16 h night during 6 w (Brunskog, Vitebsk)
III as I including 3 w with 16 h night during night prolongation (to imitate the "artificial hardening" of nursery practice) (Kalix, Brunskog, Vitebsk)
IV as I (Kalix)
C. V–VII After 8 w of continuous light, 20 °C, the scheme of Fig. 1 was followed. As a result of a mistake in the programming of a climate chamber, no night at all was given during the weeks 4 to 6 (= 3 w of continuous light). This influenced all the material in treatment No. V and some material in VI and VII.

b. *Dormancy breaking treatment (= low temperature)*
Low temperature treatment during 4 w (see Fig. 1) was given to all material but VI a and VII a.

c. *Treatments in the second growth period (= bud flushing)*
A. 16/8 h day/night, 20/20 °C
16/8 h day/night, 20/10°C
B. I–III 18/6 h day/night, 20/10 °C
IV 18/6 h day/night, 15/10 °C
C. Continuous light, 20/10 °C during 16/8 h or 16/8 h day/night, 20/10 °C

Some plants from series B and C that survived the freeze testing without damaged top shoots, were moved after bud-set to long night conditions (16 h) for a period of ca 8 weeks. After a dormancy breaking treatment (see above) they were again moved to growing conditions as in C.

Estimating stages of bud flushing

To estimate the stage of top bud flushing (bursting) the scheme of Krutzsch (1973) was used in a somewhat modified form adjusted to the development of the phytotron seedlings:

0 = Dormant bud
1 = Bud slightly swollen
2 = Bud swollen, green to grey-green in colour
3 = Bud scales bursting, needle tips emerging
4 = Needles elongated to about double bud length (compared with the photographs A4 and B4 in Krutzsch's paper, this stage is somewhat less advanced in the phytotron estimations).
5 = First spread of needles (painter's brush)
6 = Shoot elongated, basal needles not yet spread
7 = Basal needles spread
8 = All needles more or less spread, new side buds developing
9 = Completed shoot elongation, new top bud visible

Experimental design

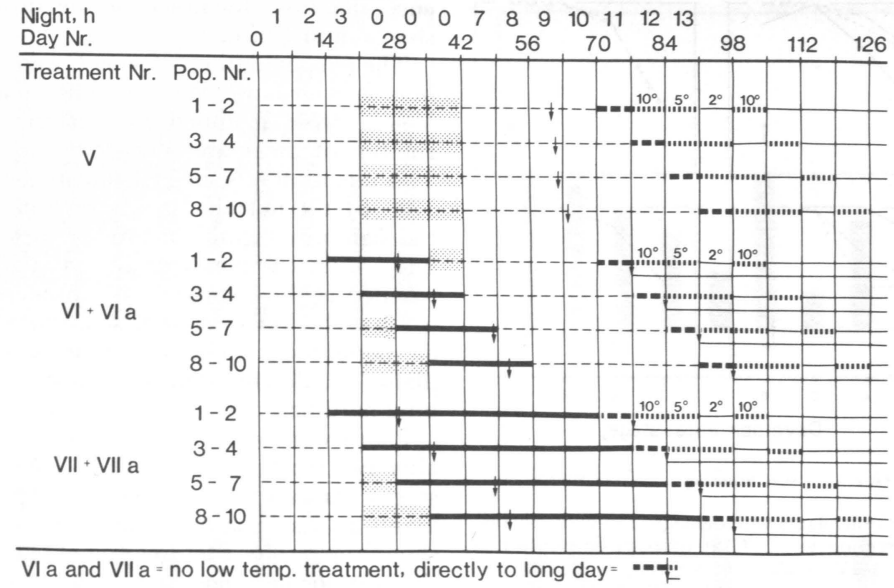

Fig. 1. Experimental design in series C. For the origin of populations 1–10, cf. Table 1.

The flushing stage was estimated every day during the most intense flushing period, otherwise three times a week.

Freeze testing

Plants for freeze testing were chosen at certain developmental stages. The temperature in the freezing room was lowered successively during a period of 16 h to the testing temperature (which lasted for 3–4 h) and successively raised. The roots of the potted plants were protected from freezing.

Testing temperatures:
A: –3 °, –5 °, –7 °C
B and C: –7 °C

After the freezing the seedlings were again given the same conditions as before. The number of plants with injured top buds (shoots) was counted after three days. Ten days later the damage caused to side shoots and to older parts of the plants was observed. The total number of new side shoots and the damaged ones was counted and the per cent damaged side shoots per plant calculated. The damage caused to older plant parts was investigated according to a scale 0–5, where 0 = undamaged older plant parts, 5 = dead plant.

Results

Frost resistance of bursting buds and developing shoots

In *series A* (cf. Dormling et al. 1977) buds and shoots in stages 2–6 were freeze tested in –3 °, –5 °, and –7 °C after bud flushing in two temperature conditions, 20/20 ° and 20/10 °C. At stage 2 even the lowest temperature caused very little harm to the buds. Stage 3 was the first sensitive stage with up to 25 per cent plants with damaged buds. The later the developmental stage, the more plants with damaged top shoots. Only a few plants at later developmental stages were injured by –3 °C, some more by –5 °C, whereas –7 °C seemed to be a really critical temperature, which was chosen for all the following tests. The different temperatures during bud flushing did not influence the susceptibility and there was no difference between the two tested populations.

In *series B* (cf. Dormling and Eriksson 1979) one more population was added. Three separate conditions, I–III, were applied during plant hardening, see above, with the aim

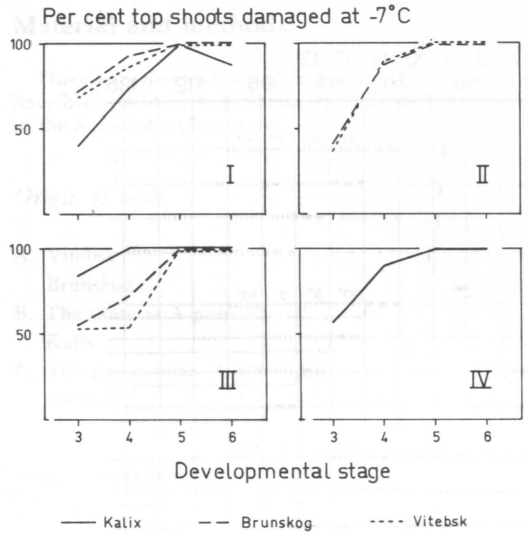

Fig. 2. Per cent plants with damaged top buds (shoots) after freezing to –7 °C at different developmental stages. I–IV = different growing conditions presented in the text. Stage 3–5: 22–30 plants/population and stage, Stage 6: 11–15 plants/population and stage. (From Dormling & Eriksson 1979.)

of producing plants with different total hardiness, I giving the least hardy, III the most hardy.

Figure 2 shows the results of the freeze testing. The results from series A were confirmed, the susceptibility of the buds and new shoots increased with the developmental stage. Taking only the two southern populations into consideration, a slightly improved survival after the treatments II and III in early developmental stages are discernible. The Kalix population shows the opposite reaction, however, which might be explained by a pretreatment that made the buds begin to open up after the "artificial hardening" (III).

Hardly any top shoots survived the freezing to –7 °C at the stages 5 and 6. Although side buds usually began to flush earlier than the top buds, the side buds mostly were damaged less than the top buds. Nearly always some side buds survived, owing to their more protected position or to a less advanced developmental stage. Only 60 and 81 per cent, respectively, of the side buds on plants tested at top bud stage 5 and 6 were killed.

The only difference between I and IV was a lower temperature during bud bursting in the latter case. That treatment did not influence the frost tolerance of the developing shoots in any direction.

The ten populations of *series C* were divided into four groups (Fig. 1). The treatments V–VII were modified to suit the growth rhythm of the material within the groups. This intention was somewhat disturbed, however, by the mistake in the programming of the night prolongation mentioned before. The bud-set in treatment V came as an immediate reaction to the change from continuous light to 7 h night, buds visible 18 to 22 days later in all populations. The intention of creating plants of different origin with clearly different total hardiness was, however, fulfilled.

Plants were tested at the stages 4, 6, 9 and after two weeks at the stage 9. The last two stages corresponded to the development of plants in the height of the summer. Fig. 3 shows the results after freezing of plants from the two northernmost populations. The new shoots were very susceptible to frost during their entire period of elongation but soon became less susceptible after the development of new top buds (after stage 9). The night length given in the second growth period (8 h) was long enough to bring about some hardening after bud-set in the plants of northern origin, as illustrated in Fig. 3. This was not the case in plants of more southern origin. Their newly formed buds bursted unless the night was prolonged. The only available possibility was to apply 16 h night as soon as the plants reached the stage 9. A freeze test (–7 °C) made fourteen days after introduction of

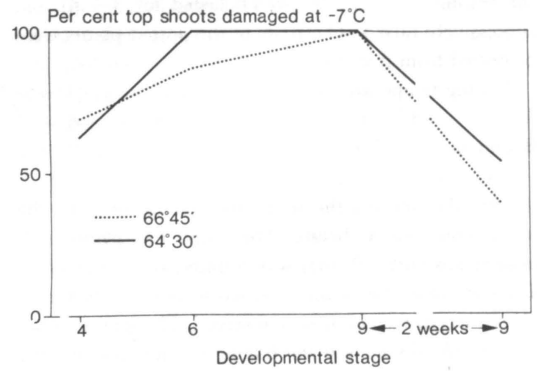

Fig. 3. Per cent plants with damaged top shoots after freezing to –7 °C at different developmental stages. Pretreatment V, populations 1–2. cf. Fig. 1. n = 16. (From Dormling & Eriksson 1981.)

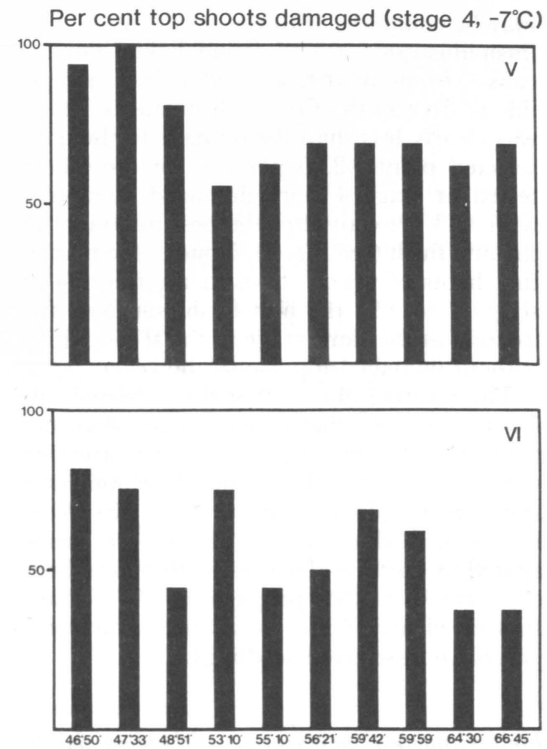

Fig. 4. Per cent plants of different populations with damaged top shoots after freezing to –7 °C at stage 4. Pretreatments V and VI, cf. Fig. 1. n = 16. (From Dormling & Eriksson 1981.)

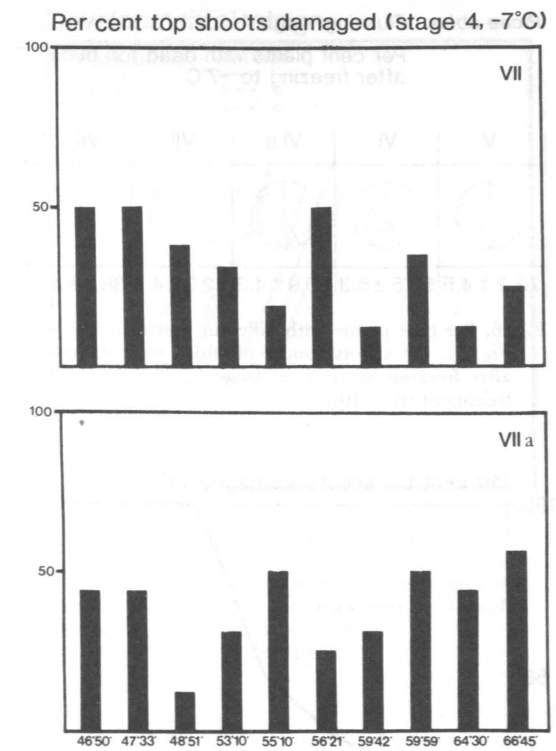

Fig. 5. Per cent plants of different populations with damaged top shoots after freezing to –7 °C at stage 4. Pretreatment VII and VIIA, cf. Fig. 1. n = 16. (From Dormling & Eriksson 1981.)

this long-night treatment gave at first no visible damage at all. In the next growth period, however, ca 20 per cent of the plants produced no normal top shoots, damage presumably caused by the "summer" frost.

Figs. 4 and 5 show the results from the material freeze tested at *stage 4*. The resistance of the new shoots was not influenced by the different conditions applied during bud flushing, 24 or 16 h light. The results from both the photoperiods, therefore, were counted together.

As in the earlier research series it was difficult to see any real difference between the populations when the plants were tested at the same developmental stage. A somewhat higher susceptibility of the plants of southern origin could possibly be discerned after the treatment giving the poorest hardiness (V, Fig. 4).

The differences between the plants tested after treatments giving different amount of hardiness (V, VI and VII) were nevertheless clear-cut. This is best illustrated by Fig. 6, were the means of all the treatments are given with their standard errors.

The t-values of the differences were the following:

V:VI 2.249*
V:VII 6.291***
VI:VII 3.541**

The *side shoots* were damaged somewhat less on plants tested after treatment VII than after the treatments V and VI: 38 per cent in VII and 46 per cent in each of the treatments V and VI. The difference was not significant, however. There were only small, non-significant differences between the plants given low temperature treatment before bud flushing and the ones taken directly from the long night treatment to growing conditions (VI:-VIa, VII:VIIa).

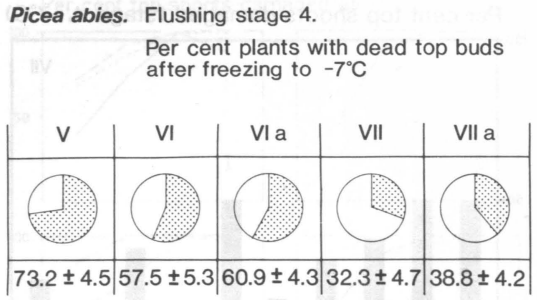

Fig. 6. Per cent plants with different pretreatments (cf. Fig. 1), the shoots (buds) of which were damaged after freezing to –7 °C at stage 4. 10 × 16 plants/treatment (n = 10).

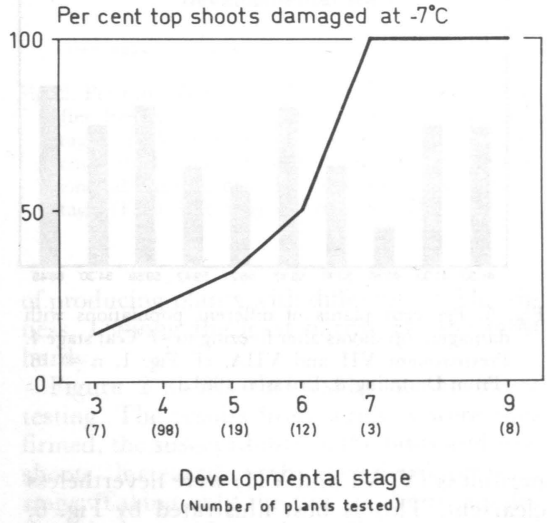

Fig. 7. Per cent plants with damaged top buds (shoots) after freezing to –7 °C at different developmental stages during their second flush (= third growth period).

At *stage 6* the susceptibility was high independently of pretreatment. 94 per cent of the plants tested at that stage (in all, 573 individuals) had damaged top shoots. Simultaneously 82 per cent of their side shoots were killed.

Some plants from all developmental stages whose top buds (shoots) were not damaged in the freezing test were grown for one more growing season. Most of them were chosen after having been tested at stage 4. After their second flush they were tested again at the same developmental stage as after the first one. The results are presented in Fig. 7 and may be compared with the results in the first flush illustrated in Figs. 2 and 3. The damage caused to the plants, selected as less susceptible to frost in the first flush in stages 3 to 6, was clearly less than the damage to the non-selected plants. 23.5 per cent of the plants tested at stage 4 were damaged compared with 32.3 per cent after the best treatment in the first flush (see Fig. 6). None of the plants, the shoots of which survived the freezing at stages 7 to 9 in the first flush, survived the freezing at the same stage in the second flush without damaged top shoots, however.

The results indicate that there existed differences between individuals rather than between populations, when the frost treatments were applied at the same developmental stage. Some plants that survived two freezings without damaged top shoots were propagated by cuttings. In a test with plants from eight clones of that propagation, five clones showed clearly better resistance than comparable non-selected seedlings.

Frost resistance of the older parts of the plants during the development of new shoots

Fig. 8 shows the frost damage score in *series B* after freeze testing of plants with different

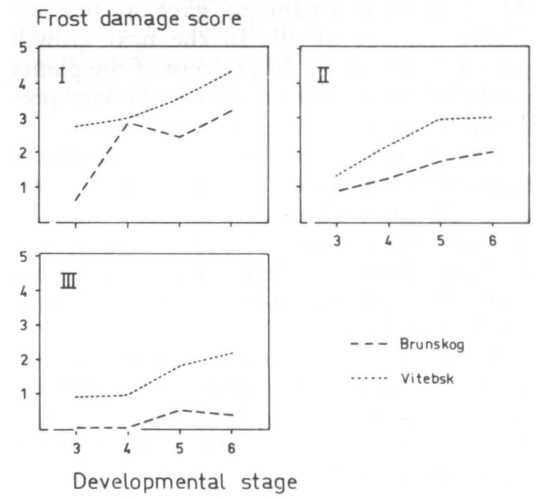

Fig. 8. Damage caused to the older parts of the plants after various pretreatments and freeze testing to –7 °C. Frost damage score: 0 = undamaged, 5 = dead plant. Pretreatments and number of plants as in Fig. 2. (From Dormling & Eriksson 1979.)

total hardiness (I–III). The older plant parts of plants belonging to the northernmost population, Kalix, were not damaged at any of the developmental stages. Plants from Vitebsk were more damaged than the ones from Brunskog. During bud bursting and the development of the new shoots, the older parts of the plants successively lost hardiness and were increasingly damaged by the frost treatments.

Also in *series C* frost damage to older plant parts occurred, especially after treatment V, which gave the least hardy plants. The night was prolonged to 10, 11, 12 or 13 h to suit the developmental rhythm of material of different origin. Two to three populations with some difference in latitudinal origin were exposed to each of the prolonged night regimes, cf. Fig. 1 and Table 1. Within each of these groups, plants from the southernmost population were the most damaged after freezing at stage 4.

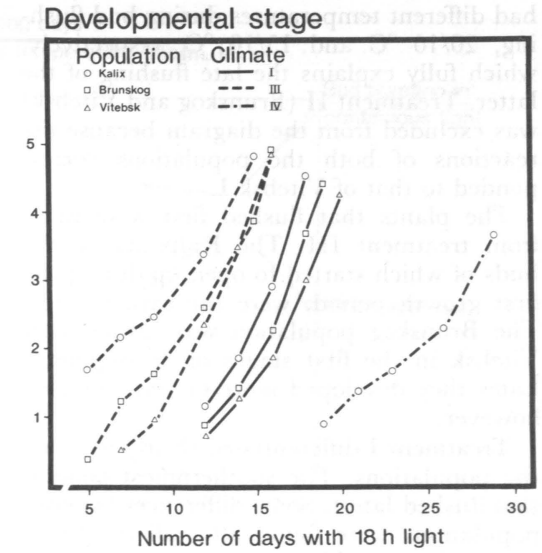

Fig. 9. Bud flushing of plants from the three populations of series B after different pretreatments, I, III and IV presented in the text. n = 42–68.
(From Dormling & Eriksson 1979.)

Rate of bud flushing

The rate of bud flushing was followed according to the scale presented earlier.

Fig. 9 illustrates results obtained in *series B*. The most striking differences were those that depended on the different treatments: in I and IV night prolongation to 10 h and in III the same but including a 3 week period of 16 h night ("artificial hardening"). I and IV

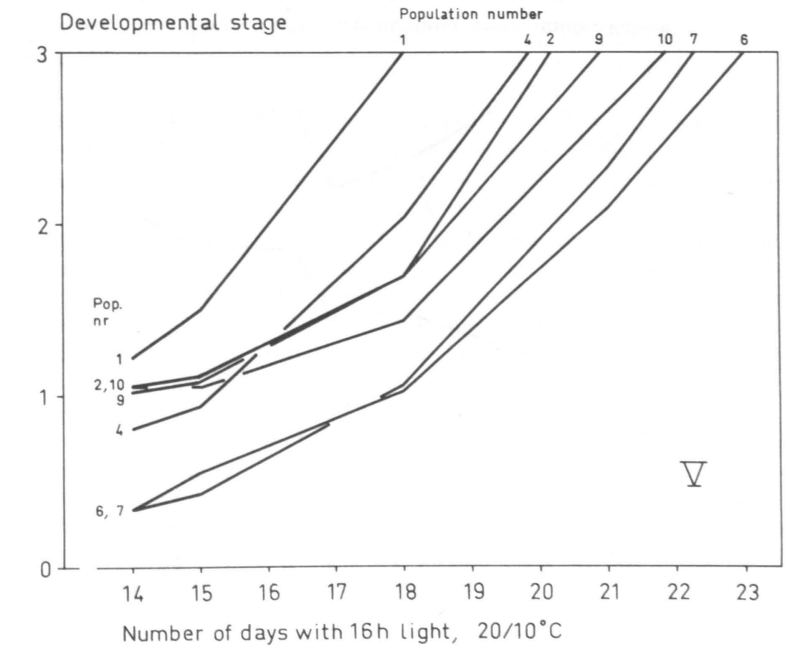

Fig. 10. Bud flushing of plants from seven populations of series C, pretreatment V, cf. Fig. 1. n = 32.

had different temperatures during bud flushing, 20/10 °C and 15/10 °C, respectively, which fully explains the late flushing of the latter. Treatment II (Brunskog and Vitebsk) was excluded from the diagram because the reactions of both the populations corresponded to that of Vitebsk I.

The plants that flushed first were those from treatment III. The Kalix-plants, the buds of which started to open up during the first growth period, were the earliest ones. The Brunskog population was earlier than Vitebsk in the first stages of development. Later they developed more or less together, however.

Treatment I differentiated clearly between the populations. The southernmost population flushed latest. Some differences between populations were found after all the treatments of *series C*. The development after treatment V is illustrated in Fig. 10. Treatment V corresponded to the treatments I and II in series B and the reactions may be compared with those from climate I in Fig. 9. Population 6 in series C and Vitebsk in series B were identical. Population 1 in C and Kalix in B were not identical but of almost the same nordic origin. Of the populations not illustrated in Fig. 10 the two missing Swedish

Table 2. Mean number of days until reaching bud flushing stage 3. Flushing in 16/8 h day/night, 20/10°C

Population		Treatment				
No.	Lat., °N	V	VI	VIa	VII	VIIa
1	66°45'	18.0	12.6	21.7	12.1	20.4
2	64°30'	20.2	13.7	23.8	13.8	16.6
5	56°21'	20.1	14.5	26.7	14.2	21.2
6	55°10'	23.0	16.2	30.4	14.9	28.3
10	46°50'	21.9	16.2	27.5	16.6	26.2

populations 3 and 5 fell within the reactions of 2 and 4 and population 8 came very close to population 10. The development was slower in series C than in series B. The main reason was probably the shorter period of long night applied after night prolongation in C: 1 week instead of 6 weeks in B. The longer photoperiod applied during flushing in B, 18 h light instead of 16 h in C, might have been of some significance, too. At stage 3 the difference between Kalix and Vitebsk was 2 days in series B (treatment I) and 5 days in series C (treatment V). Among the four remaining Swedish populations (2–5) there were no real differences after treatment V. They all reached stage 3 about 2 days later than popu-

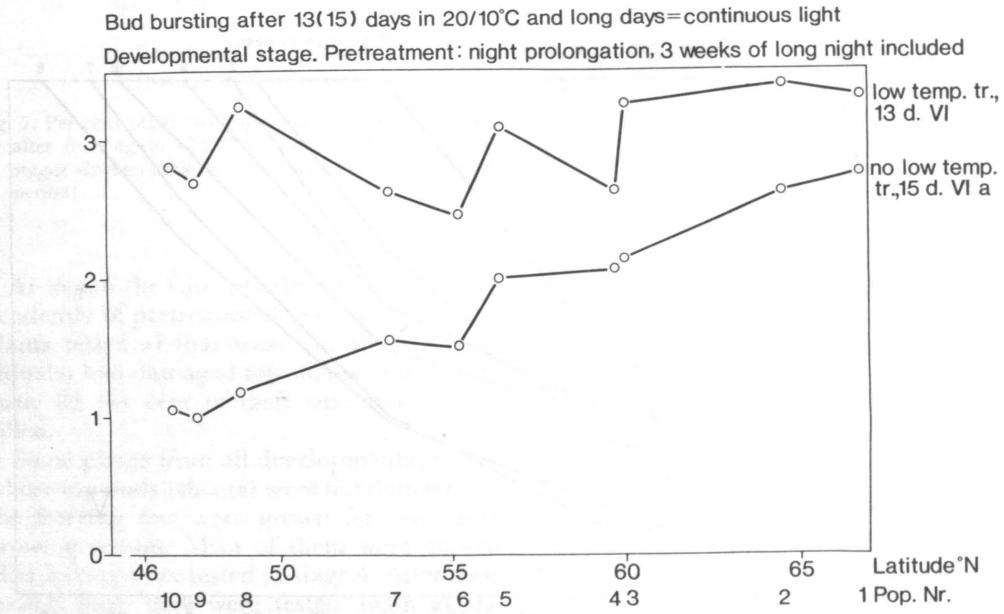

Fig. 11. Bud flushing stage of plants from the ten populations of series C. Pretreatments VI and VIa, cf. Fig. 1. Flushing in continuous light, 20/10 °C, in 13 and 15 days, respectively. n = 16.

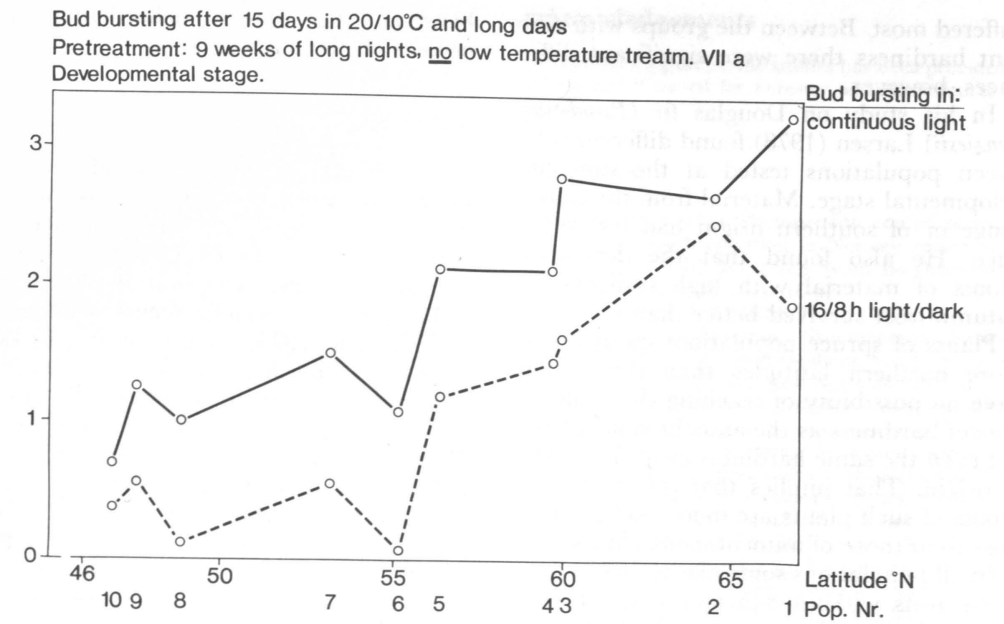

Fig. 12. Bud flushing stage of plants from the ten populations of series C. Pretreatment VII, cf. Fig. 1. Flushing in continuous light and 16/8 h day/night in 15 days. n = 16.

lation 1. The southern populations 8 to 10 from Romania and Czechoslovakia flushed earlier than the two White Russian populations 6 and 7.

Table 2 shows the number of days taken to reach stage 3 in plants of five of the populations. The values were interpolated by the aid of diagrams of the same type as the one of Fig. 10, cf. treatment V in the figure and the table. After treatments VI and VII, which gave a higher degree of hardiness, bud flushing occurred much earlier in all the populations. When the period of low temperature was excluded, as in VIa and VIIa, the time until flushing was markedly prolonged. After these treatments the differences between the populations were most pronounced, with Vitebsk (6) as the latest one. The differences after the treatments VI and VII were smaller and the southernmost population was as late as or later than Vitebsk.

Figs. 11 and 12 illustrate the development of the plants from different origin at a certain time after the start of "the growing season". After the treatments VIa and VIIa an almost clinal variation was discernible. The period of low temperature seems to rub out that variation to a certain amount; compare VI and VIa in Fig. 11. The two photoperiods applied during flushing did not change the range of the populations. The only effect of a longer photoperiod (continuous light) was a quicker development (Fig. 12).

Discussion

Resistance of bursting buds and developing shoots

The freeze testing of plants of different origin at the same developmental stage (stage 4) gave as main result on difference between the populations. A presupposition for the reaction was that the plants had about the same basic hardiness. In the experiment this aim was fulfilled by varying the treatments in accordance with the developmental rate of the populations known from earlier studies of their critical night length for bud-set (Dormling 1973). The plants from treatments V, VI and VII thus had different hardiness but there were only minor differences between the populations within the treatment groups in that respect. The variation in frost resistance between the populations within the groups was not systematic; but after the treatment with the lowest degree of hardiness (V) the southernmost populations seemed to have

suffered most. Between the groups with different hardiness there were significant differences, however.

In his study on Douglas fir (*Pseudotsuga menziesii*) Larsen (1978) found differences between populations tested at the same developmental stage. Material from the coastal range or of southern origin had less resistance. He also found that the developing shoots of material with high resistance to autumn frost survived better than the rest.

Plants of spruce populations cultivated at more northern latitudes than their origin have no possibility of reaching the same degree of hardiness as the autochtonous plants, not even the same hardiness as at their place of origin. That implies that the developing shoots of such plants are more susceptible to frost than those of autochtonous plants.

In all populations some plants survived the freeze tests without damage. Tests of these plants during their second bud flushing showed a better survival than that of unselected plants. Cuttings originating from plants tested in that way might be an alternative in reforestation on localities with a high frequency of late frosts.

At later developmental stages (6–9) the susceptibility was high, independently of pretreatment and origin. Thus it seems impossible to find spruces that are able to survive very late spring frost and summer frost without damage.

Resistance of older plant parts

During bud flushing and shoot development the whole plant is successively dehardened. Plants that had a small basic hardiness became susceptible in early flushing stages, whereas well hardened plants could stand frost during bud flushing and shoot development without damage to older plant parts. Because of varying basic hardiness of the plants, differences between populations occurred after the treatments I–III (Fig. 8). The same was true after treatment V, where the southernmost population of each group with different night prolongation was the most damaged one. This is in good agreement with results obtained with Douglas fir: Larsen (1978) found highly significant negative correlations between the rapidity of hardening and the hardening time on the one hand and the time of dehardening of the needles on the other.

Bud flushing

Late bud flushing gives good protection against damage by late spring frost, which is one of the reasons for using spruce from White Russia and other East European territories in Swedish reforestation. The time of bud flushing has been frequently studied, e.g. by Krutzsch (1975), Lindgren and Eriksson (1976) and Prescher and Persson (1981).

In order to explein differences in time of bud flushing between years, temperature (heat) sums were calculated by adding the daily mean temperatures above a certain threshold temperature, usually +6 °C, Lindgren and Eriksson (1976), Prescher and Persson (1981). The calculated sums never gave a total explanation of the differences. Results presented here show that conditions given to the plants long before the actual growing season may be responsible for differences in the time of bud flushing. Well hardened plants had earlier bud flushing than less well hardened ones. The same phenomenon was found in Douglas fir (Larsen, 1978).

Based on his findings Larsen stated (translated from German): "A quick and early hardening is connected with a late dehardening of the needles but also with an early dehardening of the buds". He also found a highly significant correlation between the flushing stage ("flushing index") and the dehardening of the buds. In contrast to these statements were the results reported by Krutzsch (1975). In plants from 227 provenances of Norway spruce he studied the lignification in the autumn, which he compared with the bud bursting. On average he found no correlation whatsoever. Among the provenances he found all combinations between early or late lignification and flushing, respectively. Ekberg (1980) pointed out the large variation within provenances and the good prospect of finding families with late flushing, which may be used as a base for further breeding.

In all studies of bud flushing it was concluded that the order of the populations was not changed between years or between material of the same origin grown on different localities (Lindgren and Eriksson 1976, Prescher and Persson 1981). Only the total rapidity was different. This agrees well with the results obtained under controlled conditions.

In forest nurseries long night treatments are used to produce hardier plants for autumn planting and cold storage. The earlier bud flushing of the treated plants may be a disadvantage but is seldom critical (Rosvall–Åhnebrink 1980).

As pointed out earlier, spruce of southern origin never reaches the same degree of hardiness as that of the autochtonous plants. One reason for the very late flushing of the introduced spruces might be incomplete bud development and hardening. Nevertheless, even after good hardening conditions, treatments VI and VII, differences existed between the Swedish populations (1–5) and the more southern ones (6–10), see Table 2.

Conclusions

- The *flushing buds* and *young shoots* had little resistance from the appearance of the new needles (stage 3) until completed shoot elongation.
- No clear difference was found between the populations if the plants (1) were freeze tested at the same developmental stage and (2) were pretreated in a way giving the same degree of basic hardiness.
- The resistance was greatly influenced by the hardening treatments applied during the past growth period, *i.e.* the degree of total hardiness and bud maturation.
- During bud flushing *the whole plant* was dehardened. If the basic hardiness was not sufficient, older plant parts were susceptible to frost.
- Spruce populations moved to the north can never reach the same high degree of hardiness as the autochtonous ones. After an autumn with unfavourable conditions this might be of significance.
- The time of *bud flushing* was influenced by the degree of bud maturation – the hardier the plants, the earlier the bud flushing.
- One of certainly several reasons for the late bud flushing of southern spruce may be incomplete bud maturation. The spruce of southern origin, however, mostly avoids spring frost damage because of its late bud flushing.
- *Further breeding* with the aim of producing spruce with resistance to late spring frost has to work along two lines (1) selecting families within good provenances and after controlled crossings with late bud flushing (2) selecting the most frost tolerant individuals after repeated freeze testing, the trees then being propagated by cuttings.

Acknowledgements

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BUD-SET PHENOLOGY AS AN INDICATOR OF CLIMATIC ADAPTATION OF SCOTS PINE IN FINLAND

JOUNI MIKOLA

Genetic variation in the timing of bud set in first-year seedlings of Scots pine was studied in several greenhouse experiments. A strong association between this characteristic and the geographic origin of material was demonstrated among Finnish natural pine populations, indicating that the variation in nursery-stage growth rhythm reflects to a great extent genetic differences in climatic hardiness and adaptation. Also in experiments consisting of full-sib or half-sib progenies of plus trees differences in bud set proved to be closely related to the origin of parental trees, although a large individual variation was found to exist within populations. In long-distance crosses bud set was intermediate to the performance of respective intrapopulation crosses or natural population samples. The effects of individual mother trees became clearly visible even in progenies born from open pollination in young seed orchards located far outside the native habitats of the clones concerned. Furthermore, progenies born from a fixed set of maternal clones in the same seed orchards showed progressively earlier bud set as the age of the orchards increased. The changes in growth rhythm of progenies could be roughly explained as a consequence of improvement in internal pollination within the respective seed orchards. These preliminary results suggest some handy applications for bud set early tests in the breeding of Scots pine.

Introduction

A strong genetic control in bud set of young plants has been revealed in a great number of boreal and temperate tree species, and the adaptive role of genetic variability in this phenomenon has been demonstrated in many studies. For instance, according to Holzer (1975) the altitudinal origin of Norway spruce (*Picea abies* (L.) Karst.) seedlots of unknown provenance can be determined by their bud set at defined photoperiodic conditions in a growth chamber, even without any comparison material of known origin and adaptation.

In Scots pine the progress of bud set can be easily and accurately observed only at the free-growth stage of the first growing season following germination. Subsequent flushes of height growth are normally almost completely predetermined, the primordia of all new leaves and internodes being present already in the dormant bud, and the developing new terminal bud is visible at the shoot apex from the beginning of shoot elongation. Therefore, in order to study annual growth rhytm differences in pine seedlings more than 1 year old, other characteristics and generally more laborious methods of observation should be used. In phytotron experiments Scots pine seedlings have been found to form terminal buds sooner or later even under continuous light conditions, indicating that photoperiodic responses in this species are not as distinct as in Norway spruce (Ekberg et al. 1979).

This paper presents some results from recent investigations on the bud-set phenology of Scots pine (*Pinus sylvestris* L.) in Finland, concerning the variation among indigenous natural provenances and among progenies of individual trees. The main objective in these studies has been to find out practical applications for bud-set early tests carried out in ordinary greenhouse conditions.

Materials and methods

This paper comprises some main results from several nursery-stage experiments grown at the Maisala tree breeding station of the Finnish Forest Research Institute (lat. 60°22′N, long. 25°00′E) between the years 1975 and 1979. These experiments were composed of varying combinations of natural stand seedlots, full-sib families of individual plus tree clones, and progenies born from open pollination of certain plus tree clones at widely differing localities and at the same locality in different years. All seed slamples from natural stands comprised a mixture of open-pollinated seeds of 30 or more individual trees.

The experiments were carried out in a greenhouse. The seeds were sown in May on fertilized peat in plastic boxes. The total number of seedlings grown per family or provenance varied from 144 to 240 in different experiments. The number of replications varied from 4 to 6 and the plot size from 24 to 48 seedlings. Some special arrangements were used in the greenhouse in order to make the irrigation as homogeneous as possible and to avoid temperatures higher than +35°C. Artificial heating or illumination was never used. In most experiments no extra fertilizers were given during the first growing season.

Bud formation was monitored in all experiments by visual observations once or twice a week from July to the end of September. Each seedling was checked individually until a terminal bud became visible. From the observation data the dates when half of the seedlings had reached this stage were graphically determined for each provenance or family (Fig. 1). The duration of height growth was calculated for each test member as the interval between sowing and the date when 50 per cent of the seedlings had formed visible buds.

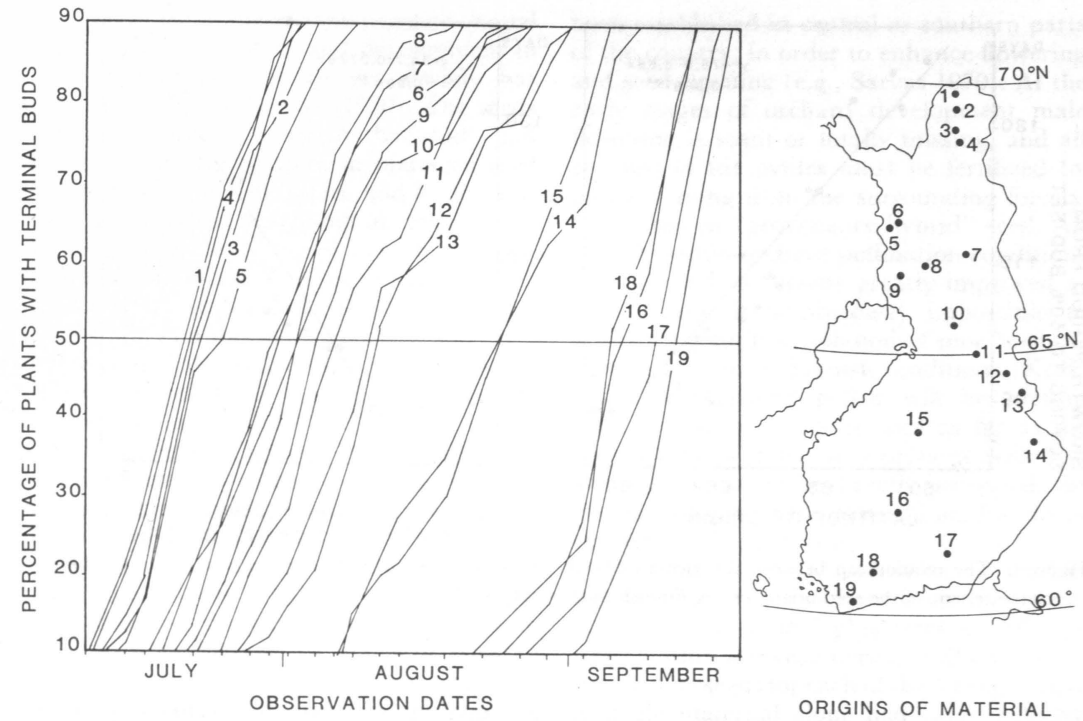

Figure 1. The progress of bud set among first-year seedlings of 19 natural Scots pine populations, and the origins of the seed samples.

Variation of bud set among natural populations

In a provenance experiment consisting of 19 samples from Finnish natural stands the initiation and termination of bud set almost invariably took place the earlier the further from north the material originated (Fig. 1). Within provenances bud set proceeded fairly linearly with time, so that the phenological order of provenances remained nearly constant through the whole period of development. In late provenances the process took progressively more time than in early ones, perhaps suggesting an increasing genetic variation within populations from north to south. However, at the beginning of September the development became much faster in all provenances in which it still was going on, maybe as a result of new accelarating external stimuli such as low night temperatures.

The relationship between timing of bud set and the origin of material among Finnish natural pine populations is further illustrated in Fig. 2. The origin of material is characterized both as the geographic latitude and as the average annual number of degree days, or heat sum, above +5°C (Kolkki 1966), of the localities from which the seeds were collected. The growth rhythm of seedlings, as measured in terms of timing of bud formation, was

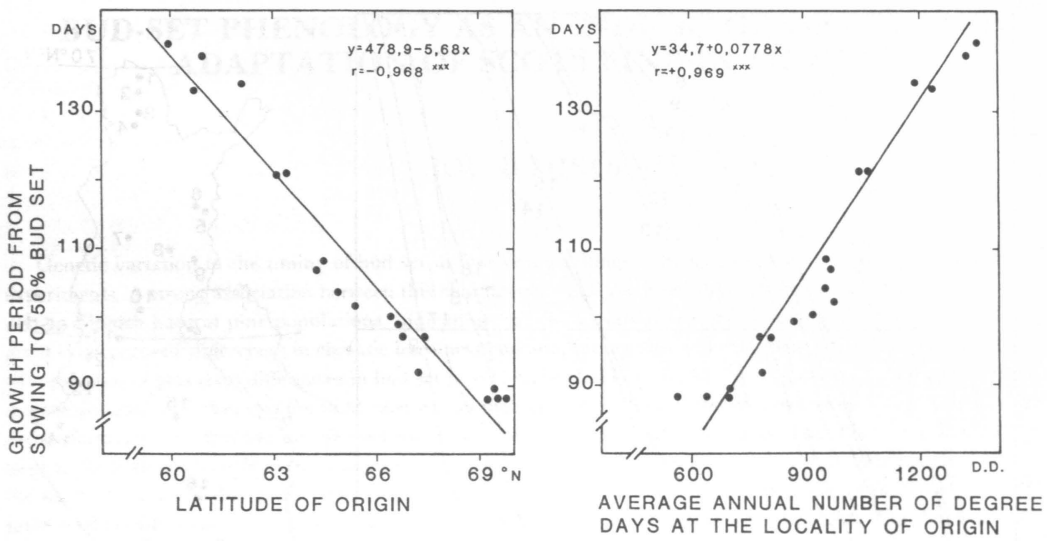

Figure 2. The relationship between the median growth period of seedlings and a geographic and a climatic characteristic of the seed origin among Finnish pine populations.

strongly correlated with both characteristics of seed origin. The equality of these geographic and climatic variables in explaining the growth rhythm differences may be a special case limited to conditions such as in Finland. In this country the variations in altitude and continentality are slight, and therefore all major changes in climate follow closely the changes in latitude.

The strong linear association between budset phenology and the features of the origin of material at the level of natural stands suggests that the variation in the timing of bud formation should almost directly reflect differences in climatic adaptation among Finnish pine populations. Although autochotonous populations may not always be the best ones as a basis of tree breeding or as a source of regeneration material (Namkoong 1969, Remröd 1974), they must be assumed to be generally well-adapted to the climatic conditions prevailing at their native habitats. Correspondingly, it should be possible to evaluate the average climatic hardiness of any pine material, e.g., a single family or an artificial population, on the basis of its bud set phenology by comparing it to the respective performance of a set of natural population samples grown in the same conditions.

Inheritance of bud set and its variation at the family level

Variation of bud set among control-pollinated full-sib families and wind-pollinated progenies of individual trees was studied in several small experiments, which cannot be separately described in this connection. Only some general findings will be summarized in the following. All these experiments included samples from natural populations as comparison materials.

Full-sib progenies of trees originating from a certain area performed, on the average, in a way similar to the natural population of their area. However, progenies of certain trees regularly expressed deviating behaviour, indicating a large individual variation within populations, in conformity with earlier studies on the inter- and intrapopulation variation of growth rhythm characteristics in boreal tree species (e.g., Hagner 1970, Dietrichson 1971).

There were some hints that crosses between phenotypical plus trees from northern Finland had a slightly later average bud set than seedlings representing respective natural populations. This may indicate that trees selected on the basis of superior growth rate

have possessed a longer than average annual active period of growth and development in their original populations, as already has been suggested by Sarvas (1970). The seeds of family materials used in this study had developed in grafted trees in managed seed orchards or clone collections, and it might be asked whether the physiological properties of the seed could have caused some fundamental difference in the bud set of family materials and natural stand samples. Anyhow, other studies on this particular problem point out that at least seed weight does not affect the bud set of Scots pine seedlings to any appreciable extent (Mikola 1980).

In long-distance crosses between northern and southern plus trees the timing of bud set was, on the average, similar to the corresponding performance of natural population samples from the areas roughly halfway between the origins of the parent trees. The same situation showed up in progenies born from open pollination of northern trees in seed orchards or clone collections located in South or Central Finland, the midpoint of bud set occuring at roughly intermediate dates between the respective timepoints in population samples from origin areas of mother trees and from localities of open pollination. Therefore, bud set in Scots pine, as well as in Norway spruce (Eriksson et al. 1978, Ekberg et al. 1979), seems to be equally determined by both parent trees over a wide range of interpopulation crosses, suggesting a multifactorial inheritance with mostly additive gene effects for this characteristic.

Bud set in seed orchard progenies of northern trees born from open pollination in different years

On the basis of the foregoing statements about intrapopulation variation in bud set, it should be expected that the average climatic adaptation of a seed orchard progeny is to a great extent determined by the genetic growth rhythm properties of the individual clones included in the orchard. A special problem concerning the adaptation of seed orchard material of Scots pine in Finland arises from the location of these orchards. All seed orchards of northern plus trees have been established in central or southern parts of the country, in order to enhance flowering and seed ripening (e.g., Sarvas 1970). At the early stages of orchard development male flowering is scant or totally missing, and all or most of the ovules must be fertilized by pollen coming from the surrounding forests, giving rise to "provenance hybrid" seed. Although within-orchard pollination conditions are expected to become greatly improved at later stages, it is obviously impossible to achieve a complete isolation of pine seed orchards, at least in Finnish conditions (Koski 1975). Background pollen will be present even in mature orchards, and as far as the proportions of different sources of pollen in fertilization cannot be directly measured, the genetic "origin" of the material produced will remain partly uncertain.

The variation in midpoints of budset among pine progenies born from open pollination of the same 7 plus trees at different times within a 9-year period is illustrated in Fig. 3. The seeds for each of the 5 progenies of a single maternal clone had been collected from the same seed orchard, but not necessarily from the same ramets of the maternal clones. Within the progeny groups of different pollination years, representing different stages of orchard development, the mother trees are shown in the same order from left to right, corresponding to the overall ranking of their progenies in the earliness of bud set. Although absolute differences among progenies were slight in this material, individual effects of the mother trees appeared surpris-

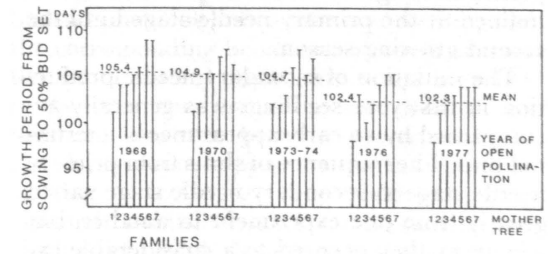

Figure 3. Variation in median growth periods among progenies born from open pollination of 7 northern Scots pine clones at 5 different years in seed orchards located in South Finlad. The maternal clones are numbered from 1 to 7 according to the overall earliness of their progenies in bud set.

ingly constant from year to year. These effects were not consistent with the order of mother tree origins from north to south, and therefore they support the previous notion that there is a large individual variation of growth rhythm within populations. The 7 mother trees originated from an area between latitudes 65 and 69°N. The northernmost tree ranked as second in its effect on the earliness of bud set in progenies, whereas the third mother was of the southernmost origin in this material. Mother tree No. 1, the progenies of which were invariably the earliest in bud set, originated from an intermediate latitude, 66°25′N. The average growth periods of progenies born in different years became abruptly shorter within the 9 years' period (Fig. 3), obviously as a result of the initiation and rapid increase of pollen production within the seed orchards. An analysis of variance on plot data of the material revealed statistically significant differences ($p<0,05$) both between mother trees and between pollination years, and no mother tree × pollination year interaction could be demonstrated.

This experiment also included material from natural populations, as a comparison for the seed orchard progenies. In this case (Fig. 4) the relationship between growth rhythm and latitude of origin among the natural stand samples was not as tight and steep as in the provenance experiment described in the foregoing (Fig. 2). The divergence must be due to differences in external conditions affecting bud set in the two experiments. In this experiment (Fig. 4) many seedlings produced secondary needles before the formation of the first terminal bud, while in the previous experiment (Fig. 2) almost all seedlings remained at the primary needle stage until the second growing season.

The initiation of secondary needle production in first-year seedlings was generally accompanied by an early appearance of a terminal bud. The frequency of shifts from primary needle stage to secondary needle stage varied greatly from one experiment to another, but whenever they occured to a considerable extent, the genetic control of bud set became partly obscured, as compared to the distinct differences among provenances or families in experiments in which all or nearly all seedlings maintained the primary needle state. A shift to the secondary needle stage during the first growing season is evidently a quite normal phenomenon in pine seedlings in ordinary nursery conditions, but in connection with the study of genetic variation in bud set it must be regarded as a disturbance, indicating the existence of serious environmental sources of variation. However, even in the provenance material shown in Fig. 4, in the presence of considerable amounts of undefined non-genetic variation, the bud set phenology of plants proved to be strongly connected to the geographic origin of the seed.

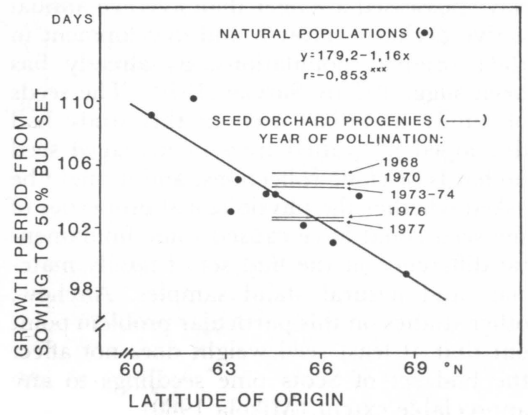

Figure 4. Average growth periods and latitudes of origin for the seed-orchard materials shown in Fig. 3, as compared to the variation of respective characteristics among 10 natural population samples. The horizontal lines represent seed-orchard progenies born from open pollination of the same set of maternal clones in 5 different years. The length of the lines shows the potential range in the average latitude of origin in seed orchard materials. The exact values would settle somewhere along these lines, depending on the proportions of external and internal pollination in the seed orchards. For further explanation, see the text.

The horizontal lines in Fig. 4 describe the average position of the seed orchard materials of different pollination years in relation to the variation of natural stand samples. The length of the lines shows the range in average latitudes of origin among seed orchard progenies, expected on the basis of varying degrees of internal pollination within the orchards and background pollination from adjacent wild stands. The seed orchards concerned were located approximately at the

latitude of 62°N. The average latitude of origin among clones included in these orchards was 67°N. Respectively, the average origin of the seed produced would correspond roughly to 64°N, if all fertilizing pollen had come from surrounding natural populations, and to 67°N, if complete internal pollination had prevailed within the orchards.

The average growth periods among seed orchard progenies of the three earliest pollinations years (Fig. 4) were very similar to the performance of natural stand material originating from latitudes around 64°N. This is close to the behaviour expected on the basis of completely external pollination. The slightly longer growth periods of seed orchard material may support the idea that northern plustrees generally possess a longer than average annual active period in their original populations. On the other hand, among seed orchard progenies of the two most recent pollination years the average growth periods correspond to the behaviour of natural populations from latitudes about 2 degrees further north. Similarly, this would require the internal pollination among plus-tree clones to have comprised more than half of all fertilizations in the seed orchads.

It is well known that in grafted trees of Scots pine male flowering starts later and at the beginning increases at a slower rate than female flowering. According to the measurements of Bhumibhamon (1978, p. 81), pollen production in Finnish seed orchards of northern clones is very scant at the early ages, but turns to a rapid increase when the height of the grafts is about 4 meters. As the grafts attain a height of 7 to 9 meters, total pollen production may reach a level of 20 kg per hectare, which has been argued to be sufficient for a complete within – orchard pollination in the presence of some isolation by flowering time between a seed orchard and the surrounding forests (Sarvas 1970, Koski 1975).

The pollen productivity conditions of the orchards from which the progeny materials in Fig. 4 were collected can be viewed against the above figures. A field inspection in 1977 revealed that the average height of grafts had been 4,5 to 5,0 meters in 1976 and 1977, when the pollination of the two most recent progeny samples took place. Thereby it seems quite likely that the phase of rapid increase in pollen production could have started just before these years, and the distinct change in average growth periods between the samples of the 3 earliest and the 2 latest years (Fig. 4) really is a consequence of abrupt improvement in pollination conditions within the respective orchards. However, as stated before, this change would indicate an internal pollination of at least 50 per cent in 1976 and 1977, but according to the figures of Bhumibhamon (1978, p. 81) total pollen productivity in these orchards should still have been at the most only 5 kg per hectare. In more recent years 1978, 1979 and 1980, Koski (1981) made pollen productivity measurements in one of the seed orchards concerned and found total figures of 1,0, 6,6 and 12,3 kg per hectare, respectively. Thus the changes in bud phenology observed in this experiment appear to be greater than expected on the basis of pollen productivity studies. Nevertheless, the discrepancy may be partly due, for instance, to yearly vatiations in the abundance of flowering, taking place apart from the general increase of flowering as a consequence of increased size of the grafts. Furthermore, the amount of pollen needed for a satisfactory internal pollination in seed orchards may be below the level of 20 kg per hectare, which Sarvas (1962) deduced to be required in normal stand conditions.

Concluding remarks

The main conclusion from the studies reported in the foregoing is that the average climatic hardiness of any Scots pine seedlot can be roughly predicted by comparing the bud-set phenology of its first year seedlings to the corresponding behaviour of a representative set of natural population samples grown in the same conditions. For seed material of northern plustrees born in young seed orchards, evidence supporting this statement is starting to accumulate from field tests, too, although published results are scant so far (Kylmänen 1980; Nikkanen, manuscript in preparation). In numerous series of experiments replicated at several localities, at the age of 5 to 10 years the survival of externally pollinated seed orchard progenies has been equal to the survival of material collected from local natural stands up to latitudes ap-

proximately halfway between the origins of the maternal clones and the seed orchard localities, but progressively inferior in experiments located at higher latitudes. Thus both nursery-stage bud phenology and survival data from field tests consistently indicate that climatic hardiness of Scots pine seedlings is determined by the genetic properties of the parent trees, irrespective of the locality where the seed developed, in contrast to the findings of Bjørnstad (1981) in Norway spruce.

The revealed distinct association between the bud set phenology of seedlings and the average latitude of origin both in natural stand materials and among progenies of individual trees suggests useful applications for bud-set early tests in Scots pine, at least for Finnish conditions. Even slight genetic differences in bud set can be detected in materials grown in ordinaty greenhouse conditions and obviously they reflect to a great extent real differences in climatic adaptation. The sharply determined variation in the timing of bud set may be revealed for a large range of seed origins at a single locality without any artificial control on photoperiod or on other environmental factors which might act as inducers of bud set in natural conditions. However, all evaluations in nursery experiments must be based on the comparative performance of standard material, the adaptation of which is known or can be taken for granted.

The appearance of secondary needles was observed to somewhat confuse the genetic growth rhythm differences among provenances or progenies. This occasional change in developmental stage in first year seedlings of Scots pine was presumably due to some temporary disturbance in the continuity of height growth, caused by transient stress factors like drought or high temperature. However, in the experiments reported here the reasons of this phenomenon remained quite unclear. Regarding practical applications for bud set early tests, it might be beneficial to find out cultural treatments or growing conditions in which the seedlings would definitely remain at the primary needle stage until the end of the first growing season.

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INDIVIDUAL VARIATION IN SEED MATURATION IN MARGINAL POPULATIONS OF SCOTS PINE

MARTTI RYYNÄNEN

Seed of Scots pine (*Pinus sylvestris* L.) from five natural stands in northern Finland was collected from single trees during several years, both good and poor ones. Seed was then x-ray radiographed and seed quality was determined as the proportion of the embryo of the embryo cavity.

The variation in seed quality between trees within some of the stands was considerable, especially in bad seed years. It seems, that in natural stands of Scots pine it should be possible to find individuals capable to produce mature seed also in years typical to northern Finland.

Introduction

In it's central populations Scots pine (*Pinus sylvestris* L.) produces fully ripened seed every 2–3 years. In the marginal population like in northernmost Finland it has seedyears with mature and fully ripened seeds less frequently. According to Renvall (1912) there are good seedyears in the forest borderline about once in century, according to some other investigators every 10–15 years. In the older papers (e.g. Renvall 1912, Heikinheimo 1921, Kujala 1927) it has been concluded that the temperature during the second summer, when the pine seed ripens, is most important for the development of the embryo and thus for the maturation of the seed. In the cold climate the summers are not warm and long enough for full seed ripening. According to Sarvas (1962) for 50 per cent ripening of pine seed temperature sum of 845 degree days is demanded (threshold value +5°C). For example in Kolari and in Sodankylä (67°20′ N) mean value of 30 years is approx. 780 d.d.

The development of pine seed from the initiation of flower bud to the mature seed requires as the matter of fact three summers. The first summer, when the flower bud initiates, is usually excluded, and it is said, that the first year in seed development is the year when the flowering or pollination takes place. It is well known, that the pollentube and male nucleus are wintering in the tip of nucellus. The cone grows rapidly early during the second summer. The fertilization takes place in midsummer, approx. 13 months after pollination. At that time the cone and the seed are almost full of size (Sarvas 1962). In the marginal population it is possible that during cold summer there is no fertilization at all.

In every ovule more than one archegon can be fertilized and thus polyzygotic or primary polyembryos are formed. In addition to this archegonial polyembryony the proembryo usually divides into four. This so-called cleavage or monozygotic or secondary polyembryony is typical for pine (e.g. Dogra 1967). Because of this the development of embryo stopps at least for a week in southern Finland and perhaps for three weeks in northern Finland.

Normally only one of the embryos develops into full size and the others disappear. If the summer is unfavourable and fertilization takes place later than usual, possibly in August, the growing season is not long enough for full seed ripening. Already Kujala (1927) has shown that in northern Finland there are usually several small embryos in the pine seed after the growing season. According to Simak (1972) frosts are harmful to the maturation of seed and they cause retarded embryo development. After that kind of summer there are several small embryos i the seed, none of these capable to germinate. After very warm summer there is only one embryo in the embryo cavity also in the pine seed of Lappland. The development of the embryo dapends on both temperature and time, and there are many possibilities to disturb the development of seed from proembryo to fully ripened ones (Sarvas 1962).

In routine x-ray analysis of Scots pine seed we found that the variation in seed quality within stand is considerable. It seems to be worth while investigating if there exist trees

with genetical character making it possible to produce mature seed also in cold summers.

Material and methods

The seed was collected from standing, single trees in natural stands in different parts of northern Finland. The cones were collected from all sides of the crown. The wings were removed from seeds by hand.

The seed was x-ray radiographed. Every tree was represented by 400 seeds, if possible, but from some trees the yield was smaller even only 100 seeds. Seed quality was calculated by embryo classes, determined as the proportion of the embryo of the embryo cavity. Expected germination percentage was calculated by means of conversion factors according to Simak (1957), slightly modified. The x-ray radiographs were examined by stereomicroscope (1971, 1972) by magnification of 9 times or on the screen of the enlarging apparatus for microfiches (1979, 1980) by magnification of 15 or 32 times.

Results and discussion

The variation in seed quality was great between years and stands (Table 1). In standard stand no. 6 (Kolari, Table 2) seed was collected also in 1971. The weather conditions were typical for Lapland in this year and mean of expected germination percentage was 48,4. There were three individuals, namely trees no. 85, 144 and 208, with seed of high quality (exp. germ. % 71, 81 and 80, resp.) also in that year. Same trees produced good seed also later, except tree no. 208, which produced no cones in 1980. There were also trees with very low seed quality, e.g. tree no. 149. The quality of seed of this tree was under the mean every time investigated.

In 1972 seed was matured as far as the timber line, even to the tree limit. In all stands investigated in that year there was only insignificant variation between trees in seed quality.

In 1979 seed was matured fairly well only in southernmost stand, Kolari Lakkarova. In all other stands the maturation of seed was very low, and mean of expected germination percentage was from 11,4 to 43,5. In that kind of year there can be a wide range of variation between trees also. E.g. at Inari, Kaamanen expected germination percentage varied from 6,4 (tree no. 61) to 50,5 (tree no. 126).

The year 1980 was fairly good for seed maturation, so the mean value of expected germination varied between 32,3 and 90,6 per cent. The variation between trees was quite small in southern stands but it came more considerable in northern stands. E.g. at Enontekiö expected germination varied from 26,4 (tree no. 34) to 86,9 (tree no.73).

In every five stands investigated there were trees which produced seed that matured better than the average and trees with poor matured seed. During bad seed years (cold summers) the quality of seed of poor producers was clearly below the mean value of the stand.

Trees that produced seed better than average during cold or medium years didn't do that during good years. This means that the real value of individual as seed producer appears during bad or average seed year. This character seems to exist nearly in the northernmost stand (Inari, Kaamanen).

Trees which produced good and poor seeds are often situated close to each other. So it seems, that there is no edafic or microclimatic reasons to differencies in seed quality. In all probability the ability of the tree to produce seed is genetically controlled.

In this connection it is worth saying that according to Linhart et al. (1979) differencies in fertility of *Pinus ponderosa* Laws. as measured by cone production, have a substantial genetic basis. But the ability of tree to produce seed has not been investigated.

Table 1. Stands and seed quality for stands.

Stand	Lat. N	Long. E	Alt.m	Number of trees	Mean value of expected germ. percentage 1971	1972	1979	1980
Kolari, Lakkarova	67°9′	24°7′	200	41	48,4	93,8	82,7	90,6
Kolari, Ylläs	67°34′	24°11′	450	11	–	76,6	13,4	63,1
Kittilä, Pallasjärvi	68°0′	24°15′	301	14	–	90,3	24,9	64,1
Enontekiö, Kaaresuvanto	68°29′	22°28′	325	27	–	81,3	11,4	56,8
Inari, Laanila	68°30′	27°27′	221	5	–	–	43,5	56,7
Inari, Kaamanen	69°8′	27°15′	150	13	–	–	17,6	32,2
Utsjoki	69°38′	27°07′	190	7	–	–	5,5	20,8

Table 2. Seed quality for extreme seed producers.

Stand and trees	Expected germination percentage 1971	1972	1979	1980
Kolari, Lakkarova St. 6				
Mean of the stand	48,4	93,8	82,7	90,6
Tree no. 85	71,0	89,7	82,1	97,2
144	81,2	94.0	92,5	97,0
208	80,4	90,9	97,3	–
149	30,6	91,9	56,7	82,1
Kittilä, Pallasjärvi St. 4				
Mean of the stand	–	90.3	24,9	64,1
Tree no. 69	–	89,3	50,0	72,4
92	–	91,5	30,9	79,6
153	–	87,2	13,1	54,0
Enontekiö, Kaaresuvanto				
Mean of the stand	–	81,3	11,4	56,8
Tree no. 15	–	83,5	29,6	83,7
16	–	83,4	21,8	79,3
33	–	94,6	–	72,2
73	–	93,2	11,6	86,9
34	–	70,8	–	26,4
Inari, Laanila St. 3				
Mean of the stand	–	–	43,5	56,7
Tree no. 229	–	–	55,1	67,9
220	–	–	18,0	44,7
Inari, Kaamanen St. 2				
Mean of the stand	–	–	17,6	32,3
Tree no. 100	–	–	38,3	49,1
126	–	–	50,5	69,2
61	–	–	6,4	17,4

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PART IV

ECOLOGICAL DIFFERENTIATION OF FOREST TREES

VARIATION IN THE ACTIVITY OF THE CATALASE ENZYME IN PROVENANCES OF SCOTS PINE

JUKKA VIDGREN and MAX. HAGMAN

The variation in the activity of the catalase enzyme was studied in young seedlings of Scots pine (*Pinus sylvestris* L.) grown from 41 different samples from the USSR and from 10 samples of Finnish origin. The activity of the enzyme expressed per milligram fresh weight of seedlings seems in general to decrease with decreasing latitude of origin between latitude 67° N. and 54° N. Below the latitude 54° N. this trend is not so clear. In all samples studied there is a considerable variation between the individual seedlings. The possibilities of using the activity of the catalase enzyme in distinquishing between provenances of Scots pine and in the selection of northern and southern types within a provenance are discussed.

Introduction

There is for several reasons a great need to identify the amount and distribution of genetic variability in the species of forest trees. For the proper conservation of gene resources it is necessary to know what amount of variation is present within and between populations of different geographic origin. Knowledge of this nature will provide possibilities for estimations of the amount of gene flow over shorter or longer distances and of the effects of isolating mechanisms of different kinds.

When seed sources are collected and seeds or plants distributed it is necessary to have methods for the identification of provenances and in more advanced breeding situations to have descriptors for each individual tree. This identification will be more and more reliable when the number of characters of uncorrelated nature increases.

In addition to the classical descriptions using anatomical, morpholocical and phenological characters, biochemical factors of different kinds have in recent times became more and more studied. Such studies have involved terpenes, phenolic compounds, glucoproteins and other compounds (See e.q. Muhs, 1981a for a review).

Isozymes have been particularly useful in identification of individuals and in the estimation of gene flow in seed orchards and natural stands (Muhs, 1981b). These studies have, however, mainly concentrated on the qualitative variation of the enzymes and the studies of the quantitative values of enzymes are not so many.

One exception is the enzyme catalase, for

which quantitative studies in forest trees were carried out already in the 1920's and for which differences between provenances were shown for Scots pine and Norway spruce (Schmidt 1930a, Schmidt 1930b, Langlet 1936, Schmidt 1966).

In these studies it was found, that in provenances from more northern and from higher sources, that is, in general from places with a more rough climate, the activity of the catalase enzyme was higher. Similar results were later obtained for *Picea abies* (L.) Karst. by Bartels (1964).

The studies of Scots pine carried out by Schmidt and Langlet were, however, done with rather limited sources of material and the volumetric method used by Schmidt somewhat complicated. For the purpose of analyzing single seeds, single endosperms and even single embryos of Scots pine, Mikola, Hongisto & Hagman developed a method on the basis of the clinical standard method of Aebi (1974). Results of these experiments with three provenances of Scots pine were reported at the meeting on Early testing in forestry held at Riga in 1980 and will be published elsewhere. The differences found between provenances encouraged us to try the same techique on a larger sample of sources. Since the late Dr. E. P. Prokazin of the Pushkino research institute near Moscow, USSR, had in 1977 sent to Finland a big collection of pine seeds we decided to take as the object of our present study to investigate the amount of variation in the activity of the catalase enzyme in these provenances which, as can be seen below, covered most of the area of distribution of the Scots pine in the USSR.

Material and methods

Material

The seed material obtained from the USSR consisted of 89 provenances of which 41 were taken for study. The origin of these samples is seen in table 1. In figure 1 the approximate localization of the sites of origin within the USSR can be seen.

In addition to the samples from the USSR, ten samples from eight different provenances in Finland were investigated. The origin of these samples is seen in table 2.

In table 1 the origin of the seed lots is given as translated from the Russian text indicating region or province and forest district (ljeshos). Since no maps of the forest districts of the USSR are available to us we have taken the forest district to be located around the place with the same name, found on common maps. The geographical coordinates here given are based on the named places and does thus not exactly show the origin of the seed. This uncertainity has to be kept in mind when the results are discussed. The Russian certificates of origin did neither contain data about the altitude of the seed source. These altitudes we have estimated from the same maps as for the latitudes and longitudes. Particularly in the montaneous regions of Central and Eastern Asia this estimation might be much in error.

It has been indicated by our Russian colleagues, that the samples obtained were to a certain degree the same as those used in the All Union Provenance Experiment laid out in the USSR some years ago. Thus the reference numbers C-1, C-2, etc. might help in the identification of the provenances for those who have access to the results of the large provenance test mentioned.

The Finnish seed lots used were collected from the standard stands of the Forest Research Institute and are thus precisely known. These samples consisted of a mixture collected from the trees growing on a sample plot of the size of 1 hectare. For two of the stands, Bomarsund and Pihtipudas, samples were available from two different collection years but from the same stands.

Methods

The seeds were sown December 1.–2. in 1980 in the glass house of the Forest Tree Breeding Station of the Forest Research Institute. The substrate was ordinary nursery peat given basic fertilizers. During the dark season December–February additional light was given daily betweed 8.00 and 16.00 by 3 Philips sodium light lamps type HDK-259-C, 400 W at a height of 1.5 m from the substrate.

Each seed lot was sown with 100 seeds and the plots were randomized over the sowing space. The collection of samples for the analysis started on March 9. and continued to May 30. 1981. In order to reduce errors caused by the fairly long sampling period the order in which the different lots were sampled was randomized.

From each lot samples consisting of single seedlings were taken, their roots removed and the fresh seedling weighed immediately to give the reference fresh weight. At the time of sampling the seedlings weighed from 5 to 70 mg. In general 10 seedlings of each lot were investigated.

The extraction of the enzymatic activity was done by homogenizing the seedling with a glass homogenizer in 4 ml 50 mM monopotassium-disodium-phosphate buffer of pH 7.3 containing 0.1 % Triton-X-100. The homogenization time was about 60 seconds and it was carried out at room temperature. The extract was separated by centrifugation for 15 min at 15 000 × g.

The estimation of the catalase activity was following the assay method of Aebi (1974). We used a single beam spectrophotometer (Carl Zeiss PMQ 2) equipped with a recorder and quarz cuvettes (d = 1 cm). The reference cuvette was filled with 0.9 ml enzyme extract and 0,1 ml phosphate buffer (without Triton-X). In the measuring cell was 0.9 ml enzyme extract. After adding and rapidly mixing of 0.1 ml of 150 mM H_2O_2 -solution into the

Table 1. Origin and catalase activity of the pine samples from the Soviet Union.

Sample nr	Seed lot nr	Origin	USSR nr	Latitude N	Longitude E	Altitude m	Catalase units /mg
1	GI-77-1613	Murmansk obl. Monthergorsk	C- 1	67° 56′	32° 55′	100– 200	30,0
2	GI-77-1614	Murmansk obl. Kandalaks	C- 2	67° 10′	32° 25′	0– 100	28.4
3	GI-77-1618	Karelskaja ASSR Tsupinsk	C- 12	66° 16′	33° 05′	0– 100	23.4
4	GI-77-1685	Krasnojarsk kraj Turuhansk	C-104	65° 50′	88° 00′	0– 200	25.6
5	GI-77-1622	Karelskaja ASSR Kem	C- 18	64° 58′	34° 40′	0– 100	17.8
6	GI-77-1615	Archangelsk obl. Pineschk	C- 3	64° 40′	43° 25′	0– 100	27.6
7	GI-77-1619	Komi ASSR Kadscheromsk	C- 13	61° 41′	56° 00′	100– 200	25.2
8	GI-77-1697	Jakutskaja ASSR Kakutsk	C-118	62° 00′	123° 00′	200– 500	21.6
9	GI-77-1620	Karelskaja ASSR Sortavala	C- 16	61° 41′	30° 40′	0– 100	25.2
10	GI-77-1621	Karelskaja ASSR Pudosh	C- 17	61° 49′	36° 40′	0– 100	20.3
11	GI-77-1696	Jakutskaja ASSR Olekminsk	C-117	60° 25′	120° 30′	200– 500	22.0
12	GI-77-1617	Vologod obl. Totemsk	C- 9	60° 00′	42° 46′	100– 200	18.0
13	GI-77-1616	Vologod obl. Tserepovetsk	C- 8	59° 10′	37° 54′	100– 200	20.2
14	GI-77-1623	Leningrad obl. Tosnensk	C- 19	59° 32′	30° 51′	0– 100	16.6
15	GI-77-1678	Krasnojarsk kraj Nishne–Jeniseisk	C- 93	58° 40′	92° 10′	0– 200 ?	23.8
16	GI-77-1675	Tomsk obl. Kolnaschevsk	C- 88	58° 20′	82° 40′	0– 100	14.8
17	GI-77-1626	Novgorod obl. Krestetsk	C- 23	58° 17′	32° 30′	0– 100 ?	11,0
18	GI-77-1625	Pskov obl. Pskov	C- 22	57° 48′	28° 20′	0– 100	12.4
19	GI-77-1700	Habarovsk obl. Ajansk	C-122	56° 30′	138° 00′	0– 1000 ?	19.4
20	GI-77-1624	Pskov obl. Velikoluki	C- 21	56° 20′	30° 30′	200– 400 ?	11.6
21	GI-77-1688	Irkutsk obl. Bratsk	C-108	56° 00′	101° 40′	500–1000	16.0
22	GI-77-1629	Vitebsk obl. Rossonsk	C- 28	55° 52′	28° 40′	100– 200	16.0
23	GI-77-1670	Kurgansk obl. Kurgansk	C- 79	55° 28′	65° 18′	0– 200	18.0
24	GI-77-1680	Krasnojarsk kraj Daursk	C- 96	55° 15′	92° 05′	200– 500	17.8
25	GI-77-1627	Litovskaja SSR Prenaisk	C- 26	54° 37′	23° 55′	0– 200 ?	9.4
26	GI-77-1654	Uljanovsk obl. Sursk	C- 58	54° 30′	46° 40′	100– 300	13.6
27	GI-77-1655	Uljanovsk obl. Melekersh	C- 59	54° 15′	49° 40′	100– 200	15.8
28	GI-77-1647	Orlovsk obl. Turgenevsk	C- 52	53° 28′	36° 46′	200– 300	14.8
29	GI-77-1628	Mogiljevsk obl. Osipovitsk	C- 27	53° 20′	28° 40′	0– 100	20.2
30	GI-77-1630	Grodnensk obl. Slonimsk	C- 30	53° 05′	25° 20′	100– 200 ?	15.2
31	GI-77-1648	Tambov obl. Tselnavsk	C- 54	53° 05′	41° 20′	100– 200	11.6
32	GI-77-1701	Kustanaisk obl. Ara-Karagaisk	C-123	53° ?	64° ?	100– 200 ?	14.6
33	GI-77-1702	Koktjetaisk obl. Urumkaisk	C-124	53° ?	69° ?	?	14.6
34	GI-77-1665	Bashkirskaja ASSR Zalairsk	C- 72	52° 13′	57° 25′	500– 700	11.0
35	GI-77-1690	Burjatskaja ASSR Zaudinsk	C-111	52° 00′ ?	109° 00′ ?	?	21.2
36	GI-77-1664	Bashkirskaja ASSR Abzjansk	C- 71A	51° 50′	56° 40′	200– 300	15.0
37	GI-77-1649	Voronesh obl. Voronesh	C- 55	51° 40′	39° 15′	100– 200 ?	14.2
38	GI-77-1663	Bashkirskaja ASSR Dubansk	C- 70	51° 30′ ?	56° 40′ ?	200– 300 ?	17.0
39	GI-77-1651	Voronesh obl. Hrenersk	C- 56	51° 08′	40° 20′	100– 200	15.2
40	GI-77-1657	Volgograd obl. Kamschinsk	C- 62	50° 05′	45° 23′	100– 200	16.0
41	GI-77-1656	Rostov obl. Veschensk	C- 60	49° 40′	41° 43′	0– 100	14.0

Table 2. Origin and catalase activity of the pine samples from Finland.

Sample nr	Seed lot nr	Origin	Latitude N	Longitude E	Altitude m	Catalase units /mg
42	Gl-62-001	Åland Bomarsund	60° 13′	20° 13′	20	20.0
43	Gl-66-021	Åland Bomarsund	60° 13′	20° 13′	20	13.7
44	Gl-65-500	Bromarv Solböle	60° 02′	23° 02′	30	14.0
45	M29-70-23	Lapinjärvi	60° 39′	26° 10′	30	15.6
46	M29-70-26	Padasjoki	61° 25′	25° 00′	115	17.2
47	Gl-65-497	Ristiina	61° 29′	27° 21′	90	22.4
48	Gl-65-495	Pihtipudas	63° 23′	26° 06′	165	19.2
49	M29-70-35	Pihtipudas	63° 23′	26° 06′	165	19.4
50	M29-70-01	Pielisjärvi	63° 04′	29° 49′	130	20.0
51	Rl-60-125	Rovaniemi	66° 20′	26° 45′	100	26.4

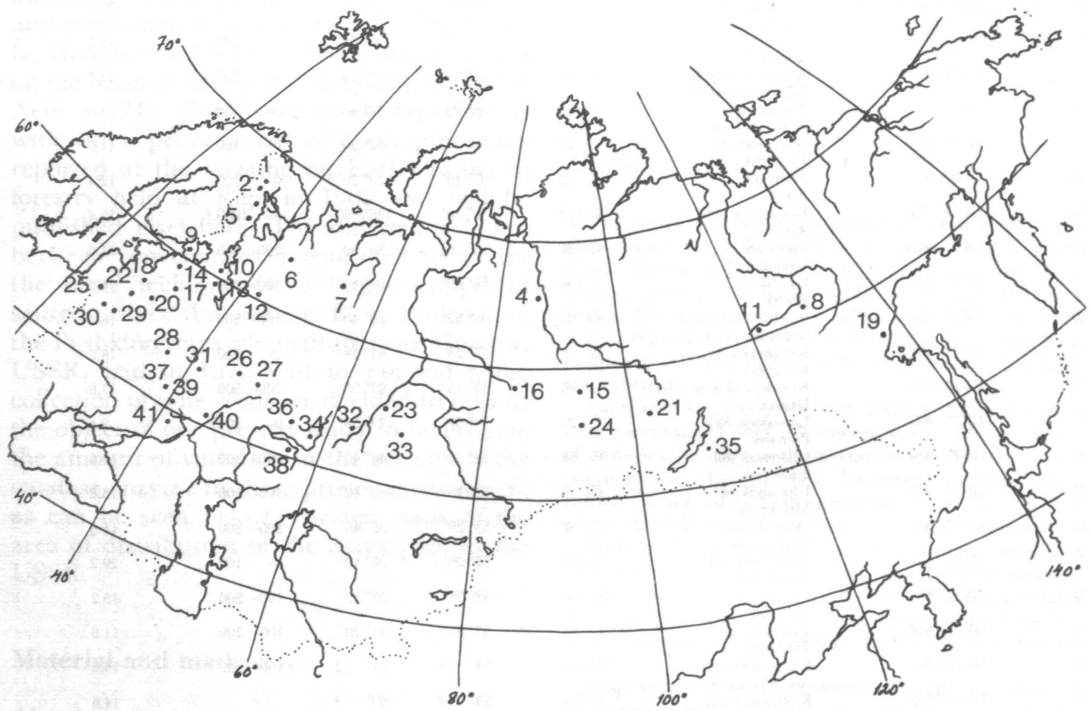

Figure 1. Geographical localization of the samples from the USSR.

cuvette the absorbance was registered at 240 nm and the reaction followed with the recorder for about 100 seconds.

The amount of catalase in the tissue extract is proportional to the first order rate constant of the reaction and one unit catalase is the amount of enzyme giving an apparent rate constant of 0.01 min^{-1}.

The catalase activity was calculated as follows. The absorbances are read for two points separated by 60 seconds and marked as A_0 and A_{60} giving the contribution of H_2O_2 to the absorbances. The reference value (the absorbance without H_2O_2) is marked as A_R. The activity in 1 ml of enzyme solution is thus:

$$\text{catalase activity} = \frac{100}{0.9} \times \ln \frac{A_0 - A_R}{A_{60} - A_R} \text{ units / ml.}$$

All assays were carried out at room temperature.

The seedling extracts (1 seedling in 4 ml buffer) were diluted 1:20 before the assay. The values were then multiplied by 80 to obtain the activity as catalase units per seedling.

Using these figures and the weight of the seedling the activity in the tables is expressed as catalase units / mg fresh weight.

Results

The mean values obtained for the different samples are tabulated in tables 1 and 2.

The highest mean values for the sources from the USSR is 30.0 in the sample from Murmansk and the lowest mean in the sample Nr 25 from the Lithuanian republic giving an activity of 9.4 units.

As seen in figure 2 where the individual values as well as the means are indicated for each sample there is a considerable variation within each seed lot. The highest individual value is 34.0 units which occurs in 4 different provenances between lat.67° and 60° N. The lowest value is 6.0 in the sample Nr 20 from Pskov with very similar values from the samples Nr 36, Abzjansk and Nr 39, Hrenersk.

The largest variation within a sample occurs in provenance Nr 21 from Bratsk in the Irkutsk oblast. Unfortunately some of the samples showed very low germination and thus the lowest variation cannot be reliably estimated due to lack of seedlings in some provenances.

When the provenances are put, as in figure 2, in an order of decreasing latitude, one can observe that between the most northern provenance and down to the provenances from about 54° there is an almost clinal decrease in the catalase activity. Further south this picture is not so clear and the results are variable. To what extent this latter observation can be the result ot the differences in altitude of the sources, is difficult to estimate due to the lack of exact figures for the height above sea level for the provenance studied.

If results from the western and eastern sources are compared for approximately the same latitude, the results are of the same magnitude. Perhaps there is an indication of somewhat higher values for the eastern sources but, as mentioned, this could also be due to differences in altitude.

In the Finnish samples (table 2) the same trend can be observed as in the samples from the USSR. The most northern provenance,

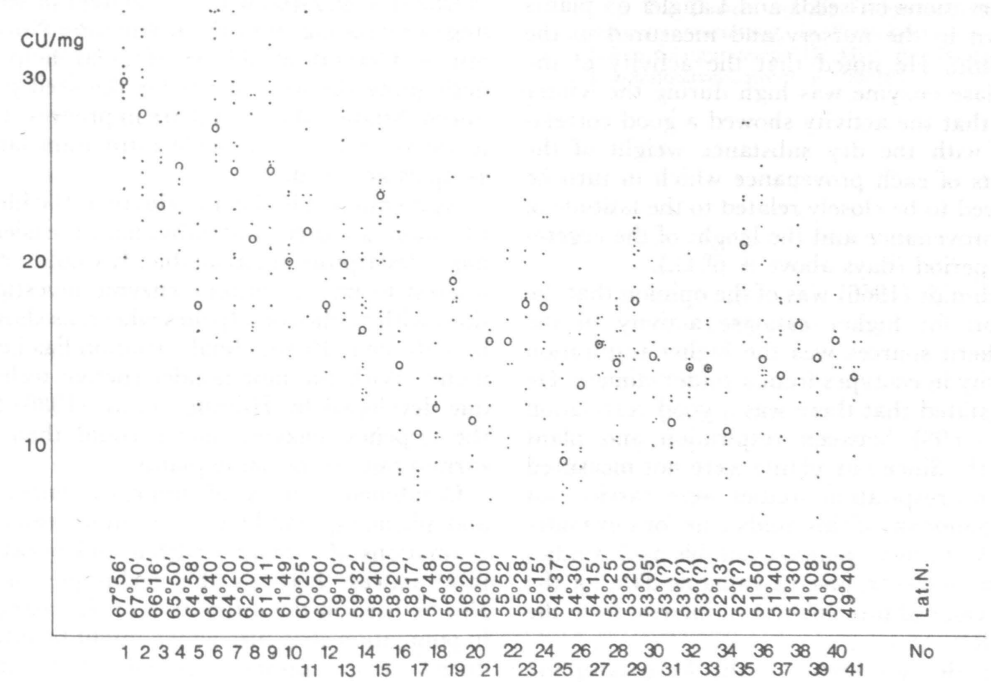

Figure 2. Catalase activity in the samples from the USSR in relation to the geographical latitude of the sample.

Rovaniemi from lat.66° has the highest value 26.4. which is well within the range of the values of the provenances from Eastern Karelia. The more southern provenances have in general lower catalase activities.

The two samples from different years in Pihtipudas agree well with each other but the two samples from the Bomarsund stand on the Åland islands are exceptional in that the difference between the years is great, the values being 13.7 and 20.0 respectively. The reason for this difference is at the present unknown.

When one compares the figures for the provenances Ristiina and Padasjoki from approximately the same latitude the more eastern provenance has the higher value. More sampling is necessary in order to prove if this is a general trend in Finland.

Discussion

The results obtained with our material of provenances from the USSR and from Finland agree well with the observations made by Schmidt and by Langlet as reported in the papers already mentioned. Schmidt made his observations on seeds and Langlet on plants grown in the nursery and measured in the autumn. He noted that the activity of the catalase enzyme was high during the winter and that the activity showed a good correlation with the dry substance weight of the plants of each provenance which in turn he showed to be closely related to the latitude of the provenance and the lenght of the vegetation period (days above + 6° C.).

Schmidt (1966) was of the opinion that the reason for higher catalase activity in the northern sources was the higher respiration activity in ecotypes from a colder climate. He also stated that there was a good correlation ($r = -0.93$) between respiration and plant growth. Since our plants were not measured and no respiration studies were carried out we cannot say if this holds true for our material. With more plants available such studies could, however, give us valuable data for the behaviour of provenances of interest from the USSR.

We do not know, if the long sampling period has induced changes in catalase content of the seedlings taken at different times although, as mentioned, we tried to reduce any such effect by taking the provenances at random. Seasonal changes in the catalase content of conifer leaves have been reported (Doyle & O'Connor 1930). A study of the activity of the catalase enzyme in needles of older plants of different provenances grown at the tree breeding station is in progress. This provenance trial will allow for repeated sampling through the seasons.

The method developed for serial studies of large samples of single seedlings will be used to estimate the variation within populations of different origin. Of particular interest for the plant breeder is if in the provenance elements of northern and southern types can be identified and e.g. the proportion of northern types can be assessed. This would be a higly valuable tool for the early testing of provenances to be used in rough climates.

Perhaps the method will also offer possibilities to estimate the amount of long distance pollination in seed orchards particularly in the case when northern seed orchards, as in Finland, for reason of seed maturation have to be located in the central parts of the country. A method for the assay of seedlings originating from the pollination Northern × Northern compared with the amount of seedlings originating from the pollination Northern × Central would be of great help in desingning the area of use for the seed produced. Studies of this kind are in progress but it seems that for a reliable estimation large samples are needed.

As mentioned in the introduction, the identification of sources and individuals is easier if more descriptors are available. It would be of interest to try to combine enzyme investigations with studies of terpenes where, as shown by Hiltunen (1976), clinal variation has been found. With the new nondestructive technique developed by Hiltunen et al. (1980) for the terpenes, enzyme assays could then be carried out on the same plants.

Combined studies of terpenes, enzymes and phenology could give us more reliable estimations of variation within and between provenances. Perhaps this technique could also be used in the estimation of the changes in population structure which might be introduced by the present methods of growing nursery plants in warm greenhouses compared with the cultivation out of doors in the natural local climate.

No attempt has yet been made to find out the causal background for the variation in catalase activity with provenance, although Schmidt's suggestions are interesting.

The case with the different results from the same stand at Bomarsund on the Åland islands is so far unexplained. The Åland islands are situated in the Baltic Sea and have a fairly cold climate in the beginning of the summer. Thus the flowering in Scots pine occurs there later than on the mainland of Finland. One possibility for the odd results could be that the pollination of the stand has taken place in one year from a distant source. One could imagin that the high catalase value could be produced by a progeny representing the cross Åland island × some northern pollen source. Such a cross could have happened if the prevailing winds on this isolated island were for a long time from the north during the flowering season. This suggestion must remain speculative until further samples from different years have been studied.

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

ALFRED E. SZMIDT

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

Introduction

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

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

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

Materials and methods

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

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

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

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

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

Results and discussion

Genetic variation within populations

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

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

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

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

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

	Al-1	Al-2	Al-3	Al-4	Al-5	Ta-1	Ta-2	Ta-3	Ta-4	Ret.	Czi.
Got-1a	1,000	1,000	1,000	0,709	0,965	1,000	1,000	0,857	1,000	0,000	0,342
b	0,000	0,000	0,000	0,291	0,035	0,000	0,000	0,143	0,000	1,000	0,658
Got-2a	0,950	0,417	0,510	0,722	0,776	0,929	0,429	0,577	0,613	0,494	0,543
b	0,000	0,166	0,010	0,000	0,000	0,000	0,000	0,021	0,062	0,000	0,074
c	0,050	0,417	0,480	0,278	0,224	0,071	0,571	0,402	0,325	0,506	0,383
Lap-1a	1,000	1,000	1,000	0,889	1,000	1,000	1,000	1,000	0,708	0,944	0,910
b	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,060
c	0,000	0,000	0,000	0,111	0,000	0,000	0,000	0,000	0,292	0,056	0,030
Lap-2a	0,750	1,000	0,668	0,704	0,977	0,429	0,429	0,272	0,000	0,000	0,000
b	0,000	0,000	0,166	0,000	0,000	0,000	0,000	0,687	0,137	0,667	0,820
c	0,250	0,000	0,166	0,049	0,023	0,571	0,571	0,014	0,201	0,333	0,030
d	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,338	0,000	0,000
e	0,000	0,000	0,000	0,247	0,000	0,000	0,000	0,027	0,324	0,000	0,150
Cat-1a	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0,000
b	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	1,000
APh-1a	0,417	0,583	0,333	0,350	0,250	0,214	0,167	0,050	0,277	0,333	0,571
b	0,583	0,417	0,667	0,650	0,750	0,786	0,833	0,950	0,723	0,667	0,429
ADh-1a	0,917	1,000	0,917	1,000	1,000	0,571	0,857	1,000	0,920	0,055	0,500
b	0,000	0,000	0,000	0,000	0,000	0,429	0,000	0,000	0,080	0,389	0,000
c	0,083	0,000	0,083	0,000	0,000	0,000	0,143	0,000	0,000	0,556	0,000
d	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,500
Est-2a	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,333
b	0,583	0,583	0,500	0,550	0,583	0,643	0,740	0,583	0,818	0,556	0,333
c	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,084
d	0,000	0,000	0,050	0,000	0,000	0,143	0,000	0,000	0,046	0,000	0,000
e	0,417	0,417	0,450	0,450	0,417	0,214	0,260	0,417	0,136	0,444	0,250
H	0,199	0,200	0,269	0,300	0,165	0.246	0.236	0,224	0,313	0,316	0,397

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

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

Genetic variation among populations

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

The values of genetic distance (D) ranging between 0,018 and 0,650 (Table 3) were generally much higher than those calculated between populations of other conifers exhibiting continuous range of distribution (Bergmann 1974; Lundkvist and Rudin 1977; Mejnartowicz 1979).

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

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

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

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

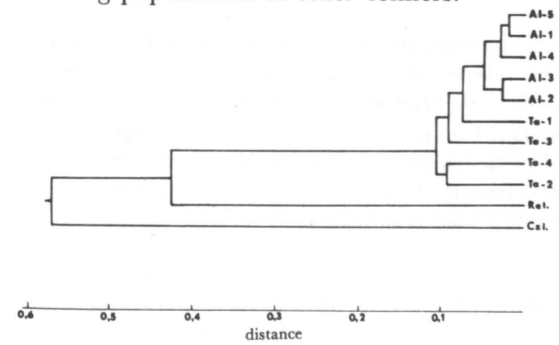

Figure 1. Dendrogram derived from Nei's genetic distance between 11 stone pine populations.

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

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

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

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

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GENETIC DIFFERENTIATION OF SCOTS PINE POPULATIONS
1. Genotypes

MARIA KRZAKOWA

The early provenience investigations described the Scots pine of the North part on Poland as a different in many morphological and physiological characters from that of the middle part of country. Eight populations, tested previously in provenience experiments, were used in this study as the representative samples of Polish Scots pine forests. At last 30 trees were randomly chose from each population. Each mother tree was examined in respect of five enzyme systems: leucine-aminopeptidase, glutamate oxalacetate-transaminase, 6-phosphogluco-dehydrogenase, alcohol dehydrogenase and glutamate dehydrogenase. Interpopulational divergences, based on genotypic frequencies, were described by Hedrick' distances. Mahalanobis' distances and Canonical Analysis.

Introduction

Description of genetic variability of Conifers has been available with the application of isoenzyme electrophoresis for detecting and analycing of the biochemical polymorphism. Isozyme investigations on Scots pine were initiated in Sweden by Rasmuson and Rudin, at the begining on the haploid needle tissue (Rasmuson and Rudin 1971, 1973), and later on the haploid macrogametophytes (Rudin 1975, 1977a,b,c,d, Rudin and Lindgren 1977, Rudin ad Ekberg 1978). Further researches were conducted in West Germany (Muller-Starck 1976, 1977, 1979, Hattemer et al., 1981) and Poland (Krzakowa et al., 1977, Krzakowa and Szweykowski 1978, Krzakowa 1979, 1980a,b, Mejnartowicz 1979, Prus-Głowacki and Szweykowski 1977, Szmidt 1979, 1980a,b,).

The regional beauty of Polish populations, especially of the North part of country, and their high flexibility in changed environments with retention on genetic value, have been noticed in the earliest provenience tests (Vilmorin 1862). Seeds of the five stands namely from Bolewice (No. 1), Rychtal (No. 6), Supraśl (No. 8) and Ruciane (No. 5), have been included to the IUFRO 1907, 1938, 1939 provenience tests (Wright and Baldwin 1953, Wright and Baldwin 1953, Wright and Bull 1963, Giertych 1979). Population from Bolewice was represented in Czechoslovakian experiments (Vincent 1953). Another four populations from Miłomlyn (No. 4), Spala (No. 7), Gubin (No. 2) and Janów Lubelski (No. 3) were also examined in a provenience test conducted in Poland Kociecki 1973).

Thus eight populations (for their distribution see Fig. 1) have been selected to this study as representative samples of Polish Scots pine forests.

Material and methods

Trees chosen for this study have been randomly sampled in almost equal number for each population: Bolewice-33 trees, Gubin-34, Janów-30, Miłomłyn 31, Ruciane-33, Rychtal-34, Spał-32 and Supraśl-32. The seeds were collected in the winter time from each tree separately. At least 6–10 macrogametophytes were assayed from every tree as an optimal number (Morris and Spieth 1978). Each macrogametophyte was homogenised in phosphte buffer pH=7. Crude extract was absorbed by paper wicks (Beckman no. 319329) which were inserted into a cut 4 cm from the cathodal end of horizontal starch gel.

Electrophoresis was conducted in 12 % starch gel (IE England) in two buffer systems: 1. Lithium boric (Scandalios 1969) for GOT and LAP at 300V for 3h: 2. Tris-citric (Shaw and Prasad 1970) for GDH, −PGD and ADH at 150V for 5h. Each tree was examined for five enzyme systems: leucine-aminopeptidase (2 loci), glutamate-oxalacetate-transaminase (3 loci), 6-phosphoglucodehydrogenase (3 loci), alcohol dehydrogenase (3 loci) and glutamate dehydrogenase (3 loci).

Populations have been compared in respect to their genotypic structure. As a consequence of the new investigations, interpopulational distances have been based here on genotype frequencies described separately. For example, in GOT, three genotypes G_1G_2, H_1H_3 and L_1I_3 were used instead of one genotype $G_1G_2H_1H_3I_1I_3$ as referred in previous papers (Krzakowa and Szweykowski 1978, Krzakowa 1979, 1980b).

Genetic diversity has been calculated by Hedrick (1974) method and the shortest genetic distances between populations illustrated by minimum spanning plotted on the maps (Fig. 1). By means of synchronous testing procedure, the hypotheses referring to differences between populations in consideration of particular loci of each enzyme system were verified, and, according to the F-statistic, not any of them have been rejected on 0.05 level as well for each locus as for each genotype separately. Multivariate techniques were used to describe interpopulational differences for each locus with calculation of Mahalanobis'distances as the measure of distinctness between populations (Anderson 1961, Calinski and Kaczmarek 1973, Calinski et al., 1975, Mahalanobis 1936, Rao 1948, Sneath and Sokal 1974). Minimum spanning, based on Mahalanobis'distances, have been plotted on the populations situated in the two first canonical axes reference system (Blackith and Reyment 1971, Morrison 1967, Rao 1964) and also on the map (Fig. 2).

Results and discussion

Genetic interpretation

Taking into account minimal number of 6–10 macrogametophytes, it is possible to identify genotype of the mother tree as homo- or heterozygote for particular loci. In the zymograms interpretation, it was assumed as a rule, that two bands (= allozymes) belonging to the same locus cannot exist together in the same macrogametophyte and in case of two simultaneously visible bands we deal with separate loci.

The variability of each enzyme system

Leucine-aminopeptidase (LAP)

The band patterns form two distinct zones. The first one is represented by four alleles locus A: A_1, A_2, A_3 and a null (= silent) allele A_o. The second zone has also 4 alleles belonging to locus C. The most common are alleles C_1 and C_2, whereas C and C_3 are rather rare. This confirms the results described earlier by other authors (Rudin 1978, Mejnartowicz et

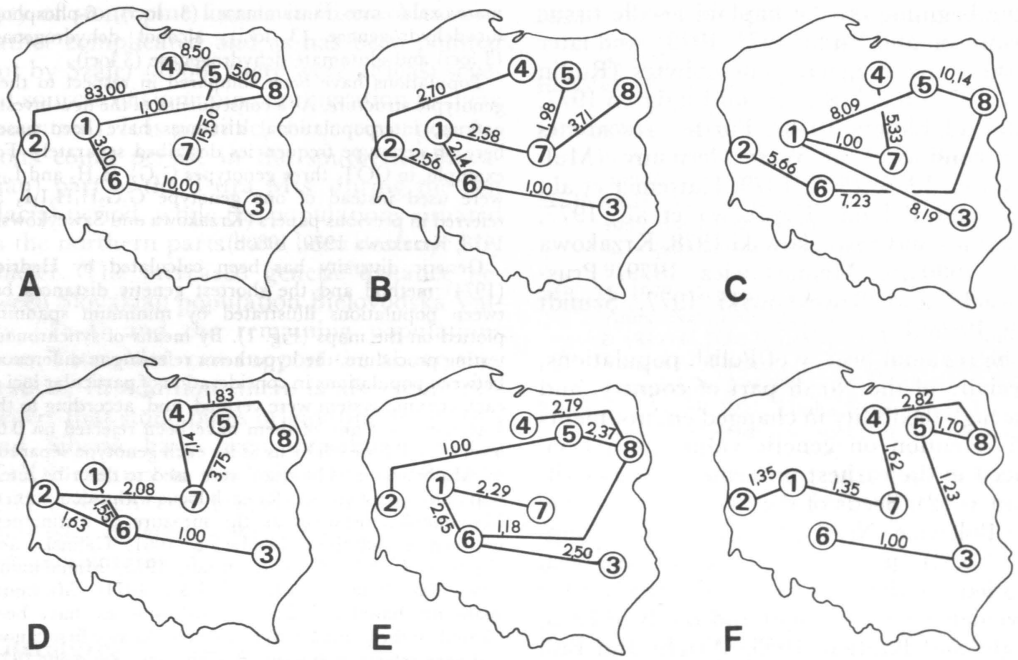

Figure 1. Dendrites of shortest connections between populations traced on the basis of the Henrick'distances for LAP-A, GOT-B, 6-PGD-C, ADH-D, GDH-E and for all enzyme systems-F, plotted on the maps. The full populations names are: Bolewice no. 1, Gubin no. 2, Janów Lubelski no. 3, Miłomłyn no. 4, Ruciane no. 5, Rychtal no. 6, Spała no. 7 and Supraśl no. 8.

al., 1978). The minimum spanning (Fig. 1A) constructed on the basis of genotypes frequency shows interpopulational divergence. Three North populations (nos. 4, 5 and 8) are closely connected and make a separate group. Populations in central Poland are rather disperse.

Glutamate oxalacetate-transaminase (GOT)

This enzyme system shows three zones of activity migrating anodally: the fastest one, locus G, with two alleles, locus H with five alleles and locus I with three alleles. This type of migration is similar to the Finnish Scots pine described earlier by Chung (1981).

The minimum spanning (Fig. 1B) shows the connection between populations of a middle part of country, and the shortest distance is between populations no. 3 and 6.

6-Phosphogluco-dehydrogenase (6-PGD)

There are three electrophoretic variants which migrate in anodal part of gel. All of them segregate as alleles of three individual loci R, S, T. The particulars of genetic interpretation were described in a previous paper Krzakowa and Szweykowski 1978/. That the North populations nos. 5 and 8 are connected, can easily be seen, on the minimum spanning (Fig. 1C). However, the shortest connection is between populations no. 1 and 7.

Alcohol dehydrogenase (ADH)

Three loci called by letters Y, Z and P can be seen on the zymograms. The first two genes Y and Z have silent alleles, whereas locus P is represented by three alleles P_1, P_2 and P_3. Dendrite, plotted on the map (Fig. 1D), shows that the shortest connection is between populations 6 and 3, and the two North populations (nos. 4 and 8) are also connected.

Glutamate dehydrogenase (GDH)

The zymograms show three zones of bands. These zones comprise three loci called by

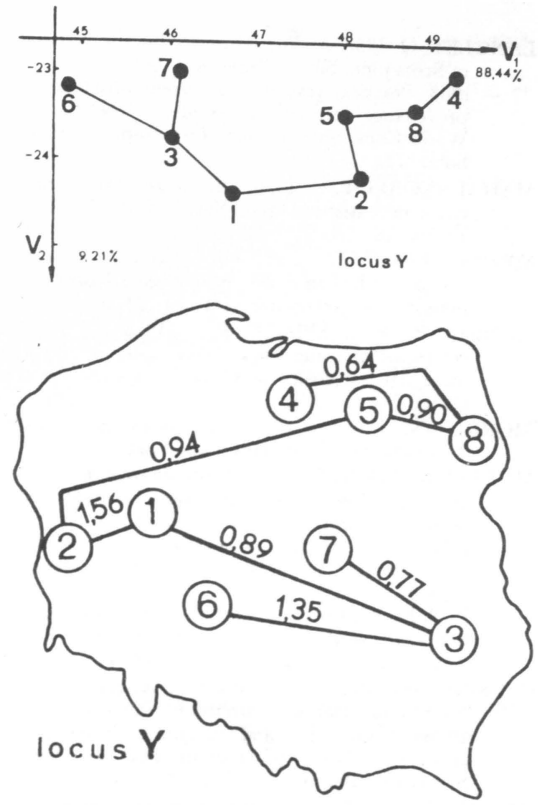

Figure 2. Scatter diagram of populations in the system of the first /V_1/ and the second /V_2/ canonical axes and minimum spanning tree of shortest connections between populations traced on the basis of Mahalanobis distances for ADH – locus Y.

letters U, W and X. Each locus has two alleles. The minimum spanning (Fig. 1E) shows that the connection between populations nos. 4 and 8 is similar to that in ADH. Interrelation of populations nos. 6 and 3 also occurs.

The variability in respect of all enzyme systems

As we can easily read from the dendrite plotted on the map (Fig. 1F), the populations characterised by 65 genotypes simultaneously, confirm the existance of two groups: North populations and populations of the middle part of Poland. Some of inerpopulational connections are of the very similar character as the connections determind in separate enzyme systems. For example the connection between two North populations no. 4 and 8 have been observed in ADH and GDH as well as between populations nos. 5 and 8 in LAP, 6-PGD and GDH. It should be noted that the connection between population from Janów Lubelski (no. 3) and Rychtal (no. 6) are almost constant. It occurs in all enzyme systems except of 6-PGD. The connection between populations nos. 1 and 7 which is also very frequent, could be seen earlier in 6-PGD, GOT and GDH enzyme systems.

The variability in each locus

Scatter diagrams of populations in the system of the two first canonical axes and dendrites constructed on the basis of Mahalanobis'distances both show populations spread in a different way. The diagrams and dendrites are not given separately here but the general scheme of connections is very similar to that of Hedrick' distances. The most interesting combination in ADH-locus Y (Fig. 2) indicates, that populations are divided into two groups with visible separateness between the North populations and populations of the middle part of country.

Genetically distinct populations of the same species which have differentiated gene pool, are called races. Races, which are geographically separated, can develop some easy to confirm discontinuities. In the case of Scots pine, the situation is more complicated. The continual distribution of this widespread species results in free exchange of its genes. Clinal variation of Scots pine, described for the first time by Langlet (1959, 1963), has been confirmed in the last years by investigations of terpenes (Tigerstedt et al., 1979, Hiltunen 1975) and isozymes (Chung 1981) variability. Since racial classification in the Scots pine probably denotes a portion of cline (Wright 1976), the concept of race as "Mendelian populations or arrays of genotypes that inhabit parts of the distribution area of a polytypic species" (Dobzhansky et al., 1977), should be considered in future investigations of Scots pine populations.

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GENETIC DIFFERENTIATION BETWEEN ADJACENT POPULATIONS OF PINUS SYLVESTRIS

URBAN GULLBERG, REZA YAZDANI AND DAG RUDIN

The possibilities are considered, in terms of Wright's theory of shifting balance, for the rapid evolution of Scots pine populations under natural conditions in Sweden.

The study is concerned with three adjacent stands in the southernmost part of the Swedish mountain range and with two such stands outside Stockholm.

In the mountain range a stand exposed to wind has later flowering periods than surrounding stands. Outside Stockholm a stand in a bog also has later flowering than the dominating stand types. It is shown that these differences cause a partial genetic isolation despite high rates of pollen entering these sites from outside.

The partial isolation and indications of diversifying selection in the adjacent stands have led us to maintain that situations could be identified for Scots pine where the theory of shifting balance is valid.

Isozyme techniques were used to study the genetic polymorphism within and between three stands from the mountain range. The result of this study points out that genetic differentiation has occurred between these adjacent stands.

Introduction

Any long term breeding programme aims both to preserve appropriate parts of existing genetic variation and to create new variation within the species in question. For Scots pine, with its short period of domestication, a proper knowledge of its processes of evolution can greatly help the breeder with both these aims.

The purpose of the study is to find out whether natural conditions of Scots pine (*Pinus sylvestris* L.), in Sweden, encourage its rapid evolution. We have examined whether the conditions in Swedish Scots pine forests are such that Wright's (1977, Ch. 13) theory of shifting balance can be applied.

Wright (1978, p. 524) states that the process of shifting balance in a structured species is the principal basis for evolution of sexually reproducing species. The shifting balance theory assumes (Wright, 1977, Ch. 13) that the relationship between genotype and phenotype is governed by a large number of segregating minor genes and by pleiotropy and multiple fitness peaks. It also assumes that the species consists of many randomly breeding local populations, demes, that are partially isolated from each other. Each evolutionary step in the process will start with a phase of random drift followed by a phase of mass selection to a new fitness peak and be completed by a phase of interdeme selection. The above conditions are necessary for the process but in addition the ecological opportunity, i.e. new environmental conditions, must occur to promote fast evolution.

With the present evidence from genetics of Scots pine it seems that the condition of partially isolated demes within which random drift can occur is difficult to fulfil. Thus Koski's (1970) study on pollen dispersal suggests large efficient population size (N_e) since a relatively big part of a stand's pollen cloud seems to be due to background pollination, that is, come from distant sources. In addition, provenance trials show a large scale clinal variation in northern Sweden for, among others, characters related to the timing of the annual growth period (Eriksson, 1982). However, the provenance studies are not designed for analysing differences between populations from the same area and Koski's study has no direct observations of the contribution to the offspring from distant pollen sources.

In recent years there have appeared studies indicating that relatively small demes can occur in Scots pine. Thus Jonsson et al. (1976) show that the timing of flowering in a seed orchard differs on different sides of a crown implying that site conditions can have a great effect on flowering time. This, in turn, can cause limited pollen migration between adjacent stands in different micro environments, that is, limited background pollination. Even Campbell's (1979) work on Douglas fir in a water-shed speaks for the existence of rather small demes in forest trees. He has shown that there are big differences in selective values for characters related to the timing of the growth period. This suggests that forest trees, like grasses (Jain and Bradshaw, 1966), could have isolated demes despite a great influx of foreign pollen.

The present study has concentrated on testing if there are adjacent populations where the timing of flowering differs. In addition the genetic relationships between three such stands have been studied by the isozyme technique.

Material and methods

Stands

Stand data are summarized in Tables 1 and 2 and in Figure 1–4. In northern Sweden, Idre, we have chosen three stands close to the timber line, one in a valley (400), one above the tree limit (500) and one in a north-facing slope (600) (Figure 1). The altitudinal differences in this region are relatively typical for the Swedish mountain regions. The timber line, however, is on a high altitude, for Swedish conditions, in this southernmost part of the mountain range. Northwards it decreases and is just around 400–500 meters above sea level in the nothernmost part of the range. It should be noted that the stand above the tree limit (500) consists of scattered trees and that it is very exposed to wind. The others (400 and 600) are parts of a continous forest.

In southern Sweden two stands have been studied in Älta, stand 700 on a hill and stand 800 on a bog (Figure 2–4). This type of site is quite common south of 60° latitude. In spring the bog generally is warmer then the hill in the day but colder in the night. During this period it also has a lower soil temperature.

From each stand in Idre and Älta we have collected cones from approximately 60 trees and the frequency of cone-bearing trees has determined the size of the stand.

In between the Idre and Älta stands we have observed flowering on single trees at a camping site close to Idre and at Åsen some 100 km south of Idre.

Observations on flowering

Tables 1 and 2 give the number of trees observed for flowering. These trees have been chosen so that at least ten male and female flowers* have been easy to observe, that is, have been within five meters from the ground. For the stand in the north-facing slope (600) this has meant that the trees studied for flowering all come from its highest part and for the stand in the valley (400) we had to choose trees outside the cone collection area.

The female stages have been classified as in Jonsson et al. (1976). Which of these that represent the start and end of the female flowers' receptive period have been determined according to Chung (1981). On each tree at least ten south-facing flowers have been chosen for observation. On a small fraction of the trees the flowering has been checked on the northern side of the tree and in its top. At each observation the frequency of flowers in different stages have been recorded, but the flowers have not been followed on an individual level.

Also when observing pollen dispersal we have used the methodology described by Jonsson et al. (1976), that is, vibration of the male flower in order to see if it sheds pollen. Frequencies of shedding and non-shedding flowers were recorded on the south-facing side of each tree.

In Älta the production of female and male flowers was recorded 1981 by classifying each tree on a ten-graded scale. The colour of the flowers was also observed.

* The term "flower" is adopted for strobili in this study.

Table 1. Background data on the observed stands. The meteorological data comes from stations that are representative for the stands.

Stand	700	800	Åsen	Idre camping	400	600	500
Size (ha)	2	4	–	–	3	3	8
Domin. height	10	10	–	–	14	14	8-10
Age[1]	~200	80-150			~150	~150	>200
Met. station	Stockholm-Bromma	Stockholm-Bromma[2]	Älvdalen II	Särna	Lofsdalen	Lofsdalen	Grövelsjön
Start[3] of growing season $\bar{x} \pm$ s.e.* 1979	101 ± 15 115	101 ± 15 115	118 ± 11 129	122 ± 8 131	127 ± 10 134	127 ± 10 134	138 ± 5 ?
Start[3] of flowering 1979							
♀ $\bar{x}$, max. min	152 ± 0.5	154 ± 0.5	-	164 ± 0.5	165 ± 0.5	165 ± 0.5	168 ± 1.5
♂ –"–	152 ± 0	154 ± 0	-	164 ± 0.5	166 ± 1	166 ± 0.5	>168
Number of trees observed	3	3	5	5	5	5	5

[1] Some exceptions
[2] Too early (see text)
[3] "0" - 1st of January
[4] Perttu et al (1978) T > 5°C

Table 2. Observations on flowering in Idre and Älta.

ÄLTA year / stand	1979 700	1979 800	1980 700	1980 800	1981 700	1981 800
Number of trees observed	3	3	3	3	39	41
Start of growing[1] season	115	[2]	112	[2]	105	[2]
Temp. sum till[1] start of flowering	220		270		240	
Start of flowering						
♀ South-facing	151.5±0.5	153.5±0.5	159±1	163±0.5	148.5±3.5	154±3
♀ North-facing	152.5±0.5	154.5±0.5	159.5±1	163.5±0.5		
♂ South-facing	152	153.5	161±1	164	148 ±2	154 ±3
♂ North-facing	152	153.5	161±1	164		

[1] See notes Table 1
[2] Later than 700

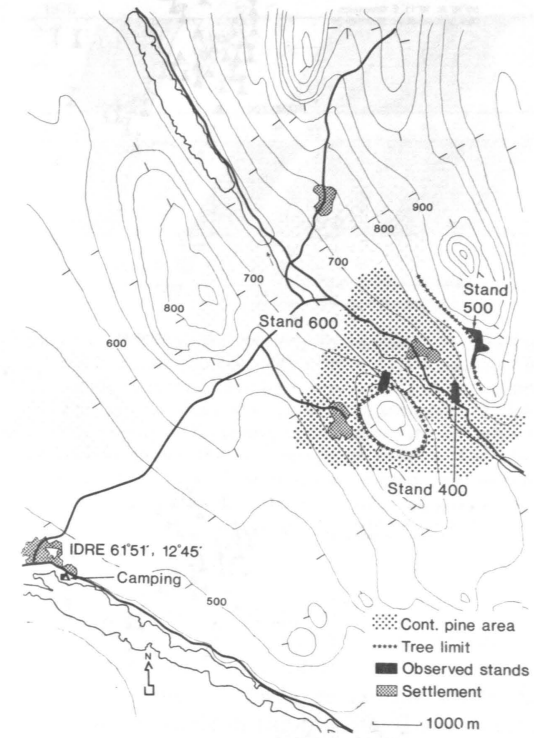

Figure 1. The Idre stands and the camping site.

Estimates of migration

No direct observations on migration have been made, but the results in Sarvas (1962), Koski (1970), Chung (1981) and from this study have been put in a model that makes an estimate possible. The model estimates the immigration to a population. It excludes the migration of seed and just considers those pollen grains that produce a viable embryo.

The model requires estimates on the distribution of the female flowers' receptive period in the population and the amount and composition of pollen in the pollen cloud during this period. In addition it assumes that the frequencies of different sizes of pollen chambers as well as the distribution of pollen grains on the ovules are known. The background pollen is assumed to have the same viability as the pollen from the neighbours.

Estimates of the receptive period are taken from this study. The composition of the pollen cloud over time has been estimated by using the results of the studies on Finnish materials mentioned above in combination with the observations made in this study. The distribution of sizes of pollen chambers have been taken from Sarvas (1962). Finally, Sarvas' (1962, Table 15) data have been used for describing pollen grain distributions on ovules.

Isozyme analyses

Cones were collected from the stands in Idre. Seed from each cone was extracted and kept in –20° C until analysed. Seeds were germinated on wet Whatman paper for five days at room temperature and macro-gametophytes were removed. Each macro-gametophyte was homogenized in tris-borate buffer (pH 7.4). Electrophoretic separation was carried out with 12 % starch gels according to the method described by Ashton and Braden (1961), and Rudin and Rasmuson (1973). For more details of isozyme sepration and staining see Rudin and Ekberg (1978), and Yazdani and Rudin (1982).

Enzyme polymorphisms detected in endosperms are listed in table 4. Mendelian inheritance for each of these loci and linkage relationships among the loci have been demonstrated by Rudin and Ekberg (1978), and Yazdani and Rudin (unpublished data).

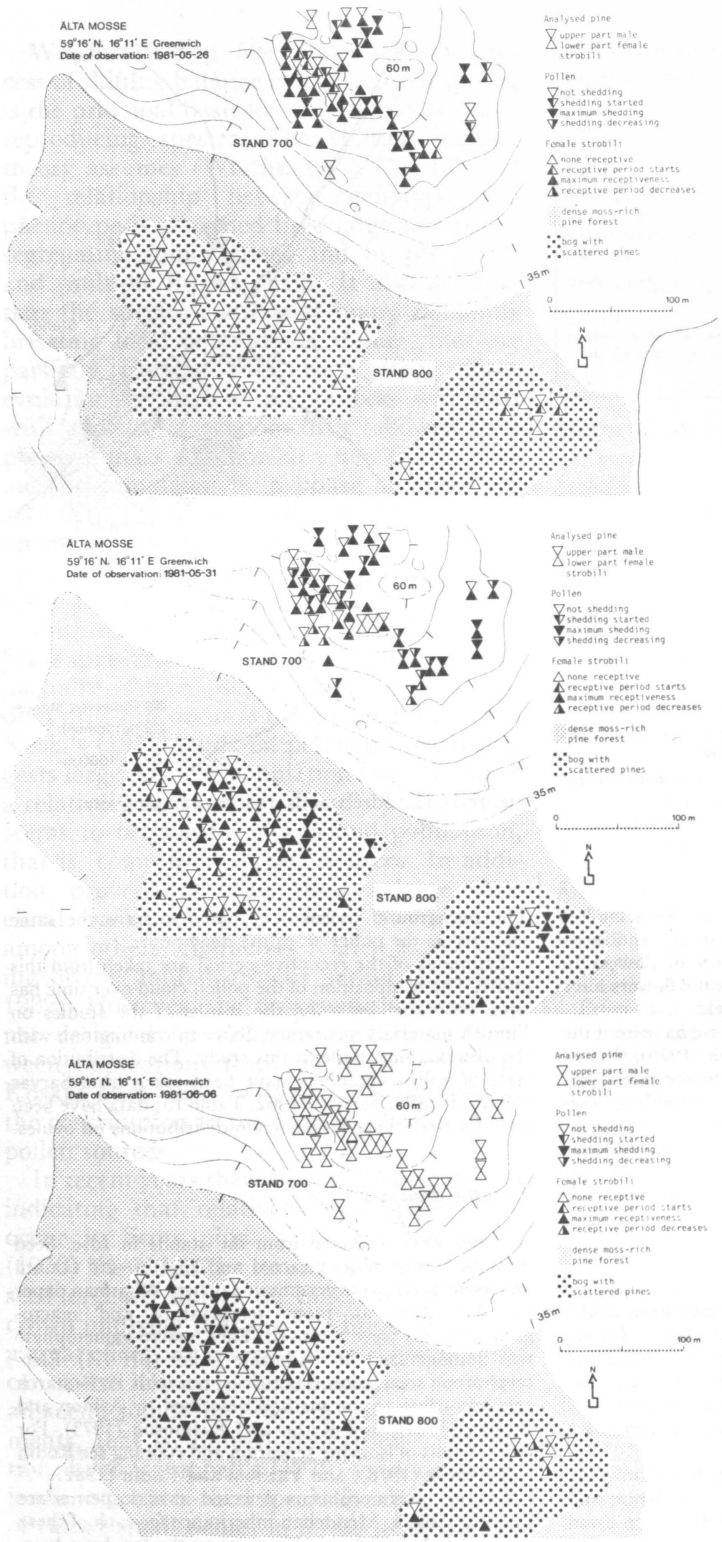

Figure 2–4. The Älta stands with the flowering for three representative days in 1981.

Statistical analyses of isozyme data

Heterogeneity of allelic frequencies among the populations at Idre was tested with the Chi-square test. Average heterozygosity ($\bar{H}$) and genetic differentiation (D_{ST}) were calculated according to Nei (1973).Wright's fixation index was computed.

Results

Flowering in Idre

The flowering periods for 1979 in the Idre stands, at Idre camping site and at Åsen, are summarized in Fiqure 5 and Table 1.

The variation in flowering time between the latest and earliest in the same tree, on the southern side, is at any flowering stage around one day for females and less than half a day for males. It is of the same order between trees when looking at their averages. However, the variation between trees is just based on five individuals per stand.

Despite the limited number of trees studied it can be stated that the flowering periods of a stand are related to the start of the growing season as could be seen from comparing the meteorological data in Table 1 with the flowering periods in Figure 5. However, the differences between localities was considerably reduced from the start of the growing season till the time for flowering. This is probably partly due to the fast development during the flowering period in 1979.

The flowering periods do not differ between the stand on the north-facing slope (600) and the one in the valley (400) despite the fact that the observed trees in the former come from its highest part. This similarity, and the fact that the stand above the tree limit (500) flowers later, indicates that wind exposure is an important factor for timing of flowering in mountain regions.

Flowering in Älta

The flowering periods for 1979 in the Älta stands are presented in Figure 5 and for 1981 in Figure 6 (see also Table 2). In addition we have observations from 1980 for these stands and for 1979 and 1980 from a stand south of the bog. The observations in 1981 give a representative picture of the flowering

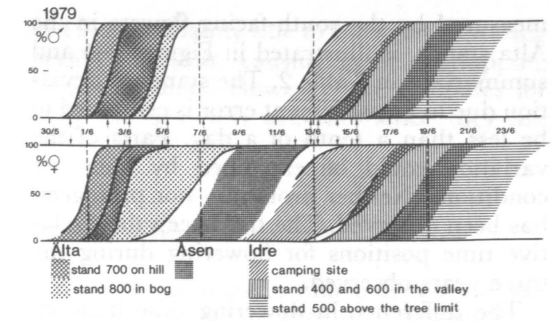

Figure 5. The flowering periods for all stands observed in 1979. Stand 400 and 600 in Idre were so similar in timing that they have been represented as one stand. The arrows show the times of observation. A field represents the flowering in one stand, the left edge of a field shows the percentage of flowers that have started the right those that have ended their period. No border lines indicates extrapolation. Compound screens show overlapping.

periods for the two populations. The trees studied in 1979 and 1980 are also among the trees observed 1981. Those on the hill are representative for the large sample while the three individuals in the bog (800) are among the earliest of those studied 1981.

The interval between the earliest and latest flower on a tree's southern side, at any flowering stage, is one day for females and half a day for males. The differences in flowering time between south- and north-facing parts of the crown, as seen from Table 2, is at maximum one day. By looking at the general appearance of the male flowers and at the occurrence of bent female flowers (cf Jonsson et al. 1976 fig 11) it has been concluded that the flowers in the top are simultaneous with south-facing flowers close to the ground.

The variation in flowering between trees,

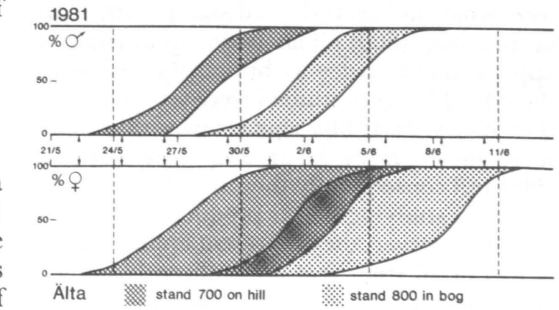

Figure 6. The flowering periods for the Älta stands in 1981. For explanations see figure 5.

measured by the south-facing flowers in the Älta stands, is illustrated in Figures 2–4 and summarized in Table 2. The standard deviation due to measurement error is estimated to be less than a tenth of a day. Parts of the variation could be explained by microsite conditions. Neither protandry nor protogeny has been observed. The trees keep their relative time positions for flowering during the three years observed.

The difference in flowering time between years is summarized in Table 2 for the two stands and the total course of events is illustrated in Figures 5 and 6 for the years 1979 and 1981, respectively. The variation in starting point is clearly related to the temperature conditions during the spring. The differences in duration of the flowering periods are mainly explained by differences in sampling. The trees observed 1979 and 1980 do not represent the whole variation observed in 1981. Partly, however, the differences are due to variable weather conditions. Thus the duration is shorter the warmer it is but, in addition to low temperatures, windy and rainy days extend the flowering period. After a couple of very windy and rainy days in 1981, the most exposed female flowers died and many of the ripe male flowers never seemed to shed any pollen.

The difference in flowering time between the two stands is also summarized in Table 2 and Figures 5 and 6. The stand on a hill south of the bog had similar flowering times as stand 700 on the other hill. No temperature recordings have been made on the different sites, but our knowledge of the climatic conditions in such sites makes us assume that the late flowering in the bog is mainly due to low soil temperatures.

The production of female flowers 1981 is somewhat higher in the hill stand (700) than in the bog (800). The male production is approximately twice as big on the hill as in the bog. The trees studied were selected since they had at least fifty ripe cones within some seven meters from the ground in the autumn 1980 and consequently they are a biased sample when studying flower production. In both stands, however, they represent approximately half of all trees that are sufficently old for flower production and the above mentioned relations therefore give an indication of the differences in male and female production between the stands. The variation within the stands for flower production partly seems to depend on the microsite, but parts of the variation cannot be explained by the observable differences in site conditions. The trees studied in all three years, six only, showed the same relationship with regard to flower production in all years.

There is no significant correlation between the flowering time of a tree and its flower production.

In both stands there is a polymorphism for colour of the male flower. Thus eight out of 51 are red, the rest yellow, in stand 700 up on the hill. In the bog there are seven red and 50 yellow. No difference in time of anthesis has been observed between trees with red and yellow flowers respectively.

Migration in Idre

We have only estimated the immigration to the stand above the tree limit (500), since this stand is well isolated from similar stands that flower simultaneously. The two other stands (400 and 600) are a part of a big continuous forest area where the flowering periods are the same. Therefore the background pollination will be simultaneous with the receptive period of these stands. This means that immigration will be equivalent to the amount of background pollination.

Figure 5 shows how the immigration from distant sources, i.e. background pollination will be reduced due to differeces in flowering periods. In Table 3 the importance of different timing of flowering periods is summarized.

The estimates are dependent on the assumptions made and on the weather conditions during the year of observation and considering the few trees observed the estimates should be used with great caution.

Migration in Älta

Table 3 gives the estimates of immigration to the bog, stand 800, in 1979 and 1981. Immigration to the hill stand has not been estimated since it is predominant in this area and therefore its immigration is equivalent to the background pollination. The high values

Table 3. Estimated immigration of pollen in Idre and Älta.

Immigration to:	Proportion of background pollination assumed		
	0.33	0.50	0.67
Stand 500 in Idre, 1979	0.10	0.15	0.20
Stand 800 in Älta, 1979	0.20	0.35	0.50
Stand 800 in Älta, 1981	0.08	0.12	0.23

Table 4. Gene frequencies in Idre for isozyme loci. A χ^2-test shows significant heterogeneities (*:p<.05;**:p<.01) between populations in GOT-B and ADH-B.

STAND	LOCUS	ALLELE FREQUENCIES						N
		1	2					
400	GOT-A	0.03	0.97					39
500		0	1.00					48
600		0	1.00					49
		1	18	2	22	3	33	
400	GOT-B	0	0.08	0.22	0.21	0.49	0	38
500		0.02	0.14	0.21	0.09	0.54	0	48
600		0.01	0.17	0.32	0.12	0.37	0.01	47
		08	1	2	22			
400	GDH	0	0.42	0.58	0			37
500		0.01	0.36	0.61	0.02			50
600		0	0.36	0.64	0			49
		01	1	2	3			
400	F-EST	0	0.82	0.05	0.13			38
500		0.01	0.71	0.14	0.15			48
600		0	0.74	0.13	0.13			47
		0	1	2	3			
400	LAP-A	0.01	0	0.97	0.01			39
500		0	0	0.97	0.03			47
600		0	0	0.98	0.02			49
		0	01	1	2	3	4	
400	LAP-B	0.01	0	0.03	0.95	0.01	0	39
500		0	0.01	0	0.94	0.05	0	47
600		0	0	0.02	0.93	0.04	0.01	49
		1	2					
400	MDH-A	0.10	0.90					40
500		0.11	0.89					48
600		0.09	0.91					48
		1-3	2-4	1-0				
400	MDH-B	0.68	0.30	0.01				40
500		0.69	0.29	0.02				53
600		0.64	0.35	0				47
		1	2	3				
400	ADH-B	0.03	0.88	0.09				38
500		0.17	0.70	0.13				46
600		0.11	0.72	0.16				49

Table 5. Population genetic parameters for the stands in Idre based on isozyme data.

Stand	Relative frequency of heterozygotes	Average heterozygosity	Fixation index
400	0.26	$\hat{H}$ = 0.276	F = 0.086
500	0.33	$\hat{H}$ = 0.318	F = -0.036
600	0.30	$\hat{H}$ = 0.314	F = 0.023

in 1979 are mainly due to the short flowering period this year, causing great overlapping between stands. However, the estimates for 1979 are also less accurate since they are based on few trees.

Isozyme studies in Idre stands

Gene frequencies for nine polymorphic loci from the stands in Idre are presented in Table 4. Alleles at a certain system were pooled so that the statistical power of the test is maximized. The Chi-square test for heterogeneity shows that there are significant differences in allelic frequencies at the GOT-B and ADH-B loci among the three populations. The stand on the north-facing slope(600) and the population in the valley(400) show differences in relative frequency for both GOT-B and ADH-B alleles. The stand above the tree limit(500) differs from the one in the valley with regard to ADH-B and from the one on the north-facing slope for GOT-B.

Population genetic parameters for the three stands are given in table 5. They show that the stand in the valley is different.

No significant deviations from Hardy-Weinberg equilibrium were detected. Finally the genetic differentiation between the stands measured as $G_{ST} = D_{ST} / \bar{H}$ is one percent.

Discussion

Sample size for flowering studies

The number of trees studied for flowering in 1979 and 1980 are very few per stand. However, they were chosen to be representative for their respective stands and which is largely confirmed by the observations in Älta 1981. We have therefore found it appropriate to use the results from these years when discussing differences between stands.

Migration

Koski (1970), observing pollen dispersal in Finnish Scots pine stands, with normal pollen production, estimated that approximately half the pollen cloud around a tree originates

from trees within 50 meters distance. His data indicates that the rest of the cloud, the background pollen, to a major part originates from stands several hundred meters away. His data on short range dispersal are largely supported by studies on actual migration using the isozyme technique (Müller, 1976; Shen et al. 1981). Stern (1972) labelling a stand of 50 × 50 m presents results that support Koski's estimates of background pollination. However, no studies, as far as we know, have estimated the actual migration from background pollination. Decreased pollen vitality or different timing from that in the stand itself could cause big differences between pollen dispersal and proper migration.

The present study concentrates on estimating the effects that timing of flowering has on migration. Assuming the same magnitude of background pollination as Koski (1970), we have estimated the pollen migration in sites with microgeographic differences and found it to be less than the rate of background pollination. This is at least true for immigration to stand types that are relatively rare in a region like the bog in Älta or the stand above the tree limit in Idre. The study also indicates that migration between stands in different climatic regions will be occasional, although pollen dispersal occurs, owing to differences in timing of flowering. The effect of pollen sources from other regions could therefore be supposed to have effects of the same type and magnitude as mutations.

As a conclusion of this study we maintain that there are conditions when Scots pine stands have low immigration rates despite the occurrence of background pollen.

Effective population size

In several studies on Scots pine the effective population size (N_e) was estimated by observing the pollen dispersal from individual trees (J. Wright, 1953; Schmidt, 1970) and it was concluded that random drift owing to small sample size will occur between neighbourhoods. Koski (1974), among others, has disagreed with these conclusions because of the effects of background pollination and he states "Genetic differentiation of neighbourhoods cannot occur through random drift".

The observations made in this study show that one could find clearly defined stands or populations that are partially isolated from background pollen. The genetic constitution of these stands will therefore probably be affected by random drift.

Diversifying selection

Jain and Bradshaw (1966) showed that divergent selection pressure in adjacent populations can maintain differentiation between them despite high migration. Much theoretical and experimental work regarding interactions between selection and gene flow in multiniche situations (for references see Brown, 1979) has been made since then in order to evaluate such situations from an evolutionary standpoint. It seems, as far as we have understood, that they are sufficient conditions for the first phases in Wright's theory of shifting balance.

The fact that the flowering periods could differ between adjacent stands certainly tells us that there is diversifying selection for viability like frost hardiness or the timing of the period of growth. Campbell (1979), studying Douglas fir, has shown that the differences in selection pressure for such characters are considerable between adjacent stands. In Scots pine evaluations of provenance trials indicate effects similar to those found by Campbell (Eriksson et al., 1976). However, Scots pine in Sweden naturally has much fewer seedlings per adult than the 2 000 estimated by Campbell and thereby less possibilities for selection to work. Estimates from Hagner (1965) indicate that the seedlings per adult after natural regeneration are 70–200 in southern Sweden and less than ten for large areas in northern Sweden.

Isozyme polymorphisms in Idre

Recently several isozyme studies have been performed on adjacent populations of coniferous trees. Genetic differences between such populations have been observed (Bergmann, 1978; Mitton et al. 1977 and 1980) as well as differences within stands (Linhart et al. 1981). Parts of the between-population differences are correlated with environmental parameters and the differences within stands seems to be associated with family structure.

We have no obvious explanation to the differences between the stand in the valley (400) and that on the north-facing slope (600). On the contrary our observations on flowering and on the period of growth speaks in favour of genetic similarity. However, there might be environmental differences, not observed by us, that have caused the genetic dissimilarity for isozymes between these stands or it can be explained by random effects causing differences associated with family structure. The presence of random effects is supported by the fact that the cold summers in these regions mostly cause very poor seed crops on an average and big differences in gametic contributions from different individuals due to microclimatic differences between the stands.

The genetic differentiation, as estimated by G_{ST}, is around one percent which is lower than generally observed for cross breeders (Brown, 1979). This can probably be explained by the fact that the environmental differences are relatively minor in Idre compared with those in the other studies.

Polymorphisms in Älta

Stern and Roche (1974) discuss the observations made on male flower colour in Scots pine. They find it probable that this polymorphism is due to a single gene and that it is maintained by the red flowers having early anthesis and the yellow ones late.

The observations on male flower colour in Älta do not disclose any differences between the two stands and there is no relation between flower colour and time of anthesis.

Conclusions

We have observed partial isolation between adjacent stands and have indications of diversifying selection on such sites. This has led us to maintain that it is possible to identify situations for Scots pine where the conditions for Wright's theory of shifting balance can occur. We therefore consider it important to continue research that tests this hypothesis and to analyse how conditions for fast evolution, caused by shifting balance situations, should be utilized in the long term breeding programme.

The following fields are of high priority in this context:

- The construction of models of the population structure of Scots pine adapted to the situations in question.
- Further studies of migration between adjacent stands in order to get better estimates and to find additional situations where partial isolation will occur.
- Further studies of migration due to background pollination in order to get direct estimates of its effect.
- Studies on natural selection for fertility components and for viability components that have their major effects from the zygote stage and a couple of years ahead.
- Selection experiments on natural populations from areas where the preconditions in the theory of shifting balance are believed to occur.
- Generating a new breeding material by cultivating selected material under "shifting balance" conditions.

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FOREST GENE RESOURCES IN SWEDEN

PETER KRUTZSCH

Modern forestry either moves selected natural populations to new sites, or uses artificial, bred material in forest cultivation thus replacing locally adapted provenances. in contrast, the objective of the Swedish Forest Gene Resource Programme is to preserve and maintain natural genetic variation in our economically important forest tree species. The work should mainly be carried out on samples of distinct natural populations with the aim to prevent genetic erosion and genetic immigration for generations to come. It is believed that the future value of Forest Gene Resources lies rather upon genetic variation among provenance-typical genotypes than in allelic multitude in unassorted genotypes.

The major problems of the Gene Conservation Programme are:

- To find an adequate number of autochthonous stands of sufficient population size which can be preserved at moderate costs.
- To find proper methods for in situ and perhaps even ex situ renewal of Gene Resource material with minimum changes of its population structure.
- To start a registration of younger stands, cultivated with well defined seed sources of today. These stands will be the future objects for studies of consequences of population transfer and domestication. In themselves they certainly will be sources of potential breeding material.

Most urgent is the need of marker systems which allow for discrimination and identification of seed sources, populations and individuals on biochemical, phenological or morphological grounds. Studies on the differentiation of populations are necessary to find measures for the distance between and the satisfactory number of "Gene Resources".

A survey of older, certainly autochthonous stands of Norway spruce and Scots pine has begun in Southern Sweden and must be continued in order to save immediately endangered material. A system for the registration of younger stands from well defined sources must be worked out carefully and properly introduced in order to gain the interest of forest owners.

In figure 1, the development of Swedish Forest Renewal, particularly for South and Central Sweden, is shown.

Active forest renewal in todays meaning is slightly older than 100 years. Before that, reforestation came as spontaneous regeneration or rather, was suppressed as forest land was turned into agricultural land. Intensive reforestation started about 1860 as a necessity, since the accessible forests at least in Southern and Central Sweden had largely been depleted by then. During a period of almost 50 years large areas were restocked, mainly by forest seeding but soon also by planting. The material used in todays terminology was provenances: Moved natural populations in contrast to natural regeneration or artificial regeneration with "local sources". Large proportions of the material were imported from Central Europe, where forest management then already was established and where seed extractories were working on a commercial scale. With the beginning of World War I, artificial reforestation decreased and was not resumed to any great extent until the end of World War II. In between, mostly natural regeneration was practiced and in artificial regeneration mostly local provenances were used. Only about 1950 a new and very intensive period of artificial regeneration came, and it is still going on.

This first century of reforestation in Southern and Central Sweden is characterized by the use of mostly rather far off provenances. After World War II, tree breeding and seed orchard work started and right now, after some 30 years of intensive work about ⅔ of our reproductive material of Scots pine is produced in seed orchards, the rest is mostly moved provenances, harvested in still autochthonous stands. For Norway spruce ½ of the material is imported from Romania and

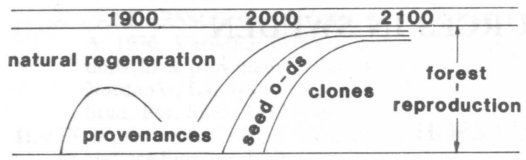

Figure 1. The development of forest regeneration in Sweden.

~ 1860 Restoration of devastated forests starts. Great importations of seed from C. Europe
~ 1910– Decline in forest planting and seeding activites.
~ 1950 New start for intensive forest cultivation. Even now importations from C. Europe.
~ 1970 Seed Orchards of Scots Pine give commercial yield.
~ 2000 Clonal Forestry (Tissue culture, Norway spruce) has become a reality.

CSR, so called South East provenances and from White Russia and Northern Poland, so called North East provenances. The rest is domestic autochthonous sources, moved to the north. Only one exotic, Lodgepole pine, Pinus contorta from British Columbia is used in Northern Sweden, it is replacing Scots pine on about 1/10 of its acreage.

Clonal forestry with cuttings of Norway spruce is about to be introduced. Clonal Forestry with Norway spruce will possibly dominate in Southern and Central Sweden by the end of this century. It will certainly dominate if tissue culture becomes successful and ageing trouble can be circumvented.

A rapid change from "natural populations" to artificial populations is going on. Firstly the change from provenances to seed-orchard material and secondly from seed orchards to clonal material by the turn of the century. Even if clonal forestry may be delayed and perhaps too difficult for Scots pine, our breeding programme and our seed orchard planning foresee clearly a 100 % artificial forest reproductive material by the end of the century.

The forest genetic situation in Southern and Central Sweden may today be described as follows:

Very few stands from "before 1860" are left. These are certainly autochthonous material, now more than 120 years old. Depending on acreage and silvicultural treatment, a natural offspring of this material must be regarded as autochthonous.

In the age class 60–120 years, cultivated stands are not identifiable as to origin. We know for sure that many of these stands are introductions from Central Europe, others are cultivations with moved Swedish provenances. The natural regenerated stands in this age class must be regarded as autochthonous.

Stands with trees in the age of 30–60 years in the majority are natural regenerations. It is probably very difficult to tell whether this material is autochthonous or not. Ever so often the pollen source may be of foreign origin and we also have natural regeneration of introduced provenances. The younger the stands of natural regeneration are, the less it is likely that they are purely autochthonous.

Finally, we have the youngest age classes of up to 30 years, the result of planting activities since 1950. Mostly Norway spruce are "provenances" either from Central Europe or Sweden, also the latter ones moved, altogether unfortunately with very little documentation on origin and use.

After one more forest rotation period, say in the year 2100, the Forest Genetic Situation is easily predicted. For Scots pine probably very little "moved provenances" in Northern Sweden will be left over in old age classes. The rest is either material from Seed Orchards, cuttings in young stands or natural regeneration. Natural regeneration, however, will be less and less frequent in practice and is by no means any longer reliably autochthonous. For Norway spruce the oldest stands in 2100 will probably be "provenances" still – very good provenances, carefully chosen and moved. The younger material, which here means ages of 100 and below, will be artificial: Either cuttings, i.e. clones or seed orchards offspring. Natural regeneration is scarce already now, there will be no chance for any autochthonous material after natural regeneration in future.

In view of this situation a Swedish Gene Conservation Programme is to be started. The aims of this programme will be:

Preservation of the genetic diversity in our commercial important tree species, if practicable at the population level. Preservation of the population structure in a certain limited number of natural populations. Preservation of a genetic "status quo" in populations as a reference for the future.

Gene Conservation should serve as a complement to current and future tree breeding and silvicultural management of our forests. Built-up and maintenance of the Forest Tree Breeders "Breeding Populations" within the entire National Tree Breeding Programme must be strongly considered in Gene Conservation Activities.

Gene Conservation will comprise a variety of activities, of which the real long-term preservation of populations is the most difficult task, if long-term is understood as a number of normal forest rotation periods. (Here it is tempting to ask for 10 generations – 1000 years or more).

This long-term conservation of Gene pools representing populations is probably only possible in "life form" i.e. within a close to nature system of forest management with forest removal and renewal on a relatively large scale. Of course, there will be no chance of an unchanged continuity as in strict copying, however, artificial means of conservation, as seed storage, tissue culture, repeated grafting or cutting do not seem to be applicable with respect to time.

It is suggested that the Gene Bank's backbone should be a number of provenance reservations. These should represent the differentiation of our natural adapted populations as closely as possible. A number of 10 reserves for Scots pine and Norway spruce would be a minimum requirement, 20 reserves each would probably be regarded as adequate.

These Provenance Reserves can still be formed within some of our Seed Source Areas, which now are approved for practical seed supply. The Seed Source Areas represent autochthonous material and are of considerable size mostly.

Provenance Reserves should have a size of at least 500 ha (a radius of 1250 m) of which most serve as a protection zone for the Centre, which is the regeneration source. Forest management will be performed in the whole reservation, with the only restriction, that no other material is used in regeneration than such harvested within the Centre. The protection zone will serve as pollen donor to the Centre, as well as it will absorb pollen from outside the reserve. Thus natural regeneration should be avoided especially in the protection zone, as some of the offspring will be hybrids.

The costs for the establishment of such reservations will not be moderate. Aside from administration, there will be additional costs for regeneration, there will be losses due to the use of unbred material instead of bred forest reproductive material, and finally in many cases costs for the premature removal of minor stands established with introduced material of the same species.

Next to this long-term provenance reservation two programmes for registration of Forest Gene Resources are suggested.

One registration aims to reinforce the provenance reservations. The very coarse network of 20 populations should, if possible, be complemented by the identification and registration of autochthonous stands outside the reservations. In this activity a survey of other reservations in the country could be included. In National and Provincial Parks, old and very old forests are usually preserved and these could certainly be of interest as Gene Resources. At the time being, no plans for the renewal of these resources should be discussed. They can hopefully be included in current breeding programmes and thus be transferred into breeding populations.

Another registration aims to identify newly established stands from natural seed sources.

Even if an increasing proportion of our forest reproductive material is derived from Seed Orchards, we still use seed harvested in autochthonous stands, and we still have some autochthonous natural regenerations. These stands, with a proper identification of locality and seed source, will certainly be a great potential as Gene Resources in future Breeding Programmes. Of interest is both locally used material as for example in natural regenerations, as well as transferred material. For Scots pine it is Northern provenances moved to the South, for Norway spruce it is South Swedish sources moved to the North, in Southern Sweden introduced Norway spruce from both South East and from North East Europe. Also sources of Lodgepole pine, *Pinus contorta* from British Columbia and the Yucon Territory, now introduced to the North of Sweden are of interest in this context.

In this registration difficulties arise from forest management, as little control on forest reproductive material is exercised. There is also risk of uncontrolled replanting activities

with different materials and the unobserved establishment of natural regeneration of the same species in planted sites.

In connection with the Conservation of Forest Gene Resources many questions urge for scientific research. In the suggested programme the need to preserve autochthonous populations is emphazised and one of the dangers threatening Gene Resources is pointed out to be hybridization of autochthonous and introduced sources. Here we need discriminating methods for genetic information on geographic origin. The classical methods of provenance research as phenological studies will be one of the Gene Bank's tools, they are, however, not sufficient. Biochemical marker systems must be developed in order to investigate more closely into the genetic structure of both individuals and populations.

Measures of genetic variation are needed to answer questions such as the number of trees needed to represent populations. In view of the well known genetic heterogeneity within our forest tree populations, the amount of differentiation between populations versus the amount of overlapping are essential with regard to the total geographic (and genetic?) range of our species.

Also in very practical questions we meet problems: How should seed-harvest in Provenance Reservations be performed? Should one collect a few cones from as many trees as possible whenever possible or, should one rather wait for a bumper crop at, say 10 years interval? Should one plant in the Reservations with thousands of seedlings per ha and thus challenge competition and selection or, should one plant just enough to secure survival in terms of forest management and thus avoid competition?

So it is obvious, in our Gene Resource Programme also activities and means should be included to initiate and promote research. The main questions will be: How to map genetic variation and how to preserve genetic variation, if possible unaltered.

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PART V

METHODS AND EQUIPMENT USED IN FOREST POPULATION GENETICS

IMMUNOCHEMICAL METHODS IN ANALYSIS OF FOREST TREE PROTEINS

WIESLAW PRUS-GLOWACKI

Immunochemical technique allow detection of the similarities in the amino acid sequence and configuration of determinant groups on protein molecules. Therefore they can provide a measure of structural correspondence among proteins of different species, different individuals of the same species and proteins from different tissues of the same organism. Comparative analysis of the protein spectra of forest trees by immunochemical methods has proven to be a useful procedure to obtain information about degree of genetic similarity between different species of forest trees and their hybrids, differentiation between populations, inheritance of some antigenic proteins, checking the identity of genes products and study of incompatibility.

The possible applications of immunochemical methods in investigations of forest trees proteins are reviewed.

Introduction

Up till now research on genetic structure of forest trees populations has been based on electrophoretic analysis of proteins and distribution of patterns secondary products of trees metabolism such as monoterpens, oilresins, polyphenol complexes and some others.

As far as population genetics of trees is concerned the importance of studies on isozymes, as direct products of gene activity is unquestionable.

It seems, however, that immunochemical investigations on proteins offer many possibilities for identification and comparison of the properties of forest trees proteins. Untill now the use of immunochemical techniques has been rather limited in this field in spite of their application in agricultural investigations.

Most of the papers published were concerned chemotaxonomy and evolution in Conifers (Saito 1968, Hagman 1977, Prager et al. 1976, Prus-Głowacki et al. 1978, 1981) and decidous trees (Brunner and Fairbrothers 1978, Petersen and Fairbrothers 1978, Villamil and Fairbrothers 1974).

Some forest scientists also investigated the problems of antigenic differentiation and antigenic proteins variation in forest trees populations as well as hybridization and introgression (Villamil and Fairbrothers 1974, Clarkson and Fairbrothers 1970, Prus-Glowacki and Szweykowski 1980, Prus-Glowacki and Rudin 1981).

The interesting examples illustrating the

application of immunochemical methods in incompatibility studies are reported in the papers by Hagman 1967, 1971.

The serological techiques as potential indicator of the ability to grafting in two juniperus species were applied by Evans and Rasmussen 1974.

Further development of immunochemical technique, especially those concerning the introduction of two-dimensional immunoelectrophoresis for qualitative and quantitative evaluation of antigenic proteins, offer to a larger degree, a wide range of possibilities of the immunochemical methods application for solving many basic problems in forest genetics.

Materials and methods

Pollen is found to be most frequent source of antigenic proteins. This material is very suitable in immunochemical analysis. It is the richest source of proteins characterised by strong antigenic properties of all material used in plant analysis, its protein spectrum is relatively simple and it contains only slight traces of substances which may have some influence on formation of non-specific precipitating complexes. The disadvantage of this type of antigens source is the fact that in comparative studies the attention should be paid for stage of development of pollen.

The advantage of seeds, the second source of protein consist in large quantity of protein, is relatively easy way of obtaining the material for investigations and strong antigenic character of storage proteins, especially in certain plant groups.

The disadvantage is the presence of some quantities of the substance causing the formation of non-specific precipitating systems and the necessity of purification of seed proteins.

The third source for obtaining antigenic proteins (though rarely used) are leaves. They are used when the above-mentioned protein sources are not available; for examples in studies of sterile hybrids or plants flowering and fruiting seldom.

The advantage of this protein source is usually very high specifity antisera obtained which makes the identification of single individual tree possible. The disadvantages include: low antigenicity of leaf proteins, their

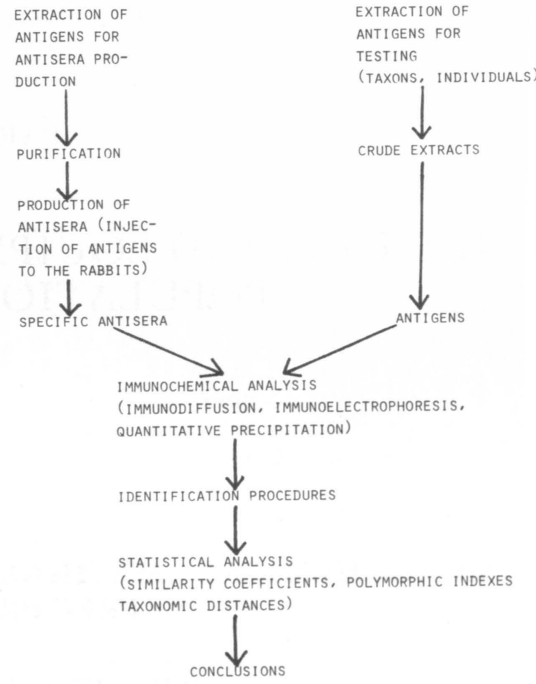

Figure 1. Diagrammetic representation the main steps of immunochemical analysis.

low concentration and the necessity to observe the same stage of development of leaves in comparative studies. In the case conifers the lather is overcome by collecting one-year old fully developed needles in winter period, when the protein metabolism is established on the minimal level.

The data obtained indicate that proteins of pollen and seeds are found to be useful especially in chemotaxonomic and phylogenetic studies, because of relatively small individual specificity of those proteins. For studies on populations, however, proteins of leaves seems to be more suitable.

Particular main steps in the procedure of immunochemical analysis are presented in figure 1.

Extraction prosedures of plant proteins for antisera production as well as for comparative analysis should be chosen according to the type of plant material under investigation. Some indications can be found in the review by Daussant 1975, and in the original papers listed in table 1.

Injections schedules leading to obtain sufficient antisera are also reported by Daussant 1975.

Table 1. Review of literature of immunochemical studies in trees and bushes.

Species	Source of antigen	Techniques	Main purpose of study	Author, year of publication
Cornaceae Nyssaceae	seeds	PB, ID	chemotaxonomy	Fairbrothers, Johnson 1964
Betula	pollen	IF, IEF	incompatibility	Hagman 1964
Magnoliaceae	seeds	PB, ID, IEF	chemotaxonomy	Johnson, Fairbrothers 1965
Magnolia sp.	seeds	PB, ID, IEF	chemotaxonomy	Pickering, Fairbrothers 1967
Betula, Pinus	pollen	ID, IEF	incompatibility	Hagman 1967
Pinus sp.	seeds	ID	phylogeny	Saito 1968
Caprifoliaceae	seeds	PB, ID	chemotaxonomy	Hillebrand, Fairbrothers 1970(a)
Caprifoliaceae	seeds	PB, ID	chemotaxonomy	—"— 1970(b)
Abies sp	seeds	PB, ID	introgression	Clarkson, Fairbrothers 1970
Betula pendula —"— *pubescens*	pollen	ID, IEF, IF	incompatibility	Hagman 1971
Alnus serrulatarigosa complex *Betula populifolia*	pollen	PB, ID	chemotaxonomy, differences among populations	Villamil, Fairbrothers 1974
Juniperus horizontalis *Juniperus chinensis*	leaf and stem tissue	IF	serological techniques as indicator for grafting ability	Evans, Rasmussen 1974
Betula, Alnus, Picea, Pinus	pollen	IF, ID	incompatibility	Hagman 1975
Pineaceae	seeds	ID, IEF, MCF,	evolution	Prager et al. 1976
Pinus sylvestris *Pinus nigra* *Pinus desinflora*	pollen	ID	incompatibility	Petricevic et al. 1977
Pinus genus	pollen	IEF	chemotaxonomy, indentification of species, clones and provenances	Hagman 1977
Cornales	seeds	ID, IEF	chemotaxonomy	Brunner, Fairbrothers 1978
Rubiaceae	seeds	PB, ID, IEF	chemotaxonomy	Lee, Fairbrothers 1978
Pinus sylvesteris *Pinus mugo* *Pinus uliginosa*	needles	QP, ID	introgression	Prus-Glowacki et al. 1978
Pinus sylvestris *Pinus mugo* *Pinus uliginosa* *Pinus nigra*	needles	QP, ID	chemotaxonomy	Prus-Glowacki, Szweykowski 1979
Corylaceae	pollen	PB, ID IEF	chemotaxonomy	Bunner, Fairbrothers 1979
Juglandaceae Myriaceae Fagaceae Anacardiaceae	pollen	PB, ID	chemotaxonomy	Petersen, Fairbrothers 1979
Pinus sylvestris *Pinus mugo* *Pinus uliginosa* *Pinus nigra* hybrids	needles	ID	introgression hybrids	Prus-Glowacki, Szweykowski 1980
—"—	needles	R IEF	quantitative comparisons of antigens	Prus-Glowacki et al. 1981
Pinus sylvestris	—"—	ID	variability of antigenic proteins in Swedish population	Prus-Glowacki, Rudin 1981

PB – Boyden procedure (quantitative)
ID – immunodiffusion
IF – immunofluorescent techniques
IEF – immunoelectrophoresis
QP – quantitative precipitation
MCF – micro-complement fixation
RIEF – rocketimmunoelectrophoresis

Methodological principles of immunodiffusion and immunoelectrophoresis as well as quantitative immunoprecipitation and the way of interpretation of results are reported in numerous publications (Ouchterlony 1967, Crowle 1961, Clausen 1971, Uriel 1971, Smith 1976, Axelsen 1977). The more precise methodological data can be found in original papers presented in table 1.

The most useful statistical method in quantitative immunoprecipitation analysis is the "serological correspondesce": ratio of precipitate quantity at the so-called reference reaction to precipitate quantity at so-called cross reaction. The value is given in procentage (Petersen and Fairhbrothers 1970).

$$Sc = \frac{\text{cross reaction}}{\text{reference reation}} \times 100$$

In the studies based on identification of individual antigenic proteins Euclidean distances are estimated according to a formula: (Sneath and Sokal 1964)

$$Djk = \left[\sum_{i=1}^{n}(xij - xik)^2\right]^{1/2}$$

where Djk denotes the taxonomic distances between "j" and "k" objecks, xij or xik – characters "i" and "j" in "j" and "k" objects and also similarity coefficients between the taxa "j" and "k" according to formula: (Sokal and Michener 1967)

$$Sj,k = \frac{a + d}{a + b + c + d}$$

where Sjk is the similarity coefficient, and the letters "a" through "d" denote presence or absence of particular precipitin lines according to the scheme:

	taxon k: +	taxon k: –
taxon j: +	a	b
taxon j: –	c	d

From antigenic similarity matrix, taxonomic distances are calculated:

$$Djk = \sqrt{1 - Sjk}$$

In the analysis of within population variation we deal with frequencies of antigenic proteins and in the case we can calculate serological similarities according to Nei 1972 formula:

$$I_N = \frac{Jxy}{\sqrt{Jx \cdot Jy}} \qquad Jxy = \sum xiyi \quad Jx = \sum x^2i \quad Jy = \sum y^2i$$

where I_N is serological similarity, xi and yi – antigenic frequencies in x and y populations.

Serological distances can be calculated by

$$DgI_N = \sqrt{1 - I_N}$$

As a graphic illustration of results minimum spanning trees can be constructed on the basis of Euclidean distances matrix (Djk), taxonomix distances (Sjk) and also serological distances (DgI_N).

For the determination of level of polymorphism in the populations under study antigenic polymorphism index according to the formula Marshall and Jain 1969 can be calculated:

$$Pi = 1/Z\left[\sum_{i=1}^{z} qi\ (1 - qi)\right]$$

where z = number of antigens in the populations
qi = frequency of antigens in single population

Examples of the application of immunochemical methods

On the basis of the results obtained from our research, some examples of the application of immunochemical methods are presented.

The investigations were concerned with some species from the genus *Pinus* and their hybrids.

Dendrite in Fig. 2. shows serological distances estimated on the basis of needles protein immunodiffusion of four pine species, i.e. *Pinus sylvestris*, *P. uliginosa*, *P. mugo* and *P. nigra*. Three of them, namely *P. sylvestris*, *P. mugo* and *P. uliginosa* were considered as putative hybrid swarm parents. The analysis revealed that *P. uliginosa* and *P. mugo* show most close relationship. *P. sylvestris* is found to be in some extend related to *P. uliginosa* and rather distant from *P. mugo*, Thus can be assumed

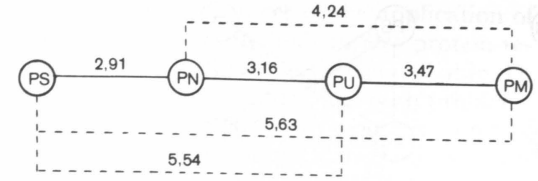

Figure 2. Diagram showing the serological similarity of *Pinus sylvestris* (PS), *P. nigra* (PN), *P. uliginosa* (PU) and *P. mugo* (PM) on the basis of double immunodiffusion. Combined sera against proteins of *P. sylvestris*, *P. mugo* and *P. uliginosa* was used (mixture 1:1:1).

that the majority of hybrid swarm individuals studied should show intermediate character between *P. mugo* and *P. uliginosa* and than between *P. sylvestris* and *P. uliginosa*.

Dendrite in Fig. 3. demonstrates serological similarities of hybrid swarm individuals to pure species, while on three dimentional diagram (Fig. 4) distribution of of pure species used as references and hybrid swarm individuals in the three first principal components space is presented.

The quantitative data based on common antigenic proteins in investigated pine species, obtained as a result of quantitative immunoelectrophoresis are presented in fig. 5. Mean value for three kinds of antisera provide evidence that *P. sylvestris* and *P. nigra* are the most closely related species with respect to antigenic properties, than *P. uliginosa* and *P. mugo* and finally *P. sylvestris* and *P. mugo* are most distant species. These data are in accordance with the results of immunodiffusion analysis.

The results of studies on differentiation of antigenic proteins in the populations of *P.*

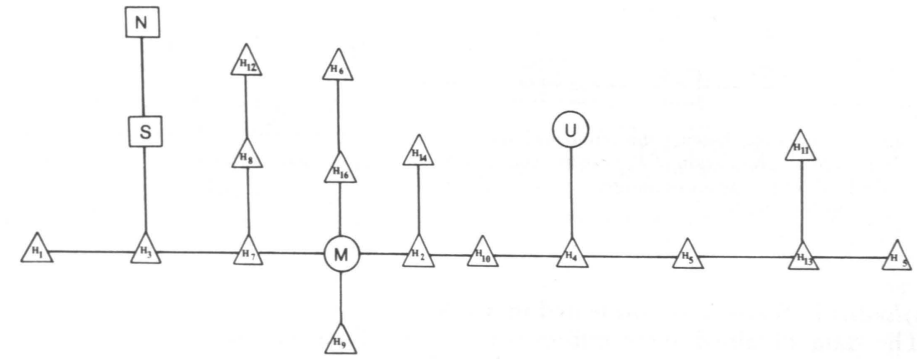

Figure 3. Dendrite showing the seroligical similarity of individuals from the hybrid swarm population to *Pinus sylvestris* (S), *P. uliginosa* (U) *P. mugo* (M) and *P. nigra* (N). Based on average results with three sera: antisylvestris, antimugo and antiuliginosa.

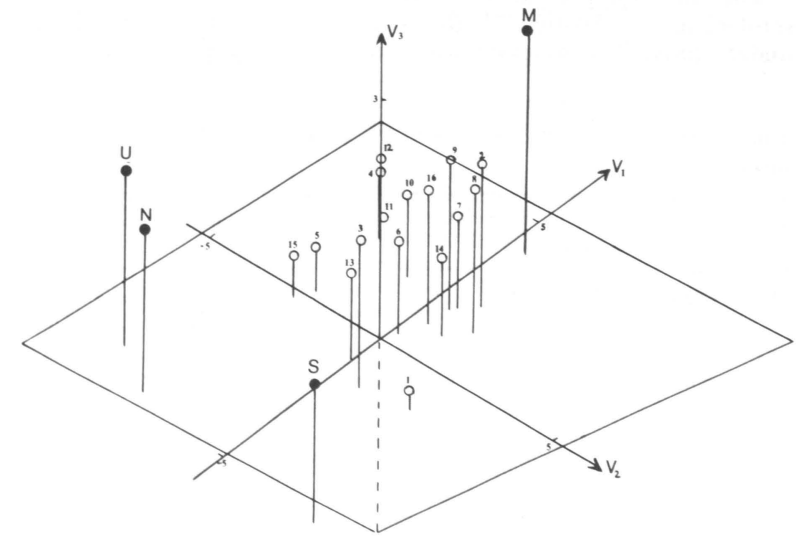

Figure 4. Three dimensional diagram showing the distribution of the hybrid swarm individuals in the three first principal component space. Points marked with letters S, M, U, N denote the populations of pure species (*P. sylvestris*, *P. mugo*, *P. uliginosa* and *P. nigra* respectively).

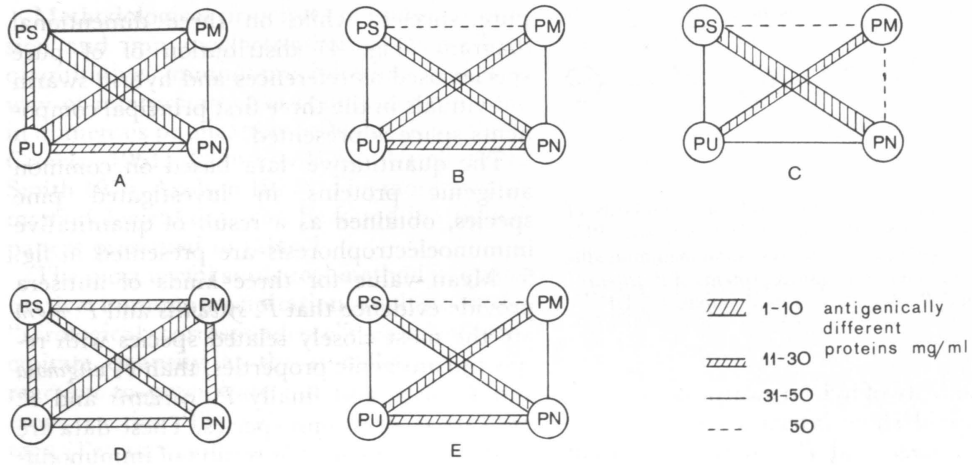

Figure 5. Diagrams showing the antigenic similarity between *Pinus sylvesteris* (PS), *P. mugo* (PM), *P. uliginosa* (PU) and *P. nigra* (PN) constructed on the basis of quantitative immunoelectrophoresis. A – antisera antisylvestris, B – antimugo, C – antiuliginosa, D – combined antisera A + B + C (mixture 1:1:1), E – average results for A, B, C and D.

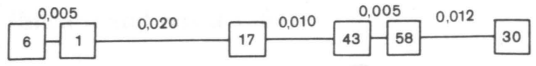

Figure 6. Diagram showing the serological similarity of six swedish population of *P. sylvestris*, constructed on the basis of frequency of antigenic proteins (Formula acc. to Nei 1972).

sylvestris in Sweden are presented in Table 2. The data obtained show differences in frequency of individual antigens as well as differentiated polymorphism of antigenic proteins.

The diagram presented in Fig. 6. shows serological similarities of the populations under study. The diagram was constructed on the basis of data in Table 2 with the use of formula by Nei 1972.

Marked similarity of two populations from the southern Sweden and an intermediate position of the population from central part of country as well as serological individuality of populations in the north of Sweden can be observed.

Final remarks

In the above mentioned examples only very few possibilities for immunchemical methods application in research on the similarities of species, introgression and population genetics are described.

Table 2. Frequency of antigenic proteins and polymorphic index in six investigated Swedish populations of *Pinus sylvestris*.

No.	Lat.	Number of tree	Frequency of antigens 1	2	3	4	5	6	7	8	9	P_i
1	56°20'	20	0.80	1.0	0.45	1.0	1.0	0.55	0.15	0	0	0.11
6	59°02'	22	0.77	1.0	0.41	0.91	0.95	0.59	0.23	0.09	0.05	0.12
17	61°45'	22	0.86	0.95	0.73	0.95	0.77	0.27	0.23	0	0	0.14
30	63°31'	22	1.0	0.95	0.95	0.86	0.82	0.05	0.23	0.23	0	0.10
43	65°05'	22	0.91	1.0	0.86	0.91	1.0	0.14	0.09	0.05	0	0.07
58	67°38'	20	0.80	1.0	0.90	0.95	0.95	0.10	0.10	0	0	0.08
		128	0.86	0.98	0.72	0.93	0.92	0.28	0.16	0.06	0.01	0.10

A few more instances of the application of immunochemical methods in tree protein research can be found in review (Table 1). Most of the papers cited, deal with problems of chemotaxonomy or phylogenesis, some of them concentrate on incompatibility in trees and several on introgression and problems connected with hybridization, whereas the methods in question are being widely used in protein studies of other plant groups. A comprehensive review of problems which are solved by means of different immunochemical techniques was reported by Daussant 1975, 1977.

Thus there is a possibility to introduce this techiques to forestry, among others, in the taxonomic studies on identification of species, varieties, races, provenances and hybrids, as well as in the genetic studies on inheritance of proteins, genes expression on haploid (macrogametophytes) and on diploid level (needles, buds and embrions).

Immunochemical techniques can offer an irreplaceable tool for studies concerned with qualitative and quantitative changes of protein spectrum, both in organo- and ontogenesis, action of citokinins on the synthesis of specific proteins and the changes introduced to protein pattern in connection with plant tumors, virus infections, woundings caused by insects and mechanical ones.

In cytological analysis they can be used for localization of enzyme synthesis in cells and tissues as well as for studies on incompatibility.

And finally, there is a potential possibility of their application for searching hardiness markers and identification of clones and seed samples. The above mentioned possibilities of the use of immunochemical methods are far from being complete. In spite of certain pitfalls and limitation connected with the discussed methods they can provide a valuable tool for solving many problems research, due to their high sensitivity and specificity.

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COMPARISON OF STARCH AND POLYACRYLAMIDE GEL-ELECTROPHORESIS AND ISOELECTRIC FOCUSING FOR ISOZYME ANALYSIS IN TWO CONIFERS

E. E. McMULLAN and A. COLANGELI

Experiences at the Pacific Forest Research Centre, Canada, with the use of starch and polyacrylamide gel electrophoresis and isoelectric focusing for isozyme analysis are discussed. Results of terpene analysis, and leucine amino peptidase analysis by isoelectric focusing, indicate that the two techniques show similar population groups though there was no individual tree correlation. The possibility of computerized data recording for isozyme separations is discussed.

Introduction

There is interest in laboratory methods for verification of seed origin for the use of seed inspectors, dealers and purchasers. Attention has been drawn to the potential of isozyme analysis as a test of seed source (Bergmann 1971, Muhs 1974). Geographically separated populations tend to accumulate different genes, with the result that enzyme polymorphisms differ in different populations (e.g. Yeng, Ching and Ching 1977). This report describes trials carried out to determine whether seed from different stands could be distinguished on the basis of isozyme polymorphisms revealed by polyacrylamide and starch gel electrophoresis, and isoelectric focusing on polyacrylamide thin layers.

Methods and Materials

Douglas fir: polyacrylamide gel-electrophoresis

Two reforestation seed lots were obtained from coastal Douglas-fir stands approximately 70 km apart in the Port Alberni district on Vancouver island (B. C. Ministry of Forests no. 965 and no. 1295), and from two interior stands in the Nelson district also about 70 km apart (B.C.M.F. no. 886 and no. 887). The coastal and interior stands were 700 km apart. Endosperm from individual hydrated seeds were crushed separately in 20 % sucrose containing bromophenol blue. Proteins were separated by electrophoresis in 7 % polyacrylamide slab-type gels with 0.01 M tris maleate buffer, pH 7.4 (LAP and EST) or 0.01 M sodium glycinate buffer, pH 8.8 (IDH and MDH) by the method of Davis (1964). Gels were stained for isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), esterase (EST), and leucine amino peptidase (LAP) by the methods of Shaw and Prasad (1970). Bands were numbered in order of increasing migration distance, and variants were labelled "c" (common), "s" (slow) and "f" (fast). Frequencies of MDH, IDH, LAP and EST apparent allozymes ("apparent allozymes": bands whose occurence showed 100 % negative correlation) were scored. Only those bands which stained sharply and consistently and showed polymorphisms were used. These were: MDH, 3 of 4 bands; LAP, 2 of 3 bands; EST, 1 of several bands; IDH, 1 band. Band frequencies were subjected to X^2 tests of independence.

Lodgepole pine: starch gel-electrophoresis

Seed was obtained during the course of OECD seed inspections from 17 collection sites separated by at least 100 km in the interior of B. C. from latitude 50°–60°. Three samples were obtained at each site, from the dealer at the time of collection, by the seed inspector during his check of the collection site, and from the dealer after extraction. Frequencies of bands of MDH, IDH, ACP (acid phosphatase) and PGI (phosphoglucoseisomerase) were recorded for 40 endosperms of each sample using starch gels.

Gels were prepared with 0.002 M citrate morpholine buffer, pH 6.1, while the electrode buffer was 0.04 M (Clayton and Tretiak 1972). Enzymes were stained as previously described.

Lodgepole pine: polyacrylamide gel-electrophoresis

Seed was obtained from two stands separated by 500 km in northern B. C. Seed was collected from 100 trees in each stand and seed from individual trees was stored separately. Endosperm were ground in 20 % sucrose containing 0.2 % bromophenol blue and subjected to electrophoresis on 9 %, 6 mm thick, vertical slab-type polyacrylamide gels containing 4.5 % cross-linker. Gels were prepared with the lithium borate/tris citrate buffer described by Brewbaker et al. (1961), diluted 1/10 in the gel and 1/100 in the electrode tanks. Gels were sliced into 3 layers and stained for ACP, LAP and EST as described.

Lodgepole pine: isoelectric focusing

Seed was collected in 10 stands along an east-west parallel across southern B. C., longitude 126° to 116°, approximate latitude 149.1° to 149.5°. These stands included one typical coastal (*P. contorta* var. *contorta*) stand, interior (*P. contorta* var. *latifolia*) stands, and geographically intermediate stands. Altitude was measured at each site at the time of collection. Cones were collected from 25 trees at each site and stored separately. Vegetative shoots were also collected from 10 of the same trees for terpene analysis. These results will be published elsewhere by von Rudloff.

Isoelectric focusing was carried out with a Bio-Rad horizontal flatbed focusing apparatus using 1 mm thick polyacrylamide gels, 5 % T, 4 % C, with 2 % pH 4–9 ampholyte and 2 % glycerol. Gels were focused for 3,5 hr at 3 watts constant power, coolant temperature 4° C. Final pH was measured with the gel in place on the cooling platform using an Ingold surface micro-electrode. Gels were re-focused a further 10 min. and stained by the methods described, except that gels were soaked in staining buffer for 10 min. prior to transfer to fresh staining solution. Endosperm of one tree from which > 1 000 seeds were obtained were used as a reference on each gel, and were also used for ACP and MDH analysis. LAP was analyzed in endosperm from 10 trees per stand. Where possible these were trees which were also sampled for terpene analysis, however occasionally cones from such trees contained empty seed. Cones from only 5 trees at site 9 contained filled seed.

Stained gels where photographed. For preliminary studies, 35 mm black and white transparencies were reduced to 1/10 and scanned using a Zeiss mechanized platform microscope densitometer interfaced to a computer, programmed to record density at 0.5 μ intervals.

Results and discussion

Douglas fir: polyacrylamide electrophoresis

Apparent allozyme frequencies in endosperm from the four seed-lots are shown in Table 1. Nineteen of 28 (68 %) of the X^2 tests of independence of apparent allozyme frequencies in pairwise coastal/interior seed-lot comparisons were significantly different ($p <= 0.05$), while 2 of 14 coastal/coastal and 2 of 14 interior/interior comparisons (14 % in eash case) were significantly different. These results indicated greater differentiation bet-

Table 1. Douglas fir. P.A.G.E. Frequency of apparent allozymes in endosperm of mixed-tree seed-lots.

Provenance		MDH-2 s f c Tot	MDH-3 s f c Tot	MDH-4 s f c Tot	LAP-2 s f c Tot	LAP-3 s f c Tot	EST-2 s f c Tot	IDH s f c Tot
Coastal, number	965	0 0 69 69	10 4 94 108	12 5 88 108	7 4 43 54	13 11 30 54	0 5 67 72	8 6 94 108
Coastal number	1295	0 0 72 72	6 2 64 72	4 9 59 72	4 6 44 54	8 12 34 54	0 7 65 72	15 14 79 108
Interior number	886	0 0 72 72	4 0 68 72	11 1 60 72	0 5 49 54	0 4 50 54	0 0 72 72	4 1 103 108
Interior number	887	2 4 66 72	3 3 66 72	9 2 61 72	0 0 54 54	0 2 52 54	0 0 70 72	2 1 105 108

Table 2. Lodgepole pine, starch-gel electrophoresis, frequency of apparent allozymes in endosperm of mixed-tree seed-lots.

Collection area	*	MDH-1 s f c	MDH-2 s f c	MDH-3 s f c	MDH4 s f c	GPI-1 s f c
Lynx Lake	A	0 0 40	6 0 34	5 0 35	0 0 40	0 1 39
	B	0 0 40	7 2 31	7 0 33	0 0 40	0 2 38
	C	0 0 40	4 3 33	3 0 37	0 0 40	0 3 37
Chetwynd-1	A	0 0 40	15 0 25	1 0 39	0 1 39	1 0 39
	B	0 1 39	5 1 34	2 0 38	0 0 40	1 4 35
	C	0 0 40	8 0 32	4 0 36	0 1 39	0 1 39
Jackfish Lake	A	0 1 39	0 0 40	0 0 40	0 0 40	0 0 40
	B	0 0 40	1 2 37	0 0 40	0 0 40	0 0 40
	C	0 0 40	1 0 39	0 0 40	0 0 40	0 0 40
Wonowon	A	0 0 40	4 0 36	3 0 37	0 0 40	1 2 37
	B	0 0 40	1 0 39	0 0 40	0 0 40	1 1 38
	C	0 0 40	5 0 35	1 0 39	0 0 40	1 2 37
Stevens Meadow	A	0 0 40	1 2 37	0 1 39	0 1 39	0 3 37
	B	0 0 40	2 0 38	2 0 38	0 1 39	0 0 40
	C	0 0 40	3 0 37	0 0 40	0 0 40	0 1 39
Hefley Lake	A	0 0 40	2 1 37	0 2 38	0 0 40	2 0 38
	B	0 1 39	2 0 38	0 0 40	0 2 38	0 0 40
	C	0 0 40	1 0 39	0 1 39	0 1 39	0 0 40
Lac la Hache	A	0 0 40	8 4 28	0 0 40	0 0 40	0 0 40
	B	0 0 40	1 0 39	0 0 40	0 0 40	0 0 40
	C	0 0 40	4 0 36	1 0 39	0 0 40	1 1 38
Likely	A	1 0 39	9 0 31	0 0 40	0 0 40	0 0 40
	B	0 0 40	11 0 29	0 0 40	0 0 40	1 0 39
	C	3 1 36	10 0 30	0 0 40	0 0 40	0 1 39
100 Mile House	A	0 0 40	8 1 31	0 0 40	0 0 40	3 2 35
	B	0 0 40	8 0 32	0 2 38	0 1 39	0 0 40
	C	0 0 40	4 0 36	0 1 39	0 0 40	1 3 36
Lac le Jeune	A	0 1 39	4 1 35	0 0 40	0 2 38	2 0 38
	B	0 0 40	1 2 37	4 0 36	0 2 38	1 1 38
	C	1 0 39	5 1 34	0 0 40	0 0 40	0 4 36
Chase Creek	A	0 0 40	3 0 37	0 0 40	0 0 40	1 2 37
	B	0 0 40	5 1 34	0 0 40	0 0 40	2 0 38
	C	2 0 38	2 0 38	0 0 40	0 0 40	0 1 39
Muskeg Lake	A	2 0 38	2 0 38	0 0 40	0 0 40	2 0 38
	B	1 0 39	0 0 40	0 0 40	0 0 40	0 0 40
	C	0 0 40	3 1 36	0 0 40	0 0 40	0 0 40
Chetwynd-2	A	1 0 39	1 0 39	1 0 39	0 0 40	1 1 38
	B	1 1 38	0 0 40	1 0 39	0 0 40	0 3 37
	C	0 0 40	1 2 38	0 0 40	1 1 38	0 3 37
Hudson Hope	A	0 1 39	5 1 34	2 1 37	0 0 40	2 0 38
	B	0 0 40	2 3 35	2 2 36	1 0 39	1 0 39
Takysis Lake	B	0 0 40	6 2 32	0 1 39	2 0 38	1 1 38
Upper Liard	A	0 0 40	0 0 40	0 0 40	0 0 40	0 1 39
	B	9 0 31	0 1 39	0 0 40	0 0 40	1 2 37
Lone Butte	A	0 0 40	3 5 32	0 0 40	0 1 39	2 0 38

* A = collection sample
B = check sample
C = post-extraction sample

ween the more widely separated coastal and inland populations. The possible use of this technique was pursued on a wider scale with lodgepole seed collections.

Lodgepole pine: starch gel-electrophoresis

Starch blocks were used as more seeds could be screened for more enzymes using this technique. IDH and ACP showed no polymorpism. For the one PGI and 4 MDH bands which did show polymorphism, apparent allelic frequencies were scored. Results are shown in Table 2. Eighty of the 680, or 12 % of the X^2 tests of significance of differences of two-way comparisons between collection areas were significantly different, while 5 of 75, (7 %) of 3-way comparisons between site, storage and extraction samples within collection areas were significantly different. Five per cent of such comparisons are expected to be significantly different due to random error. Again there was a trend to more differences between more separated populations, though less enzyme polymorphism was detectable than in the previous study in which polyacrylamide gels were used.

Interpretation of between and within site differences was complicated by the fact that reforestation seed lots are not collected for the purpose of population sampling. As pointed out by O'Malley and Yeh (1977), such seed lots may be biased by a few heavily-bearing trees and not be representative samples of the reproductive output of the stand.

Lodgepole pine: polyacrylamide gel-electrophoresis

ACP staining showed two, and EST staining three, variable bands. LAP staining revealed two well resolved bands which showed no polymorphism. .

Lodgepole pine: isoelectric focusing

ACP staining revealed bands at approximate isoelectric points (pI's) of 6.8, 6.5, 5.9, 5.0, and 4.7. Six less well resolved bands appeared at approximate pI 4.2–4.6. The resolution of ACP was better than with either electrophoretic technique used.

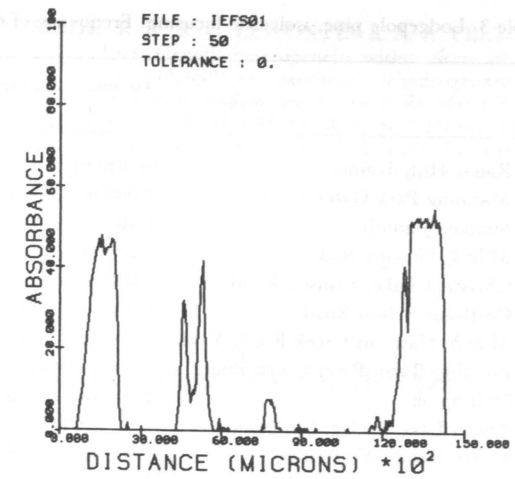

Figure 1. Computerized microdensitometric scan, MDH. Absorbance at distance > 120 microns due to electrode wick. Approximate pI of major peaks 5.4, 5.6, 6.7, 7.1 and 8.2.

MDH showed well resolved bands at approximate pI's of 8.2, 7.1, and 6.7, and 2–4 poorly resolved bands with pI's 5.3–5.8. An example of the computer output of a microdensitometric scan of one sample stained for MDH is given in Fig. 1. The use of computer interfaced microdensitometry appears promising for overcoming the problem of recording band patterns from large numbers of samples and storing them for easy reference for seed source identification. Studies are continuing at the Pacific Forest Research Centre to develop means of standardising these records.

LAP staining revealed five patterns of bands. The frequency of these patterns is summarized in Table 3. Pattern A had two bands, approximate pI's 4.3 and 4.7; B had 3 bands, 4.3, 4.4, and 4.7; C had 2 bands, 4.3 and 4.4; D had 3 bands 4.3, 4.4, and 4.8; E had 1 band, 4.7. Different endosperm from some trees showed two different patterns. Some patterns were found only at one site. Endosperm with no band at 4.7 or 4.8 (C-type) were found only in one Tofino tree. Some trees from this coastal stand also had characteristic terpene patterns. However, the tree with the unusual LAP pattern had terpenes similar to interior trees, while a tree with typical coastal terpenes had the common A and B type LAP patterns found in trees across B. C. Thus the two techniques indi-

Table 3. Lodgepole pine, isolectric focusing. Frequency of endosperm LAP patterns in single tree collections.

Site	Elevation	Approximate longitude	Numbers of Trees with Pattern A+A	B+B	A+B	C+C	B+D	A+E	B+E
1. Radar Hill, Tofino	10–100 m	125.7	2	2	5	1			
2. Manning Park Gates	1 000 m	121.2	7	3					
3. Sunday Summit	1 400 m	121	6	2	2				
4. Mile 1, Phoenix Road	1 200 m	118.5	4	2	4		1		
5. Christina Lake, Paulson Road	800 m	118.2	1	5	2				2
6. Castlegar-Salmo Road	1 000 m	117.3	4	2	4				
7. Mile 5, Hawkins Creek Road, Yahk	1 100 m	115.9	4	1	5				
8. Pudding Burn, Perry Creek Road, Cranbrook	1 100 m	116	3	4	3				
9. Thorn Lake Bridge, Christian Valley Road, Westbridge	800 m	118.8	1	2	1			1	
10. Mile 10, Brenda Mine Road	1 100 m	119.7	3	1	6				

Pattern A – LAP bands at 4.3, –, 4.7
Pattern B – LAP bands at 4.3, 4.4, 4.7
Pattern C – LAP bands at 4.3, 4,4, –
Pattern D – LAP bands at 4.3, 4.4, –, 4.8
Pattern E – LAP bands at –, –, 4.7

cated similar population groups, but either technique by itself under-estimated the distinctiveness of this population.

Greater enzyme polymorphism was revealed by polycrylamide gel than starch gel electrophoresis, and considerably more again by isoelectric focusing. The possibility that variation in proteins may be obscured by overlap of bands is frequently pointed out (e.g. Rudin and Brune 1976) and the problems such overlap creates for phylogenetic interpretations of isozyme analysis have been discussed (e.g. Singh, Lewontin and Felton 1976, Brown and Moran 1979). The "apparent allozyme" frequency data reported in this study represent only the limited part of the actual enzyme polymorphism which could be detected by the electrophoretic techniques used. However, these electrophoretically extreme protein types appeared to be useful for differentiating seedlots. For seed source identification, a protein separation technique which provides maximum resolution would probably be advantageous, allowing differentiation of smaller samples. Studies are continuing at the Swedish University of Agricultural Sciences, Umeå, Sweden, to determine the biochemical characteristics and mode of inheritance of isozymes in lodgepole pine detectable after isoelectric focusing.

Acknowledgements

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DETERMINATION OF VAPOURIZABLE TERPENES OF PINUS SYLVESTRIS

R. HILTUNEN, RIITTA LÖYTTYNIEMI, S. RÄISÄNEN and P. M. A. TIGERSTEDT

The vapours in the air-space around a Scots pine seedling and a tree were analysed by gas chromatography-mass spectrometry (GC–MS). The vapours were collected on a Tenax GC adsorbent and desorbed by n-hexane. The reproducibility of the analysis expressed as the relative standard deviation was 6.4 %. The relative standard deviation for repeated GC–MS analysis of a single needle oil was 4.2 %.

Introduction

Monoterpenes have been used as markers for genetic research on inheritance and also for problems related to population and ecological genetics of pine trees. Monoterpenes have also been used as a tool in studies of general adaptation and growth of certain genotypes of pine. In addition studies have been carried out to investigate whether correlations exist between monoterpenes of host trees and the primary orientation of forest injurious insects. In many connections some pitfalls have been pointed out in terpene analyses which to some extent restrict the application of this method to above-mentioned purposes.

One of the weaknesses is steam distillation which has been shown to give rise to certain artefacts. A serious problem is the time of storing, prehandling of material, distillation and gas chromatographic analysis thus making impossible to analyse large series of samples that are needed for purposes of beforementioned studies. Very often volatile oils also contain other compounds than terpenes, particularly when volatile oil is obtained by solvent extraction. In this case the results of terpene analyses should be checked by comparing them with authentic reference compounds.

Many of the disadvantages, such as time-consuming prehandling of the material, steam distillation and identification problems can be avoided when a gas chromatographic-mass spectrometric technique is used. The advantages of this method are among others a high selectivity owing to the detection of a characteristic ion and a high sensitivity due to detection by electron multiplier. By this method the constituents of the volatile oil can be analysed directly and quickly from the air-space around the plant (Hiltunen et al. 1980). In the studies of the orientations of injurious insects terpenes can be analysed by this technique in air-space around a test tree in its natural environment as "odours of nature".

The aim of this work was to continue our recent studies of utilizing mass fragmentography in the direct analysis of the terpene composition of vapours in the air-space around Scots pine. Particularly in this study the aim was to investigate the trapping of the terpenes in air-space on an adsorbent.

Material and methods

A volatile oil of pine needles (*Pinus sylvestris* L.) obtained by steam distillation (TS-1) was used as a test solution in this study. Ten microliters of TS-1 was added into a glass-stoppered tube (30 ml). After a 15 min equilibrium time at room temperatre 1.2 l gas was drawn from the head-space through an adsorption tube. Gas was sucked by a Sipin personal sampler pump (Model SP-15, Anatole J. Sipin Co., N.Y., U.S.A.) with the speed of 110 ml/min. Replacing air into the head-space tube was drawn through a similar adsorption tube in order to prevent impurities from the laboratory air. The gas was passed through two similar adsorption tubes in series to secure the trapping of all the terpenes in the gas.

Six consecutive samples were drawn from the vapour surrounding the 5-years old pine seedling in the laboratory (PSV). A 20 mg needle sample was taken from the same seedling from which terpenes were extracted by using 1 ml of n-hexane (PSN). Again six separate gas chromatographic-mass spectrometric (GC–MS) analyses were carried out. The six repetitions were made to estimate instrumental errors. For comparison vapour and needle oil measurements were done in nature on a 15-year old pine tree (PTV and PTN).

Laboratory gas samples were of 1.2 litre, samples in nature 3.5 litre. Laboratory air temperature was approximately +25°C but outdoor temperature was only −2°C therefore more gas was required in the latter case. As in above the gas was passed through two tubes in series.

Adsorption tubes were prepared by filling 300 mg of activated (200°C, 16 h) Tenax GC (80/100 mesh, Ohio Valley Specially Chemical, Inc., Marietta, Ohio, U.S.A.) adsorbent into a Pasteur pipette (11 cm × 6 mm I. D.). Tubes were secured at both ends with silanized glass-wool plugs. Prior to GC–MS analysis the terpenes were eluted from the tubes with 1.3 ml of n-hexane and the eluate was concentrated to about 20 ul.

Instrumentation: Gas chromatographic separations were carried out on a free fatty acid phase (FFAP) (62 m × 0.35 mm I.D.) glass capillary column with following conditions: injection port temperature 250°C, interface temperature 260°C and oven temperature 55°C, after solvent had been eluted the oven was programmed for 4°C/min to 180°C. The samples were injected into the GC using the split sampling technique.

Gas chromatographic-mass spectrometric analyses were obtained on a Carlo Erba Fractovap 2300 gas chromatograph coupled to a Jeol D 100 mass spectrometer by means of a Pt-Ir-tube (30 cm × 0.30 mm O.D., 0.15 mm I.D.) into the ion source without a helium separator. The MS parameters were as follows: electron energy 26 eV, electron multiplier voltage 1.5–1.6 kV, recorder input 0.5 v, ionisation current 300 uA and ion source temperature 220°C. The ion monitor was adjusted to m/z 93 and resolution to 500.

Quantitative determinations are based on peak areas which were measured with a Hewlett-Packard 3390 A peak integrator connected to the mass spectrometer. The coefficient of variation values given for populations, clones and F_1-generations are published elsewhere (Hiltunen 1976). So also the values for GLC analyses (Chung 1981).

Results and discussion

The fragment ion m/z 93 was chosen for GC–MS analyses because it is common for all of the monoterpenes present in pine oil. In addition this fragment is the most abundant for most of the terpene components (Hiltunen et al. 1980).

The coefficient of variation for repetitive injections of needle oil (Table 2, Fig. 2) and

Table 1. Statistical data for monoterpenes in air-space around a pine seedling (PSV).

Constituent	Mean (%)	S.d.	cv (%)
1. Tricyclene	2.82	0.087	3.1
2. α-pinene	67.95	0.241	0.4
3. Camphene	9.01	0.116	1.3
4. β-pinene	4.38	0.069	1.6
5. Sabinene	1.05	0.041	3.9
6. 3-carene	10.70	0.216	2.0
7. Myrcene	1.63	0.045	2.8
8. Limonene	0.95	0.059	6.2
9. β-Phellandrene	0.87	0.061	7.0
10. trans-β-ocimene	0.24	0.085	35.2
11. Terpinolene	0.40	0.029	7.3
		Mean	6.4

Table 2. Statistical data for monoterpenes in Scots pine needle oil (PSN).

Constituent	Mean (%)	S.d.	cv (%)
1. Tricyclene	2.77	0.087	3.1
2. α-pinene	68.40	0.082	0.1
3. Camphene	6.55	0.050	0.8
4. β-pinene	3.47	0.031	0.9
5. Sabinene	0.87	0.052	6.0
6. 3-carene	12.80	0.111	0.9
7. Myrcene	2.36	0.049	2.1
8. Limonene	0.77	0.052	6.8
9. β-phellandrene	0.61	0.080	13.2
10. trans-β-ocimene	0.61	0.045	7.3
11. Terpinolene	0.80	0.036	4.5
		Mean	4.1

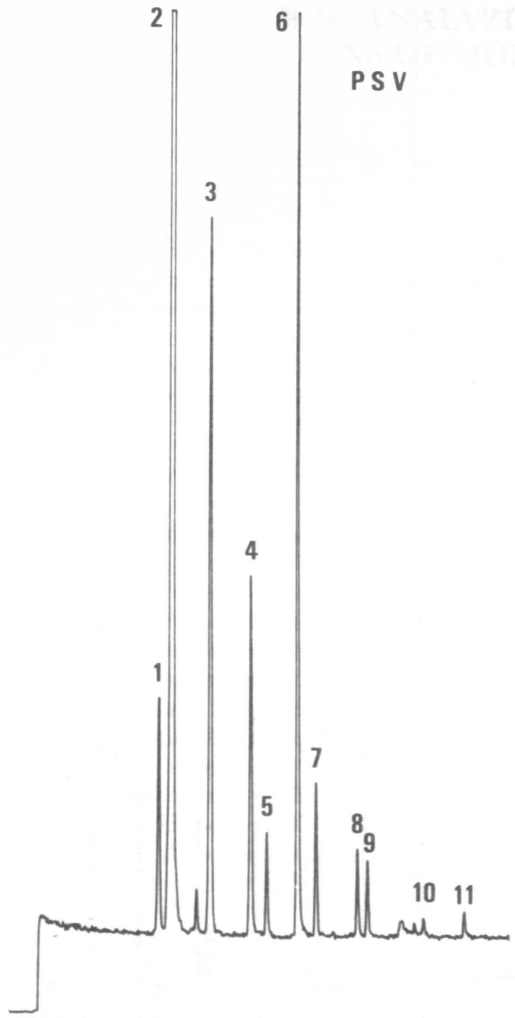

Figure 1. Selected ion chromatogram (m/z 93) of terpene vapour in air-space around a pine seedling in the laboratory. (PSV). See Table 1 for key to compounds.

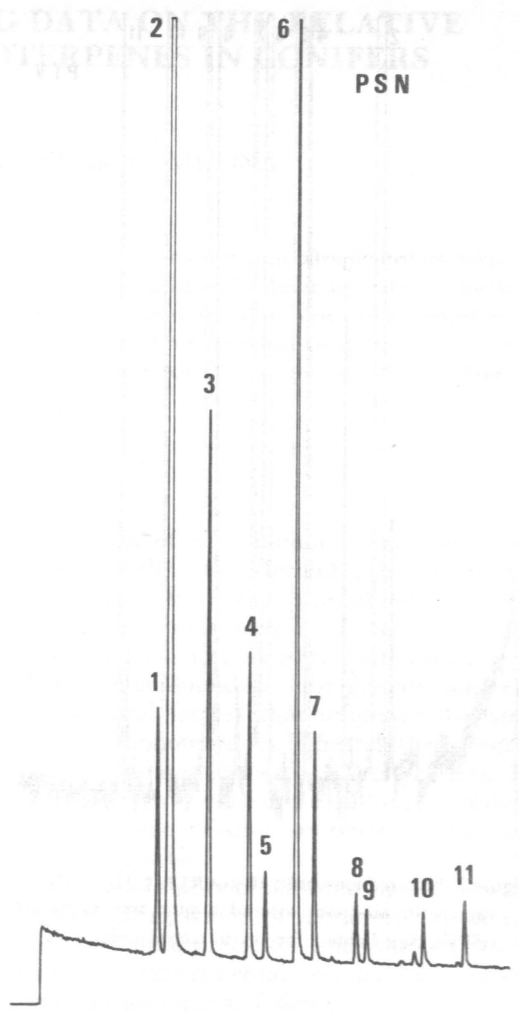

Figure 2. Selected ion chromatogram m/z 93) of needle oil (PSN). See Table 2 for key to compounds.

trapped vapour (Table 1, Fig. 1) is as low as 4.2 % and 6.4 %, respectively, on average for the eleven components. The results obtained by GC–MS are approximately the same as calculated on the basis of GC-Flame ionization Detector (FID) analyses (3.3 %). For comparison purpose, the corresponding values after GC–FID analyses of the samples taken from populations, clones and F_1-generation are 55 % (N = 242), 44 % (N = 143) and 57 % (N = 163), respectively. These high cv-values encompass the biological variation and the errors arising from the handling of the material and GC analyses. On the basis of this the GC–MS technique is adequate for the purposes mentioned in the introduction because the biological variation is close to ten times greater than the analytical error. The precision of vapour analyses obtained by the method discussed here is surprisingly high when taken into consideration that the amounts of volatile fractions in these analysses are only 1/1000 part of that in GC–FID analyses.

The analyses of two adsorption tubes in series indicated that all of the terpenes in the

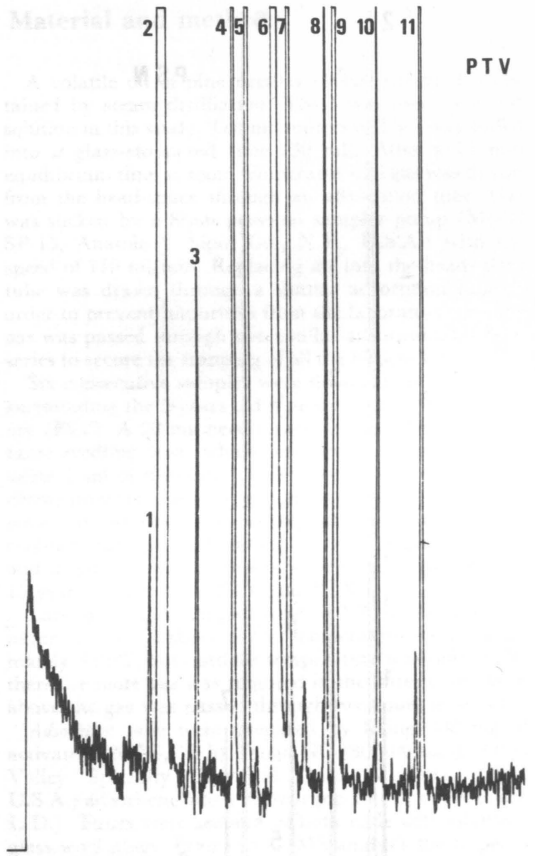

Figure 3. Selected ion chromatogram (m/z 93) of terpene vapour in air-space around a pine tree in nature (PTV). See Table 2 for key to compounds.

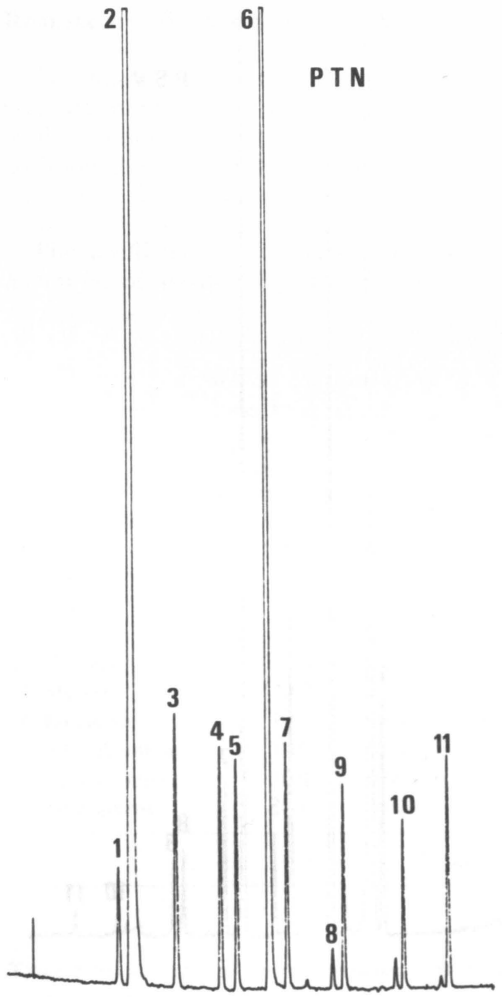

Figure 4. Selected ion chromatogram (m/93) of needle oil (PTN). See Table 2 for key to compounds.

head-space (TS-1) can be trapped on the first tube when 1.2 l of gas is passed through the tubes. The more precise breaktrough values for terpenes are not studied in this preliminary work. Figure 3 is the result of GC–MS analysis of the terpenes in the vapours around a tree in nature at −2°C. The needle of the same individual is analysed by GC–MS in figure 4. The two fragmentograms clearly give the same information of the monoterpene composition. The time required per analysis in GC–MS is only 1/4 of that in GC–FID including all sample preparations. It also shows, that GC–MS can be applied to quick and reliable analyses directly from vapours of natural growing individuals.

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METHODS FOR ANALYZING DATA ON THE RELATIVE PROPORTIONS OF MONOTERPENES IN CONIFERS

D. V. SHAW, R. YAZDANI and O. MUONA

The interpretation and testing of data on the relative proportions of monoterpenes is often limited by unknown modes of inheritance and correlations among individual monoterpene variables. Multivariate statistical methods designed for analysis of normally distributed variables, that are to some degree correlated, are suggested as an alternative to conventional methods of analysis. Two multivariate methods, principal component analysis and discriminant function analysis, are demonstrated with Swedish populations of *Pinus sylvestris*. The assumptions necessary for use of such multivariate methods are discussed.

Introduction and background

In a recent review article, Squillace cited over 300 references on the topic of monoterpene composition in coniferous trees (Squillace 1976), most of which were published within the last 20 years. These studies demonstrate that the monoterpene composition of oleoresin samples is often variable both within and between populations of trees (Tobolski and Hanover 1971; Wilkinson et al. 1971) and that this composition is under strong genetic control (Squillace 1980; Squillace and Fisher 1966, Hiltunen 1975). Because monoterpene composition is affected little by environmental variation it has proven to be a useful character for studying similarities and differences among populations. Most recent publications aimed at assessing genetic similarities or differences among populations of trees study either allelic variants at electrophoretically detectible loci (which presumably reflect variation close to the DNA level) or metric characters (which result from the combined action of many loci and the environment). Because monoterpenes are more simply inherited than most commonly studied metric characters, they may reflect a kind of variation that is not measured in conventional electrophoretic or metric character studies.

The most common procedure for analyzing monoterpene composition is the collection of exuded oleoresin, determination of monoterpene composition by gas-liquid chromatography, and expression of this composition as the proportion of each monoterpene constitient relative to the total. Methods are available for determining the actual quantity of each constituent per unit weight of tissue, but the resulting measures are often quite variable, as they are confounded with genetic and environmental factors that influence the total yield of monoterpenes. The relative proportions of monoterpenes are less affected by these sources of variation and consequently it is only these relative proportions that are highly heritable.

The availability of information on only the relative proportions of monoterpenes limits both the interpretation and the statistical testing of experimental results. Two important cautions are as follows:

1) The mode of genetic control for individual monoterpene quantity is often unknown or untested. When strong bimodality occurs in the distribution of individual monoterpene proportions, sample trees can be assigned to phenotypic classes, e.g. high or low proportions of a specific constituent. Experiments designed to study the genetic control of such phenotypic classes have been successful when large numbers of trees were analyzed, usually indicating single-locus inheritance with dominant and recessive alleles (Squillace 1980), but also suggesting the action of modifier loci (Hiltunen et al. 1975). When bimodality is not strong in the distribution of individual monoterpene proportions the division into phenotypic classes may be imprecise. Furthermore, the assumption of simple modes of inheritance when modifier loci are affecting classification may lead to the use of inappropriate statistical tests.

2) The observed proportions of different monoterpene constituents are usually correlated, for two different reasons. First, when the actual quantity of a given monoterpene produced by a single tree is large, all others constituents must represent a smaller fraction of the total. The use of relative proportions for describing the monoterpene composition thus results in a set of variables that are necessarily correlated. The expected correlation between proportions "due to constraint" (Squillace 1976) is negative and can be predicted as:

$$r_{M_1M_2} = -\sqrt{\frac{\bar{M}_1\bar{M}_2}{(1-\bar{M}_1)\ (1-\bar{M}_2)}}$$

in which $\bar{M}_1$ and $\bar{M}_2$ are the mean proportions of any two monoterpenes in the total sample and $r_{M_1M_2}$ is the correlation coefficient between these two monoterpenes (after Mosimann 1962). Because of this correlation, strong bimodality in the proportion of one monoterpene may cause an apparent, or "mirror image", bimodality in other nonvariant monoterpenes (Squillace 1976). A second kind of correlation between monoterpene proportions may arise due to the final monoterpene constituents sharing a common biosynthetic pathway, or because they are formed from a common intermediate product (Hiltunen 1975). For example, if genetic variation occurs for a precursor from which two monoterpenes are formed, then they may both reflect this pattern of variation, and will be positively correlated. In fact, positive correlation between monoterpene proportions has been taken as evidence for common biosynthesis (Squillace 1976).

From the above discussion it is clear that individual monoterpene proportions cannot be treated as independent variables when used to make comparisons either between individuals within populations or between populations. An alternative to conventional methods of phenotypic classification and subsequent analysis based on discrete models, is to use statistical methods designed to analyze normally distributed variables, which are to some degree correlated. Such multivariate statistical methods have been used to make between-population comparisons for monoterpene composition (Flake et al. 1973; Sturgeon 1979) and to investigate the biosynthesis of monoterpenes (Hiltunen 1975). In this paper we demonstrate two multivariate methods with samples from Swedish populations of *Pinus sylvestris,* and outline some of the assumptions and advantages of these methods.

Methods and results

Oleoresin samples were collected from an open-pollinated provenance trial located near Sävar, Sweden (64° N latitude). The 340 trees sampled represent ten provenances scattered throughout Sweden (from 57° to 67°). However, much of our report concentrates on the comparison of sample trees from two provenances – 31 trees from provenance 1 and 39 trees from provenance 30 – which are located at 57° and 60° respectively. These two provenances were chosen for demonstrative purposes. Oleoresin samples were analyzed by gas-liquid choromotography and the relative proportions of nine monoterpenes (α-pinene, β-pinene, sabine, △-3-carene, myrcene, α-terpinene, limonene, β-phellandrene, terpinolene) and remaining fraction (consisting of water and five trace monoterpene constituents) were scored and used as variables for our study. Table 1 given the means and standard deviations for these ten variables in the sample of 320 trees.

One assumption necessary for the use of multivariate statistical methods is that the input variables are normally distributed. A first step in our method of analysis is the transformation of raw proportions with an arcsin-square root function. Such a transformation renders the variances of proportions independent of the mean, usually improving the fit to normality. A Chi-square test for fit to a normal distribution indicated that 7 out of 20 transformed distributions (from provenances 1 and 30) were significantly non-normal. This non-normality may arise in part from the previously mentioned bimodality that can occur in the distribution of some monoterpene proportions. Violation of the normality assumption is not viewed as a serious threat to the validity of multivariate methods (Morrison 1967). We should also note that normalization of original monoterpene proportions by transformation does not overcome either of the two sources of correlation previously discussed.

The first multivariate method used to treat our transformed proportions was principal component analysis (PCA). Such an analysis has several functions, the most important of which for our purposes is the derivation of a new set of independent variables (principal components) from the original set of input

Table 1. Mean proportions and standard deviations for nine monoterpenes and a remaining fraction.

	$\bar{x}$	S.D.
α-pinene	.102	.057
β-pinene	.134	.133
sabinene	.024	.009
Δ-3-carene	.393	.225
myrcene	.079	.082
α-terpinene	.004	.004
limonene	.085	.112
β-phellandrene	.126	.108
terpinolene	.035	.002
remainder	.023	.008

Table 2. Loadings on the original (transformed) variables for the three most important principal components.

Variable	Loadings PC 1	PC 2	PC 3
α-pinene	–0.75	–0.11	0.02
β-pinene	–0.67	–0.51	0.10
sabinene	0.90	0.11	0.03
Δ-3-carene	0.94	–0.16	–0.11
myrcene	–0.20	0.09	0.94
α-terpinene	0.83	–0.06	0.02
limonene	–0.72	0.13	–0.46
β-phellandrene	–0.14	0.94	0.00
terpinolene	0.95	–0.09	–0.01
remainder	0.76	0.00	0.02

variables (in our case, the transformed monoterpene proportions). Values for this new set of variables are obtained from linear functions of the input variables. This function of PCA can be viewed as a transformation of the original correlated variables to a set of independent variables that contain all of the original information. Because principal components are independent they can be used as variables in a range of statistical test (e.g. ANOVA, Students' t test, etc.) and perhaps for relative measures of genetic similarity and distance.

PCA may also be used to efficiently reduce the dimensionality of the data set. If several of the original variables are highly correlated, then most of the total variance may be accounted for with a smaller number on independent principal components. In such a case, very little information is lost when only a few of the most important principal components are used in further analyses.

As mentioned earlier, strong positive correlations between monoterpene proportions may indicate common biosynthesis. The importance of several original monoterpene variables to a single principal component may be similarly interpreted. Such an interpretation of results is a commonly used third function of PCA, but is not necessry for the use of principal components as variables in statistical tests.

The entire data set of 340 trees was subjected to PCA. The three most important (or first three) principal components explain 55 %, 12 % and 11 % of the variance in the original set of 10 transformed proportions. This result, that 78 % of the original variance is explained with just 3 independent variables, indicates a good deal of correlation among original monoterpenes proportions. Table 2 gives the multiplication coefficents, or loadings, for the linear functions that convert transformed proportions into the first three principal components. Loadings on original variables can vary from –1.0 to 1.0, and values that deviate from zero indicate the importance of a variable to the principal component score. We will not give a detailed biological interpretation of our results but should mention that the pattern of variation established by the first 3 principal components would be expected from the synthetic pathways established for *P. sylvestris* by Hiltunen (pers. comm.). For example, the four constituents with high positive loadings in the first principal component (sabinene, △-3-carene, α-terpinene, and terpinolene) belong to a single pathway while the three with high negative loadings (α-pinene, β-pinene and limonene) belong to a competing pathway. β-phellandrene and myrcene, which are important to the second and third principal components respectively, each have at least one additional independent synthetic pathway.

All of the first three principal components differ significantly between provenances 1 and 30 (Table 3) as indicated by a t test. When the distributions of these principal component scores within each provenance were tested for fit to a normal distribution 3

Table 3. Results of a t test for differences in principal component scores between provenances 1 and 30.

	t	d.f.
PC 1	2.27*	60
PC 2	–2.81**	54
PC 3	6.66***	59

out of 6 showed significant deviation. Principal component scores did not deviate as strongly from normality as did transformed monoterpene proportions. The central limit theorem states that, under rather general conditions, sums of several variables tend towards a normal distribution regardless of the original distribution of these variables (Lindgren 1968). This theorem would predict an improvement in fit to normality for principal components relative to the transformed proportions. The effect of deviations from normality on the validity of statistical test will be discussed later.

A second multivariate method employed was discriminant function analysis (DFA). As with PCA, DFA can be used to treat data consisting of a set of correlated variables. The objective of DFA is to derive a single variable (the discriminant function score) which allows the best discrimination between sample groups (in our case provenances 1 and 30). This single variable is obtained from a linear function of all input variables (the discriminant function). The importance of each input variable to this function depeds on the difference in means between groups for each variable, and the within-group variances and covariances of all input variables. DFA thus provides a composite measure of between-group differences based on all input variables. Such a measure may be particularly useful for monoterpene analysis when principal component scores do not differ significantly between sample groups. Either the original (transformed) proportions or a smaller number of principal components can be used as input variables for DFA. A function derived using transformed proportions has the advantage of using all available information. However, the use of principal components as input variables allows discrimination based on only those factors contributing most to the total variation. As in PCA, the DFA assumes that input variables are normally distributed, but deviations from assumption may not seriously affect the validity of the method (Spielman and Smouse 1976).

Another useful parameter provided by DFA is the probability of correct classification of samples into groups, based on the generated discriminant function. This parameter is in some sense a measure of the similarity of groups.

We did DFA for samples from provenances 1 and 30 using: 1) all 10 transformed proportions, 2) the first 6 principal components (which account for 90 % of the total variance), 3) the first three principal components (which account for 78 % of the total variance). Loadings for these three discriminant functions are given in Table 4. As in PCA, loadings that deviate from zero imply importance the input variable for discrimination between groups. Notice in the function based on 6 principal components that PC 3 is more heavily loaded than PC 2 or PC 1, despite the fact that PC 3 accounts for less of total variation than do PC 1 or PC 2. This may indicate that much of the variance accounted for by PC 1 and PC 2 occurs within each population.

Discriminant function scores for the analysis of provenances 1 and 30 obtained from the function based on 10 transformed monoterpene proportions are plotted in Figure 1. Similar distributions were obtained for analyses based on 6 and 3 principal components. In all three analyses there are significant differences between the two provenances for discriminant function scores, and in no case did the within population distribution of discriminant function scores deviate significantly from normality.

The probability of correctly classifying samples can be estimated with several methods (Giri 1977), the simplest of which is the resubstitution and subsequent reclassification of the samples used to generate the discriminant function. Upon resubstitution, 80 % of the samples from provenances 1 and 30 were correctly classified when either 10 transformed proportions or 6 principal components were used to generate the discriminant function; 77 % of the samples were correctly classified with the function generated with 3 principal components. Reducing the number of input variables for DFA to 6 principal components thus has essentially no effect on the power to discriminate between provenances 1 and 30, while reduction to just 3 principal components causes a substantial loss of discrimination power.

Table 4. Loadings for discriminant functions derived with 10 transformed proportions, 6 principal components and 3 principal components.

Variable	Loading
$DF_{(10)}$	
α-pinene	–0.131
β-pinene	–0.272
sabinene	–0.136
Δ-3-carene	–0.406
myrcene	–0.159
α-terpinene	0.797
limonene	–0.304
β-phellandrene	0.237
tepinolene	–0.245
remainder	0.193
$DF_{(6)}$	
PC 1	–0.217
PC 2	0.084
PC 3	–0.670
PC 4	–0.139
PC 5	–.0159
PC 6	–0.385
$DF_{(3)}$	
PC 1	–0.656
PC 2	–0.545
PC 3	0.092

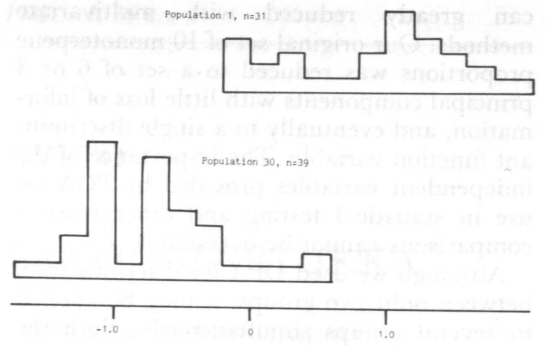

Figure 1. The distributions of discriminant function scores, for the function generated with 10 transformed monoterpene proportions.

While resubstitution of samples provides a relative parameter of between-group similarity, this method is somewhat circular, as the same observations are used both to generate the discriminant function and to evaluate its performance. Such circularity may result in an over-estimation ofthe power of DFA for discriminating between untested samples from the same groups. Another method for estimating the probability of correct classification derives from the statistical technique known as jack-knifing (Miller 1974). In a very simple version of this method we divided the samples from provenances 1 and 30 into two parts each, used the first part to generate a discriminant function, and classified the remaining samples using this function. While 83 % of the samples used to generate the function were correctly classified upon resubstitution only 73 % of the remaining samples were correctly classified. In this case, the proportion of samples correctly classified upon resubstitution provides an overly optimistic estimate of the true power of correct classification, although better concordance between the two methods would be expected for discriminant functions generated with larger numbers of samples. More complicated jackknifing procedures can be used to obtain confidence intervals for this classification parameter.

Discussion

Our preliminary results indicate that multivariate methods can be quite useful in treating data on the relative proportions of monoterpenes in coniferous trees. Such methods overcome both kinds of correlation that can complicate monoterpene data sets. Furthermore when test statistics designed for analysis of normally distributed characters can be used, the need for knowledge about the genetic mechanisms that determine monoterpene composition is diminished. The deviations from normality observed for principal component scores are somewhat disconcerting and caution should be used when interpreting such test results, especially when significance levels are borderline. However, tests for differences of means, such as F ratio or Student's t test, are quite robust to deviations from normality (Scheffé 1955).

The complexity of monoterpene data sets

can greatly reduced with multivariate methods. Our original set of 10 monoterpene proportions was reduced to a set of 6 or 3 principal components with little loss of information, and eventually to a single discriminant function variable. The importance of the independent variables provided by PCA for use in statistical testing and other relative comparisons cannot be overstated.

Although we used DFA for discrimination between onlu two groups, it may be applied to several groups simultaneously. Both the discriminant function score and the proportion of samples correctly reclassified to original groups may be useful parameters for assessing patterns of variation in monoterpene composition.

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YHTEENVETO

METSÄPUIDEN POPULAATIOGENETIIKKA

Helsingissä 1981 pidetyn sympoosion tutkimusraportit

OSA I.

Metsäpuiden risteytymisjärjestelmät

Kaikki 4 raporttia käsittelevät pääasiallisesti pölytyksen koostumusta ja erityisesti itsepölytyksen osuutta. Kokeellinen aineisto on pääasiallisesti männystä. Ensimmäisessä raportissa (*Koski*) kiinnitetään aluksi huomiota siihen, että havupuilla itsepölytys, itsehedelmöitys ja itsehedelmöityksestä syntyneiden jälkeläisten osuus eivät merkitse samaa. Varsinkin lukuarvoja käytettäessä on täsmällisesti ilmaistava mitä asiaa tarkoitetaan. Lukuarvoja käytettäessä niitä helposti pidetään tarkkoina ja varmoina, vaikka tähän on harvoin edellytyksiä. Pienistä näytteistä johtuen todettuja arvojen tilastolliset luotettavuusrajat ovat yleensä väljät. Biologisista syistä johtuen vuotuiset erot pölytyksen kokoonpanossa ja itsepölytyksen osuudessa voivat olla suuria. Vapaapölytyksestä syntyvät osittaisen itsepölytyksen todellista suuruutta ja siitä aiheutuvaa sisäsiitosdepressiota ei voida täsmällisesti selvittää ilman valvottuja itsepölytyksiä. Havupuiden monialkiojärjestelmä ja geneettinen taakka muuttavat lukusuhteita merkitsevästi. Tätä ei yleensä oteta huomioon erilaisia geneettisiä merkkiominaisuuksia kuten isoentsyymejä käytettäessä. Kirjoituksen lopussa on laskelmia itsehedelmöitysalkioiden osuudesta siemensadossa eri asteisen itsepölytyksen jälkeen. Laskelmat osoittavat, että yleensä 20 % itsepölytysosuus johtaa vielä hyvin alhaiseen sisäsiitososuuteen, mutta itsepölytyksen osuuden noustessa yli 50 %, sisäsiitoksen osuus nousee jyrkästi.

Toinen raportti (*Rudin* ja *Ekberg*) käsittelee eräässä männyn siemenviljelyksessä vapaapölytyksestä syntyneen siemensadon geneettistä rakennetta. Aineisto on peräisin Ruotsin Långtorassa sijaitsevasta siemenviljelyksestä N:o 48. Vartteet olivat tutkimuksen aikana 10–12 vuotiaita ja 3,5–5,5 m pitkiä. Isoentsyymitekniikan avulla tutkittiin pölytyssuhteita 5 kloonin siemensadossa. Siemenviljelyssiemenen virallista hyväksymistä varten ehdotetaan kloonikohtaisen selvityksen pohjalta määritettäväksi: 1. tuhatjyväpaino, 2. itämistarmo, 3. heterotsygotia-aste, 4. isäpopulaation koostumus, 5. taustapölytyksen osuus, 6. itsesiitoksen osuus ja 7. eri kloonien suhteelliset osuudet siemensadossa. Tulokset osoittavat itsesiitoksen osuudessa eroja sekä kloonien että latvuksen eri osien välillä. Keskimääräiseksi itsesiitososuudeksi todettiin 16 %. Kloonien välistä risteytymistä voitiin tutkia vain yhden, harvinaisen alleelin omaavan, kloonin avulla. Tämän osuudeksi lähinaapurien pölytyksessä todettiin 0,8–3,0 %. Lopuksi pohditaan tekijöitä, jotka voivat lisätä itsesiitoksen osuutta. Tällaisina luetellaan:

1. alhainen geneettinen taakka,
2. hede- ja emikukintojen lähekkäinen sijainti,
3. hede- ja emikukinnan samanaikaisuus kyseisessä kloonissa,
4. vieraspölytyksen pieni määrä ja
5. alhainen tuulen nopeus kukinnan aikana.

Kaksi muuta raporttia (*Ziehe* ja *Müller-Stark*) käsittelevät yksilöiden välisen emi- ja hedekukinnan runsauden vaihtelun sekä osittaisen itsehedelmöityksen vaikutusta. Ziehen työssä asiaa on tarkasteltu yleisesti laskemalla algebrallisesti teoreettiset poikkeamat Hardy-Weinberg tasapainosta. Puiden väliset erot hede- ja emikukinnan määrissä pyrkivät

lisäämään heterotsygoottien osuutta, kun taas osittainen itsesiitos pyrkii lisäämään homotsygoottien osuutta. Mainittujen kahden tekijän yhteisvaikutuksen suhteen viitataan eräisiin aikaisemmin julkaistuihin raportteihin, joissa laskelmat on todettu monimutkaisiksi. Müller-Stark on isoentsyymitekniikan avulla selvittänyt erään männyn siemenviljelyksen siemensadon geneettistä rakennetta ja verrannut sitten tuloksia Ziehen kehittämiin lausekkeisiin. Kohteena oleva siemenviljelys koostuu 36:sta kloonista, joista kustakin on 25 vartetta. Kukkimisvuonna (1975–1976) siemenviljelys oli 16 ja 17 vuotias. Tuloksista voitiin todeta, että eri vuosien suhteelliset osuudet itävien siementen emoina ja isinä erosivat merkitsevästi panmiktisen populaation runsaussuhteista. Kahden tutkitun vuoden siemensatojen välillä todettiin myös merkitseviä eroja eri kloonien osuuksissa.

OSA II.

Metsäpuiden populaatiorakenne

Vasta 1980-luvulla on julkaistu tutkimuksia, jotka pyrkivät selvittämään metsiköiden sisäistä geneettistä rakennetta. Voidaankin syyllä kysyä, minkä takia ei aikaisemmin ole suoritettu mainitunlaatuista tutkimusta, sillä tällaiset työt antavat metsägenetiikkaa soveltavalle metsänjalostukselle mahdollisuuksia tarkempaan jalostuksen hyödyntämiseen.

Metsänjalostajat ovat kyllä olleet melko hyvin perillä erilaisten ominaisuuksien periytyvyydestä, mutta esimerkiksi kysymykset luonnonmetsien sukurasituksesta ja metsikön sisällä olevien yksilöiden sukulaisuudesta ovat olleet spekuloinnin varassa. Vähitellen on nyt syntymässä kuva luonnonmetsän populaatiorakenteesta. Voidaan jopa väittää, että metsikön sisäiset populaatiogeneettiset tutkimukset ovat johtamassa parempaan yleiskäsitykseen kasvipopulaatioiden geneettisestä rakenteesta. Suurin osa aikaisemmista tutkimuksista on tehty heinäkasveilla, lähinnä kauralla, ohralla ja maissilla. Metsäpuut ovat edellisiä kiitollisempia tutkimuskohteita siksi, että samoja puita, tai jopa kahta eri sukupolvea voidaan tutkia samanaikaisesti ja toistuvasti samasta metsiköstä. Lisäksi havupuiden siemenen haploidi siemenvalkuainen tai naarasgametofyytti antaa mahdollisuuden hyvin tarkkaan geneettiseen analyysiin eri entsyymien geneettisestä periytymisestä.

Metsäpuut, varsinkin havupuut, ovat ristipölyttäjiä. Itse- tai sukulaispölytysten jälkeen syntyy yleensä jälkeläistö, joka kärsii sisäsiitosdepressiosta, kasvu on hidasta ja kilpailukyky heikkoa. Lisäksi metsäpuut ovat useimmiten tuulipölyttäjiä ja siitepöly voi lentää pitkiäkin matkoja tuulen mukana. Nämä kukkimiseen liittyvät seikat muodostavat taustan koko populaatiotutkimukselle.

Ennenkaikkea tulisi metsäpuilla selvittää miten populaatiot poikkeavat Hardyn ja Weinbergin populaatiotasapainosta ja miten voimakkaasti populaatiossa syntyy epätasapainoja, jotka johtuvat esimerkiksi geneettisestä ajautumisesta, populaation osittumisesta, geenivirrasta tai valinnasta. Näiden seikkojen teoreettiseen analyysiin paneudutaan *Muonan* työssä. Luontaisen metsän uudistuminen tapahtuu tavallisesti siten, että vanhojen siemenpuiden alle syntyy hyvänä siemenvuonna ja edullisten ilmasto-olosuhteiden vallitessa hyvin tiheä taimisto. Vuosien mittaan taimisto harvenee, rehevin ja nopeakasvuisin yksilö valtaa ankarassa kilpailussa kasvualustan ja voi syrjäyttää kymmeniä kituvia, kuolemaan tuomittuja yksilöitä. Luonnonvalinta muokkaa näin metsäpuiden populaatiorakennetta. Tarkat populaatioanalyysit jotka toisaalta on suoritettu Douglaskuusella (*Shaw* y.m.) toisaalta männyllä (*Tigerstedt* y.m.) viittaavat siihen, että taimikilpailu on todella ratkaiseva tekijä metsäpuiden populaatiorakenteen muodostuksessa. Kilpailussa häviää nähtävästi suurin osa sukusiitoksen kautta syntyneistä yksilöistä. Viimeksimainitussa mäntyä koskevassa työssä on myös kartoitettu vanhan männikön ja sen alla olevan taimiston maantieteellistä rakennetta. Analyysein on mm. voitu todeta, että siemenpuiden alla kasvava taimisto ei näytä koostuvan yläpuolella olevien puiden sisar- tai puolisisarperheistä kuten voisi olettaa. Nähtävästi siemenet kulkeutuvat tuulen mukana jonkun matkaa emopuista. Luonto huolehtii tällä tavalla metsikön sisällä mahdollisimman tehokkaasti ristipölytyksestä jotta sukurasituksen määrä pysyisi pienenä. Toisaalta tutkimukset osoittavat, että taimistossa geenit jakautuvat epätasaisesti tai ryhmittäin mikä puolestaan voisi johtua siementen parvimaisesta lennosta ja laskeutumisesta. Populaatiotutkimukset

johtavat todella arvokkaisiin tietoihin metsänviljelyn kannalta. Erikoisesti kun metsää viljellään istuttamalla poistetaan metsikön kehityksestä jokseenkin kokonaan luontainen valintapaine. Metsänjalostajan olisi erityisen tarkasti huolehdittava taimiaineiston geneettisestä laadusta, ennenkaikkea on taimiaineksesta pyrittävä poistamaan heikkoja taimia jotka todennäköisesti ovat perimältään poikkeuksellisen homotsygoottisia. Kilpailun kannalta kylvöä on pidettävä istutusta parempana toimenpiteenä.

Vellingin ja *Pöykön* julkaisuissa on tarkasteltu 10–20 vuotta vanhojen männyn jälkeläiskokeiden antama tieto emo- eli pluspuiden jalostusarvoista. On myös laskettu kasvu- ja laatuominaisuuksien periytyvyysarvoja sekä ominaisuuksien välisiä korrelaatioita. Tässä todetaan mm, että pituus- ja paksuuskasvun periytyvyys on yleensä alle 30 %. Laatuominaisuuksien, kuten oksien määrän sekä oksakulman, periytyvyysarvot ovat korkeampia, noin 40–50 %. Tutkimukset osoittavat, että mäntypopulaatioissa esiintyy runsaasti kvantitatiivista geneettistä vaihtelua sekä kasvun että laadun suhteen. Osa tästä vaihtelusta on additiivisten geenien aiheuttamaa ja sitä voidaan näinollen hyödyntää siemenviljelysten avulla. Velling tarkastelee erästä 20-vuotiasta männyn jälkeläiskoetta ja hänen tuloksistaan on mm. laskettavissa, että kuutiokasvun jalostushyöty on 12.9 %. Kaikissa laatuominaisuuksissa on todettavissa jalostushyötyä noin 2–10 %. Tulokset antavat aihetta suureen optimismiin arvioitaessa metsänjalostuksen mahdollisuuksia lisätä puun arvokasvua. Luontaiset metsäpopulaatiot ja niissä oleva geneettinen vaihtelu on paras mahdollinen lähtökohta metsänjalostukselle. Huolehtiminen riittävän geneettisen vaihtelun säilyttämisestä populaatioissa kuuluu metsägenetiikan tärkeimpiin tehtäviin.

OSA III.

Metsäpuiden sopeutuminen ympäristöolosuhteisiin

Eliön sopeutuneisuus ympäristöönsä koostuu kaikista niistä geneettisesti säädellyistä ominaisuuksista ja elintoiminnoista, jotka tekevät mahdolliseksi sen olemassaolon tietyissä ekologisissa olosuhteissa. Yksilön tasolla sopeutuneisuuden voidaan katsoa kuvastavan geneettistä erikoistumista tarkoin rajattaviin ympäristöoloihin. Populaation tasolla optimaalinen sopeutuneisuus merkitsee myös geneettistä valmiutta menestyä vaihtelevissa olosuhteissa. Ajallisesti tai paikallisesti sattumanvaraisesti vaihtelevassa kasvuympäristössä elävälle populaatiolle edullisin sopeutumisstrategia on ilmeisesti erilaisten, eri olosuhteisiin erikoistuneiden yksilöiden tuottaminen, eikä niinkään pyrkimys johonkin yksittäiseen, kaikissa mahdollisissa olosuhteissa toimeentulevaan joustavaan yksilötyyppiin. Lisäksi geneettisesti vaihtelevan populaation kokoonpano voi luonnonvalinnan seurauksena muuttua sukupolvesta toiseen: keskimääräinen sopeutuneisuus vallitseviin olosuhteisiin voi parantua ja olosuhteiden muuttuessa se voi kehittyä tarvittavaan suuntaan.

Symposion tässä osassa pidetyt esitelmät käsittelivät laajalti metsäpuiden ekologista genetiikkaa, niiden luontaisten populaatioiden sopeutumisstrategioiden ja geneettisten rakenteiden teoreettisesta tarkastelusta sopeutuneisuuteen vaikuttavien erillisten fysiologisten ilmiöiden vaihteluun ja hyväksikäyttöön metsänjalostustyössä. Ensimmäisissä kolmessa kirjoituksessa tarkastellaan puupopulaatioiden geneettistä rakennetta kokonaisuutena sekä jalostuksen ja metsänviljelyn sitä mahdollisesti muuttavia vaikutuksia.

Lundkvist tarkastelee metsäpuille edullisimpia populaatiorakenteita ekologisen genetiikan yleisten hypoteesien kannalta. Koska puiden kasvuympäristö vaihtelee suuresti jo lyhyillä etäisyyksillä ja vuodesta toiseen, optimaalinen sopeutuneisuus niiden ristisiittoisissa luonnonpopulaatioissa edellyttää tiettyä tasapainoa ekologisen erikoistumisen ja joustavuuden välillä. Joustavuus voi perustua osaksi populaation geneettiseen monimuotoisuuteen (geneettiseen homeostasiaan) ja osaksi puuyksilöiden tai genotyyppien fysiologiseen mukautuvuuteen (yksilölliseen homeostasiaan), ts. kykyyn toimia ja kehittyä samantapaisesti erilaisissa ympäristöissä. Viljelymetsät voivat muodostua geneettiseltä rakenteeltaan suuresti luonnonmetsistä poikkeaviksi. Tähän ei välttämättä sisälly epävarmuutta tai haittaa sopeutuneisuuden kannalta, sillä viljellyiltä metsiköiltä ei tarvitse edellyttää valmiutta evoluutioon. Puun tuotannon kannalta edullisin geneettinen rakenne

onkin ilmeisesti aivan erilainen kuin luonnonmetsissä vallitseva, populaatioiden säilymisen kannalta optimaalinen rakenne. Metsänjalostuksen kannalta olisi tärkeätä selvittää, miten paljon geneettistä vaihtelua voidaan kaventaa ekologisen joustavuuden kustannuksella viljelyvarmuutta vaarantamatta ja millainen geneettinen vaihtelevuus olisi puuntuotannon kannalta optimaalinen. Tärkeäksi tutkimustehtäväksi todetaan myös varhaistestimenetelmien kehittäminen, joilla eri genotyyppien sopeutuneisuus ja tuotoskyky voitaisiin arvioda lyhytaikaisen kasvatuksen perusteella.

Eriksson esittelee Ruotsissa tehtyjä metsäpuiden ekologis-geneettisiä tutkimuksia. Niissä kaikissa on havaittu vähittäistä klinaalista erilaistumista eri alueiden populaatioiden välillä sekä laajaa geneettistä yksilövaihtelua populaatioiden sisällä. Nämä piirteet vastaavat populaatiorakennetta, jonka teoreettisesti voidaan odottaa kehittyvän vuodesta toiseen ilmastollisesti vaihtelevissa kasvuolosuhteissa. Kasvualustan heterogeenisuuden merkitys puupopulaatioiden geneettisen rakenteen ja optimaalisen sopeutuneisuuden kannalta on tärkeä tutkimuskohde, jota toistaiseksi on vasta hyvin vähän selvitetty.

Lindgren tarkastelee painon perusteella tapahtuvan siemenen lajittelun vaikutuksia syntyvän taimiaineksen geneettiseen kokoonpanoon. Lajittelun todetaan vähentävän taimiaineiston geneettistä monimuotoisuutta, koska raskaat ja kevyet siemenet ovat suurelta osin peräisin eri emopuuyksilöistä. Geneettisen vaihtelun kaventuminen voi rajoittaa syntyvän viljelymateriaalin sopeutuneisuutta vaihteleviin kasvuolosuhteisiin, ja siten siemenen lajittelua esim. taimitarhakylvöjen yhteydessä ei voida pitää suotavana.

Seuraavat neljä esitystä käsittelevät metsäpuiden vuotuisen kehityksen fysiologisia osailmiöitä, jotka ovat tärkeitä ilmastoonsopeutuneisuuden kannalta.

Skrøppan kirjoitus käsittelee kuusen latvakasvainten pituuskehityksen ajoittumisen geneettistä vaihtelua ja sen merkitystä vuotuisen kokonaiskasvun kannalta. Geneettisiä eroja todetaan esiintyvän niin maantieteellisten alkuperien välillä kuin yksittäisistä metsiköistä alkunsa saaneiden jälkeläistöjenkin välillä. Itä- ja keskieurooppalaisten kuusiprovenienssien suuremman pituuskasvun norjalaisiin alkuperiin nähden todetaan johtuvan yleensä niiden pitemmästä vuotuisesta kasvuperiodista, mutta metsiköiden sisällä esiintyvät kasvuerot johtuvat usein enemmänkin vaihtelusta päivittäisessä kehitysnopeudessa kasvujakson aikana.

Dormling esittelee kasvukammiokokeissa tehtyjä havaintoja kuusen hallankestävyydestä kasvainsilmujen aukeamis- ja pitenemisvaiheessa. Etelästä siirretyt kuusialkuperät aloittavat kasvunsa yleensä paikallisia alkuperiä myöhemmin ja säästyvät siksi usein paremmin keväthalloilta. Samassa kehitysvaihessa olevissa taimissa paleltumisalttius on kuitenkin sitä suurempi mitä heikommin ne ovat talveentuneet edellisen kasvukauden lopulla. Eteläiset alkuperät ovat siten Skandinaviassa itseasiassa herkempiä hallatuhoille kuin paikallinen kuusi, sillä pitemmästä vuotuisesta kehitysjaksosta johtuen niiden talveentuminen jää aina heikommaksi.

Mikolan esityksessä todetaan männyntaimien latvasilmun muodostuksen ajallisen vaihtelun niiden ensimmäisenä elinvuotena määräytyvän tarkoin siemenen alkuperän maantieteellisen sijainnin ja sillä vallitsevien lämpöolojen mukaan. Tulosten perusteella päätellään, että silmunmuodostuksen ajoittumista voitaisiin yleisesti käyttää ilmastollisen sopeutuneisuuden indikaattorina sekä varhaistestiperusteena alkuperältään ja kestävyydeltään puutteellisesti tunnetulle materiaalille sopivien viljelyalueiden määrittämisessä.

Ryynänen käsittelee siemenen tuleentumisen vaihtelua Pohjois-Suomen mäntymetsissä. Yksilöllisiä eroja todetaan esiintyvän saman metsikön puiden välillä, erityisesti huonoina siemenvuosina, jolloin tuleentuminen jää yleisesti vajavaiseksi.

OSA IV.

Metsäpuiden ekologinen erilaistuminen

Langlet kirjoitti vuonna 1971 perinpohjaisen katsauksen nimeltään "200 vuotta geeniekologiaa". Siinä hän toteaa, että varsinainen provenienssitutkimus alkoi jo 1700-luvun loppupuolella. Maailman metsäammattikunta on ollut hyvin perillä puulajien rotueroista, joskin niiden geneettinen perusta on ollut

aivan meidän päiviimme saakka hämärän peitossa. Provenienssitutkimus on jokseenkin sama asia kuin geeniekologia ja molemmat käsitteet sopivat ekologisen genetiikan piiriin. Voidaan ylpeydellä todeta, että metsämiehet ovat olleet maailman johtavia alan tuntijoita ja soveltajia jo pitkään ennenkuin perinnöllisyystiede ryhtyi selvittämään näitä asioita. Tyypillistä tälle tutkimukselle oli se, että vuosisatamme parhaat geneetikot eivät yleensä tunteneet metsämiesten provenienssitutkimuksia.

Tässä esitetyt neljä ensimmäistä kirjoitusta paneutuvat metsäpuiden ekologiseen erilaistumiseen uusin menetelmin, joissa tarkastellaan entsyymien aktiviteettia ja vaihtelua. Entsyymien avulla päästään mahdollisimman lähelle geenien toimintaa ja entsyymierot, isoentsyymit ja allotsyymit, ovat useimmiten todellisia perimässä olevia DNA:n aakkoseroja.

Voidaan todeta, että *Vidgren* ym. tutkimuksessaan tavallaan seuraavat *Langletin* jälkiä. He käyttävät tutkimuksissaan katalaasi-entsyymin aktiviteettia männyllä kuten Langlet, mutta aineisto on laajempi ja mittausmenetelmät parempia kuin vuonna 1936. Katalaasi-entsyymin aktiviteetti osoittaa selvää ekologista vaihtelua, joka on korrelaatiossa leveysasteen kanssa. Kuitenkin aivan etelässä 45.–49. leveysasteen välillä korrelaatio loppuu.

Szmidt käyttää tutkimusaineistonaan sembramäntyä. Hän analysoi kahdeksaa eri entsyymilokusta elektroforeesin ja isoelektrisen fokusoinnin avulla. Analyysit tehdään siemenen haploidisesta endospermistä, minkä ansiosta on helppo päätellä, mitkä isoentsyymit kuuluvat samaan allotsyymisysteemiin. Tuloksia analysoidaan Nei:n geneettistä etäisyysmittaa (D) käyttäen. Analyysit osoittavat selvästi, että sembramännyn siperialaiset populaatiot eroavat eniten geneettisesti eurooppalaisista populaatioista, mutta viimeksimainitutkin eroavat merkittävästi toisistaan. Geneettinen etäisyys näytää mm. johtuvan populaatioiden eristäytymisestä ei niinkään suoranaisesta maantieteellisestä etäisyydestä.

Samantapaisiin tuloksiin päädytään myös *Krzakowan* mäntyä koskevassa tutkimuksessa, jossa analysoidaan Puolassa kasvavia männyn populaatioita. Metsäntutkijat, erityisesti metsägeneetikot, ovat jo kauan eri tavoin pyrkineet selvittämään eroavatko hyvin lähellä toisiaan, mutta eri biotoopeissa kasvavat saman puulajin metsiköt geneettisesti toisistaan. Pohjois-Amerikassa on tutkittu mm. Ponderosa-mäntyä ja tuijaa, meillä taas lähinnä mäntyä. Aikaisemmissa kokeissa on voitu todeta, että ilman tarkkoja geneettisiä merkkiaineita, kuten entsyymit, on jokseenkin mahdotonta erottaa toisistaan geneettiset ja fysiologiset erot, jotka voivat johtua ns. fysiologisesta sopeutumisesta. Tutkijat *Gullberg* ym. ovat hakemassa asiaan selvityksiä analysoimalla ruotsalaisia männyn populaatioita, jotka kasvavat lähellä toisiaan suolla ja kankaalla tai vuoren laella ja sen rinteillä. He toteavat, että eri biotoopeissa, mutta lähellä toisiaan kasvavat mäntymetsiköt eroavat jonkinverran toisistaan kukkimisaikojen suhteen. He päättelevät, että erot kukkimisessa ovat siksi selviä, että ilmiö voisi aiheuttaa myös metsiköiden välistä geneettistä erilaistumista, vaikka geenivirta niiden välillä olisi huomattavaa. He analysoivat myös ko. populaatioita siementen endospermeissä olevien entsyymien suhteen ja toteavat, että metsiköiden välillä esiintyy geenitaajuuseroja. He toteavat tulostensa perusteella, että luontaisissa metsissä ilmenee eri biotooppien välillä jatkuvaa erottavaa valintapainetta. Tämä valintapaine on heidän mukaansa johtanut männyllä biotooppien mukaiseen ekologiseen erilaistumiseen. Tulos on erittäin huomionarvoinen ja ansaitsee lähempää tarkastelua ja vielä laajempaa tutkimusta lähitulevaisuudessa. Vastaavia tuloksia on saatu 1960- ja 1970-luvulla eri heinäpopulaatioissa suoritetuilla morfologisilla ja fenologisilla vertailuilla.

Tämän osaston viimeisessä työssä *Krutzsch* tarkastelee metsänviljelyn vaikutuksia metsien geenivarantoon Ruotsissa. Hän toteaa, että on pikaisesti ryhdyttävä toimenpiteisiin riittävän laajojen luontaisten metsiköiden säilyttämiseksi, jotta niissä oleva geneettinen sopeutuminen säilyisi tulevaisuudessa. Hän korostaa myös, että viljelymetsät olisi aina rekisteröitävä alkuperältään, jotta tulevaisuudessa olisi mahdollista käyttää niitä metsänjalostuksessa.

Yhteenvetona on todettava, että uudet tutkimukset metsien sisäisestä geneettisestä vaihtelusta antavat aihetta yhä suurempaan tarkkuuteen tulevien viljelymetsien siementä valittaessa.

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KIRJOITUSTEN LAATIMISOHJEET

Silva Fennica-sarjassa julkaistaan lyhyitä metsätieteellisiä tutkimuksia ja kirjoituksia kotimaisilla kielillä tai jollakin suurella tieteellisellä kielellä. Julkaistavaksi tarkoitettu käsikirjoitus toimitetaan kahtena kappaleena seuran sihteerille painatuskelpoisessa asussa. Seuran hallitus ratkaisee asiantuntijoita kuultuaan, hyväksytäänkö kirjoitus painettavaksi.

Kirjoitusten laadinnassa noudatetaan Silva Fennica 4 (3):ssa (1970) annettuja sekä toimittajan erikseen antamia ohjeita. Suureissa, yksiköissä, symboleissa ja kaavoissa sekä oikoluvussa noudatetaan suomalaisia standardeja SFS 2300, 3100, 3101 ja 2324.

Kirjoitusten alkuun tulee julkaisun kielellä lyhyt tiivistelmä tutkimuksen tuloksista (ladottuna korkeintaan 20 riviä). Samoin laaditaan lyhyt mutta riittävä englanninkielinen summary ja myös englanninkielinen kirjastokortti, joka pituudeltaan on korkeintaan 18 konekirjoitusriviä. Sisällysluetteloa ei käytetä. Mahdolliset kiitokset esitetään johdannon lopussa ja ne ladotaan normaalia pienemmällä kirjasimella.

Kuvat on laadittava mieluiten yhdelle palstalle sopiviksi (lev. n. 6,5 cm). Kuvien sisällä olevat tekstit on kirjoitettava siirtokirjaimin, tekstityslaitteella tai muuten siististi. Useita osakuvia sisältävät kuvat tai monen kuvan sarjat on suunniteltava siten, ettei taitto vaikeudu. Kuvaoriginaalien tulee olla korkeintaan kokoa A4. Mikäli isompia kuvia joudutaan käyttämään, on asiasta sovittava toimittajan kanssa. Valokuvien on oltava teknisesti moitteettomia, kiiltävälle paperille vedostettuja. Värikuvia ei yleensä hyväksytä. Kuvien otsikkotekstejä ei missään tapauksessa saa kirjoittaa kuvaoriginaaleihin, vaan ne kirjoitetaan erilliselle liuskalle. Taulukkotekstit kirjoitetaan kuitenkin ao. taulukon yläosaan, eikä niistä erillistä luetteloa tarvita.

Taulukot laaditaan mahdollisimman paljon lopullista painatusasuaan muistuttaviksi. Taulukoiden viivoituksen on oltava yhdenmukainen ja harkittu, yleensä pari johtoviivaa riittää. Vain pienet, yhdelle palstalle sopivat asetelmat ovat sallittuja, suuremmista tulee tehdä taulukko. Taulukot ja kuvat numeroidaan juoksevasti ja sijoitetaan tekstiosasta erilleen kukin omalle liuskalleen. Kuvien ja taulukoiden toivotut paikat merkitään käsikirjoituksen marginaaleihin. Jos vieraskielisessä summaryssä viitataan kuviin ja taulukoihin, tulee viitatuissa kuvissa ja taulukoissa olla vieraskieliset otsikot ja selitykset. Muut kuvat ja taulukot saavat olla yksikieliset.

Matemaattiset kaavat, ylä- ja alaindeksit sekä erikoismerkit on kirjoitettava selkeästi, niin että jokainen merkki on yksiselitteinen. Matemaattiset kaavat on muokattava sellaisiksi, että ne mahtuvat palstan leveydelle (n. 6,5 cm). Leveämmät kaavat on katkaistava soveltuvasta kohdasta ja jatkettava seuraavalle riville.

Tekstin lähdeviittaukset kirjoitetaan aikaisemmasta poiketen pienin kirjaimin. Milloin tekijöitä on kolme tai useampia, mainitaan tekstissä vain ensimmäinen (esim. Heikurainen ym. 1961). Jos julkaisulla on kaksi tekijää, pannaan nimien väliin ja-sana painatuskielellä. Sulkeiden sisässä olevat viittaukset erotetaan toisistaan pilkulla (esim. Aho 1976, Elo ja Virtanen 1979, Suk ym. 1980).

Kirjallisuusluettelossa julkaisujen tekijät kirjoitetaan isoin kirjaimin, milloin tekijänä on henkilö. Jos tekijöitä on useita, nimet erotetaan pilkulla, paitsi kaksi viimeistä, jotka erotetaan &-merkillä. Tekijäin etunimistä käytetään vain alkukirjaimia. Mikäli sama ensimmäinen tekijä on kirjoittanut useampia julkaisuja, nimeä ei toisteta vaan se korvataan yhtäläisyysmerkillä. Toisen tekijän suhteen ei näin kuitenkaan tehdä. Tutkimusten nimet kirjoitetaan lyhentämättä. Tavallisista julkaisusarjoista käytetään lyhenteitä, jotka on painettu Silva Fennica 5(2):ssa (1971). Harvinaisia tai poikkeuksellisia sarjoja ei lyhennetä. Julkaisun numeron yhteydessä ei mainita vol.- tai n:o -sanoja. Sivunumerot erotetaan kaksoispisteellä volyymistä tai julkaisun numerosta. Esimerkkejä:

GUSTAVSEN, H. G. 1976. Miten puut reagoivat lannoitukseen varttuneissa metsiköissä? Metsä ja Puu 4: 15–18.

— & LIPAS, E. 1975. Lannoituksella saatavan kasvunlisäyksen riippuvuus annetusta typpimäärästä. Summary: Effect of nitrogen dosage on fertilizer response. Folia For. 246: 1–20.

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Sääsähkeohjeet 1982. Ilmatieteen laitos. Helsinki.

Englanninkielisten tekstien kääntämisestä ja pätevän kieliasiantuntijan tekemästä tarkastamisesta huolehtii kirjoittaja. Seura voi maksaa tarkastamiskustannukset valtionvarainministeriön antamien ohjeiden mukaisesti.

Lähempiä tietoja antaa seuran julkaisujen toimittaja.

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