METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 875, 2002
FINNISH FOREST RESEARCH INSTITUTE, RESEARCH PAPERS 875, 2002

0?.12.02

Managing breeding stock in the initiation of a long-term tree breeding program

Seppo Ruotsalainen

PUNKAHARJUN TUTKIMUSASEMA – PUNKAHARJU RESEARCH STATION

METLA

0.12.02

Managing breeding stock in the initiation of a long-term tree breeding program

Jalostusaineiston hallinta pitkäntähtäyksen metsänjalostusohjelman käynnistämisessä

Seppo Ruotsalainen

Akateeminen väitöskirja

Esitetään Helsingin yliopiston maatalous-metsätieteellisen tiedekunnan luvalla julkisesti tarkastettavaksi Viikissä luentosalissa B2, perjantaina 13. päivänä joulukuuta 2002 kello 12.

Academic dissertation

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Viikki, Auditorium B2, on 13th of December, at 12 o'clock.

Ruotsalainen, Seppo. 2002. Managing breeding stock in the initiation of a long-term tree breeding program.
Metsäntutkimuslaitoksen tiedonantoja 875. Finnish Forest Research Institute, Research Papers 875., 95 + 61 p.
ISBN 951-40-1860-5. ISSN 0358-4283.

Publisher: The Finnish Forest Research Institute
Punkaharju Research Station

Accepted by: **Kari Mielikäinen**, Research Director 20.11.2002

Supervisor: Professor **Dag Lindgren**
Swedish University of Agricultural Sciences,
Umeå, Sweden

Reviewers: Dr. **Gary R. Hodge**
North Carolina State University
Raleigh, USA

Dr. **Tore Skrøppa**.
Norwegian Forest Research Institute,
Ås, Norway

Opponent: Professor **Tim Mullin**
North Carolina State University
Raleigh, USA

Author's address:
Seppo Ruotsalainen
Punkaharju Research Station, Finlandiantie 18,
FIN-58450 Punkaharju, Finland
tel. +358 (0)15 7302 286, fax +358 (0)15 644 333
email: seppo.ruotsalainen@metla.fi

Hakapaino Oy
Helsinki 2002

To Anni, Matti and Jouni

Abstract

The initiation of a tree breeding program was investigated on the basis of literature studies, deterministic simulations and empirical data analysis. The issues covered structuring the breeding stock, the importance of genetic diversity for long-term genetic improvement, and the selection and deployment of plus trees in the first cycles of a breeding program.

The selection of Scots pine plus trees has resulted in some concentration of the selected plus trees, but this is unlikely to seriously decrease the gene diversity of the breeding stock. Plus trees with a higher selection intensity had progeny with somewhat better height growth.

When making selection decisions for the next breeding cycle, either the parent trees (backward selection) or their best open pollinated offspring (forward selection) are often available. In the best ranking plus tree families, backward selection yields higher genetic gain than forward selection from open pollinated progenies when one generation is considered, whereas the opposite is true for the lower ranking families. However, forward selection is usually not to be recommended for practical tree breeding in such a situation, as it means either increased costs or decreased genetic gain per unit of time.

Between family genetic gain in the second cycle breeding stock can be increased by about 10 % without decreasing the gene diversity or increasing the costs, if the breeding population size is increased by about 20 % and the plus trees are allowed to contribute to the next cycle in the ratio 3:2:1, depending on their genetic value.

Division of breeding stock into reproductively isolated sublines is a method to manage the inbreeding in the forest regeneration material. If sublining is performed according to the genetic value of the plus trees, the obtainable genetic gain can be increased by 18 % if only half of the sublines are contributing to the multiplication population.

The current Finnish Scots pine breeding program contains a large amount of genetic diversity, which could be better utilised by proper structuring of the breeding stock. Rather small populations (less than 100 trees) are sufficient to retain enough genetic variation for long-term genetic improvement.

Keywords: breeding strategy, long-term tree breeding, population structure, genetic gain, genetic diversity, selection

Seloste

Jalostusaineiston hallinta pitkäntähtäyksen metsänjalostusohjelman käynnistämisessä

Metsänjalostuksessa pyritään parantamaan metsäpuiden taloudellisesti merkittäviä ominaisuuksia vaikuttamalla niitä sääteleviin perintötekijöihin. Tätä toimintaa voidaan kuvata jalostussyklillä, missä jalostustoiminnan keskeiset toimenpiteet, valinta ja uusien perintötekijäyhdistelmien luominen, vuorottelevat sukupolvesta toiseen.

Tässä tutkimuksessa tarkastellaan metsänjalostusohjelman aloittamiseen liittyviä keskeisiä kysymyksiä: pluspuiden valintaa ja hyödyntämistä ensimmäisillä jalostuskierroksilla, tarvittavaa jalostusaineiston organisointia sekä geneettisen monimuotoisuuden merkitystä pitkän aikavälin jalostuksessa. Näitä kysymyksiä tutkittiin kirjallisuuden avulla, tietokonesimuloinneilla ja empiirisen aineiston pohjalta.

Männyn pluspuuvalinta, jossa valintaperusteena on käytetty puiden kasvua ja laatua, on käytännössä johtanut jossain määrin ryhmittäiseen lopputulokseen, niin että valitut pluspuut jakautuvat epätasaisesti eri metsiköihin ja alueille. Ryhmittäisyyden syyt jäivät epäselviksi, mutta se ei johdu ainakaan metsiköiden välisistä perinnöllisistä eroista. Tämä ryhmittäisyys ei myöskään merkittävästi vaikuta jalostusaineiston geneettiseen monimuotoisuuteen.

Maantieteellisesti rajatun alueen pluspuujälkeläistöjen pituuskasvussa vaihtelu oli valtaosin metsiköiden sisällä yksittäisten puiden välillä, metsiköiden välisen vaihtelun ollessa vain n. 20 % periytyvästä geneettisestä vaihtelusta. Valinnanintensiteetiltään keskinkertaisten pluspuiden jälkeläisten pituuskasvu oli 1-2 % parempi kuin metsikön keskimääräisten puiden jälkeläisten pituuskasvu. Korkeampi valinnanintensiteetti johtaa suurempaan valintahyötyyn, mutta samalla nopeasti kasvaviin kustannuksiin. Tästä syystä pluspuuvalinnassa kannattaa tyytyä kohtuulliseen valinnanintensiteettiin ja investoida sen sijaan esim. jälkeläistestaukseen.

Siirryttäessä seuraavalle jalostuskierrokselle voidaan usein käyttää joko alkuperäisiä pluspuita (taaksepäin valinta) tai niiden vapaapölytteisiä jälkeläisiä (eteenpäin valinta). Parhaissa perheissä taaksepäin valinta antaa suuremman jalostushyödyn jalostuskierrosta kohti, kun taas muissa perheissä eteenpäin valinta on parempi vaihtoehto. Käytännön jalostustilanteessa eteenpäin valinta johtaa kuitenkin tällaisessa tilanteessa yleensä joko lisääntyneisiin kustannuksiin tai aikayksikköä kohti laskettuna taaksepäin valintaa alhaisempaan jalostushyötyyn, joten se ei välttämättä ole suositeltava ratkaisu. Taaksepäin ja eteenpäin valinnassa yhtä suuren jalostushyödyn antavan rajakohdan sijainti riippuu mm. periytyvän vaihte-

lun osuudesta ja pluspuiden valinnassa saavutetusta jalostushyödystä. Rajakohdan lähettyvillä taaksepäin ja eteenpäin valinnan tuottamien jalostushyötyjen ero on vähäinen, joten ratkaisut voivat niissä perheissä perustua paljolti käytännön näkökohtiin.

Simulaatiotutkimuksessa toisen jalostuskierroksen perhevalinnan jalostushyötyä voitiin lisätä 10 % samalla geneettisen monimuotoisuuden ja kustannusten tasolla, kun jalostuspopulaation kokoa kasvatettiin 20 % ja pluspuut osallistuivat toisen jalostuskierroksen peruspopulaation muodostamiseen jalostusarvojensa mukaisesti lukusuhteissa 3:2:1. Suurin jalostushyödyn lisäys saavutettiin jo lisäämällä muutama puu jalostuspopulaatioon ja lisäämällä samalla muutaman parhaan puun osuutta seuraavan kierroksen geneettisessä kokoonpanossa. Toisaalta optimikohdan ympäristössä pluspuiden määrän ja niiden geneettisen panostuksen suhteen vaihtelulla ei ole suurta vaikutusta jalostushyötyyn. Näin ollen jalostuspopulaatioon valittavien pluspuiden määrää voidaan kasvattaa tai pienentää laskennallisesta optimista, jos muut tekijät sitä puoltavat.

Sukusiitoksen aiheuttama metsänviljelymateriaalin elinkykyisyyden alentuminen voidaan estää jakamalla jalostusaineisto lisääntymisen suhteen eristettyihin osalinjoihin. Perinteisesti osalinjajako on tehty satunnaisesti, niin että osalinjat ovat geneettisesti likipitäen samanarvoisia. Jos osalinjajako tehdään pluspuiden jalostusarvojen mukaisesti, voidaan siemenviljelyksen tuottamaa jalostushyötyä kasvattaa käyttämällä sen perustamiseen vain osalinjojen parhaimmistoa. Esimerkiksi jos siemenviljelyksen perustamiseen käytetään vain puolet osalinjoista, on jalostushyödyn lisäys 18 %. Toisaalta, mikäli halutaankin hyödyntää kaikkia osalinjoja, ei hävitä jalostushyödyssä mitään verrattuna satunnaiseen osalinjajakoon. Jalostusarvojen mukainen osalinjajako tarjoaa näin ollen joustavan mahdollisuuden jalostusaineiston tehokkaaseen hyödyntämiseen.

Männynjalostuksessa Suomessa käytetty aineisto on geneettisesti hyvin monimuotoinen ja tarjoaa hyvät mahdollisuudet monia sukupolvia jatkuvalle jalostustoiminnalle. Jalostusta voidaan tehostaa oikealla jalostusaineiston ja työpanosten kohdentamisella ja ottamalla tehokkaan jalostustoiminnan kohteeksi selvästi nykyistä pienemmät jalostuspopulaatiot. Vielä alle sadan puun jalostuspopulaatio on riittävän kokoinen säilyttämään geneettistä vaihtelua tehokkaan pitkän aikavälin jalostuksen tarpeisiin omalla jalostusvyöhykkeellään. Muut pluspuut jälkeläisineen muodostavat arvokkaan jalostuksen geenivarannon, jota voidaan hyödyntää mm. geenikartoituksessa ja merkkigeeniavusteisessa valinnassa.

Avainsanat: jalostusstrategia, pitkäntähtäyksen metsänjalostus, populaatiorakenne, jalostushyöty, geneettinen monimuotoisuus, valinta

Foreword

This work has its roots in the middle of the 1990's when I became involved in discussions with my colleagues Jouni Mikola, Matti Haapanen, Tapani Pöykkö and Martti Venäläinen concerning the restructuring of the Finnish forest tree breeding strategies. It awoke my interest in this issue and brought me into contact with Professor Dag Lindgren, who had studied the optimisation of breeding strategies for a considerable time. This cooperation intensified during 1998, which was the year I spent as a visiting researcher in Dag Lindgren's department at the Swedish University of Agricultural Sciences, Umeå. My visit was partly financed by the Nordic Academy for Advanced Study (NorFa). Dag Lindgren became my supervisor in my efforts, which have finally led to the present doctoral thesis. My thesis is one of the close to ten theses concerning the problem of balancing genetic diversity and gain in tree breeding that Dag Lindgren has supervised. The theses with the closest connection to my work are those by Run-Peng Wei (1995), Erik Andersson (1999) and Ola Rosvall (1999).

I am greatly indebted to all the above-mentioned people for helping me through discussions and writing, to penetrate deeper into the secrets of forest tree breeding. I am especially grateful to Dag Lindgren who, in addition to being my supervisor and co-author in three of the enclosed articles, also gave me the possibility to work at his department. I also thank my other co-authors, Bengt Andersson and Torgny Persson for their constructive cooperation.

At the most painful phases of trying to knit a coherent net of the many separate threads, I got valuable comments from my colleague and wife Anni Harju, my supervisor Dag Lindgren and colleagues Tuija Aronen, Teijo Nikkanen, Pertti Pulkkinen and Matti Rousi. The manuscript was also checked in a constructive way by the reviewers Dr. Gary Hodge and Dr. Tore Skrøppa.

The staff from the National Register of Forest Genetics at the Forest Research Institute, and especially Raija Koski, performed a considerable amount of work in searching for and checking a wide range of information concerning the plus trees. Also Juhani Hahl, Osmo Luostarinen, Ole Oskarsson and Reijo Rauniomaa provided valuable information concerning the early stages of plus tree selection. I must also mention the excellent service which I have received from the staff of the library of the Forest Research Institute, whenever I have ordered the articles – often from rather odd series – I have needed, or thought I will need, in my studies. During the practical writing I received valuable technical help from Tiina Tynkkynen and Esko Oksa. The language was checked by John Derome.

Most of the work was performed at the Punkaharju Research Station of the Finnish Forest Research Institute, although the work has its oldest roots in the time when I was working at the Kolari Research Station, and the main body of the work was initiated in Umeå. These three places have provided me with excellent working facilities, for which I am grateful. Thanks are also due to my employer, the Finnish Forest Research Institute, for all the support I have received in my work.

All the above-mentioned people and organisations helped me to realise my thesis and to improve its quality. You all deserve my sincere thanks.

Punkaharju 18th November 2002 Seppo Ruotsalainen

Contents

Abstract 4
Seloste 5
Foreword 7
Contents 9
List of original articles 10
1 Introduction 11
2 Aim of the thesis 15
3 Breeding cycle and population structure 16
3.1 Description of the populations 16
3.2 The importance of genetic diversity 21
3.2.1 Genetic diversity and long-term breeding 21
3.2.2 Rare alleles 24
3.2.3 Genetic diversity and production populations 26
3.2.4 Inbreeding 31
3.3 The size of breeding population 31
3.4 The breeding program in function 34
4 Material and Methods 40
4.1 General outline 40
4.2 Material 40
4.3 Methods 42
4.3.1 Analysis of the plus trees and their progenies (**I**) 42
4.3.2 Comparison of backward and forward selection (**II**) 42
4.3.3 Group coancestry and status number (**III, IV**) 43
4.3.4 Contribution to the second generation (**III**) 44
4.3.5 Stratified sublining (**IV**) 45
4.3.6 Application to a real breeding stock 45
5 Results 48
5.1 Selection of founders to a breeding program 48
5.2 Constructing the first cycle breeding population 52
5.3 Founder contribution to the 2nd cycle 55
5.4 Sublining the breeding stock 57
6 Discussion 59
6.1 Representativity of the plus trees 59
6.2 Selection efficiency 61
6.3 Constructing the first cycle breeding population 63
6.4 Founder contribution to the second cycle 67
6.5 Sublining the breeding stock 71
6.6 Constituents for a breeding program 73
7 Conclusions 80
References 81
Articles I-IV

List of original articles

This thesis is based on the following original articles, which are referred to in the text by the Roman numerals I-IV. Some unpublished results are also presented. Articles II-IV are reproduced with the publishers' permissions.

I Ruotsalainen, S., Andersson, B. and Persson, T. The effect of plus tree selection intensity on the growth of the progeny of *Pinus sylvestris*. (submitted to Silvae Genetica).

II Ruotsalainen, S. and Lindgren, D. 1998. Predicting genetic gain of backward and forward selection in forest tree breeding. Silvae Genetica 47:42-50.

III Ruotsalainen, S. and Lindgren, D. 2001 Number of founders for a breeding population using variable contribution. Forest Genetics 8:57-67.

IV Ruotsalainen, S. and Lindgren, D. 2000. Stratified sublining: a new option for structuring breeding population. Canadian Journal of Forest Research 30:596-604.

Ruotsalainen has carried out the statistical analyses of the material in **I** after initial data processing by the coauthors. Studies **II-IV** are based on initial ideas provided by Prof. Lindgren, which were further developed by Ruotsalainen who also performed the numerical analyses. Ruotsalainen wrote the first version of the manuscript in all the studies and completed the final versions on the basis of comments from and discussions with the co-authors.

1 Introduction

Tree breeding means improvement of the economic value of the yield by influencing the genetic constitution of the regeneration material. Although the selection of species and provenances is classified as tree breeding in a wide sense, tree breeding is often defined more strictly as consisting of selection and crossing at the individual tree level (Eriksson and Ekberg 2001). New tools for achieving genetic improvements, based on biotechnology, are currently under development. In this thesis the emphasis is on traditional tree breeding that exploits within-population genetic variation. Economically important characters are usually influenced by a large number of genes, and they therefore have a quantitative inheritance.

In all breeding and the domestication of crop plants and animals, the initial phases were merely more or less intentional selection towards plants and animals better suited for the purposes of man (McKinley and van Buijtenen 1998). The technique resembled evolution directed by natural selection. More intentional and sophisticated breeding plans were not adopted until hundreds of generations of such domestication had occurred. Forest tree breeding, although still in its infancy when judged by the number of generations, has the advantage of learning from the long experience of breeding in other fields, and has thus the privilege to directly enter the era of scientifically directed breeding (Namkoong et al. 1988).

However, although the same genetic laws hold for both forest trees and agricultural plants, as well as for all other living organisms, the construction of breeding plans differs in many important aspects that depend on the breeding objects. At the present time, the breeding of agricultural plants is almost always based on previously bred material, whereas the breeding of forest trees is still relatively close to the unimproved natural populations. The much shorter generation time and often autogamous reproduction system of crop plants also have an influence on the breeding strategy applied.

Although the present form of forest tree breeding started in many countries in the 1950's, it took some 20-30 years before the need for special, advanced generation breeding programs was recognised (van Buijtenen 1975, Zobel and Talbert 1984, Shelbourne 1986). Tree improvement programs have often been started to satisfy the immediate requirements for good quality regeneration material. When such programs are at a later stage extended into long-term breeding programs covering several generations, problems are unavoidable (Burdon and Shelbourne 1972, Werner et al. 1981, Zobel and Talbert 1984). Earlier, also second-

generation breeding was performed merely by repeating selection in the first-generation progeny in order to obtain a new seed orchard generation. However, the shortcomings of this method were soon realised (Weir 1974, Pitkäntähtäyksen... 1989). The small numbers of initially selected plus trees and the crossing schemes used have often meant that new selections have to be made from unimproved forests (Burdon and Shelbourne 1972, Werner et al. 1981, Pitkäntähtäyksen... 1989).

It is rather natural that the practical work load of a new discipline and branch of forestry initially took so much attention and energy, that planning for future generations was given a low priority. The most important task was to satisfy the immediate needs of seed procurement. In the early phases the testing period was also considered to be much longer than it is nowadays, which further decreased the urgency of long-term planning (Johnsson 1964).

Advanced generation selection was first described with the term recurrent selection by Stonecypher (1969). The method was first used in plant breeding in the 1940's. Recurrent selection is the planned selection of individuals and families, repeated over several generations within consecutive recruitment populations (Bridgwater and Franklin 1985). Although simple recurrent selection can rely on open-pollinations among selected trees, more complicated and effective methods are usually applied. These normally include at least controlled crossing and genetic testing. In addition to obtaining genetic improvement in selected characters, another dominating feature in tree breeding is the avoidance of inbreeding, as severe inbreeding depression has been observed in many forest trees (Bridgwater and Franklin 1985, Williams and Savolainen 1996).

All serious breeding efforts involve large economic investments, and thus careful planning is necessary (Zobel and Talbert 1984). In fact, optimising the use of resources is one of the most important tasks in planning breeding strategies (Juga et al. 1999), but this has often not been properly emphasised (Lindgren et al. 1997b). A tree breeding plan has to provide genetic gain within a reasonable time scale to be economically successful. However, it must also yield continuous improvements to be sustainable in the long run (Zobel and Talbert 1984). High short-term genetic gain is obtained by intensive selection in the early stages of a program, but the preservation of genetic variation is a prerequisite for sustainable later improvements. The solution to this inherent contradiction between short-term and long-term breeding goals is the separation of different functions to different populations (van Buijtenen 1975, Zobel and Talbert 1984, White 1987, Ruotsalainen 1999).

To be successful, long-term tree breeding must include more than just simple repetition of short-term economically oriented selection. (Kang 1982, Namkoong et al. 1988) Its scope lies in questions that are important

for providing genetic diversity for the needs of continuous improvement in future generations in unpredicted environments (Kang and Nienstaedt 1987). Due to the scarcity of knowledge, breeding programs are often, at least in the early stages, based more on skill or craft and intuition than on science (Zobel and Talbert 1984, Mayo 1987, Nienstaedt and Kang 1987). The breeding objectives of long-term breeding are usually more general than those in short-term breeding, and can even involve characters that are not aimed at in short-term breeding (e.g. precocious flowering) (Kang et al. 1998). A long time horizon and uncertainties about future economic and technical developments make any detailed economic analysis of long-term tree breeding plans useless (Kang and Nienstaedt 1987, Kang et al. 1998), although such can be performed for short-term breeding activities (e.g. Porterfield et al. 1975, Palmer et al. 1998, Ahtikoski 2000).

The huge amount of knowledge that has accumulated about the behaviour of crop plants, and the ease of making crosses, facilitate rather detailed designing of the crop plant by combining desired characters in one variety (Mayo 1987). This kind of ideotype breeding has also been suggested for forest trees (Kärki 1985, Dickmann 1991, Pulkkinen 1991, Pöykkö 1993), but the practical applications are still far away. A breeding program for an agricultural crop plant usually starts with screening a large array of existing varieties in field trials. When the interesting material has been recognised, a crossing and selection scheme over many generations is constructed. The final phase is to evaluate the results in field tests (Mayo 1987).

In allogamous agricultural plants, the reproductive system of which is similar to that of most forest trees, hybrid breeding strategies have been developed for utilising heterosis. This kind of breeding strategy has been little used with forest trees, because several generations are needed to develop the hybrids. The Illinois maize selection program, which has continued over ninety generations, has shown that traditional recurrent selection strategies can yield genetic improvement comparable to hybrid breeding strategies in agricultural crop plants, too (Mayo 1987, Dudley and Lambert 1992). The closest resemblance between the breeding strategies of agricultural crops and forest trees is found in allogamous forage crops, where synthetic varieties are commonly used. In contrast to the pure line varieties used with autogamous plants, synthetic varieties are in fact collections of good genotypes that interbreed freely with each other (Mayo 1987). This is rather analogous with the commonly used, open pollinating forest tree seed orchards.

At first sight it may sound strange that forest tree breeding has more in common with animal breeding than with the breeding of most crop plants. Animals are outcrossing like most forest trees and both have a considera-

bly long generation time. Outcrossing usually implies severe inbreeding depression, which necessitates the use of a rather large number of individuals and thus a population approach to the breeding. Consequently, many concepts and techniques in tree breeding have been adopted from animal rather than from plant breeding (Stonecypher and Arbez 1976). The avoidance of inbreeding in the case of forest trees is further emphasised by their early stage in domestication, and thus low degree of purging of deleterious alleles.

Due to the long generation time of forest trees it is useless to make detailed breeding plans that stretch for many generations ahead. It is enough to assure that the breeding strategy is constructed in a sustainable way, and that it does not lead to a dead end in the future generations. Therefore the greatest emphasis also in studies concerning long-term breeding should be in the first few cycles of breeding. The influence of the rapidly developing field of biotechnology on tree breeding strategies can, at the moment, only be guessed, but it seems probable that its main effect will be to speed up breeding and make it more effective, rather than to change breeding strategies qualitatively into something totally new (Plomion et al. 1996, Aronen 1999, Zhong 2001). Thus the analysis of traditional tree breeding strategies can still be considered relevant.

2 Aim of the thesis

The aim of this thesis is to elucidate the fundamental dilemma of producing maximum genetic improvement with minimum loss in genetic diversity in long-term tree breeding. The issue was studied by reviewing relevant literature, using deterministic simulations and analysing empirical material. The specific questions studied were:

1) What is the impact of phenotypic plus tree selection on the genetic gain and diversity of the first generation breeding stock?
2) What is the optimal way to combine backward and forward selection when moving to the second breeding cycle?
3) How should the trees of the first breeding cycle contribute to the second cycle?
4) How should the material be arranged to control the level of inbreeding in production forest?
5) What is the importance and best way of managing genetic diversity in a long-term tree breeding program?

3 Breeding cycle and population structure

3.1 Description of the populations

The population hierarchy and the functions of the populations follow the conceptual framework of breeding cycle suggested by White (1987) (Fig. 1). For the sake of clarity the following treatise assumes discrete generations, although also in the case of overlapping generations, as e.g. in the suggested rolling front breeding method (Borralho and Dutkowski 1998), the same elements are present but in a more diluted form. The populations are defined functionally – their physical appearance is of minor importance, and in fact some functionally distinct populations can be physically overlapping. The most pervasive idea presented in the framework is the separation of populations in the breeding cycle from those responsible for conveying the genetically improved material to operational plantations.

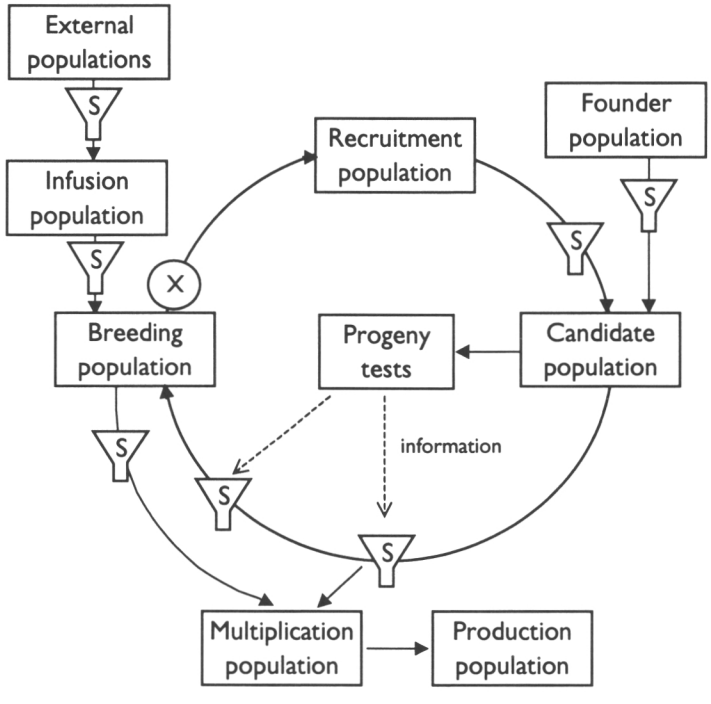


Figure 1. The structure and functioning of breeding cycle and attached populations (S=selection, X=crossing) (modified after White 1987).

This distinction has arisen as a response to the dilemma between intensive selection for high short-term gain and preserving genetic diversity in order to ensure high long-term gains. The importance of this separation was noticed only when the tree breeding plans reached such a degree of maturity that second generation selections became topical (e.g. Burdon and Shelbourne 1972, van Buijtenen 1975, Weir and Zobel 1975).

The ideas and concepts of population hierarchy began to develop in the late 1960's (Stonecypher 1969, Burdon and Shelbourne 1972, Franklin and Squillace 1973, Libby 1973). However, the terminology is rather ambiguous, sometimes even contradictory, and has remained such in spite of some efforts to harmonise it (e.g. Zobel and Talbert 1984, White 1987). Thus it was considered important in this connection to make explicit definitions of the applied population terminology (Table 1).

Breeding population is the most frequently used of all these population concepts, but it has been used in two different meanings - a strict and broad sense. To make things worse, it has seldom been explicitly stated in which meaning the concept is applied. Here the strict definition is used, which means that a breeding population is only one part of the breeding cycle – those trees which contribute to the next generation (Zobel and Talbert 1984, White 1987). The broader definition of breeding population includes all the populations in a breeding cycle, although they should be better called collectively breeders' populations (Namkoong and Koshy 1997). Especially the earliest occurrences of the term (e.g. Burdon and Shelbourne 1972, Zobel et al. 1972, Lindgren and Gregorius 1976) were either in the broad meaning or not explicitly defined.

Conceptually *recruitment population* has been clearly defined by Zobel and Talbert (1984) and White (1987), but it has earlier usually been called base population. The reason for using a different term here is that base population has several other uses in the fields of genetics and breeding. It is often used for the unselected natural population from which some experimental lines are derived (Falconer and Mackay 1996), or for the starting material of a breeding program, irrespective of its genetic level or background (Stonecypher 1969, Werner et al. 1981, Breeding poplars for... 1985). Thus it was considered best to leave base population for use only in basic genetic studies, and in a breeding cycle use recruitment population according to **IV**.

The third alias *candidate population* in breeding cycle is defined by White (1987), although he uses the name selected population. It is the least used of the population terms of the breeding cycle, but its use can clarify the structuring of the breeding plan, if there are clearly different stages in the selection process. However, if only phenotypic selection is used, or if the selection is otherwise made in one step, it is not reasonable to speak of a separate candidate population. In such a case the breeding

cycle consists only of alternation of recruitment and breeding populations.

Table 1. Definitions of the population terminology applied in the study, their classification into different categories and relevant references (often the earliest).

Term	Category[a)]	Definition and reference
Founder population	I	The initial set of individuals from which selections will be taken to establish a breeding program (e.g. the wild forest). Gullberg and Kang (1985b)
Recruitment population	B	The population in the breeding cycle from which selections for candidate population are made. The beginning of new breeding cycle. (**IV**)
Candidate population	B	Phenotypically selected individuals from recruitment population to be possibly later included in the breeding population (depending on progeny test results etc.). Ruotsalainen (1999)
Breeding population	B	Individuals which transmit their genes to next generation recruitment population in the breeding cycle. Zobel and Talbert (1984)
Multiplication population	O	Individuals used to multiply the genetically advanced material for commercial purposes (e.g. seed orchards). Kang and Nienstaedt (1987)
Production population	O	Individuals used to produce the commodities forestry is aimed to, i.e. operational plantations. Kang and Nienstaedt (1987)
Gene resource population	I	Population used to maintain large genetic variation of species for the future breeding purposes. Eriksson and Ekberg (2001)
Infusion population	I	A gateway for importing material to the breeding cycle from outside the breeding program. White (1987)
Breeding stock	U	All populations included in breeding cycle
Sublines	L	Subunits of the breeding stock which are kept unrelated (Burdon and Namkoong 1983)
Nucleus breeding group	L	Genetically advanced, intensively managed part of the breeding stock. Cotterill et al. (1989), Ruotsalainen (1999)
Main breeding group	L	Genetically less advanced part of the breeding stock, which is managed less intensively. Cotterill et al. (1989), Ruotsalainen (1999)

[a)] following classification is used: B = breeding cycle, I = input to breeding cycle, O = output from breeding cycle, U = upper level, L = lower level

The three populations (i.e. recruitment, candidate and breeding populations) making up the breeding cycle are called here the *breeding stock*. This is close to the use of the term by Gullberg and Kang (1985a), except that they also included multiplication population in the breeding stock. This does not usually have any practical importance, as the multiplication population is commonly a selected subset of the breeding population.

The following populations lie outside the breeding cycle, but are essential for a successful breeding program.

In order to replace the ambiguous use of the term base population in connection with starting a breeding program, the term *founder population* was adopted from Gullberg and Kang (1985b). Founder population is identical with first generation recruitment population, if the breeding program is started from zero level, without any earlier breeding efforts. If a new breeding program is based on earlier programs, the founder population can be formed from any population in the breeding cycle, or from a mixture of them (e.g. Skrøppa 1982, Nienstaedt and Kang 1987). Synonymously, founder population has also been called source population (Breeding poplars for... 1985, Kang and Nienstaedt 1987).

Gene resource population is used to maintain the genetic diversity of the species over long time periods for the future needs of tree breeding (Eriksson and Ekberg 2001). This is accomplished in natural populations (in situ) or in plantations or clone archives (ex situ). Gene conservation population is sometimes used in a rather similar meaning, but mainly referring to natural stands (Lindgren and Gregorius 1976).

When material from gene resource populations or other outside sources is introduced to the breeding stock, there is often a need to carry out some selection or even more profound breeding before the material is allowed to enter the breeding stock. This is done in the *infusion population* (White 1987). The importance of infusion population depends on the situation. If there is only a small difference between the materials to be fused, then special infusion populations can be unnecessary, but the additional material can e.g. be used as parents when creating a recruitment population. On the other hand, if the additional material is unadapted or lags considerably behind the breeding stock in genetic value, there may be a need to enlarge the infusion population into a small breeding program covering several generations.

The function of *multiplication population* is to serve as a source of genetically improved material for practical forestry. Examples of the physical forms of this are seed orchards or hedges for cutting production. This population is often called by tree breeders egocentrically as the production population, but when looked at from a wider perspective, it must be admitted that the production of seeds or other propagules is only a method to create the real *production populations*, i.e. operational planta-

tions. Thus the terminology used by Kang and Nienstaedt (1987) was considered to be most suitable here. Propagation (Barnes 1995) or propagule population (Eriksson and Ekberg 2001) is also used as a synonym for multiplication population.

Another kind of terminology is needed when the breeding stock is divided into subunits, either in order to provide genetically unrelated parents for seed production in multiplication populations or to enhance the genetic improvement in part of the breeding stock. When the purpose of the division is to avoid inbreeding in operational plantations by providing unrelated parents to seed orchards, the method is called sublining and the groups *sublines*. The sublines are usually genetically at same level, are bred for the same objectives, and are kept reproductively isolated through the generations (Burdon et al. 1977, Burdon and Namkoong 1983, van Buijtenen 1984). This means a faster build-up of inbreeding within sublines, but this inbreeding can be totally removed in commercial seed production by allowing only one tree from each subline to participate in reproduction. Sublining also offers administrative advantages, as they often form units of suitable size for practical operations (Wellendorf 1986). Sublines have also been called breeding groups (van Buijtenen 1984).

If the division into subunits is intended to lead to different genetic values of the groups, it can be regarded as being more a question of hierarchical breeding stock structures (Cotterill et al. 1989, Cramer and Kannenberg 1992, White et al. 1993), or more generally, a fine-tuning of the population hierarchy in the breeding cycle. In such a case the subunits are usually not reproductively isolated, but there is some change of the material between the tiers.

In the nucleus breeding system the genetically best part of the breeding population is called the nucleus (population) whereas the genetically lower ranking majority forms the main breeding population (Cotterill et al. 1989). As the same division is present in all populations in the breeding cycle, though less pronounced due to the exchange of material between the levels, it is more appropriate to designate the subunits as *nucleus* and *main breeding groups* (Ruotsalainen 1999). In nucleus breeding there can thus be seen an orthogonal division of breeding stock in two directions: sequentially different populations, which are then divided into two separate tiers according to the genetic value. This breeding system can in fact be described as consisting of two parallel, loosely connected breeding cycles. Typically the nucleus contains some 10 % of the top ranking trees of the breeding population, whereas the remaining 90 % is allocated to the main breeding group (White 1992). Sometimes the nucleus is denoted as the elite population (e.g. White et al. 1993, Williams and Hamrick 1996).

3.2 The importance of genetic diversity

3.2.1 Genetic diversity and long-term breeding

Genetic variation is of utmost importance for genetic improvement. According to Fisher's fundamental theorem of natural selection, the rate of increase in mean fitness is directly related to the additive genetic variance of fitness at that time (Wallace 1981, Eriksson and Ekberg 2001). However, there exists an inherent dilemma in every breeding program: in order to make genetic improvements the genetic diversity must be reduced (Namkoong and Koshy 1997). For example, striving for high production has led to a situation in which more than 50 % of all the poplar plantations in the world have been established with only a few high productive clones (Zsuffa et al. 1993).

Genetic variation at the phenotypic level depends on the existence of different alleles of the genes affecting the considered character, i.e. on genetic diversity (Fig. 2). The diversity at the genotypic level and the genetically determined phenotypic variation can be designated as genetic variability. It must also be noted that, although in rough lines genetic variation parallels genetic diversity, the relationship is far from exact (Libby et al. 1997). Neither does high diversity necessarily mean better adaptability – it just offers potential for it (Lambeth and McCullough 1997).

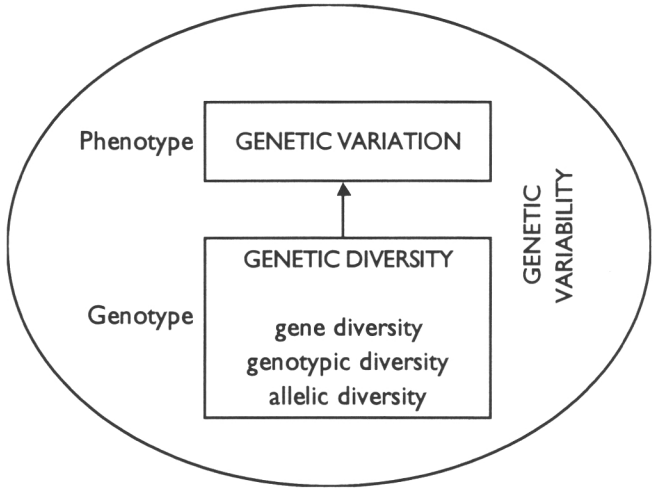


Figure 2. Classification of concepts of genetic variability

Most adaptive traits of forest trees show clinal genetic variation within the species range, so that population means change gradually with geo-

graphic distance. This variation pattern is caused by effective gene flow over long distances, usually by wind pollination, and gradually changing selection pressures (Stern and Roche 1974, Mitton 1995, Eriksson and Ekberg 2001). Due to effective gene flow and low selection pressures for disruptive selection, the within-population genetic variation is mainly between trees; the stand component is of minor importance (Park and Gerhold 1986, Eriksson et al. 1987, Simpson 1998, **I**). To ensure successful artificial forest regeneration, the planting stock or seed must be relatively well adapted to the site. Practical methods for administering this are seed transfer rules or delineation of planting and breeding zones (Namkoong et al. 1988, Mitton 1995). In the following treatise the question of genetic diversity is studied mainly within such a limited area that the adaptational differences of the material as regards climate can be regarded as negligible.

Genetic diversity has many indicators which measure different aspects of the genetic variability in the population. The allelic diversity can be described e.g. by the number of polymorphic loci, number or frequency of alleles in loci, and the proportion of heterozygous loci (Rosvall 1999), whereas gene diversity is defined as the probability that alleles are different by descent (Rosvall 1999, Kang 2001). In metric characters the genetic diversity is manifested as quantitative variation, which is described statistically by an analysis of variance (Rosvall 1999). The genetic diversity can be expressed at the individual, population or higher level. These diversity levels differ in importance as regards the different aims of a breeding program (Table 2). Observed heterozygosity describes the diversity within an individual and number of genotypes measures the genetic diversity between individuals within a population, whereas expected heterozygosity or gene diversity functions at the population level.

Table 2. The importance of the level of expression of genetic diversity for different aims of a tree breeding program (* = moderately, ** = highly important).

Aim	Within individual	Within population	Between populations	Between species
Short-term gain		**	**[1)]	**[1)]
Long-term gain		**	*	*
Evolution		**	**	*
Stability and resistance	*	*	**	**
Unexpected risks		*	*	**
New goals		**	*	*

[1)] refers to selection of the correct provenance and species

There are several technical methods (e.g. biochemical markers, pedigree analysis, metric characters) to obtain data for genetic variability analyses. Diversity can also be measured at different life stages, and is strongly dependent on the species (Savolainen and Kärkkäinen 1992). Thus it is understandable that different views of the importance of genetic diversity prevail. The discrepancies can be alleviated by recognising that different measures are suitable for different purposes (Table 3). For instance, indicators measuring the retaining ability of rare alleles are more relevant in gene conservation purposes, whereas in active tree breeding the genetic variance of target traits is the most important, as can be inferred from Fisher's fundamental theorem of natural selection (Wallace 1981).

Table 3. The significance of different types of genetic variability in fulfilling the different aims of a tree breeding program (see Table 2 for explanation of symbols).

Aim	Allele richness	Genotypic richness	Individual heterozygosity	Gene diversity	Quantitative variation
Short-term gain		*	*	*	**
Long-term gain				**	**
Evolution	**				**
Stability and resistance		**	*		**
Unexpected risks	**				
New goals	**				**

The existing genetic variation is mainly caused by the common alleles (above about allele frequency 0.1), which are present already in a very small sample of individuals (Danell 1993, Yanchuk 2001). In fact, theoretically, a full sib family which contains alleles from its two parents only contains more than 50 % of the total genetic variance in a population. The decrease in additive genetic variation due to random drift caused by finite population size is described by equation (1) (Danell 1993)

$$V_A(t) = (1 - \frac{1}{2N_e})^t V_A(0), \qquad (1)$$

where $V_A(t)$ and $V_A(0)$ are additive genetic variances in generations t and 0, respectively and N_e is effective population size. The dependence of additive genetic variance on population size is similar to that between gene diversity and population size (Kang 2001).

In long-term breeding the balance between gene diversity and genetic gain can be sought by means of group merit selection, where the selection index combines both these targets by giving a cost factor for the relatedness. (Lindgren and Mullin 1997, Rosvall and Andersson 1999). The task is thus to select such a set of individuals that the index value is maximised. There is a problem of how to weight relatedness against the genetic gain but, as a general rule it can be stated that avoidance of relatedness should be given the greater weight the longer the time horizon is in the breeding program (Andersson 1999).

The population structure of a breeding program enables adjustment of the genetic diversity of each population separately according to their specific requirements (Table 4). Consequently the need for genetic diversity for each specific target can best be measured by a specific diversity criterion (Table 3).

Table 4. The roles of different populations in a breeding program (see Table 2 for explanation of symbols).

Aim	Gene resource population	Breeding stock, main	Breeding stock, nucleus	Multiplication population	Production population
Short-term gain		*	**	**	
Long-term gain		**	*		
Evolution	**				
Stability and resistance					**
Unexpected risks	**	*			
New goals	*	**			

3.2.2 Rare alleles

Rare alleles contribute little to the present quantitative variation. They arise mainly as unfavourable mutations or they are evolutionary relics, although a minority of them can also be or become favourable (Lindgren and Gregorius 1976, Williams et al. 1995, Libby et al. 1997). Homozygotisation of rare recessive deleterious alleles with inbreeding is supposed to be one reason for inbreeding depression. Examples of unfavourable rare alleles can be found e.g. in the resistance of *Pseudotsuga menziesii* to damaging biotic agents (Chen et al. 2001) or the survival of *Pinus taeda* (as reviewed by Mitton 1995), in which homozygotes for rarer allele had lower values than homozygotes for common allele. Rare recessive alleles can be important for qualitative variation and thus be important from the point of view of man. Resistance to pathogens in agricultural crops is often connected to such alleles (Lindgren and Gregorius 1976, Burdon

2001). A good example of a rare recessive allele with a potentially great economic value is *cad-n1*, which changes the lignin metabolism of *Pinus taeda* so that the lignin content of the wood is decreased (MacKay et al. 1997).

Artificial selection by man represents in fact a more or less drastically changed new environment, where those alleles that are not favoured by natural selection can become valuable in improving yield or quality (Eriksson and Ekberg 2001). For instance, good volume growth, although having some adaptive fitness, is probably not the key factor in natural selection, but is one of the most important traits in artificial selection. Volume production is genetically independent or only weakly correlated with adaptive traits (McKeand and Svensson 1997). Thus rare alleles that promote volume growth may exist. These breeding objectives set by man can be described in terms of crop ideotype, which involves the redirection of plant resources to those parts of the tree which are economically the most valuable (Mayo 1987, Dickmann 1991). When brought to the maximum, this development may mean a total reconstruction of the tree (Mann and Plummer 2002).

In spite of these potential direct applications, the most important function of rare alleles in a breeding program is to contribute to the future variation. When continuous directional selection brings the selected common alleles close to fixation, the originally less common alleles come within the reach of the selection, and thus in turn important for genetic variation. Therefore, for long-term breeding and for evolutionary needs,

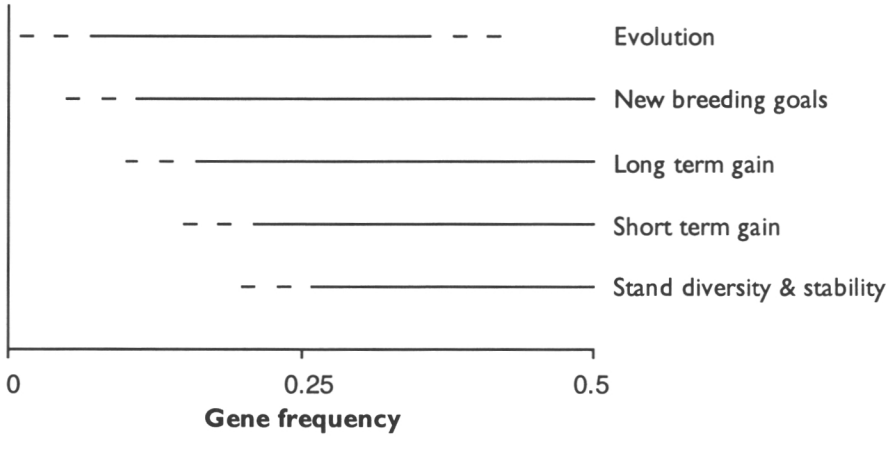


Figure 3. The importance of alleles of different frequencies for different aspects of a tree breeding program (modified after Danell 1993).

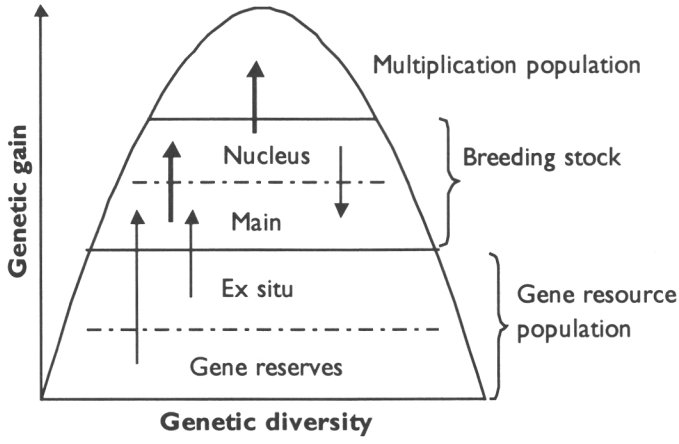


Figure 4. The population hierarchy and gene flow between populations in a tree breeding program.

rarer alleles existing in the population should also be preserved (Danell 1993). However, another opinion treats rare alleles more as unfavourable relics or newly arisen mutations that make little contribution to the genetic improvement of the population (Lindgren and Gregorius 1976).

As a summary, it can be stated that the importance of rare alleles increases as a function of distance to the practical application (Fig. 3). Thus a solution for preserving alleles of different frequencies is the population hierarchy; the importance of rare alleles and genetic diversity increases with decreasing level in the population hierarchy (Fig. 4).

3.2.3 Genetic diversity and production populations

In the production populations the importance of genetic diversity differs in many aspects from that in the breeding stock. As production populations are dead ends from the point of view of a breeding program, they do not need to contain more genetic variation than is necessary for their rotation time. Variation is thus essential in the breeding stock but not necessarily in the operational plantations derived from them (Namkoong and Koshy 1997, Eriksson and Ekberg 2001). In fact, from the harvesting point of view small variation in the economically important characters is desirable, because uniformity means easier management, processing and marketing.

Theoretically, it can be expected that both heterozygosity at the tree level and genetic variation at the population level are connected with higher stability and genetic gain (Lundkvist 1982). However, it has been difficult to show any general association between heterozygosity and growth or fitness (e.g. Savolainen and Hedrick 1995). The detrimental ef-

fect of increased homozygosity after inbreeding on growth and fitness has been clearly demonstrated (Koski 1973, Sorensen and Miles 1974, Lundkvist et al. 1987, Williams and Savolainen 1996), but in outcrossed material the relationship between heterozygosity and fitness, growth or stability is far from unequivocal.

Natural selection seems to favour heterozygous trees, or trees homozygous for common alleles (Mitton 1995). The germination conditions of beech (*Fagus sylvatica*) nuts determine the level of heterozygosity of the resulting seedling population: optimal raising conditions in a greenhouse favour homozygotes, whereas harsher field conditions favour heterozygotes (Ziehe and Hattemer 1998). Trees of *Pseudotsuga menziesii* and *Pinus lawsonii* heterozygous for several isoenzymes are more susceptible to insect damage than homozygous trees (Chen et al. 2001, Vargas et al. 2002), in *Pinus montezumae* there is no association between heterozygosity and susceptibility (Vargas et al. 2002), and in *Pinus edulis* heterozygous trees are more resistant (Mitton 1995).

Resistance to insect damage in Douglas fir seems to be connected to the presence of the most common alleles (Chen et al. 2001). In *Fagus sylvatica* a higher frequency of heterozygous trees has been observed among trees resistant to air pollution stress than among the susceptible ones (Ziehe and Hattemer 1998). Association between growth and heterozygosity can be positive (Mitton et al. 1981), negative (Strauss and Libby 1987), missing (Knowles and Mitton 1980), or vary between stands (Chen et al. 2001). In some species heterozygosity is connected to less variability in growth, in others to higher variability, which can depend on differing reproductive strategies and resource allocation (Knowles and Mitton 1980, Mitton et al. 1981). Thus it seems that the importance of heterozygosity is still poorly understood, but there are indications that it can be connected with individual tree adaptability in a variable environment (Ziehe and Hattemer 1998, Rajora 1999).

Studies concerning genetic diversity in multiplication populations, or in production populations derived from them, have yielded variable results (Muona and Harju 1989, Bergmann and Ruetz 1991, Williams et al. 1995, Schmidtling et al. 1999, El-Kassaby 1999, Rajora 1999, Macdonald et al. 2001). As a rough generalisation it can be stated that genetically improved material has the same or slightly increased gene diversity compared to natural populations, but with somewhat reduced allelic diversity. The variable results can be explained by a differing allelic distribution or sampling procedures. The selection of phenotypic plus trees in most cases thus seems to be an effective method for sampling the genetic variability of natural populations, which is important for long-term tree improvement.

As the connection between individual tree heterozygosity and adaptation or stability is not well understood, a more straightforward method to improve adaptability could be to test material in different environments and to select those with the best average performance (Finlay and Wilkinson 1963, Eberhart and Russell 1966, Namkoong 1999). Testing for stability is especially important when rather limited number of vegetatively propagated clones are utilised in operational plantations. In fact, the extent of deployment of clonal material can be bound to the testing intensity (Lindgren et al. 1990, Venäläinen 1992, Lindgren 1993). It can also be asked, whether it is better to use genetically rather narrow-based, but well-tested material, than untested material with large genetic variation. It must be noted that large variation in the economically important characters inevitably also means the existence of less favourable individuals in the population. On the other hand, strict requirements for testing can lead to a lower number of tested clones, which reduces the obtainable genetic gain. High testing costs can lead to pressures to accept most of the tested clones, which can in fact increase the total risk (Lindgren et al. 1990).

The importance of within population genetic diversity in production populations often seems to be overemphasised (Lindgren 2002a). There are many examples of forestry practised sustainably with clearly reduced diversity both with clones and family-wise plantations of generatively propagated material (Libby 1982, Zobel and Talbert 1984, Zsuffa et al. 1993) and, similarly natural stands established by a single clone do exist. For instance, quaking aspen (*Populus tremuloides*) can vegetatively spread within a number of years across areas several hectares large (Perala 1990). On the other hand there are also examples of serious damage caused by the extensive use of a single genotype in clonal forestry (Toda 1974, Breeding poplars for... 1985). It is hypothesised that the importance of genetic variation in a production population increases with rotation time, environmental heterogeneity and a lack of evidence of performance from tests (Eriksson and Ekberg 2001). However, there are examples that are contrasting to these general trends. For instance, vegetative propagation and single genotype stands of sugi (*Cryptomeria japonica*) is more frequent at the timberline than on more favourable sites (Taira et al. 1997).

The rationale of using genetically variable regeneration material can be divided into three categories: risk spreading, reduction of biotic damage and more efficient use of site resources (Heybroek 1982, Lindgren 1993). These concepts are especially applicable in clonal forestry where genetic uniformity can be driven to the utmost extreme. Extensive use of one or a few clones can be economically disastrous if some – even unlikely – disease or other failure occurs (Heybroek 1982). Large monoclonal plantations are also believed to lead either to higher general disease levels, if

the clone is susceptible, or to the development of a supervirulent disease if the clone is resistant (Heybroek 1982). Development of durable population-based disease resistance involves management of the whole pathosystem. An important part of this are the spatial and sequential discontinuities created by mixtures of different genotypes (Breeding poplars for... 1985). A mixture of genotypes can theoretically better utilise the growth potential of the site through different nutrient etc. requirements and compensatory growth in the case of failures for some clones (Heybroek 1982, Lindgren 1993).

There are two kinds of risk in a tree breeding program: known and unexpected (Hubert and Bastien 1999, Burdon 2001). The known risks can be handled with testing as discussed above, and the unknown risks with risk spreading and population hierarchy. The higher unexpected risks connected with low diversity and high yield are limited to the relatively short-lived multiplication and production populations, whereas the long-term breeding stock is managed with a low risk option (Fig. 4). Along with accumulating knowledge, more and more of the unknown risks become known and thus controllable (Kleinschmit et al. 1993).

The risks involved in the deployment of forest regeneration material can be considered at the level of a forest owner, region or the whole global planting area (Lindgren 1993). Two possible strategies can be utilised in risk spreading: either one can plant the genetically different entries in separate blocks, or mix them at one site. At the region or global level these alternatives are equal, as long as the total utilisation of a single genotype is at an acceptable level, but what is an acceptable risk at a higher level may be too high for a forest owner. Thus he may select the utilisation of mixtures. The creation of diversity at a larger scale has the advantage that the plantations are more uniform and thus facilitate silvicultural operations (Heybroek 1982, Libby 1982, Zobel and Talbert 1984, Zsuffa et al. 1993). At the same time the plantations of single clone or single parent progenies also function as valuable genetic tests if they are documented properly.

The choice between separate block and mixture strategies depends on e.g. the rotation age, silvicultural practices, and the relative importance of the different reasons behind favouring within population genetic diversity. For instance, if the prevention of fungal attacks is of greatest importance, then genotype mixtures are probably to be favoured (Heybroek 1982, Prakash and Thielges 1986). However, mixing genotypes is not a universal solution to increase disease resistance; its use should be based on profound knowledge of the host-pathogen interaction (Heybroek 1982, Lindgren 1993). If the host range of the pathogen or pest is very wide - in many cases it can be over species or genus limits (Davidson et al. 1999) -

then there are limited possibilities for improving the stand resistance by using genotype mixtures (Lindgren 1993).

The mixing of genotypes offers theoretical advantages in more effective utilisation of the site resources by reducing genotype-environment interaction. This is called population buffering in the agricultural literature (e.g. Eberhart and Russell 1966). However, there is little evidence of more effective site utilisation by mixtures in forestry (Kleinschmit et al. 1993, Lindgren 1993, Zsuffa et al. 1993, DeBell and Harrington 1997). From the point of view of genetic diversity, a mixture of genotypes can in fact lead to a worse outcome than a mosaic of pure genotypes, as some of the genotypes are totally or partially eliminated due to lower competing ability (von Wuehlisch et al. 1990, DeBell and Harrington 1997). A prerequisite for successful cultivation of mixtures is the selection of genotypes capable of good performance in mixed stands.

Recommendations concerning the number of clones needed to manage the risks in clonal forestry vary widely, but somewhere between 10 to 40 clones seems to be a limit above which the increase in clone number does not essentially reduce the risk (Libby 1982, Bishir and Roberds 1999, Burdon 2001, Sonesson et al. 2001). If most genotypes are susceptible to the new damaging agent, then increasing the number of genotypes will not improve the mean performance of the stand. In fact, in such a case an increase in the number of genotypes increases the probability that some of them will be affected, and thus possibly render the whole plantation silviculturally unacceptable (Bishir and Roberds 1999). If a high incidence of damages is to be expected, the best strategy might be to use a mosaic of single genotype plantations, although also the rationality of the whole operation could be questioned. On the other hand, if only a small proportion of genotypes are affected, then not many genotypes are needed to surpass the required probability of stand success (Namkoong 1999).

If these numbers are compared to generatively propagated regeneration material on a gene diversity basis, a seed orchard with total isolation from contaminating pollen should contain a higher number of clones than there are in the vegetative reforestation material in order to obtain equal gene diversity, when the variation in ramet number and flowering are considered (Lindgren and Mullin 1998, Nikkanen and Ruotsalainen 2000). When the effect of background pollination is considered, seed orchard crops have about the same gene diversity as the seed orchard clones (Nikkanen and Ruotsalainen 2000). However, when genetic diversity is measured by the number of genotypes (genotypic diversity), then the offspring of a seed orchard with only a few clones is by far the more diverse than clonal mixtures.

The possibilities in operational plantations to safeguard against unexpected risks seem to be limited to risk spreading. As the enhancement of

resistance and yield stability through the genetic diversity seems to be rather poorly supported by experimental evidence, deployment can equally well be done in single genotype mosaics (Sonesson et al. 2001).

3.2.4 Inbreeding

One special feature connected with the inevitably increasing relatedness in closed breeding populations of forest trees is the inbreeding depression typical of outcrossing species (Lundkvist et al. 1987, Williams and Savolainen 1996). This is of minor concern in the populations of the breeding cycle, although high levels of inbreeding can also restrict the success of sexual reproduction and interfere with genetic testing. In operational plantations the situation is quite different, because there the yield can be lowered due to inbreeding depression and thus the genetic gain through breeding operations is at least partly negated. Higher breeding population sizes can reduce this problem, but other measures are probably more effective in dealing with it. Division of the breeding population into reproductively isolated sublines always enables production of outcrossed propagules when the multiplication population is constructed using only one member from each subline (McKeand and Beineke 1980, White 1992). It is also rather probable that tolerance of inbreeding, i.e. low inbreeding depression, will be among the future breeding goals, either deliberately or unintentionally (Danell 1993).

It has been suggested that selfing or milder forms of inbreeding could be used as a breeding tool to purge deleterious alleles from the breeding stock (Kang 1982, Namkoong et al. 1988, Williams and Savolainen 1996) or to increase between family variance (Lindgren and Gregorius 1976). Inbreeding could also be used to reveal recessive favourable alleles (Burdon 2001). The results concerning the effect of inbreeding on the resistance of a herbaceous plant, *Silene alba*, to the fungus *Microbotryum violaceum* are promising in this respect (Ouborg et al. 2000).

3.3 The size of breeding population

There is large possibility for subjectivity in determining the breeding population size in advanced generation breeding programs because genetic studies give only very rough estimates (White 1992). From the point of view of genetic diversity, the size of the breeding population is the most critical of all the populations in the breeding cycle, because it forms the bottle neck which determines the fate of the alleles. Other population sizes are mainly of interest from the point of view of costs, although a

very small recruitment population can also be limiting for genetic diversity.

The question of the size of the breeding population for forest trees has been studied theoretically using simulation models with some simplifying assumptions. Empirical studies are restricted to more easily handled experimental organisms with a short generation time (Falconer and Mackay 1996). The size of the breeding population can be studied from several perspectives. The first category of studies concerns the size needed to obtain the maximum ultimate genetic gain, the second category the size for maximum gain in a few (5 to 10) generations, and the third the size needed to maintain neutral alleles in the population for a broad genetic base for flexibility (White 1992). As indicated by the discussion above, these aspects can best be dealt with using separate populations for different purposes (Table 4). The short-term genetic gain in the first generations is a question relevant for the nucleus breeding group in the breeding stock, whereas the long-term genetic gain is the target for the main breeding group. Preservation of neutral alleles to provide flexibility for possible future challenges is best handled in the main breeding group and the gene resource population.

Studies concerning the ultimate genetic gain after many generations of selection have shown that effective population sizes of 20 to 60 are needed to obtain 50 to 90 % of the maximum attainable gain (White 1992). When the goal is set at 5 to 10 generations, which is still a very long time horizon for most forest trees, then even smaller population sizes give satisfactory results. Effective breeding population sizes of the order of 20 to 80 have been regarded as adequate also in many other studies (Danell 1993, Rosvall 1999, Yanchuk 2001). From the point of view of avoiding inbreeding depression in the first generations at least, effective population sizes of 40 to 50 are needed (Danell 1993, Rosvall 1999). A population with an effective population size of 10 in fact contains 95 % of the additive genetic variation of an infinite population, and the additive genetic variation is not greatly influenced by the sampling process even with small population sizes ($N_e = 20$) in some ten generations (Eq. 1).

The population sizes needed to maintain neutral alleles depend on the allele frequency and targeted number of generations. A population size of over 400 is needed to preserve a rare allele ($p = 0.01$) for 80 generations with a probability of 95 %. These population sizes can be considered as minimum estimates. Larger population sizes are advisable if the basic assumptions in the model (e.g. no dominance, no epistasis, no linkage, no family selection) are violated (White 1992). The length of the breeding cycle also affects the requirements for breeding population sizes, if time schedules are expressed in years. In an intensive breeding program with a short generation interval, larger populations are needed than when slower

cycling is used (Wei and Lindgren 2001). It should also be noted that this discussion concerns effective population sizes, which can be considerably lower than the census numbers if there is relatedness or differences in contribution between the trees in the breeding population. For example, the use of family selection leads to total rejection of poorly performing families and thus faster accumulation of relatedness. This can be counteracted by initiating a breeding program with a large breeding population in order to afford strong family selection to achieve larger initial gains.

A small population size also means a less predictable selection response as there are more opportunities for random drift to affect the outcome (White 1992). This property of small populations can be utilised in breeding to increase the total variation in the population by dividing it into smaller sublines. The increased unpredictability of selection in small populations is caused by the increased probability of fixing also rare alleles in them (Eriksson and Ekberg 2001). In fact small marginal populations can be important for speciation in evolution (Stern and Roche 1974). The unpredictability is the stronger, the smaller the sublines are. This reasoning is one of the arguments for the suggested subline size of four in a *Pinus taeda* breeding program (McKeand and Bridgwater 1998). Small sublines mean a conservative and flexible strategy – it is always possible to merge small sublines into larger ones, but the opposite is not true.

There is growing evidence that mutations and the breakage of gene complexes can provide enough genetic diversity also in rather small breeding populations (30 – 50) to counteract the inevitable loss of genetic diversity through random drift in the change of generations (Namkoong et al. 1988, Danell 1993, Burdon and Carson 1999, Eriksson and Ekberg 2001). This view is also supported by the continuous progress in long-time selection experiments e.g. with maize (Dudley and Lambert 1992).

The required size of breeding population also depends on the abundance, importance and degree of adaptedness of the species. Larger population sizes are motivated in the case of extensively planted economically important exotic species, which can be anticipated to lack good adaptation to potential damaging agents (Burdon and Carson 1999). In contrast, because native trees are better adapted to the local ecosystems and are thus not facing such large unexpected biological risks, smaller breeding population sizes may suffice. Breeding population size alone is perhaps not enough when considering the biological and genetic consequences of genetic diversity of a breeding program, but also other populations in the breeding stock are of importance. For instance, the size of the recruitment population, and the degree of selection it has been exposed to, can be decisive in determining the resistance of breeding stock to some damaging agent. Usually there are several geographically or oth-

erwise separate, but still linked breeding populations, which can to some extent enable a change of material, thus increasing the breeding population size de facto. This is an aspect that can be interpreted as a multiple population approach (Namkoong et al. 1988, Eriksson 2001). Also quality and functioning of the existing gene conservation program affects the required breeding population sizes (Yanchuk 2001).

In summary it can be stated that calculations based on preservation or fixing of rare favourable or neutral alleles lead to higher breeding population sizes (from several hundreds to thousand), whereas from the point of view of the obtainable genetic gain clearly smaller population sizes (less than a hundred) are sufficient (Kang and Nienstaedt 1987, White 1992, Danell 1993, Kang et al. 1998, Rosvall 1999, Eriksson and Ekberg 2001, Yanchuk 2001). This is also much the same distinction that can be seen between the breeding population sizes based on theoretical studies and those based on practical experience.

In practice, the breeding population sizes applied in many advanced generation breeding programs are of the order of 300 to 400, with ranges from less than 200 to close to 1000 (White 1992). These figures are more in line with the needs of genetic conservation than of genetic improvement. As examples of breeding programs that are more in line with theoretical calculations of genetic gain, the proposed breeding strategies for Scots pine and Norway spruce in Sweden, (Strategi för framtida... 1995) and for conifers in British Columbia (Yanchuk 2001), have breeding population sizes of less than 100. Very large breeding populations could be advocated as a safety measure when dealing with such new undomesticated crop plants as forest trees. However, with accumulating knowledge the precautions can be relieved and population sizes diminished to boost genetic improvement and reduce costs (Namkoong et al. 1988).

3.4 The breeding program in function

Allegorically speaking, the breeding cycle is the engine of the tree breeding program, which uses genetic variation as raw material to make the end product - genetically improved forest regeneration material. The construction of the machine determines how effective it is in reaching its goal in the short and in the long term. If the raw material, i.e. the genetic variation, is depleted rapidly then the initial gains can be large, but the long-term goals are endangered. In such cases new, lower quality raw material must be imported from outside the breeding cycle. It is also possible that new end products are set for the breeding machine, which can lead to reconstruction of the machine and a need for new raw material.

In the breeding cycle the different phases and populations follow each other consecutively generation after generation, but each time at a higher genetic level. Quantitative genetic variation is created anew in each generation when the trees in the breeding population are intermated to form the next generation recruitment population (Fig. 5). In the breeding cycle, the recruitment population represents the highest level of genetic variation, which is depleted in the selection steps leading through candidate population back to a new breeding population (White 1987).

The selection phases in the breeding cycle can be considered to be sieving processes in which the frequency of the favourable alleles is increased step by step, generation by generation (Fig. 5a). Thus genetic gain is correspondingly accumulated. This approach mainly assumes additive gene effects, and thus selection for general combining ability (McKeand and Bridgwater 1998). The increase in genetic value is made at the cost of genetic variation (Fig. 5b).

The intermating of the selected genotypes to form the recruitment population restores the genetic variability (when expressed as quantitative genetic variation or genotypic diversity), but leaves the mean genetic value of the population untouched (Fig. 5). Allelic or gene diversity is not affected by the rearrangement of the genetic constitution, but it is steadily decreasing in the breeding stock due to the genetic drift driven by the limited numbers of parents and offspring. However, the decrease in genetic diversity is counteracted by stabilising natural selection, and in the long run by mutations and the breakdown of epistasis.

To make the selection process effective, a sufficiently large recruitment population growing in a proper environment must be produced. It is often preferable to divide the recruitment population, and especially the progeny tests used to derive parental breeding values, into several sites differing in power and direction of the selection pressures. The long-term genetic advance is greatest with rather modest selection intensities (Kang et al. 1998).

Multiplication populations are side steps from the breeding cycle, and can be considered as dead-ends from the point of long-term breeding since they lead only to the material exploited in the operational plantations. As the multiplication population is a selected sample of the breeding population, its genetic value is higher than that of the breeding population, but its genetic variability is somewhat lower (Fig. 5).

The mean genetic value of the production population is the same as that of the multiplication population (assuming no migration from outside), but the level of genetic diversity depends on the propagation method used. If the operational plantations are established with sexually propagated material, then they contain high levels of genetic variability because each individual is genetically unique (thus high genotypic diver-

sity). This is a similar situation to intermating the trees in a breeding cycle. However, if vegetatively propagated material is used in establishing plantations, then the level of genetic diversity remains at the most at the same level as in the multiplication population. In practice there is always some rejection of clones due to poor success in vegetative propagation, which decreases genetic diversity (Namkoong 1999).

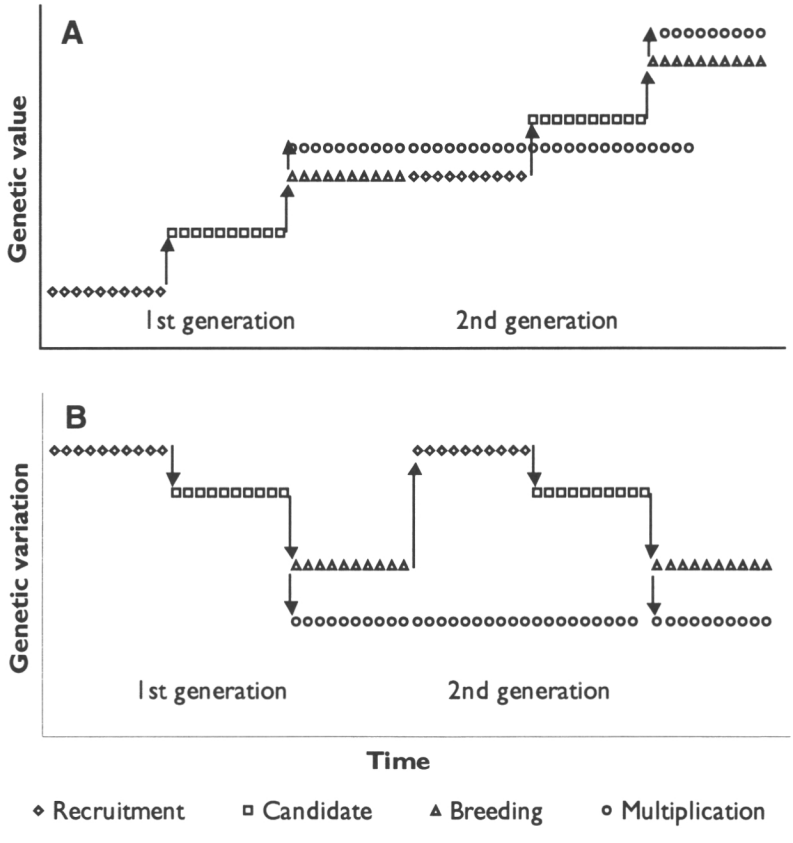


Figure 5. Schematic description of changes caused by advancing breeding in the genetic value (A) and genetic variation (B) in the central populations of a breeding program.

The production population is the last step in the dead end branch separating from the breeding cycle as the multiplication population. However, in practice also production forests can contribute to the genetic resources of the breeding stock if they contain some valuable alleles, although there are usually no systematic methods for this kind of allele harvesting. Operational plantations can become a valuable gene resource in the occurrence of some severe damage, when resistant material should be gathered

from all available sources (Burdon and Carson 1999). Thus ultimately there are no definite dead ends in the population network of a tree breeding program.

Gene resource populations also usually reside outside the breeding cycle. There are different kinds of gene resource population. In situ gene reserves are intended for preserving the genetic variation present in the species in a natural state, and can be called gene conservation populations in the meaning of Lindgren and Gregorius (1976). Gene resource populations also include clone collections, experiments and provenance tests with possibly valuable genetic material. In some breeding programs such actively managed gene resource populations are well integrated into the breeding cycle and can even be regarded as the lowest level of the breeding stock (Shelbourne et al. 1989, Burdon and Carson 1999). Genetically, gene resource populations are at a lower level than the breeding stock, but have higher genetic diversity.

The role of gene resource populations, and especially that of gene reserves aimed at preserving the original genetic variation of the species, in the context of tree breeding plan has not yet been fully settled. The whole ideology and especially practical operations in genetic conservation are rather young: for instance, the first gene reserve forest in Finland was established in 1992 (Yrjänä et al. 2000). In later generations of tree breeding there will be an inherent problem of how to utilise the gene resource populations: as the breeding stock is advancing in genetic value, the gap between it and the gene resource populations is widening. Thus any introductions from the resource populations to the breeding stock become less attractive (Zheng et al. 1998). As a whole, gene conservation can have several functions: safeguarding the possibilities for adaptation and responding to drastically changed selection goals, preservation of rare unknown genes, and carrying the ethical responsibility of species and population existence (Eriksson and Ekberg 2001).

Clone collections and specialty archives are rather static forms of gene resource management. They only preserve potentially valuable genotypes, which are not included in the breeding stock proper. The most active form of gene resource management can be utilised in gene resource plantings, which are often used in connection with breeding exotic tree species. For instance, in New Zealand gene resource plantings of *Pinus radiata* have been established using introductions from the original populations in order to broaden the genetic basis of the breeding stock. These plantings are treated to achieve "appreciable genetic improvement for basic silvicultural traits without materially eroding the genetic base" (Burdon and Carson 1999).

Selection for the same goals as in the breeding stock helps to bridge the gap in genetic value between the breeding stock and gene resource

population, but it can also lead to the loss of genetic diversity which could be valuable in the future. If the genetic variability in gene resource plantations, and also in gene reserves, is given the highest priority, then their management can be directed to increase the genetic variability, e.g. by applying such regeneration methods which also allow in natural conditions inferior trees to contribute to the next generation. Especially if there is a considerable gene flow from genetically narrowly based operational plantations to gene resource populations (e.g. Burdon and Carson 1999), it may be worth considering silvicultural operations that oppose the selection goals in the breeding stock. Current directions for the management of gene reserves predominantly advocate following the same guidelines as for regeneration in commercial forests (e.g. Koski et al. 1997).

The importance of gene resource populations for practical tree breeding is highly dependent on the species and conditions. Most in situ gene reserves can be regarded as a rather passive long-term insurance for an unexpected disastrous event, when large genetically diverse material is needed for screening for special genetic resistance against the damaging agent. At the present time, such gene resource populations function more in fulfilling the ethical aspects of preserving original genetic variability than as an integral part of a tree breeding plan. Their importance in coping with an unexpected damaging agent can also be questioned, because in some cases not even the whole gene pool of the species contains the needed resistance factors. For instance, resistance against chestnut blight (*Endothia parasitica*), a fungal disease which has totally eliminated adult American chestnut since its arrival from Asia about a century ago, has not been found in the affected species, but must be sought from Asian species with long coevolution with the pathogen (Burnham et al. 1986). Equally well, in the case of another aggressive pathogen, Dutch elm disease (*Ophiostoma* sp.), the most promising way to overcome the problem might be genetic modification of the host with genes transferred from totally unrelated organisms (Gartland et al. 2001).

Well-integrated gene resource populations are needed in the case of small breeding populations, exotic species without long coadaptation to the ecosystem, and a lack of possibilities for introduction from other breeding zones or programs. In a way, the earlier described nucleus breeding strategy is an application with a large gene resource population (main breeding group) that underpins the breeding population (nucleus). This structure will eliminate the problem of a large gap in genetic value between the breeding and gene resource populations. Breeding strategies have been suggested also with three (*Pinus taeda*; McKeand and Bridgwater 1998), or four genetically different levels (*Pinus elliottii*; White et al. 1993, Hierarchical Open Ended breeding system for corn; Kannenberg

1984, Cramer and Kannenberg 1992). The central idea in stratifying breeding stock according to its breeding value is to direct financial investments and breeding actions according to the genetic value of the material (White 1992, McKeand and Svensson 1997). The intensively managed nucleus provides material for a multiplication population and has thus a rather direct influence on the operational plantations (White 1992). The genetically lower tiers are needed to provide new genetic variability through planned gene flow to the upper levels (Fig. 4, p. 26).

Another solution involving total integration of the breeding and gene resource population is offered by the multiple population breeding system (MPBS), in which the separate small populations with diverse origins and selection goals simultaneously take care of both genetic advancement and diversity, and consequently no special actions for gene conservation are needed (Namkoong et al. 1988, Eriksson 2001, Eriksson and Ekberg 2001, Ståhl and Jansson 2002). In a manner similar to small sublines, they also capitalise the property of small populations to increase the probability of fixing rare alleles (Eriksson and Ekberg 2001). The population differences in MPBS stem, in addition to genetic drift that is present also in sublines, from differential selection pressures (Kang and Nienstaedt 1987). However, such actively managed gene resource populations can seldom be large enough to secure the preservation of the low frequency alleles.

The main advantage of MPBS in comparison to hierarchical systems is that there will be no gap in genetic value between the breeding stock and gene resource populations, which can, in practice, hamper later gene introductions to the higher levels (Eriksson et al. 1993). The method also emphasises the wide sampling of genotypes in establishing the populations. One basic presumption in MPBS is that natural populations are not necessarily optimally adapted; there may be several adaptive solutions with equal fitness (Eriksson et al. 1993). MPBS is especially suitable for breeding exotic species, where no autocthonous well-adapted populations exist. The purpose of the small subpopulations is to enhance the potential for rapid evolution and to preadapt populations e.g. to climatic change (Eriksson et al. 1993). On a higher level (e.g. the whole country), division into different breeding zones can be regarded as some kind of MPBS: different zones having different adaptational and breeding goals.

4 Material and Methods

4.1 General outline

The thesis consists of several parts. The importance of genetic diversity and the methods for managing the contradiction between gain and diversity were studied by reviewing the relevant literature. The effect of plus tree selection for the first breeding cycle was studied empirically by analysing existing plus tree and progeny test data. Deterministic simulation was used to study the selection method for the second breeding cycle, and how the founders contribute to the second cycle. Finally, a new method of sublining the breeding stock was developed to study the possibilities of enhancing genetic gain, while enabling the selection of unrelated clones for seed orchards. The results were applied to a real Finnish Scots pine breeding situation, and discussed in the context of the breeding cycle concept (White 1987).

A basic assumption of the thesis is that the species in question is of such economic importance that a rather sophisticated tree breeding program is considered necessary. The breeding objectives are assumed to be given and the biology of the species to be sufficiently well-known. The propagule production will be based on generative reproduction with conventional clonal seed orchards. A species that fits well with these qualifications is Scots pine in Finland.

4.2 Material

As the thesis was mainly based on computer simulations, physical research objects were used only in study **I** and in the application part, where Scots pine plus trees[1] and their progeny tests formed the basic material. The plus trees used as female parents for the plants in the progeny tests are part of the large body of Finnish Scots pine breeding stock that has mainly been selected from mature natural stands during a period of over twenty years (1940's to 1970's) (Oskarsson 1972). The studied trees in **I** were used to complement northern Swedish Scots pine breeding stocks

[1] In this study the term 'plus tree' refers to a tree specially selected for breeding purposes; a member of the candidate or breeding population. The word 'tree' is used to describe the members of all populations in a breeding program irrespective of their size or developmental stage.

and were therefore progeny tested in northern Sweden. There were a total of 242 plus trees allocated to two different Swedish breeding stocks: 137 trees to Tpop4, and 105 trees to the more southern Tpop7 (Table 2, Fig. 1 in **I**).

The studied variables described the conditions of the growing site of the plus tree, the stand or the plus tree itself. Site variables included latitude, longitude, altitude and effective temperature sum. The stands were divided into special seed collection stands and other stands. On plus trees themselves the height, selection intensity for height, selection age, and the emphasised character in selection of the plus trees were utilised in the analyses.

Four field trials testing progenies of Tpop4 and five trials with progenies of Tpop7 were studied (Table 1 in **I**). The trials belong to the operational progeny test series of tree breeding managed by the Forestry Research Institute of Sweden. From each plus tree, 15 to 16 one-year-old potted seedlings were planted in each completely randomised single tree plot trial. The progeny material was produced using controlled crossing with pollen mixes from Swedish Scots pine plus trees. The progenies were thus considered as half sibs. Height was measured on each tree after 13 growing seasons from sowing for Tpop4, and after 11 growing seasons from sowing for Tpop7 (Persson 1999a, b).

All data concerning the plus trees in study **I** and in the application part were drawn from the databases of the National Register of Forest Genetics, administered by the Finnish Forest Research Institute.

The observed regularities in **I** were tested with an independent material from Scots pine breeding zone no. 2 (Fig. 8a, page 49). The material from the same breeding zone was also studied more comprehensively as regards the geographic distribution of the plus trees. Scots pine breeding zone no. 2 was selected for a more detailed investigation because it has the highest number of tested plus trees of all the Finnish Scots pine breeding zones (Venäläinen et al. 1994), and is closest to entering the next breeding cycle. The studied plus tree characters in breeding zone no. 2 were the same as in I, except that the absolute plus tree height was not utilised.

The progeny height measurements in breeding zone no. 2 were unpublished material from the Finnish Forest Research Institute[2]. They are based on a large number of progeny tests of variable age (on the average 12 years) and test conditions.

[2] Data kindly provided by Mr. Juhani Hahl, who is responsible for Scots pine breeding.

4.3 Methods

4.3.1 Analysis of the plus trees and their progenies (I)

The plus trees have been selected on the basis of a combined visual criterion consisting of growth, stem quality and general vigour (Sarvas 1953). In some special cases either growth or quality has been specifically emphasised in the selection and this has been noted in the plus tree record. The difference in height between the plus tree and its neighbours has been standardised to obtain the selection intensity (*i*) (Eq. 2)

$$i = S / sd \qquad (2)$$

where *S* is the selection differential and *sd* is the standard deviation of height in the surrounding stand.

The plus tree selection method used can be described as individual or ocular selection (Oskarsson 1995) according to the definition of Simpson (1998). The plus trees were then measured and selection differentials and intensities were calculated, but the result of the measurement did not affect the selection of the tree; it only noted the outcome of the selection.

The height growth of the plus tree offspring was analysed with analysis of variance according to a linear model (Eq. 2 in **I**) to reveal stand and tree effects. Correlation and regression analyses were performed to study the association between the progeny height and plus tree selection intensity and other plus tree characteristics. These analyses were performed using the SAS statistical software package (SAS Institute Inc. 1989, 1996).

4.3.2 Comparison of backward and forward selection (II)

Genetic gains for backward and forward selection were compared using deterministic computer simulation. Normally distributed selection intensity values were predicted for each plus tree and the best of its offspring using a quantity called normal order statistics. Breeding values for the parent trees were further derived from these predicted selection intensity values using equations 2 and 5 in **II**. A plus tree that was acting as a mother formed a family together with its progeny. Either the plus tree or its best offspring was selected from each family. The normal order statistics for a population of 100 plus trees were generated by the SELEINT2[3] program developed by professor Dag Lindgren (Lindgren 2002b).

[3] the program is available at
http://www.genfys.slu.se/staff/dagl/Breed_Home_Page/.

The genetic gain for each of the compared selection alternatives was calculated as the mean of the breeding values of the individual plus trees or their best offspring. In addition to these pure selection alternatives, two cases with combined backward and forward selection were also studied. Backward selection was also compared with forward selection at the family level in order to determine in which families backward selection was the preferred choice. Heritability and selection intensities were altered in order to study their effects on the outcome of comparison between backward and forward selection. In addition, two genetic levels of male parents (unimproved and equal to female parents) and two family selection ratios (no selection and 50 % culling) were also studied.

4.3.3 Group coancestry and status number (**III, IV**)

Gene diversity was measured in this study by means of group coancestry (Θ) (Cockerham 1967) or, equivalently, by status number (N_S) (Lindgren et al. 1996, 1997a, Lindgren and Kang 1997), which is a measure derived from group coancestry. It is a pedigree-based concept and assumes that some sort of reference population is defined, to which the studied population is referred (for tree breeders this is typically the wild forest from which the initial plus trees were selected). Group coancestry is the probability that two alleles taken at random from the gene pool, with replacement, are identical by descent (Cockerham 1967). Group coancestry can be calculated with different formulae in different situations and with different assumptions, but the general basic formula is given in Eq. (3) (Lindgren and Mullin 1998)

$$\Theta = \sum_{i=1}^{n} p_i \sum_{j=1}^{n} p_j \Theta_{ij}, \qquad (3)$$

where p_i and p_j are probabilities for sampled alleles originating from genotypes i and j, respectively, and Θ_{ij} is the coancestry between genotypes i and j.

Status number is derived from group coancestry as (Lindgren et al. 1996).

$$N_S = \frac{1}{2\Theta} \qquad (4)$$

Status number can be regarded as the effective size of the population, equivalent to the census size of a population of non-inbred, unrelated individuals, with the same group coancestry (Kang 2001). It can be understood as the allele-carrying capacity of a population in terms of the expected number of founders that would be required to provide the same level of gene diversity, if the founders were equally represented and if no genetic drift had occurred (Rosvall 1999).

Group coancestry describes the loss of gene diversity compared to the base population. Gene diversity (GD) can thus be expressed as

$$GD = 1 - \Theta = 1 - \frac{0.5}{N_S} \qquad (5)$$

The different concepts of effective population sizes and indicators of genetic diversity are discussed in Kjær (1996), Rosvall (1999) and Kang (2001).

4.3.4 Contribution to the second generation (III)

The contribution of first generation plus trees to next generation was studied for a hypothetical situation with a large number of tested plus trees (candidate population). Controlled crosses of the best plus trees were made in order to generate the second generation recruitment population. The number of crosses in which a plus tree was involved was related to its breeding value: they were used in zero (rejected tested plus trees with a low breeding value), one (the plus trees with the lowest accepted breeding values), two (intermediate plus trees) or three crosses (fraction with the highest breeding values) (Fig. 1 in **III**). All the families were of equal size. Only one selection was subsequently made from each family. The number of crosses and number of selected offspring were therefore equal. This formulation was relatively easy to use as a basis for the calculations.

This variable (3:2:1) contribution method was compared to the conventionally used equal contribution method, in which all members of the breeding population pass equal number of offspring to the next breeding population (in stable situation two), and any fluctuations in this number are accidental. In this study, genetic gain was regarded as being proportional to the selection intensity related to the choice of plus tree parents. Cost was kept constant by fixing the number and size of the families. The gene diversity was described using group coancestry or status number (N_S). In the basic comparison the gene diversity was set equal between the two compared contribution methods, although increased and decreased diversity levels were also studied. The lowering effect of a higher contribution of the best plus trees to the gene diversity was compensated by increasing the size of the breeding population (Fig. 1 in **III**). Also the borderlines between differently contributing plus tree fractions could be altered in order to retain the gene diversity.

The comparisons were accomplished with deterministic computer simulations. Maximum obtainable genetic gains were calculated with dif-

ferent candidate population sizes (N_{R1}), fraction divisions and levels of genetic diversity (Table 1 in **III**).

4.3.5 Stratified sublining (**IV**)

A stratified sublining system was studied by means of a deterministic infinitesimal model. In stratified sublining, the sublines were initially formed by positive assortative grouping of parents according to their breeding values. The breeding population was assumed to be very large and the population structure to be regular: this was done for analytical convenience in using a deterministic approach. A breeding population of N unrelated, non-inbred genotypes with a normal distribution of breeding values was subdivided into $N/4$ sublines (i.e. each subline was composed of four trees). In stratified sublining, the assignment of the plus trees to sublines was done according to their ranking for breeding value, whereas in parallel sublining they were randomly assigned to sublines. Subsequent matings were made among the two best individuals from the two families within the sublines, and the sublines maintained at size four (Fig. 2 in **IV**). The matings were made according to the balanced, double first cousin method (Fig. 1 in **IV**)

Generation turnover was depicted by means of discrete breeding cycles (White 1987). In each cycle, the trees selected for crossing constituted the breeding population. The offspring of the trees in the breeding population were referred to as the recruitment population from which the new breeding population members were selected. Stratified and random allocation to sublines were compared by evaluating the genetic value of the seed orchards that each approach could provide. The seed orchards were established by first selecting the best individual from each subline, and then a given best proportion ($N/8$) from the selections. The genetic gain of the seed orchard was the sum of three terms: the accumulated average gain in the breeding population by selection in previous generations, the expected gain by selecting the best in each subline as a seed orchard candidate, and the gain by selecting the best among the candidates to serve as seed orchard parent (see **IV** for details). The simulation was continued for ten generations.

4.3.6 Application to a real breeding stock

In applying the studied principles to the material of Scots pine breeding zone no. 2, the breeding stock was divided into two parts according to a nucleus breeding strategy. This differs from the current long-term breeding plan for Scots pine (Pitkäntähtäyksen… 1989), but is in line with recent intentions to restructure the breeding plan (Haapanen et al. 1999, Mikola 2002). In the breeding population comparisons the size of the

whole breeding population was set to 288 and the nucleus size to 48. These sizes are slightly lower than those suggested by Mikola (2002), but they were selected to simplify the calculations.

The material used for the applied part of the work was taken from the operational Finnish forest tree breeding program and the obtained data were thus to some degree already processed. The plus tree data from breeding zone no. 2 were treated similarly as in **I**, with the few exceptions concerning measured characters as mentioned in Material. The analyses included analysis of variance for testing differences in progeny height growth between different groupings of plus trees, and correlation and regression analyses for the dependencies between progeny height growth and several plus tree characters. χ^2-test for goodness of fit was performed to measure whether there were differences in the performance of progeny height growth of plus trees from different stands or stand types. In order to study the applicability of selection intensity as a predicative indicator, the division of the candidate population to rejected, main breeding group and nucleus was also carried out according to the selection intensity. These analyses were performed with SPSS® Base 10.0 statistical software (SPSS Base... 1999).

The geographical distribution of the plus trees was studied visually, and the effect of concentration of plus trees to the same stands was analysed under different assumptions of relatedness within a stand. The required group coancestries and status numbers were calculated as described earlier.

The genetic values for the plus trees were weighted performance level values (Hatcher et al. 1981), in which weighing was done with the number of tests and test reliability (Venäläinen 1993, Venäläinen et al. 1994, Venäläinen and Ruotsalainen 2002). These values were standardised to N(0,1) scale in order to reconstruct the selection intensity values (corresponding to the normal order statistics in **II**) for the plus trees. The genetic values for the plus tree and its best offspring were obtained as described in **II**, but they were expressed on an absolute scale to make the results more comprehensible.

The genetic gain of phenotypic plus tree selection in breeding zone 2, when measured in 10-year-old open pollinated progeny tests, is 0.6 dm (1.8 %) (Venäläinen et al. 1994). Additive genetic standard deviation (σ_A = 3.0 dm) was derived according to Eq. (6) using data from tables 1 and 3 in Haapanen (1996).

$$\sigma_A = \frac{\sqrt{4\sigma_f^2}}{\bar{x}_H}\bar{x}_{BZ2} \qquad (6)$$

where σ_f^2 is the family component of variance (Haapanen 1996), $\bar{x}_H$ is the test mean in material according to Haapanen (1996), and $\bar{x}_{BZ2}$ is the mean of progeny tests in breeding zone no. 2 in material according to Venäläinen et al. (1994). The genetic value of plus tree *i* (GV_i) was thus predicted by Eq. 7 as the sum of the mean of the reference material, genetic gain of phenotypic plus tree selection (twice that expressed in open pollinated progeny tests) and a plus tree-wise factor indicating the deviation of the plus tree mean from the population mean (G_{2i} is genetic gain from plus tree selection depending on the ranking of the plus tree according to **II**).

$$GV_i = 32.7dm + 1.2dm + G_{2i} \times 3.0dm \qquad (7)$$

Other parameters needed in the calculations were given the following values: individual heritability (h^2) 0.2, and family size (n) 100.

To estimate the maximum time delay allowed for an alternative breeding program (2) an equation for the acceptable time delay (ATD) was defined as

$$ATD = \frac{G_{2e} - G_{1e}}{G_{1e} - G_{1b}} t_1, \qquad (8)$$

where *G* is the genetic value of the breeding population at the beginning (subscript *b*) or the end (subscript *e*) of the breeding cycle for the alternative breeding programs (subscripts *1* and *2*) and t_1 is the generation length in breeding program 1. In other words, the ATD is the additional genetic gain obtained by the alternative breeding program divided by the mean annual genetic gain of the base program.

Single pair mating (SPM) was used to study the effect of sublining of breeding stock as it forms the first stage of sublining and is the simplest mating system and thus suitable for a model study. Single pair mating was made both assortatively and randomly. These correspond to the crossing method used in creating stratified and parallel sublines according to **IV**. The two mating systems were compared in terms of variances between family means and as genetic values of seed orchards, which could be drawn from the two mating systems when assuming selection of best offspring from each family and a heritability value of 0.2. The alternatives were compared at different seed orchard sizes. The random mating was repeated 10 times and the mean of these separate runs was compared to the assortative mating alternative.

5 Results

5.1 Selection of founders to a breeding program

About 80 % of the additive genetic variation of height growth was within the stands in 11 to 13 years old progeny tests in the breeding stocks Tpop4 and Tpop7 of *Pinus sylvestris*, the rest being between the stands. The plus trees selected from the seed collection stands did not produce progeny with better height growth than the plus trees from other stands (**I**). Slight positive correlation was observed between parental selection intensity and offspring height growth ($r = 0.143$, $p = 0.0951$ and $r = 0.247$, $p = 0.0120$ for Tpop4 and Tpop7, respectively). The progeny height increased by 1.9 cm (0.7 % of the population mean) and 2.5 cm (1.2 %) with an increase of one unit of selection intensity of the plus trees in Tpop4 and Tpop7, respectively (Table 5). There was no clear indication of dependence between plus tree age at selection and progeny height growth. Plus trees with a more northern origin had progenies with better height growth. However, this was, in addition to selection intensity the only other plus tree character connected to the progeny height growth (**I**).

In Scots pine breeding zone no. 2, no statistically significant effects of selection criteria on the progeny height growth could be detected ($p = 0.731$ for F-test in one-way analysis of variance). Neither was there any statistically significant differences between the progenies of plus trees originating from different stands, as deduced on the proportion of the tested plus trees qualifying for the breeding population ($\chi^2 = 10.125$, $p = 0.519$). Neither was there any difference in the performance of plus trees from seed collection stands and other stands ($\chi^2 = 0.887$, $p = 0.346$).

The progeny height increased by 5 cm (1.5 % of the population mean) with an increase of one unit of selection intensity (Fig. 6) The dependence was statistically highly significant ($r = 0.175$, $p = 0.000$). The temperature sum of origin did not influence progeny height growth, but the age of the plus tree at the selection was negatively correlated with progeny height growth ($r = -0.140$, $p = 0.006$). This effect was partly independent of the negative correlation between selection intensity and selection age ($r = -0.153$, $p = 0.002$), so that in a regression model with both these characters as independent factors an increase in selection age of 10 years decreased progeny height by 2 cm (Table 5). In the material of this breeding zone, statistically significant dependencies appeared between selection year and several plus tree characters. Plus trees which were selected later were younger ($r = -0.198$, $p = 0.000$), were from colder areas ($r = -0.356$, $p = 0.000$) and had a higher selection intensity ($r = 0.183$, $p = 0.000$).

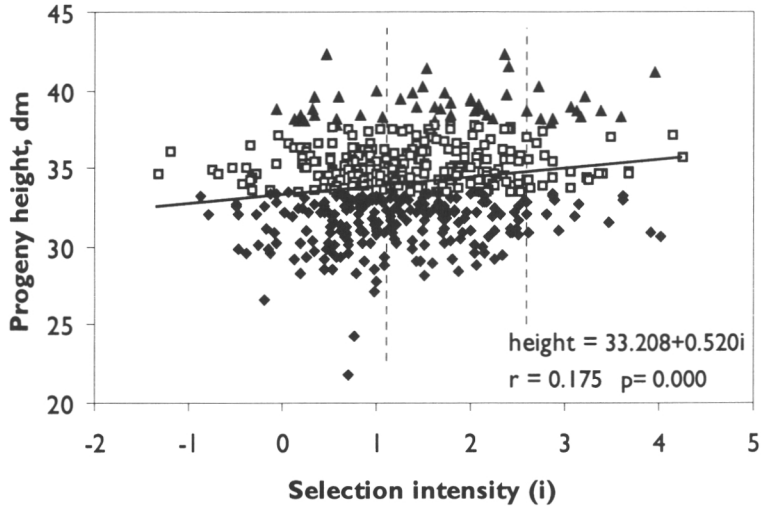


Figure 6. Dependence of progeny height on plus tree selection intensity in Scots pine breeding zone no. 2. (▲ = nucleus tree, □ = main breeding group tree, ◆ = rejected tree). Vertical dashed lines denote division of the population into nucleus (right) and main (middle) on the basis of selection intensity.

Table 5. Parameters for regression equations describing dependence of estimated plus tree genetic value (in dm) in the Scots pine breeding stocks (Tpop4 and Tpop7, from I) and breeding zone no. 2 (BZ2) on selection intensity and selection age of the plus tree.

Material	Intercept	Selection intensity		Selection age, years		R^2
		b	p	b	p	
Tpop4	25.6	0.19	0.095			0.020
Tpop7	20.1	0.25	0.012			0.061
BZ2	33.2	0.52	0.000			0.031
BZ2	35.3	0.33	0.027	-0.019	0.016	0.032

When the candidate population of breeding zone no. 2 was divided on the basis of selection intensity into nucleus (the best 48 trees), main breeding group (next 240 trees) and rejected trees (rest 402 trees), the obtained genetic gain was 4 % for the nucleus and 1 % for the main breeding group. These values can be compared to the gains obtained on the basis of progeny testing (15 % and 4 %, respectively) (Fig. 7). 12 plus trees were correctly classified as nucleus trees on the basis of their selection intensity. This is more than twice as many plus trees as would be

achieved by random allocation. On the other hand, 15 nucleus trees would have been totally culled from the breeding population (Fig. 6).

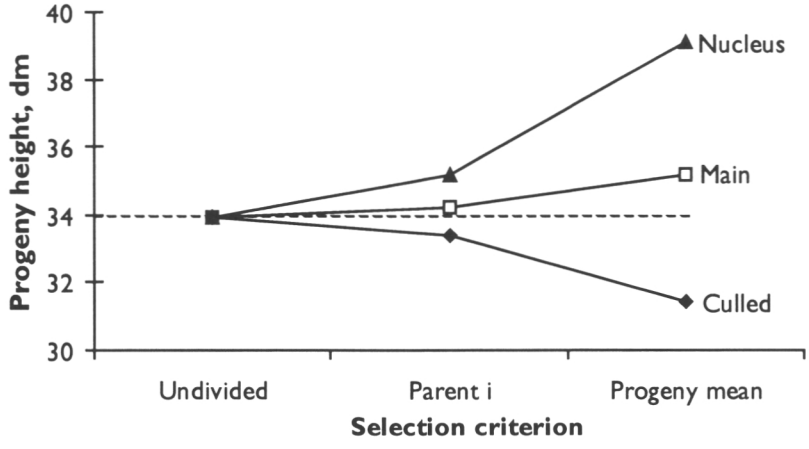


Figure 7. Progeny mean height when division into different groups is based on different criteria in Scots pine breeding zone no. 2. (Symbols as in Fig. 6).

Table 6. Effect of common ancestry within the stand on the absolute (N_S) and relative (N_r) status number of the whole breeding population (N = 288) and its nucleus (N = 48) in Scots pine breeding zone no. 2. The probability that two trees originated from the same stand was 0.012 for the whole breeding population and 0.011 for the nucleus. Different degrees of coancestry were assumed between the trees within a stand.

	Generations since common ancestor	Coancestry among trees within the same stand	N_S	N_r
Whole breeding population (N=288)	1	0.125	154.9	54
	2	0.03125	237.1	82
	3	0.0078	273.3	95
	4	0.00195	284.2	99
	5	0.00049	287.0	100
Nucleus (N=48)	1	0.125	42.7	89
	2	0.03125	46.6	97
	3	0.0078	47.6	99
	4	0.00195	47.9	100

There were rather many stands with several plus trees in the breeding population of breeding zone no. 2: the maximum in the whole breeding population was 16 (5.6 % of total number of trees) and in the nucleus 4

(8.3 %). A total 33 % of all the plus trees in the breeding zone came from stands with 5 or more trees. The effect of this grouping on the status number of the breeding population or its nucleus depended on the assumed coancestry among trees in the same stand. The effect of the observed level of grouping on the status number was low, unless very close relatedness (trees half sibs) within a stand was assumed (Table 6.).

The geographic distribution of the phenotypic plus trees in breeding zone no. 2 showed clear signs of clustering, with large empty areas in the south-central and northern areas (Fig. 8b). Tree culling according to the progeny test results when constructing the breeding population and nucleus enlarged the empty areas to some extent, but did not lead to a severely skewed distribution (Figs. 8c, d).

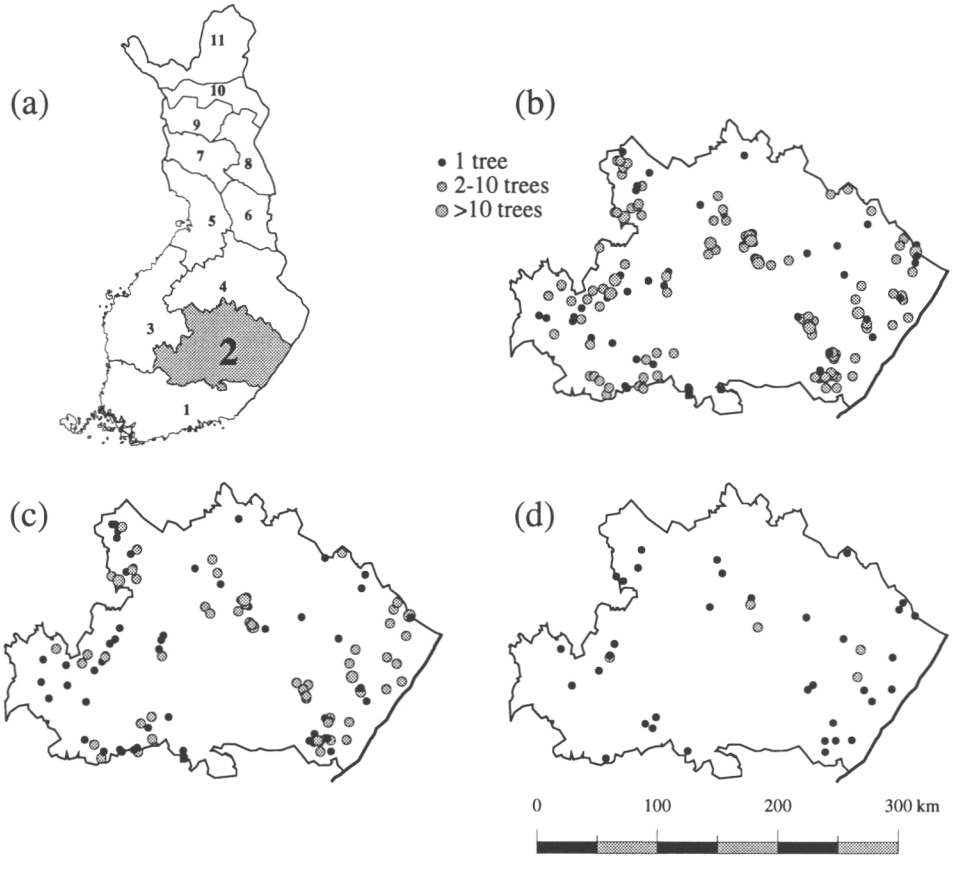


Figure 8. Geographic distribution of Scots pine plus trees in breeding zone no. 2 in Finland. a) Scots pine breeding zones, b) all phenotypic plus trees (n = 690), c) plus trees in whole breeding population (n = 288), d) plus trees selected to the nucleus of the breeding population (n = 48)

The standwise concentrations of plus trees in breeding zone no. 2 were no exceptions, when compared to the situation in the whole country. In northern Finland especially, large numbers of plus trees have been selected from the same stands, the maximum being 100 plus trees selected from stand no. 548 (Table 7). This corresponded to 17 % of all the plus trees in the breeding zone. The number of plus trees per hectare remained below 5 in all but one seed collection stand.

Table 7. The Scots pine seed collection stands with the greatest number of selected plus trees (data from the National Register of Forest Genetics)

Stand No	Breeding zone	Commune	Size of the stand, ha	No of plus trees	% of all trees in the zone
174	5	Pudasjärvi	75.0	100	17
187	9	Sodankylä	250.0	99	12
757	8	Savukoski	33.0	75	13
630	7	Ylitornio	33.0	67	6
548	9	Sodankylä	6.0	64	8
596	6	Puolanka	14.0	62	11
585	7	Pello	12.0	53	5
594	6	Puolanka	25.0	53	9
42	2	Tohmajärvi	13.0	51	6
586	9	Kittilä	25.0	51	6

5.2 Constructing the first cycle breeding population

The higher the ranking of the plus tree, the more favourable it was to select backwards (Fig. 1 in **II**). Depending on the combination of parameter values, backward selection was superior to forward selection for open pollinated progeny in 1 to 57 % of the top ranking families. High genetic gain in the phenotypic plus tree selection in the forest, low heritability and small progeny size, favoured backward selection (Table 3, Figs. 2, 3 and 4 in **II**). As the order of superiority between backward and forward selection strongly depends on the rank of the family, the result of selection at the population level was dependent on the degree of culling families. If the size of the candidate population was large in relation to the breeding population, only top-ranking families were needed, and thus backward selection gave the highest genetic gain on the average. In the opposite case, where most families had to contribute to the breeding population, forward selection was the best overall solution (Table 2 in **II**). In order to reach the highest genetic gain, a combined strategy can be ap-

plied by selecting backward in the best families and forward in the lower ranking families. When the better half of the families was contributing to the breeding population, the combined selection strategy gave, in the maximum case, 7 % higher genetic gain than any of the methods separately (Table 2 in **II**). The average difference between the plus tree and its best offspring was often, however, small.

Backward selection in families with plus tree fathers was usually not favourable for more than a few percent of the families. Only when heritability was low could it be favourable to select 10 % of the top-ranking mother trees (Table 3 in **II**). This relationship was not dependent on the genetic gain in phenotypic plus tree selection. If the phenotypic selection had yielded no gain at all, then forward selection from open pollinated and polycross families would, of course, give equal results, which highly favour forward selection.

The distribution of the predicted genetic values of plus trees in breeding zone no. 2 followed rather closely the theoretically expected values of the normal distribution, with only a few irregularities (Fig. 9). The 54 highest ranking plus trees in the candidate population had higher breeding values than their best open pollinated offspring (Fig. 9). This represented 7.8 % of all of the plus trees tested, and 18.8 % of the trees in the breeding population. Combined selection of either the original plus tree or its best open pollinated offspring led to an increase of the first cycle breeding population mean by 0.9 dm from 36.4 dm, which was obtained when using only backward selection. This was 23 % of the genetic gain obtained with backward selection, including the gain from phenotypic plus tree selection. The mean genetic value of the nucleus was not influenced

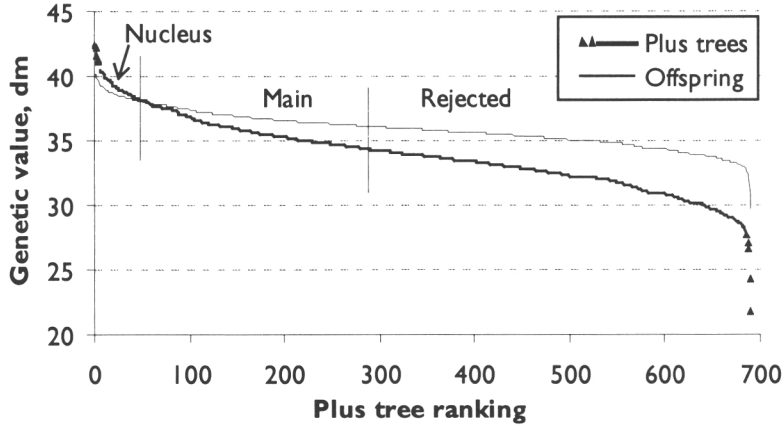


Figure 9. Standardised genetic values for the candidate trees in Scots pine breeding zone no. 2 with the predicted values of their best offspring in progenies of size 100.

by combined backward and forward selection, because the applied nucleus size was such that all recruitments to nucleus could be made backward. When assuming within-family selection of one from 100 with a heritability of 0.2, the expected means of the second cycle breeding population would be 39.0 dm for backward selection and 39.9 dm for combined backward and forward selection. These values gave an acceptable time delay (Eq. 8) of 8.3 years.

The genetic diversity was affected by the mating design and family structure. When total background pollination was assumed, the gene diversity was higher in open pollinated progeny than in full sib families (Table 8). If the plus trees were non-inbred and unrelated, then the status number of the parent population and a population consisting of one open pollinated offspring from each tree was equal, provided that none of the selected offspring shared a common male parent. Selecting a plus tree and its offspring gave a lower status number than selecting an equal number of unrelated trees, but the decrease was lower when the offspring originated from open pollination than if they also shared the other parent (Table 8).

Table 8. Status numbers of a breeding population of size N = 50 consisting of families of different size with and without the parent tree.

Family size	Status number			
	Parent included in family		Parent not included in family	
	Half sib offspring	Full sib offspring	Half sib offspring	Full sib offpring
1	50.0[a]		50.0	50.0
2	33.3	33.3	40.0	33.3
3	27.3	25.0	33.3	25.0
4	23.5	20.0	28.6	20.0
5	20.8	16.7	25.0	16.7

[a] It is not relevant to speak of family structure when only the plus tree is selected

Maternal half sibs in a polycross or wind-pollinated progeny may share a father. The gene diversity of the selections rapidly decreased with increasing proportion of common paternity within the assumed maternal half sib families (Table 9). The decrease was especially pronounced when selecting within half sib families with high degrees of common paternity. When all the selections shared the same father, the status number of the population was close to 4, which is the theoretical limit for a half sib family.

Table 9. Status numbers of a breeding population of size N = 50 consisting of either parent trees and open pollinated offspring or members of open pollinated families. Different degrees of common paternity are assumed.

Percentage of offspring with common paternity	Status number		
	Mother and offspring	Two half sibs	Five half sibs
0	33.3	40.0	25.0
5	30.3	39.4	24.8
10	27.8	37.0	23.8
20	23.8	29.4	20.4
50	16.7	11.8	10.0
100	11.1	3.7	3.5

5.3 Founder contribution to the 2nd cycle

Maximum genetic gain in the 2nd generation breeding stock with constant gene diversity was obtained by increasing the size of the 1st generation breeding population by about 20 %, and allowing the trees to contribute to the 2nd generation in the ratio 3:2:1 according to their genetic value. The optimum breeding population size increased with increasing gene diversity, and also slightly with increasing candidate population (referred to as recruitment population in **III**) size (number of tested plus trees). The size of the highest contributing fraction of trees in the breeding population increased with decreasing gene diversity and increasing candidate population size (Table 2 in **III**).

The additional genetic gain obtained by variable contribution was greater with lower gene diversity and a smaller candidate population size. In the case where the candidate population size (N_{R1} in **III**) was twice the breeding population size, the between-family genetic gain could be increased by 14 % with this method without affecting the gene diversity (Table 3 and Fig. 4 in **III**). Most of the additional genetic gain was obtained after the first few additional plus trees had been added to the breeding population. The response curve of genetic gain against breeding population size was rather flat close to the maximum (Fig. 6 in **III**).

In the case of unaltered gene diversity, the size of the highest contributing breeding population fraction (3 offspring) was equal to the increase in breeding population size from the initial stage of equal contribution (Fig. 3 in **III**). The lowest ranking half of the breeding population contributed only one offspring to the next generation breeding stock, and the remaining mediocre one-third contributed two offspring. If the targeted

gene diversity was changed from the starting situation, the breeding population size and the proportions of differently contributing fractions were also changed. Decreased gene diversity led to a smaller breeding population size and to an increasing proportion of trees contributing three offspring. Increased gene diversity also increased the breeding population size, but decreased the size of the highest contributing fraction (Fig. 3 in **III**).

Table 10. Sizes of differently contributing fractions of the breeding population, status numbers and genetic values in the 2nd cycle recruitment population (RP) in Scots pine breeding zone no. 2 with different mating and selection alternatives. N_{R1} = 690, Nr = 0.667

N_2	n_3	n_2	n_1	N_1	N_{S2}	2nd cycle RP means, dm	
						Backward	Back+ Forward
288	0	288	0	288	192	36.4	37.3
288	59	111	177	347	192	36.8	37.5
200	42	74	126	242	133.3	37.5	38.0
150	32	54	96	182	100	38.0	38.3
100	21	36	66	122	66.67	38.6	38.7
48	0	48	0	48	32	39.4	39.4
48	11	15	33	59	32	39.6	39.6

Note: N_{R1} = 1st generation candidate population size, Nr = relative 2nd generation status number, N_2 = 2nd generation breeding population size, n_3, n_2, n_1 = number of 1st generation breeding population trees contributing 3, 2 and 1 offspring to the next generation, respectively, N_1 = 1st generation breeding population size, and N_{S2} = status number of the 2nd generation breeding population.

Application of the variable contribution method (**III**) to the breeding stock of Scots pine breeding zone no. 2 increased the first generation breeding population size from 288 to 347 (20 %) (Table 10). The obtained increase in genetic value was only a few centimetres but, when related to the total genetic gain, it corresponded to a 9 % increase in pure backward selection and a 6 % increase in combined backward and forward selection. Decreasing the breeding population size without changing the relative status number increased the genetic value of the next generation recruitment population. The increase was 6 % (from 36.4 dm to 38.6 dm) when the targeted status number of the second cycle breeding population was 66.7. In the second cycle recruitment population nucleus created with equal contribution, the genetic value was 8 % (39.4 dm vs. 36.4 dm) higher than in the base alternative, and when variable contribution was also applied to the nucleus the genetic value further increased slightly. However, the increase obtained by variable contribution was

smaller in the nucleus than in the whole breeding population (0.2 dm vs. 0.4 dm). The improvement obtained by variable contribution was smaller in the breeding stock consisting of both backward and forward selected material than in purely backward selected material.

The status number in the second cycle breeding population after double pair mating and balanced recruitment was 192, and in the nucleus 32 on the condition of unrelated non-inbred plus trees. These figures were 67 % of the respective census numbers. The status number of the second cycle breeding population was the same irrespective of whether plus trees or their open pollinated offspring were used.

5.4 Sublining the breeding stock

The group coancestry increased steadily within a subline consequently leading to a decrease in the gene diversity. Inbreeding started to accumulate from generation four onwards, as matings between relatives started (Table 3 in **IV**). This build-up of coancestry and inbreeding followed the same course in both the stratified and parallel sublining system. Differences between the stratified and parallel sublining arose in the second and third generations, when the among-subline variance decreased in parallel sublining, but increased in stratified sublining (Fig. 3 in **IV**). This difference offered an option for a higher selection difference and thus a higher genetic gain for multiplication populations in stratified sublining in contrast to parallel sublining. This occurred in spite of the fact that the genetic gain of the breeding population as a whole remained unchanged (Fig. 4 in **IV**).

For the case studied, stratified sublining gave 18 % higher genetic gain than parallel sublining in the multiplication population with contributions from the better half of the sublines (Fig. 5 in **IV**). The difference in genetic gain corresponded approximately to an advantage of one generation in the cumulative genetic gain.

The variances of the family means in the second cycle recruitment population of Scots pine breeding zone no. 2 were twice as high with stratified sublining as with parallel sublining (2.84 dm vs. 1.34 dm in backward selection, and 1.28 dm vs. 0.62 dm in combined backward and forward selection, respectively). Stratified sublining enabled the establishment of seed orchards with a higher genetic value than parallel sublining. The advantage when using backward selection was 1.3 dm with the smallest seed orchard size, and decreased with increasing seed orchard size (Table 11). The advantage corresponded to a 14 % increase in genetic gain when only the best 9 sublines were utilised for the seed or-

chard, but only about a 5 % increase when the best half of the sublines were used. If all 144 families were utilised to construct the seed orchard, then both sublining alternatives generated seed orchards with equal genetic value. The advantage of stratified sublining was smaller with combined backward and forward selection than with pure backward selection.

Table 11. Effect of sublining method on the genetic value (dm) and increase in genetic gain by stratified sublining as a function of seed orchard size (number of sublines utilised) in Scots pine breeding zone no. 2 (N = 288).

No. of sublines utilised	Backward selection		Combined backward and forward selection		Increase in genetic gain, %[a)]	
	Parallel	Stratified	Parallel	Stratified	Backward	Combined
9	41.7	43.0	41.9	43.0	14.4	12.0
18	41.2	42.3	41.4	42.3	12.9	10.3
36	40.6	41.4	41.0	41.4	10.1	4.8
72	39.9	40.3	40.5	40.7	5.6	2.6
144	39.0	39.0	39.9	39.9	0.0	0.0

[a)] Compared to the increase from the mean of the unselected population (32.7 dm) to the corresponding parallel sublining alternative

6 Discussion

6.1 Representativity of the plus trees

An essential first step in starting a tree breeding program is selection of the founders (Zobel and Talbert 1984). In this study a situation is considered where the breeding program is started from zero level without any earlier breeding activities. Thus the founders are phenotypical plus trees selected from wild forest or unimproved plantations and, in the context of the breeding cycle, they form the candidate population of the first generation. The selected plus trees have to be phenotypically superior to their neighbours in respect to one or several characters. In the early days of plus tree selection very high criteria were set for the plus trees, leading to a small number of selections per unit of cost and time used (Oskarsson 1995, Puri 1998, Lee 1999). Later the opinions changed when the plus trees were understood more as the starting material for a breeding program than the final product as such (Oskarsson 1995, Simpson 1998, Lee 1999). It has also been emphasised that, in plus tree selection, balanced sampling of the genetic material should be given high priority (Kang and Nienstaedt 1987). In later years less rigorous selection leading to higher productivity of the selection work has also been utilised in Finland. In some cases this process was also accelerated by the need for more material for clonal seed orchards (Oskarsson 1995).

Relatedness of the selected trees is not wanted because it will cause inbreeding in the regeneration material and diminish the future gains by decreasing the genetic variability. Selection of related trees is precluded by setting limitations for the number of trees selected from a single stand, or for the distance between selected plus trees (Lindgren and Gregorius 1976, Zobel and Talbert 1984, Puri 1998). In spite of such precautionary measures, it is clear that the geographic distribution of the selected plus trees is far from even both at the national level (Yrjänä et al. 2000) and within a breeding zone (Fig. 8). For instance, up to 100 plus trees have been selected in a single Finnish Scots pine stand (Table 7), which is equivalent to 17 % of the total number of selections in that breeding zone. There are also several other cases where more than 10 % of the material in a breeding zone originates from one stand only.

In the more comprehensively studied Scots pine breeding zone no. 2, the apparently skewed recruitment to the breeding population or its nucleus did not severely reduce the gene diversity unless a very close relatedness between the trees within the stands was assumed (Θ_{ij} = 0.125, meaning that trees from the same stand are half sibs) (Table 6). The group

coancestry (Θ) within Scots pine stands probably varies between stands, but it is considered to be at much lower level, about 0.02 (Rosvall 1999, Rosvall et al. 2001). Not even the effect of the stand representing 17 % of all the selected plus trees in its zone would be detrimental for the gene diversity if this level of group coancestry is assumed. The overall probability that plus trees originate from the same stand remains at a rather low level unless the number of contributing stands is very low. This explains the low impact of the observed plus tree concentrations on the gene diversity. The question concerning the effect of plus tree concentrations on the genetic diversity could be better elucidated by using DNA marker techniques to quantify the degree of relatedness within a stand (e.g. Lambeth et al. 2001). Even a moderate relatedness between the selected trees will make the inevitable decrease in gene diversity during the generations much less drastic (Andersson 1999).

The reasons for the clustering and uneven geographic distribution of the originally selected phenotypic plus trees are difficult to explain. The plus tree selection was performed in cooperation with the local professional foresters (Oskarsson 1995), so perhaps their activities and perceptions of good stands and trees have directed the selection work. One partial explanation could be that an important goal in Finnish tree breeding has been, in addition to genetic improvement, the establishment of seed orchards on a large scale in order to secure seed production for northern Finland (Oskarsson 1995). This has probably resulted in the emphasis on finding the required number of plus trees at the expense of an even geographic distribution. This is in line with the fact that the majority of the concentrations occur in northern Finland.

On the whole, the geographic distribution of the plus trees can be regarded as satisfactory. As far as the effective gene flow of Scots pine is concerned, there are no reasons to assume that some important genetic variation has escaped sampling, although there are some holes on the distribution map of plus trees. However, it might be wise to restrict to some extent the proportion of trees selected from a single stand for the breeding population in order to maintain better geographic coverage, at least in the main breeding group (Kang and Nienstaedt 1987). There seemed to be no indications that selection for height would have favoured some stands (**I** and breeding zone 2) but, in order to avoid the possible occurrence of random bias, some consideration should be given to the representativity of the breeding stock for the whole breeding zone. In some breeding zones it can become difficult to retain the alleles of coastal Scots pine in the breeding stock due to their poor productivity (Velling and Nepveu 1986). These trees may, however, possess genetic adaptations to coastal conditions that could prove to be valuable in the future.

6.2 Selection efficiency

No statistically significant differences were detected in progeny height growth between the stands in the two sets of empirical material (**I** and Scots pine, breeding zone no. 2). As the largest proportion of the genetic variation in most forest trees at the population level is between trees (Park and Gerhold 1986, Eriksson et al. 1987, Simpson 1998), this was to be expected. The genetic gain of seed collection stands has very seldom been tested. Seed lot tests of loblolly and slash pine seed production areas revealed little or no improvement in growth rate, but clear improvement in form and pest resistance (Rudolf et al. 1974). Seed production areas represent, however, a slightly higher degree of genetic improvement than seed collection stands, as they are upgraded by leaving only the best trees in the stands. In the present study, the plus trees selected from seed collection stands did not produce progeny with better height growth than plus trees from other stands. This is in accordance with expectations when we consider the within- and between-stand distribution of the genetic variation in height growth, as cited above. However, this does not totally abolish the possible positive effect of selection of seed collection stands, because the other plus trees were also mainly selected from stands with some degree of selection (Sarvas 1953).

There was a slight increase in progeny height growth with increasing selection intensity of the parent plus trees (**I** and Scots pine breeding zone no. 2). This result is rather similar to that reported for Scots pine in southern Finland (Tynkkynen 1998). However, it may not be advisable to strive for higher selection intensities: this will result in rapidly increasing costs, because there is an exponential relationship between selection intensity and the number of trees to be evaluated (Porterfield et al. 1975). It would be more efficient to direct more resources to progeny testing than to strive for ambitious goals in phenotypic selection for height growth (Namkoong 1969).

The plus trees selected with an average selection intensity ($i \approx 2$ in **I**, and 1.4 in breeding zone no. 2) resulted in progeny with about 1 to 2 % better height growth than the trees with zero selection intensity. This is a lower gain than is normally obtained for Scots pine plus tree selection when plus tree progenies are compared to natural stand offspring (Wilhelmsson et al. 1993, Venäläinen et al. 1994, Andersson et al. 2002). The difference could be partly explained by the fact that this comparison omits the better physiological condition (e.g. heavier seed) and lower inbreeding level of a seed orchard. These effects have been estimated to account for about 4 % of the gain in height growth of plus tree progenies of Scots pine (Wilhelmsson et al. 1993).

In spite of the low correlation between selection intensity and progeny performance, selection intensity could be used for the preliminary grouping of material within the breeding stock, as the example in Scots pine breeding zone no. 2 shows. One situation where information about selection intensity could be utilised is when seed orchards are established with untested plus trees. By favouring plus trees with the highest selection intensity, the expected genetic gain could be slightly increased compared to that for random deployment.

The economically essential genetic differences in height growth are best expressed in trees with an age close to the rotation age (Zobel and Talbert 1984, Simpson 1998). It can be assumed that, in over-aged stands the differences caused by a differential life-time growth rhythm are gradually disappearing. This is shown by the decrease in selection differential with age in Scots pine plus tree selection (Tigerstedt and Malmivaara 1970). This hypothesis was supported by material from southern Finland, where increasing age of the plus tree decreased progeny height growth, but not in northern Finland. The negative correlation observed in Scots pine breeding zone no. 2 is similar to that obtained by Tynkkynen (1998) for partly the same material. In Estonian Scots pine breeding material no correlation was observed between plus tree age and progeny height growth, but there was a positive correlation between plus tree age and progeny diameter (Kurm et al. 2000). There are, however, so many uncontrollable background variables in the studied materials that further discussion is unproductive. At the most it can be stated that over-aged stands should be avoided in plus tree selection, as is commonly recommended (Sarvas 1953, Simpson 1998, Zobel and Talbert 1984).

The plus trees selected more recently had a higher selection intensity. This is contrary to expectations, because the rigorous standards set for a plus tree in the early years of plus tree selection were relaxed because large numbers of plus trees were needed (Oskarsson 1995). It may be that the early rigorous plus tree selection placed more emphasis on quality characters or other aspects irrelevant for genetic improvement in height growth, since the early selected plus trees did not have a higher selection intensity for height or better progeny height growth. There can be a common – conscious or not – tendency to select nice looking trees at the expense of growth. In an evaluation of a loblolly pine breeding program in southeastern USA, it was observed that selection for crown width was given far too much attention in the phenotypic plus tree selection relative to its economic importance (Porterfield et al. 1975). As the more recently selected plus trees were younger than the earlier selections, the negative correlation between selection age and selection intensity ($r = -0.153$, $p = 0.002$) could partly explain the positive relationship between selection year and selection intensity.

Although selection in advanced generations will be mainly concentrated in progeny tests and other artificially regenerated stands, selection from natural stands may become relevant for some new species that are currently not included in the breeding programs. However, studies concerning selection efficiency in cultivated stands should be given higher priority. One could expect that the equal age and more even growing conditions would lead to more efficient selection in progeny tests than in natural forests. Although even-aged natural stands were preferred in plus tree selection, in many cases this aim was not fulfilled (**I**). An interesting question is how effective is the phenotypic selection for timber quality in natural forests. There was a weak indication that plus trees selected with quality as the main selection criterion produced shorter progeny than trees selected simultaneously for height and quality (**I**).

6.3 Constructing the first cycle breeding population

There is an inherent dilemma embedded in progeny testing: when the progeny test results for ranking the parents are available, the next generation trees are also within reach (Falconer and Mackay 1996). Thus it is possible to make selections in two generations: either backward selection among the parents according to the progeny test results, or phenotypic forward selection among the progeny. Which alternative to use depends on the expected genetic gain, obtainable genetic diversity, the planned use of the selections, and also practical considerations.

The difference in genetic gain between a plus tree and its best offspring depends on the ranking of the parent tree and the genetic value of the other parent (Hodge and White 1993, **II**). In open pollination the probability of pollen coming from a genetically better tree than the female parent decreases with increasing genetic value of the female parent. Thus the expected breeding value of the best plus trees is higher than that of their best offspring (Lindgren 1986, Hodge and White 1993, **II**). On the other hand, lower ranking plus trees have a higher probability of having offspring with a breeding value exceeding that of their own parent. Thus backward selection is to be preferred among the best plus trees, and forward selection in the lower ranking trees.

The point of equality between backward and forward selection depends on several factors (**II**). The genetic gain in phenotypic plus tree selection (expressed as the selection intensity in the forest) influences the balance between backward and forward selection by affecting the relative breeding value of the pollen pool. When selection in the forest has been ineffective, the unselected pollen cloud has almost as high a genetic value

as the plus trees themselves, thus making forward selection from open pollinated progeny a useful option. In contrast, with a higher intensity of selection in the forest, the breeding value of the background pollen pool is decreased in relative terms, thus favouring backward selection (Lindgren and Werner 1989, **II**). If the background pollen comes from populations unadapted to the target area of the selections, there is no possibility to use forward selection without hazarding the adaptation of the breeding stock. Such a situation occurs in the northern breeding zones in Finland, because the seed orchards have been moved to more favourable climate to improve seed crops and quality (Sarvas 1970), but the background pollinated progenies are unadapted to the breeding zones of the plus trees (Nikkanen 1982, Rousi 1983, Mikola 1993, Pulkkinen et al. 1995).

Low heritability and small families favour backward selection because, in such a case, phenotypic forward selection is not effective. In fact, low heritability is one of the main factors favouring progeny testing and thus backward selection (Falconer and Mackay, 1996). Small families have the consequence that the selection intensity is reduced when selecting the best, and they thus reduce the gain of forward selection. This makes backward selection a more preferable method (**II**). The greatest effect was at the smallest progeny sizes (10 to 50).

In a typical case, about 20 % of the best plus trees had a higher breeding value than their best open pollinated offspring (**II**). The practical consequences of this depend on the size of the breeding population compared to the candidate population. If the size of the breeding population is less than 20 % of the size of the candidate population, then all selections can be made backward. On the other hand, if considerably more than 20 % of the phenotypic plus trees are needed, then the use of the best open pollinated offspring instead of the lower ranking plus tree itself can be a good alternative. Combining the generations in an optimal way increased the genetic gain by more than 10 % compared to either pure backward or pure forward selection. The superiority of combining generations decreased with decreasing proportion of families needed for the next breeding cycle, because in this case backward selection was the preferred one in all or most of the families. This is in agreement with combining generations in seed orchard establishment (Hodge and White 1993). The difference in the breeding values of the plus tree and its best offspring was rather small in a large number of the families around the critical value. Thus in these families the question of whether to select backward or forward could be solved on grounds other than the breeding values.

In the studied empirical material (Scots pine breeding zone no. 2) only 7.8 % of the tested plus trees had a higher breeding value than their best open pollinated offspring. The main reason for the lower percentage of backward selections than in a typical case in the theoretical study was the

low gain in phenotypic plus tree selection (1.8 % for open pollinated progeny). This is a low value compared to many of the published ones (Cornelius 1994b, Rosvall et al. 2001), but is based on an empirical study from exactly the same breeding zone (Venäläinen et al. 1994). Such genetic gain corresponds to a heritability value of 0.09, with a selection intensity value of 1.36, which is the average for the studied material. This may also seem low compared to the published single site values (Cornelius 1994a, Haapanen et al. 1997), but not much lower than an unbiased estimate made over several sites (Haapanen 2002). However, one must consider that the published heritability values are based on planned experiments, which have been treated intentionally to decrease environmental variation, whereas environmental variation in the forest where plus tree selection took place has probably been larger. Thus the difference between realised heritability and the published values is not unexplainable.

The calculations made with the real values indicated that the number of plus trees in which backward selection was the better alternative almost exactly coincided with the suggested nucleus size (54 vs. 48). Thus a straightforward recommendation could be that one should use backward selection in the nucleus and forward selection in the main breeding group. The optimal combination of backward and forward selection would lead to a 23 % increase in genetic gain in the first cycle breeding population. The increase was higher than that generally obtained in **II**, but in agreement with it when we take into account the low realised genetic gain in breeding zone no. 2.

The genetic gain per breeding cycle is not the most important criterion when comparing breeding alternatives. Gain per time unit and costs are of greater importance in final decision-making. When forward selection is compared to backward selection, it is important to consider in which cycle the forward selected offspring are included (Fig. 10). They can either be incorporated into the present cycle breeding population together with the best progeny tested phenotypic plus trees (alt. B in Fig. 10), or into the candidate or breeding population of the next cycle together with the offspring originating from crosses between the trees in the nucleus (alt. A in Fig. 10). The first alternative can lead to a time delay compared to the use of the progeny tested, phenotypical plus trees which are usually already present in clone archives or seed orchards ready for crossing. To get the next generation trees to the same state needs selection, and possibly also the establishment of graft collections and flower stimulation. In Scots pine, especially initiation of male flowering can involve a long waiting time (Koski 1975). The acceptable time delay (ATD) for no loss in genetic gain per year in the studied case of Scots pine breeding zone no. 2 was 8.3 years. Thus, for combined backward and forward selection

to be a defendable alternative in genetic terms, the cycle must not be lengthened by more than about eight years. From the point of view of economic constraints the time delay must be clearly less in order to jus tify the use of forward selection.

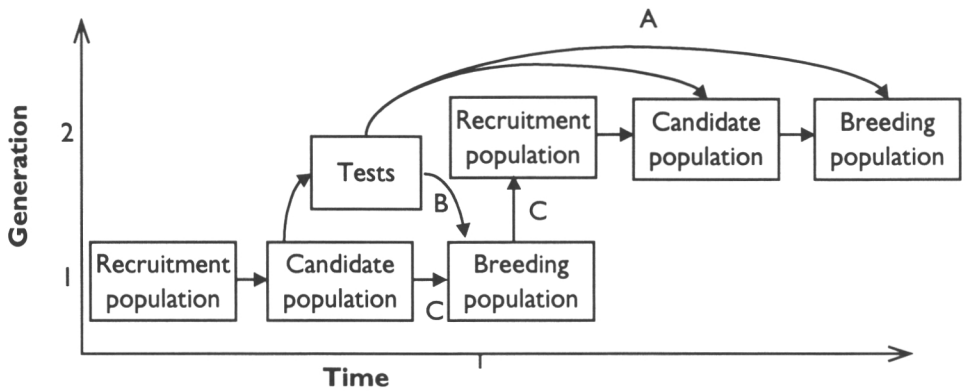


Figure 10. Different alternatives to incorporate forward selected plus tree progenies to the breeding cycle.

If the forward selected, open pollinated offspring are incorporated into the next cycle (A in Fig. 10), then there will be no time delay, but clearly a loss in genetic gain. For instance, if in the studied case (breeding zone no. 2) crossing and within family selection are used in the nucleus (C), but forward selection from open pollinated families is considered sufficient in the main breeding group (A), then the genetic value of the population will be 37.2 dm. This can be compared to 39.0 dm, which would be obtainable if the whole breeding population had been treated in a similar way as the nucleus (only alternative C), or to 39.9 dm after optimal combination of backward and forward selection (both B and C). However, inclusion of forward selections directly to the next cycle would save a considerable amount of breeding effort and thus costs. Thus, if the primary function of the main breeding group is to act as gene resource, and active breeding is concentrated in the nucleus, then this can be a justifiable alternative.

The biological characteristics of the species in question and other practical restrictions have a strong influence on the optimal solution. When creating breeding populations of radiata pine in South Australia, slight family selection was followed by forward selection within families (Cotterill 1984). On the other hand, forward selection within a late flowering species like Sitka spruce in Britain was not a justified option when composing breeding populations (Lee 1993). Within-family selection is inferior to backward selection in Scots pine progeny tests, and the opti-

mum selection age is much higher for within-family selection than for backward selection (Haapanen 2002). This practical finding can also reduce the scope for forward selection when planning breeding strategies. However, if progeny tests of suitable age already exist, the situation can be different. The lower precision of the genetic information in phenotypic forward selections can also limit their use in a multiplication population (Hodge and White 1993).

In spite of possibility of some practical difficulties it is probable that, in advanced breeding, different generations will be mixed so that the genetically best material from each family are selected irrespective of the generation. The generation turnover in the best families can be slower than that in the lower ranking families (Lindgren 1986). Mating within a limited population creates relatedness and thus decreases gene diversity although, on the other hand it is essential for constructing new genetic combinations in order to advance the genetic value. Generation turnover is not the main target of tree breeding, but genetic improvement without too large sacrifices in genetic diversity. The optimal generation time is a compromise between achieved genetic gain and diversity lost (Wei and Lindgren 2001).

Mating within a closed population of constant size leads to the loss of alleles and a decrease in gene diversity. In this respect, forward selection among open pollinated offspring is a better alternative than selection from control pollinated progenies. However, the gene diversity of a population of open pollinated offspring does not exceed that of the unrelated non-inbred parent generation if the population size is kept constant. The decrease in genetic gain connected with open pollination is avoided if the pollen cloud originates from the seed orchard or clone archive itself. On the other hand, the build-up of unknown and uncontrolled coancestries and thus decreased genetic diversity are new disadvantages. This problem can be alleviated by using DNA identification techniques to avoid the selection of related trees (Lambeth et al. 2001).

6.4 Founder contribution to the second cycle

Balanced within family selection is regarded as the method to maximise long-term response to selection, given a constant breeding population size (Dempfle 1975). In forest tree breeding programs this has led to truncating family selection and within-family selection with equal selection intensity within families (e.g. Danell 1995, Rosvall et al. 1998). In recent years, breeding strategies have been developed which give greater weight to the genetically more advanced material, e.g. an open nucleus breeding

scheme (Cotterill et al. 1989, White et al. 1993, McKeand and Bridgwater 1998). A further step in maximising the genetic gain is to make the limit between rejected and accepted breeding stock members less abrupt, and to increase the contribution of the trees continuously with increasing genetic value (**III**). This has similarities with differential fitnesses in natural selection, whereas the traditionally used truncating selection is unnatural in the sense that there are only two groups of genotypes, selected and rejected, without any fitness differences within the groups.

The obtained results showed that by manipulating the size of the breeding population and the contributions of its members, genetic gain could be increased with the same gene diversity and costs as in a case with equally contributing breeding population members (**III**). The increase was about 10 % of the between-family genetic gain. However, it should be noted that the increase was smaller when it was related to the total genetic gain, but as the amount of within-family genetic gain depends for instance on family size and heritability, the total effect could not be unequivocally estimated.

The additional genetic gain was obtained by allowing the best trees in the breeding population to contribute more to the next generation than those with a lower breeding value. The impending increase in relatedness in the next generation was counteracted by increasing the number of accepted trees and letting the lower ranking trees contribute less than they would in the case of equal contribution. In terms of gene diversity each unrelated, non-inbred tree is equally valuable, but as regards genetic gain the value of the tree increases with its ranking (Fig. 9). The differences between the included trees are greatest in small candidate populations, which was reflected as greater advantage of the method in small populations (**III**). The effect of variable contribution on genetic values means elevation of the genetic level of the whole breeding stock in connection to the generation shift, whereas with equal contribution the next generation recruitment population has the same genetic value as the previous generation breeding population (Fig. 11).

Several studies have shown that the relative merits of different selection systems change if comparison is made at the same level of inbreeding instead of the same number of selections (Quinton et al. 1992, Wei 1995, Meuwissen 1997). When inbreeding (or coancestry) is considered, a reduced weight is given to family information in order to decrease the selection of related individuals. However, an opposite phenomenon was observed in this study: the introduction of variable contribution means giving some weight to the family information, instead of totally omitting it, as is done when using balanced within-family selection alone. This indicates that there is an optimum combination of between- and within-family selection required to achieve maximum genetic gain, which is

contrary to the traditional view of Dempfle (1975), but in line with Villanueva and Woolliams (1997). Based on a simulation model in which the weight given to family information differed between generations, Wray and Goddard (1994) speculated that in early generations it would be beneficial to use family selection in order to eliminate the poorest families, whereas in later generations within-family selection should be used.

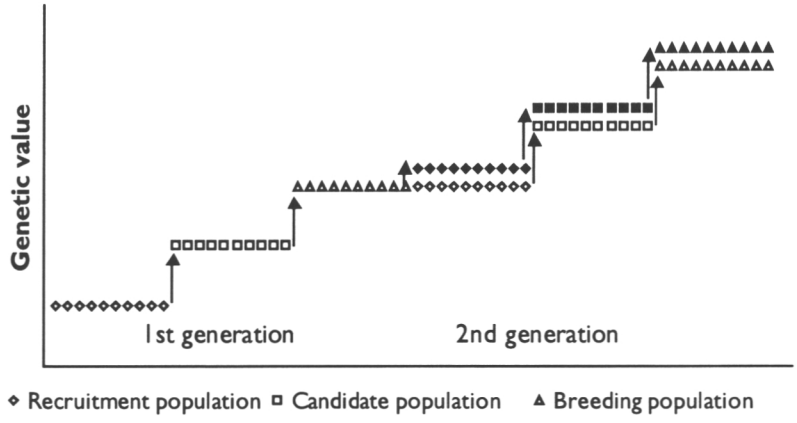


Figure 11. Schematic description of changes in the genetic value of breeding stock with advancing breeding with equal (open symbols) and variable contribution (filled symbols) of plus trees to the recruitment population (cf. Fig. 5).

Using genotypes in relation to their genetic value has also been suggested in other connections as a means of obtaining maximum genetic gain with a fixed genetic diversity (Toro and Nieto 1984, Lindgren 1986, Lindgren and Matheson 1986, Lindgren et al. 1989), and the results are in agreement with those of this study (**III**). The question of the best possible contribution of the breeding population members to the next generation has also been studied earlier in both animal (Meuwissen 1997) and tree breeding (Kerr et al. 1998, Andersson et al. 1999, Rosvall 1999). These studies differed from the present one in respect to the restrictions set for genetic diversity and costs but, despite these shortcomings, their results are in fairly good agreement with this study.

Increased contribution of the best trees results in the best fraction of the breeding population having a higher internal relatedness than the lower fractions. This may lead to increased inbreeding if repeated over many generations. Several studies with stochastic simulations have shown that selection methods implementing the contribution of parents in relation to their breeding values also produce, after many generations of se-

lection, higher genetic gain than equal contribution, with equal or lower inbreeding (Toro and Nieto 1984, Toro et al. 1988, Wray and Goddard 1994, Lindgren and Mullin 1997, Meuwissen 1997, Rosvall 1999, Rosvall and Andersson 1999). Similar results have been obtained empirically in a six-generation selection experiment with *Drosophila melanogaster* (Sanchez et al. 1999). Continuously improving the genetic gain by optimising the genetic contributions of the parents thus seems to be possible.

The greatest increases in the genetic gain were obtained when the first plus trees were added to the breeding population and the few highest ranking plus trees were simultaneously allowed to contribute an extra offspring to the next generation (**III**). Closer to the maximum genetic gain, the change in gain was small with additional plus trees. Similarly, in a simulation study carried out by Brisbane and Gibson (1995) it was observed that close to the maximum genetic gain inbreeding could be decreased with only a minimal decrease in genetic gain. Thus it is possible to use even more than the optimum number of plus trees without any great decrease in genetic gain, if motivated e.g. by better geographic representation of the founder population.

For the sake of analytical convenience, only one selection from each family was allowed in this study and the variable contribution was accomplished by a different number of matings. It is also possible to make several selections from the same families or to select both parent and its offspring for the next breeding cycle in cases where they have approximately equal expected breeding values (**II**). However, unbalanced mating is a better method than unbalanced selection for increasing the contribution of the best trees, as it results in less relatedness between the selected trees (Rosvall 1999). When weighing up the alternative selection schemes one should keep in mind the different degrees of coancestry between differently related individuals (Table 8). Half-sibs are to be preferred to parent-offspring pairs or full sibs in this respect.

In the empirical material from Scots pine breeding zone no. 2, the increase in population size needed to obtain the maximum genetic gain was in accordance with **III**, but the increase in genetic gain was somewhat lower than in a comparable case in **III**. This was caused by the much larger breeding population sizes than those used in **III**, which means that restriction of the maximum contribution to three offspring limited the realised gain. In order to approximate the optimal parental contributions in such large breeding populations as in this study, a method such as linear deployment (Lindgren et al. 1989) could be used. The even lower increase in the combined backward and forward selection alternative with variable contribution was caused by the non-normality of the combined distribution of the breeding values: the difference between the highest and

lowest accepted breeding values was smaller than in the normal distribution (Fig. 9). One consequence of this was that manipulation of the genetic contributions of the selected trees had less effect on the genetic gain.

6.5 Sublining the breeding stock

Traditionally sublining has been made in parallel, i.e. with random assignment of the plus trees to sublines. In fact, this has been sometimes included in the definition of sublines (Burdon and Namkoong 1983). However, it was demonstrated that genetic gain from deployed material could be increased considerably (more than 15 %) if the breeding program utilised stratified instead of parallel sublining (**IV**). The method of sublining did not affect the mean genetic value of the breeding population, but only that of the multiplication populations derived from it. The greatest relative increase in multiplication population gain was obtained in the second generation. Thereafter, the relative gain decreased because the absolute level of genetic gain increased, although the absolute superiority of the stratified sublining method remained almost unchanged. The increase in genetic gain was roughly of the same order of the genetic gain obtained in one generation. The superiority of stratified sublining resulted from the different behaviour of the among-subline variance in the two sublining alternatives. In the second and third generation, the among-subline variance decreased under parallel sublining, whereas it increased in stratified sublining. The greater among-subline variance under stratified sublining made it possible to obtain greater genetic gain when only a selected portion of the sublines was utilised in establishing seed orchards.

The observed increase in genetic gain is related to the effect of positive assortative mating (PAM) (Falconer and Mackay 1996, Rosvall 1999). In the second generation, it is in fact the same. In later generations, however, stratified sublining differed from continued PAM, because the isolation was maintained, and the ranking of individuals did not affect the mate selection once the sublines had been fixed. Stratified sublining is likely to give a lower increase in the theoretical genetic gain than might be obtained by continued PAM. On the other hand, the individuals selected for multiplication population are totally unrelated, which cannot be achieved in PAM. In a simulation study comparing PAM to random mating, Shepherd and Kinghorn (1994) obtained an increase of 24 % in genetic gain, which is comparable to the value for this study (**IV**).

Stratified sublining is a combination of traditional sublining and hierarchical breeding population structures (Cotterill et al. 1989, Cramer and Kannenberg 1992, **III**). The features distinguishing it from traditional

parallel sublining were discussed earlier, and the main difference compared to hierarchical breeding populations is a lack of gene flow between the genetically different levels. Stratified sublining, however, can be questioned on the grounds that such a breeding strategy does not utilise genetic material from the lower-ranking sublines. It can be argued that the lower ranking sublines could equally well be totally discarded without any essential loss in genetic gain.

The arguments for preserving the lower ranking sublines stem from the desire to maintain genetic diversity and to keep several breeding options available. It is not exactly known how many clones will be needed in a seed orchard in the future. Thus sublining gives the option to use only the best genetic material if the number of clones needed is lower than the number of sublines, because material is assigned to the sublines according to its genetic value. In parallel sublining the best material is mixed with the poor material and there is therefore no possibility to utilise only the best. If all sublines are needed also in the future, then the two sublining alternatives give equal genetic gain in multiplication populations, as was also found in the simulation with empirical material from Scots pine breeding zone no. 2. Thus stratified sublining is an option that gives possibilities for gain with no risk of loss. The small subline size makes them prone to genetic drift, which can even lead to large rank changes between the sublines in coming generations. Genetic drift is a motivation to establish more than the required number of sublines also in parallel sublining (White 1992).

It is also possible to breed in the lower ranking sublines for other goals than in the higher sublines. A modification that would greatly improve the economic efficiency of stratified sublining is to adjust the management intensity according to the genetic level of the subline. This would make the system more reminiscent of the hierarchical open-ended breeding system (HOPE) (Cramer and Kannenberg 1992). Stratified sublining also facilitates easy removal of lower ranking material from the breeding stock if smaller population sizes are found to be appropriate. As sublining in general is a useful method to preserve gene diversity (Lindgren et al. 1996) and control inbreeding (McKeand and Beineke 1980, van Buijtenen 1984), stratified sublining is especially recommendable for its flexibility in meeting unforeseen future challenges.

In the present study (**IV**) the initial breeding population was assumed, in order to simplify the calculations, to consist of normally distributed individuals. In practical applications the trees belonging to the lowest part of the distribution would be discarded. This would decrease the superiority of stratified sublining, but not totally eliminate it. Culling more than 50 % of the candidate population explains the lower increase in genetic gain achieved through stratified sublining in Scots pine breeding zone no.

2 than in the theoretical study **IV**. If a smaller proportion of the sublines is used, then higher genetic gains can be achieved than those predicted here. This trend was clearly visible in Scots pine breeding zone no. 2. The gain could be further increased by allowing several trees from fewer top-ranking sublines to be included in the seed orchard.

6.6 Constituents for a breeding program

The breeding population size and gene diversity in the studied Scots pine breeding zone no. 2 can be regarded as rather high. A breeding population of about 50 unrelated trees seems to be sufficient for at least 10 generations, and probably much longer if selection is kept reasonably balanced (Danell 1993, Rosvall 1999, Yanchuk 2001). This allows the use of a breeding plan which emphasises genetic gain at the cost of gene diversity. The outlined adoption of the nucleus breeding system (Haapanen et al. 1999, Mikola 2002) is a long step in that direction. The development could be further boosted by giving the nucleus a central role in the breeding strategy and treating the main breeding group more as a backup measure. The sufficiency of the material is further ensured by the fact that breeding zone no. 2 is only one of several Scots pine breeding zones in southern Finland. When southern Finland is treated as a whole, the breeding strategy can be interpreted to have features of a multiple population breeding system (Namkoong et al. 1988, Eriksson 2001).

The division of breeding stock into the nucleus and main breeding groups is the first step in manipulating the contributions of the plus trees according to their breeding values (Fig. 12.). The basic mating methods could be double pair mating in the nucleus and single pair mating in the main breeding group. When nucleus trees are further crossed with the best trees of the main breeding group, the mating plan as a whole will resemble the variable contribution method (**III**). The matings between the different breeding groups can be accomplished e.g. by using the higher ranking trees in the main breeding group for pollinating the nucleus trees. It can even be possible to utilise pollen mixes in order to give to as many trees as possible a chance to contribute to the next generation, if the offspring prove to be competitive. Identification of the selections might be made afterwards using DNA identification methods (Lambeth et al. 2001). The strong gene flow from the nucleus to the main breeding group will assist in narrowing the gap in genetic value between the nucleus and main breeding groups.

It is advisable to divide the breeding stock into sublines according to the breeding values of the trees (**IV**). Subline division is a recommend-

able method for controlling inbreeding in the future multiplication populations (Burdon et al. 1977, McKeand and Beineke 1980), and doing it according to the breeding values will increase the obtainable genetic gain of the future seed orchards without any sacrifices in the overall genetic gain. Such sublining stratified according to the breeding values resembles positive assortative mating and is identical with it in the first generation. Positive assortative mating and a higher number of crosses with the best trees has been adopted e.g. in the breeding strategy of *Picea sitchensis* in Britain (Lee 2001). The combining of sublining and gene flow between different breeding groups can cause some problems, but they can be solved by proper planning. Stratified sublining enables the establishment of seed orchards and other multiplication populations with a high genetic value if low genetic diversity is allowed.

If untested forward selections of the best families are used as seed orchard ortets, those having the highest selection intensity should be favoured in order to increase the genetic gain (**I**).

The greatest genetic advancement in the breeding cycle takes place when the progeny testing has been finished and selections for the breeding population can be made (Fig. 5). This is also the most proper time for establishing new generation seed orchards (Fig. 12.). However, when we take into account the existence of many breeding zones, several breeding objectives and changes in demand for improved seed, in practice the establishment of new seed orchards is a more or less continuous process. It is also probable that, in the long run, the distinct generation or cycle structure will disappear from tree breeding, and the breeding strategy will develop in the direction of rolling front breeding, which gives a considerably higher genetic gain per year than distinct generation breeding (Borralho and Dutkowski 1998). The adjustability in the productive life span of a seed orchard also offers some flexibility for the establishment time table. Thus it is not necessary to bind long-term breeding and seed orchard establishment tightly together.

The advanced generation breeding (both short- and long-term) is mainly performed in the nucleus of the breeding stock. The main breeding group functions more as a safety net, with its greatest importance in the early breeding cycles. The existence of a main breeding group consisting of a large number of tested plus trees also offers possibilities for the formation of several nuclei, each stressing a different selection goal. This would make the population structure resemble what has been described as amoeba breeding (Shelbourne et al. 1989, see also Lee 1999), or essentially MPBS on a small scale.

The current breeding population sizes in Finnish Scots pine breeding are so high that they can be considerably reduced without sacrificing any

long-term genetic gain. The reduction can be made gradually in some of the coming generations, as suggested by Namkoong et al. (1988).

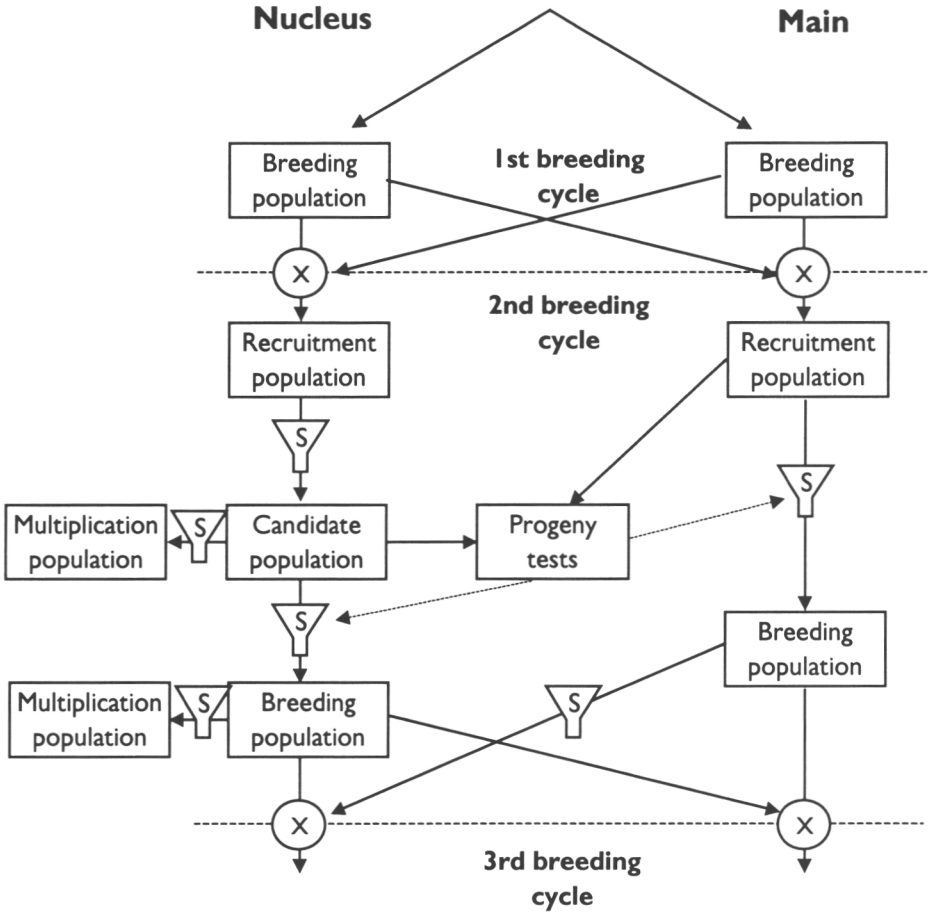


Figure 12. A proposed open nucleus breeding plan for Scots pine breeding zone No 2 (—— = transfer of material, - - - = information, X = crossing, S = selection) (after Ruotsalainen 1999).

Smaller population sizes decrease costs and shorten the generation time, thus increasing the feasibility of the breeding program. The reduction could most conveniently be made by assimilating the genetically most advanced part of the main breeding group to the nucleus, and moving other interesting genotypes to the gene resource population. In the selection operations, one should keep in mind genetic diversity and geographic representativity, in addition to genetic gain (Kang and Nienstaedt 1987). Geographically balanced recruitment is advisable as a means for safeguarding against both unknown selection pressures and public concerns.

Forward selection in existing open pollinated progeny tests does not seem to be a very competitive alternative, as it means either a time delay and increased costs or a loss in genetic gain per time (**II**). The use of forward selection cannot be motivated as a method to increase gene diversity, unless the original plus trees are highly related or inbred. A different justification for using background pollinated material is that it may sometimes offer a possibility to introduce valuable new genetic material to the breeding stock. If the number of selected phenotypic plus trees is considerably lower than in the studied breeding zone, then fulfilment of the breeding population can be made by selecting material from the progenies of the plus trees. However, the progenies of low ranking plus trees should be avoided even in such situations, because selections from these progenies can be inferior to randomly selected wild trees (Toro and Nieto 1984). Alternatively one can select both a parent and its best offspring, although this will increase coancestry.

The breeding population size is most crucial for the maintenance of genetic diversity in the breeding stock, because it forms a bottleneck in the breeding cycle. Other populations, especially the recruitment population, are important in the sense of harvesting new mutations or testing the existing genetic variability against selective forces (e.g. Burnham et al. 1986). At a constant mutation rate, the probability of new mutations is directly proportional to the size of the population. Of course a large population size is not enough; there must also be resources for screening the variability in order to harvest the potentially useful alleles. The environments in which recruitment populations and progeny tests are growing are of great importance for revealing the genetic variability caused by both old and novel genetic variation. Within-site variation should be minimal, and the sites should provide good growing conditions so as to ensure good expression for genetic differences (Haapanen 2002). On the other hand, there should also be differences between test sites in order to secure breeding for sufficiently wide adaptation. In fact, some testing should also be done outside the targeted utilisation area so that the material would be prepared for any future threats.

The importance of gene resource populations will increase over time. This does not refer as much to the in situ genetic reserves, which can mainly be seen to fulfil ethical purposes, as to those collections of plus trees from earlier generations, progeny tests, collections of special forms, and in fact all the material which has a recorded ancestry or can otherwise have importance in future breeding activities. Although it may merely be a question of definition, it can be said that the functions of the main breeding group will be overtaken by the gene resource population. Probably there will not be as great a concern about differences in the genetic levels of the nucleus and main breeding groups, because the development

of artificial gene transformation techniques enables the utilisation of interesting alleles also when they are embedded in an otherwise inferior genetic background. What is most important is to have a large sample of genotypes and families with known ancestry available for the search for such interesting alleles (Kang et al 1998).

A representative sample of progeny tests should be maintained, also after they have fulfilled their major function in breeding value estimation, in order to facilitate e.g. the search for quantitative trait loci or usable major genes. A minimum requirement could be to retain at some location the offspring of each plus tree accepted to the breeding population. Such preserved progeny tests are also valuable in following the long-term performance of the plus trees. They may prove to be especially valuable if a new damaging agent emerges. Even special forms of trees, although mainly considered as dendrological curiosities (e.g. Oskarsson and Nikkanen 1999), can be an interesting source in the search for major genes affecting the structure and metabolism of trees.

In all research related to trees it is advisable to use material derived from breeding programs, unless a wild origin is the absolute prerequisite for the study. This will give synergistic benefits, as some populations and trees will become profoundly known and, in the best case lead to the discovery of valuable alleles (e.g. MacKay et al. 1997).

There are also a number of factors affecting the construction of a successful breeding plan that should be known in greater detail. Stratified sublining is a potentially promising method for increasing the genetic gain obtainable from a breeding program, but further studies are needed to obtain the maximum benefit from its application. What is especially worth studying is how the allocation of resources according to the genetic value of the subline could improve the competitiveness of the method. This would probably be best studied using stochastic simulation (e.g. POPSIM, Mullin and Park 1995). However, planning the matings according to the stratified subline method is possible already during the creation of second generation recruitment populations without excluding any future alternatives. Studies concerning the effect of mild inbreeding and breeding for reduced inbreeding depression are important for long-term tree breeding. To better understand the importance of gene diversity for tree breeding, its amount and function should be studied in natural populations (Andersson 1999).

Purging recessive deleterious alleles, which has been suggested as a breeding method (Kang 1982, Namkoong et al. 1988, Williams and Savolainen 1996), is rather inefficient without strong inbreeding, as most of the recessive alleles are hidden in heterozygous form. A better solution would be backward selection of founders for the breeding program after selfing the candidates, and then selecting those showing the lowest in-

breeding depression. This possibility, as well as the long-term effects of inbreeding, should be studied in a special experimental population with intensive inbreeding and rapid generation turnover (Lindgren and Gregorius 1976, Williams and Savolainen 1996). Studies should also be carried out whether there is correlation between inbreeding depression and the breeding value of the tree. This kind of testing and selection according to inbreeding depression would be especially important if very small sublines are used.

It is still unknown to what extent the novel developments in the field of biotechnology will change tree breeding. The magnitude and speed of the change depends on, among other things, the species in question, the economic resources, and the attitude of society to these techniques. The first techniques to be applied in practice will probably be vegetative propagation and marker aided selection (Aronen 1999). Their main advantage will be in speeding up breeding and making selection and testing more effective (Plomion et al. 1996, Högberg et al. 1998). In the long-term, genetic transformation will probably be the technique having the greatest influence on tree breeding. However, public acceptance of genetically modified forest trees is not necessarily easily achieved, as recent discussion has shown (e.g. Strauss et al. 2001, Cauley 2001, Mann and Plummer 2002).

The stability of the transformed trees and the evaluation of the risks need long field testing; the longer testing time, the more alien is the transformed genetic material (Aronen 1999, Strauss et al. 2001, van Raemdonck et al. 2001, Mann and Plummer 2002). Perhaps the first practical applications will be genetically transformed trees, where the introduced gene comes from the same or closely related species. The use of genetic transformation for enriching a favourable rare allele, instead of traditional breeding techniques, could have the advantage that rest of the genome can be left untouched, thus maintaining the genetic diversity at a higher level. As a whole, the effect of biotechnology will probably not be as dramatic as has often been anticipated, but it will be incorporated into the traditional tree breeding strategies as a new tool and extension for solving specific questions (Karnosky et al. 1998, Strauss et al. 2001, Zhong 2001). The greatest advantage will be obtained when the new techniques are applied to genetically advanced material (Karnosky et al. 1998).

Constructing a tree breeding plan means dovetailing genetic gain and diversity, time, financial and other resources, feasibility and robustness into a functioning machinery. Many factors have unfavourable dependencies which complicate planning. Another difficulty is that many of the essential parameters are often unsatisfactorily known. Especially there are uncertainties in the selection goals and about the availability of resources in the future. This underlines the importance of making breeding plans

adjustable and robust so that they are tolerant to changes in basic premises and can easily be modified according to new requirements.

The presented examples of modifications to a breeding strategy applied to Scots pine breeding zone no. 2 demonstrate that there is plenty of room for operations that increase genetic gain (both short- and long-term) within acceptable limits of genetic diversity, without essentially affecting the costs. Some of the suggestions mean increased complexity in the practical operations, which can be considered a drawback (Cotterill 1986, Rosvall 1999). However, good documentation of the practical breeding plan and a well functioning organisation can help overcome such obstacles and result in some extra genetic improvement. In creating a functioning tree breeding strategy, one must attain a balance between the need for simplicity and the optimistic view expressed by Namkoong et al. (1988) in their statement: "The only major cost in applying even sophisticated breeding systems is intellectual".

7 Conclusions

The aim of this study was to examine the fundamental dilemma between preserving genetic diversity for future use and deploying it for current genetic improvement. The following results, which are mainly applicable to the Finnish Scots pine breeding situation, were obtained.

1) The phenotypic plus tree selection used to construct the first generation candidate population has yielded some geographically slightly unbalanced material, but this has not led to any considerable decrease in gene diversity. The selection method has been moderately effective in improving the height growth of the offspring.

2) Backward selection among the best ranking plus tree families yields higher genetic gain than forward selection from open pollinated progenies, whereas the opposite is true for the lower ranking families. However, forward selection from open pollinated progenies is seldom to be recommended in practical tree breeding, as it usually means either increased costs or decreased genetic gain per unit of time. This is especially true in connection with the smaller breeding population sizes recommended below.

3) The genetic value of the next generation breeding stock can be increased by letting the plus trees contribute to it in relation to their breeding values.

4) It is possible to avoid inbreeding depression in the forest regeneration material, and at the same time retain the option to increase its genetic value, if the breeding stock is divided into reproductively isolated sublines according to the breeding values of the plus trees. The first step in this process is to mate the plus trees in breeding value order when the material for next generation is being created.

5) Breeding population sizes can be decreased from the current ones to less than 100 in order to increase the efficiency of tree breeding, without decreasing the genetic diversity to such an extent that the long-term sustainable genetic improvement would be jeopardised. The remaining progeny-tested lower ranking plus trees should be preserved, together with their offspring, as a valuable gene resource for e.g. the needs of genetic mapping or the search for valuable alleles for future molecular breeding.

References

Ahtikoski, A. 2000. The profitability of Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth) next-generation seed orchards in Finland. (Doctoral thesis) Helsingin yliopisto. Metsäekonomian laitos. Julkaisuja 7. 148 p + app.

Andersson, B., Elfving, B., Ericsson, T., Persson, T. & Gregorsson, B. 2002. Performance of improved Pinus sylvestris in northern Sweden. (submitted to Scandinavian Journal of Forest Research)

Andersson, E. W. 1999. Gain and diveristy in multi-generation breeding programs. (Doctoral thesis) Acta Universitatis Agriculturae Sueciae. Silvestria 95. 42 p. app.

Andersson, E.W., Sánches Rodriguez, L. & Andersson, B. 1999. Group coancestry controlled selection in a *Pinus sylvestris* L. breeding program. Theoretical and Applied Genetics 99:73-80.

Aronen, T. 1999. Biotekniikan sovellukset tulevat – muuttuvatko metsänjalostuksen mallit? [The applications of biotechnology are coming – will the models of tree breeding change?] (in Finnish) Metsätieteellinen aikakauskirja 2/1999:255-260.

Barnes, R. D. 1995. The breeding seedling orchard in the multiple population breeding strategy. Silvae Genetica 44:81-88.

Bergmann, F. & Ruetz, W. 1991. Isozyme genetic variation and heterozygosity in random tree samples and selected orchard clones from the same Norway spruce populations. Forest Ecology and Management 46:39-47.

Bishir, J. & Roberds, J. H. 1999. On the numbers of clones needed for managing risks in clonal forestry. Forest Genetics 6:149-155.

Borralho, N. M. G. & Dutkowski, G. W. 1998. Comparison of rolling front and discrete generation breeding strategies for trees. Canadian Journal of Forest Research 28:987-993.

Breeding poplars for disease resistance. 1985. FAO forestry paper 56. 66 p.

Bridgwater, F. E. & Franklin, E. C. 1985. Forest tree breeding: strategies, achievements and constraints. In: Cannell, M. G. R. & Jackson, J. E. (eds.) Attributes of trees as crop plants. Titus Wilson & Son Ltd, Kendal, Great Britain, pp. 36-48.

Brisbane, J. R. & Gibson, J. P. 1995. Balancing selection response and rate of inbreeding by including genetic relationships in selection decisions. Theoretical and Applied Genetics 91:421-431.

Burdon, R. D. 2001. Genetic diversity and disease resistance: some considerations for research, breeding, and deployment. Canadian Journal of Forest Research 31:596-606.

Burdon, R. D. & Carson, M. J. 1999. Conservation and management of genetic resources of commercial forests in New Zealand: Challenges of institutional changes and new technology. In: Mátyás, C. (ed.) Forest

Genetics and Sustainability. Kluwer Academic Publishers. pp. 235-246.

Burdon, R. D. & Namkoong, G. 1983. Short note: Multiple populations and sublines. Silvae Genetica 32:221-222.

Burdon, R. D. & Shelbourne, C. J. A. 1972. Breeding populations for recurrent selection: conflicts and possible solutions. New Zealand Journal of Forestry Science 1(2):174-193.

Burdon, R. D., Shelbourne, C. J. A. & Wilcox, M. D. 1977. Advanced selection strategies. Proceedings of Third World Consultation on Forest Tree Breeding, Canberra, Australia 21-26 March 1977. pp. 1134-1147.

Burnham, C. R., Rutter, P. A. & French, D. W. 1986. Breeding blight-resistant chestnuts. In: Janick, J. (ed.) Plant breeding reviews. Volume 4. AVI Publishing Company. Westport, Connecticut. USA. pp. 347-397.

Cauley, H. 2001. FSC says risks are still too great. Journal of Forestry, December 2001:8-9.

Chen, Z., Kolb, T. E., Clancy, K. M., Hipkins, V. D. & DeWald, L. E. 2001. Allozyme variation in interior Douglas-fir: association with growth and resistance to western spruce budworm herbivory. Canadian Journal of Forest Research 31:1691-1700.

Cockerham, C. C. 1967. Group inbreeding and coancestry. Genetics 56: 89-104.

Cornelius, J. 1994a. Heritabilities and additive genetic coefficients of variation in forest trees. Canadian Journal of Forest Research 24:372-379.

Cornelius, J. 1994b. The effectiveness of plus-tree selection for yield. Forest Ecology and Management 67:23-34

Cotterill, P. P. 1984. A plan for breeding radiata pine. Silvae Genetica 33:84-90.

Cotterill, P. 1986. Breeding strategy: don't underestimate simplicity! Proceedings of a joint meeting of IUFRO WPs on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, October 13-17. 1986. pp. 8-23.

Cotterill, P.P., Dean, C., Cameron, J. & Brindbergs, M. 1989. Nucleus breeding: a new strategy for rapid improvement under clonal forestry. In: Gibson, G. I, Griffin, A. R. & Matheson, A. C. (eds.) Breeding tropical trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Proc. IUFRO Conf. Pattaya, Thailand. November 1988. Oxford Forestry Institute, Oxford, UK and Winrock International, Arlington, Virginia, USA. pp. 39-51.

Cramer, M. M. and Kannenberg, L. W. 1992. Five years of HOPE: the Hierarchical Open-Ended corn breeding system. Crop Science 32:1163-1171.

Danell, Ö. 1993. Tree breeding stategy: are we too concerned conservationists but inefficient breeders? In: Lee, S. J. (ed.). Progeny Testing and Breeding Strategies. Proceedings of the Nordic Group for Tree

Breeding, Edinburgh, Scotland, 6-10 October, 1993. Forestry Commission, Roslin. pp. 80 - 94.

Danell, Ö. 1995. Breeding programs in Sweden: 1. General approach. Skog-Forsk. Arbetsrapport nr 302. 4 p.

Davidson C. B., Gottschalk, K. W. & Johnson, J. E. 1999. Tree mortality following defoliation by the European gypsy moth (Lymantria dispar L.) in the United States: a review. Forest Science 45:74-84.

DeBell, D. S. & Harrington, C. A. 1997. Productivity of *Populus* in monoclonal and polyclonal blocks at three spacings. Canadian Journal of Forest Research. 27:978-985.

Dempfle, L. 1975. A note on increasing the limit of selection through selection within families. Genetical Research, Cambridge 24: 127-135.

Dickmann, D. I. 1991. Role of physiology in forest tree improvement. In: Koski, V. (ed.) Biological systems in tree breeding. Joint meeting of IUFRO working parties in Tuusula, Finland. Silva Fennica 25:248-256.

Dudley, J. W. & Lambert, R. J. 1992. Ninety generations of selection for oil and protein in maize. Maydica 37:81-87.

Eberhart, S. A. & Russell, W. A. 1966. Stability parameters for comparing varieties. Crop Science 6:36-40.

El-Kassaby, Y. A. 1999. Impacts of industrial forestry on genetic diversity of temperate forest trees. In: Mátyás, C (ed.) Forest Genetics and Sustainability. Kluwer Academic Publishers. pp. 155-169.

Eriksson, G. 2001. Conservation of noble hardwoods in Europe. Canadian Journal of Forest Research 31:577-587.

Eriksson, G. & Ekberg, I. 2001. An introduction to forest genetics. Uppsala, Sweden. 166 p.

Eriksson, G., Ilstedt, B., Nilsson, C. & Ryttman, H. 1987. Within and between-population variation of growth and stem quality in a 30-year-old *Pinus sylvestris* trial. Scandinavian Journal of Forest Research 2:301-314.

Eriksson, G., Namkoong, G. & Roberds, J. H. 1993. Dynamic gene conservation for uncertain futures. Forest Ecology and Management 62:15-37.

Falconer, D. S. & Mackay, T.F.C. 1996. Introduction to Quantitative Genetics. 4th ed. Longman Group. London and New York. 464 p.

Finlay, K. W. & Wilkinson, G. N. 1963. The analysis of adaptation in a plant-breeding programme. Australian Journal of Agricultural Research 14:742-754.

Franklin, E. C. & Squillace, A. E. 1973. Short-term progeny tests and second generation breeding in slash pine. Canadian Journal of Forest Research 3:165-169.

Gartland, K. M. A., Gartland, J. S. & Fenning, T. M. 2001. The Biochemist. August 2001:21-23.

Gullberg, U. & Kang, H. 1985a. A model for tree breeding. Studia Forestalia Suecica 169. 8 p.

Gullberg, U. & Kang, H. 1985b. Application of a model for tree breeding to conifers in southern Sweden. Studia Forestalia Suecica 170. 8 p.

Haapanen, M. 1996. Impact of family-by-trial interaction on the utility of progeny testing methods for Scots pine. Silvae Genetica 45: 130-135.

Haapanen, M. 2002. Evaluation of options for use in efficient genetic field testing of *Pinus sylvestris* (L.) (Doctoral thesis) Finnish Forest Research Institute, Research Papers 826. 67 p. + app.

Haapanen, M., Velling, P. & Annala, M.-L. 1997. Progeny trial estimates of genetic parameters for growth and quality traits in Scots pine. Silva Fennica 31(1):3-12.

Haapanen, M., Mikola, J., Ruotsalainen, S. & Venäläinen, M. 1999. Combining genetic gain and diversity by means of open nucleus breeding system. In: Skrøppa, T. (ed.) Proceedings of the 1998 Joint meeting of Nordic group for the management of genetic resources of trees and Nordic Arboretum Council, Biri, Norway, June 25-27, 1998. Aktuelt fra skogforskningen 3/99:26.

Hatcher, A.V., Bridgwater, F.E., & Weir, R.J. 1981. Performance level - standardized score for progeny test performance. Silvae Genetica 30:6 184-187.

Heybroek, H. M. 1982. Monoculture vs. mixture: interactions between susceptible and resistant trees in a mixed stand. In: Heybroek, H. M., Stephan, B. R. & von Weissenberg, K. (eds.) Resistance to diseases and pests in forest trees. Proceedings of the third international workshop on the genetics of host-parasite interactions in forestry, Wageningen, the Netherlands, 14-21 September 1980. Pudoc, Wageningen, pp. 326-341.

Hodge, G. R. and White, T. L. 1993. Advanced generation wind-pollinated seed orchard design. New Forests 7:213-236.

Högberg, K.-A., Ekberg, I., Norell, L. & von Arnold, S. 1998. Integration of somatic embryogenesis in a tree breeding programme: a case study with *Picea abies*. Canadian Journal of Forest Research 28:1536-1545.

Hubert, C. & Bastien, C. 1999. Gain génétique, risque économique, risque écologique: quels liens? Abstract: Genetic benefits, economic risks, ecological risks: how are they connected? Revue Forestière Francaise 4-1999:496-510.

Juga, J., Maijala, K., Mäki-Tanila, A., Mäntysaari, E., Ojala, M. & Syväjärvi, J. 1999. Kotieläinjalostus. [Breeding of domestic animals] (in Finnish). Suomen kotieläinjalostusosuuskunta. Jyväskylä. 294 p.

Johnsson, H. 1964. Forest tree breeding by selection. Clonal seed orchards – seedling seed orchards, progeny tests. Silvae Genetica 13:41-49.

Kang, H. 1982. Components of a tree breeding plan. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including Multiclonal Varieties, Sensenstein, September 6-10 1982. Lower Saxony Forest Research Institute, Department of Forest Tree Breeding, Staufenberg-Escherode, Germany, s. 119-135.

Kang, H. & Nienstaedt, H. 1987. Managing long-term tree breeding stock. Silvae Genetica 36(1):30-39.

Kang, H., Lascoux, M. & Gullberg, U. 1998. Systematic tree breeding. In: Mandal, A. K. & Gibson, G. L. (eds.) Forest Genetics and Tree Breeding. CBS Publishers & Distributors, New Delhi, pp. 38-62.

Kang, K.-S. 2001. Genetic gain and gene diversity of seed orchard crops. (Doctoral thesis) Acta Universitatis Agriculturae Sueciae. Silvestria 187. 75 pp. + app.

Kannenberg, L. W. 1984. Utilization of genetic diversity in crop breeding. In: Yeatman, W, Kafton, D. & Wilkes, G. (eds.) Plant Genetic Resources. A Conservation Imperative. Westview Press, Inc. Boulder, Colorado. USA. pp. 93-109.

Kärki, L. 1985. Genetically narrow-crowned trees combine high timber quality and high stem wood production at low cost. In: Tigerstedt PMA, Puttonen P. & Koski, V. (eds) Crop Physiology of Forest Trees. Helsinki University Press, Helsinki, pp 245-256.

Karnosky, D. F., Shin, D., Podila, G. K., Chiang, V. L. C. & Riemenschneider, D. E. 1998. Biotechnology in Forest Tree Improvement. In: Mandal, A. K. & Gibson, G. L. (eds.) Forest Genetics and Tree Breeding. CBS Publishers & Distributors, New Delhi, pp. 252-268.

Kerr, R. J., Goddard, M. E. & Jarvis, S. F. 1998. Maximising genetic response in tree breeding with constraints on group coancestry. Silvae Genetica 47(2-3):165-173.

Kjær, E. D. 1996. Estimation of effective population number in a *Picea abies* (Karst.) seed orchard based on flower assessment. Scandinavian Journal of Forest Research 11:111-121.

Kleinschmit, J., Khurana, D. K., Gerhold, H. D. & Libby, W. J. 1993. Past, present, and anticipated applications of clonal forestry. In: Ahuja, M. R. & Libby, W. J. (eds.) Clonal Forestry II. Springer-Verlag. pp. 9-41.

Knowles, P. & Mitton, J. B. 1980. Genetic heterozygosity and radial growth variability in *Pinus contorta*. Silvae Genetica 29: 114-118.

Koski, V. 1973. On self-pollination, genetic load, and subsequent inbreeding in some conifers. Communicationes Instituti Forestalis Fenniae 78(10). 42 p.

Koski, V. 1975. Natural pollination in seed orchards with special reference to pines. In: Faulkner, R. (ed.) Seed orchards. Forestry Commission. Bulletin 54. pp. 83-91.

Koski, V., Skrøppa, T., Paule, L., Wolf, H. & Turok, J. 1997. Technical guidelines for genetic conservation of Norway spruce (*Picea abies* (L.) Karst.). EUFORGEN. 42 p.

Kurm, M., Möls, T., Tamm, Ü. & Jõepera, E. 2000. Kõrguse ja rinnasdiameetri päritavuse uurimine harilikul männil. Abstract: Heritability of stem height and BHD in Pinus sylvestris L. Metsanduslikud uurimused XXXII:32-42.

Lambeth, C. C. & McCullough, R. B. 1997. Genetic diversity in managed loblolly pine forests in the southeastern United States: perspective of the private industrial forest land owner 1997. Canadian Journal of Forest Research 27: 409-414.

Lambeth, C., Lee, B.-C., O'Malley, D. & Wheeler, N. 2001. Polymix breeding with parental analysis of progeny: an alternative to full-sib breeding and testing. Theoretical and Applied Genetics 103:930-943.

Lee, S. J. 1993. Breeding strategy for Sitka spruce in Britain. In: Lee, S. J. (ed.).Progeny testing and breeding strategies. Proceedings of the Nordic Group for Tree Breeding, Edinburgh, Scotland. 6-10 October 1993, pp. 95-107.

Lee, S. J. 1999. Improving the timber quality of Sitka spruce through selection and breeding. Forestry 72:123-133.

Lee, S. J. 2001. Selection of parents for the Sitka spruce breeding population in Britain and the strategy for the next breeding cycle. Forestry 74:129-143.

Libby, W. J. 1973. Domestication strategies for forest trees. Canadian Journal of Forest Research 3:265-275.

Libby, W. J. 1982. What is a safe number of clones per plantation. In: Heybroek, H. M., Stephan, B. R. & von Weissenberg, K. (eds.) Resistance to diseases and pests in forest trees. Proceedings of the third international workshop on the genetics of host-parasite interactions in forestry, Wageningen, the Netherlands, 14-21 September 1980. Pudoc, Wageningen, pp. 342-360.

Libby, W. J., Bridgwater, F., Lanz, C. & White, T. 1997. Genetic diversity in commercial forest tree plantations: introductory comments to the 1994 SRIEG meeting papers. Canadian Journal of Forest Research 27:397-400.

Lindgren, D. 1986. How should breeders respond to breeding values? Proceedings of a joint meeting of IUFRO WPs on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, October 13-17. 1986. pp. 361-372.

Lindgren, D. 1993. The population biology of clonal deployment. In: Ahuja, M. R. & Libby, W. J. (eds.) Clonal Forestry I. Springer-Verlag. pp. 34-49.

Lindgren, D. 2002a. Advantages of clonal propagation. In Welander, M. & Zhu, L. H. (eds.) Proceedings of Workshop on high quality birch – clonal propagation and wood properties. August 27-28, 2001. Swedish University of Agricultural Sciences, Alnarp, pp 98-109.

Lindgren, D. 2002b. Tree breeding tools (TBT). In: Haapanen, M. & Mikola, J. (eds.) Integrating Tree Breeding and Forestry. Proceedings of the Nordic Group for Management of Genetic Resources of Trees, meeting at Mekrijärvi, Finland, March 23-27, 2001. Finnish Forest Research Institute, Research Papers 842: 59-70.

Lindgren, D. & Gregorius, H. R. 1976. Inbreeding and coancestry. Proceedings of the IUFRO Joint meeting. Advanced generation breeding. Bordeaux. June 14-18 1976. pp. 49-69.

Lindgren, D. & Kang, K. S. 1997. Status number - a useful tool for tree breeding. Research Report of Forest Genetics Research Institute, Korea 33:154-165.

Lindgren, D. & Matheson, A. C. 1986. An algorith for increasing the genetic quality of seed from seed orchards by using better clones in higher proportions. Silvae Genetica 35:173-177.

Lindgren, D. & Mullin, T. J. 1997. Balancing gain and relatedness in selection. Silvae Genetica 46: 124-129.

Lindgren, D. & Mullin, T. J. 1998. Relatedness and status number in seed orchard crops. Canadian Journal of Forest Research 28:276-283.

Lindgren, D. & Werner, M. 1989. Gain generating efficiency of different Norway spruce seed orchard designs. In: Stener, L.-G. and Werner, M. (eds.). Norway Spruce; Provenances, Breeding and Genetic Conservation. Proceedings of the IUFRO working party meeting, S2.02-11, in Sweden 1988. Institutet för skogsförbättring. Rapport nr 11:189-206.

Lindgren, D., Libby, W. S. & Bondesson, F. L. 1989. Deployment to plantations of numbers and proportions of clones with special emphasis on maximizing gain at a constant diversity. Theoretical and Applied Genetics 77: 825-831.

Lindgren, D., Gustafsson, L., Hulthén, H. & Lundkvist, K. 1990. Klonskogsbruk. [Clonal forestry] SLU. Skogsvetenskapliga fakulteten. Rapport nr 9. Umeå. 61 p. (in Swedish)

Lindgren, D., Gea, L. & Jefferson, P. 1996. Loss of genetic diversity monitored by status number. Silvae Genetica 45:52-59.

Lindgren, D., Gea, L. D. & Jefferson, P. A. 1997a. Status number for measuring genetic diversity. Forest Genetics. 4:69-76.

Lindgren, D., Wei, R.-P. & Lee, S. J. 1997b. How to calculate optimum family number when starting a breeding program. Forest Science 43(2):206-212.

Lundkvist, K. 1982. Genetic structure in natural and cultivated forest tree populations. Silva Fennica 16:141-149.

Lundkvist, K., Eriksson, G., Norell, L. & Ekberg, I. 1987. Inbreeding depression in two field trials of young *Pinus sylvestris* (L.). Scandinavian Journal of Forest Research 2: 281-290.

Macdonald, S. E., Thomas, B. R., Cherniawsky, D. M. & Purdy, B. G. 2001. Managing genetic resources of lodgepole pine in west-central Alberta: patterns of isozyme variation in natural populations and effects of forest management. Forest Ecology and Management 152:45-58.

MacKay, J. J., O'Malley, D. M., Presnell, T., Booker, F., Campbell, M. M., Whetten, R. W. & Sederoff, R. R. 1997. Inheritance, gene expression, and lignin characterization in a mutant pine deficient in cinnamyl alcohol dehydrogenase. Proceedings of the National Academy of Sciences 94:8255-8260.

Mann, C. C. & Plummer, M. L. 2002. Forest biotech edges out of the lab. Science 295: 1624-1629.

Mayo, O. 1987. The theory of plant breeding. 2nd edition. Clarendon Press. Oxford. 334 p.

McKeand, E. & Beineke, F. 1980. Sublining for half-sib breeding populations of forest trees. Silvae Genetica 29: 14-17.

McKeand, S. E. & Bridgwater, F. 1998. A strategy for the third breeding cycle of loblolly pine in the southeastern U.S. Silvae Genetica 47: 223-234.

McKeand, S. & Svensson, J. 1997. Sustainable management of genetic resources. Journal of Forestry 95(3):4-9.

McKinley, C. R. & van Buijtenen, J. P. 1998. Principles and techniques of selection in tree breeding. In: Mandal, A. K. & Gibson, G. L. (eds.) Forest Genetics and Tree Breeding. CBS Publishers & Distributors, New Delhi, pp. 1-15.

Meuwissen, T. H. E. 1997. Maximizing the response of selection with a predefined rate of inbreeding. Journal of Animal Science 75:934-940.

Mikola, J. 1993. Provenance and individual variation in climatic hardiness of Scots pine in northern Finland. In: Alden, J., Mastrantonio, J. L. & Ødum, S. (eds.) Forest Development in Cold Climates. Proceedings of a NATO Advanced Research Workshop. Laugarvatn, Iceland, June 18-23.1991. Plenum Press. New York and London. pp. 333-342.

Mikola, J. 2002. Long-term tree breeding strategy in Finland: integration of seed production and breeding. In: Haapanen, M. & Mikola, J. (eds.) Integrating Tree Breeding and Forestry. Proceedings of the Nordic Group for Management of Genetic Resources of Trees, meeting at Mekrijärvi, Finland, March 23-27, 2001. Finnish Forest Research Institute, Research Papers 842: 12-13.

Mitton, J. B. 1995. Genetics and the physiological ecology of conifers. In: Smith, W. K. & Hinckley, T. M. (eds.) Ecophysiology of coniferous forests. Academic Press. pp. 1-36.

Mitton, J. B., Knowles, P., Sturgeon, K. B., Linhart, Y. B., Davis, M. 1981. Associations between heterozygosity and growth rate variables in three western forest trees. In: Conkle, M. T. (ed.) Proceedings of the symposium on Isozymes of North American forest trees and forest insects. July 27, 1979, Berkeley, California. USDA Forest Service. Pacific Southwest Forest and Range Experiment Station. General technical report PSW-48. pp. 27-34.

Mullin, T. J. and Park, Y. S. 1995. Stochastic simulation of population management strategies for tree breeding: a new decision-support tool for personal computers. Silvae Genetica 44:132-141.

Muona, O. & Harju, A. 1989. Effective population sizes, genetic variability, and mating system in natural stands and seed orchards of *Pinus sylvestris*. Silvae Genetica 38:221-228.

Namkoong, G. 1969. Choosing selection intensities for seedling seed orchards. Silvae Genetica 18:174-176.

Namkoong, G. 1999. Plantations vs. naturally regenerated forests: Risks and stability in using bred, cloned, or biotech products. In: Mátyás, C. (ed.) Forest Genetics and Sustainability. Kluwer Academic Publishers. pp. 147-154.

Namkoong, G. & Koshy, M.P. 1997. Managing the genetic variance. In: Mátyás, C. (ed.) Perspectives of forest genetics and tree breeding in a changing world. IUFRO World Series Vol. 6 pp. 9-16.

Namkoong, G., Kang, H. C. & Brouard, J. S. 1988. Tree Breeding: Principles and Strategies. Monographs on Theoretical and Applied Genetics 11. Springer-Verlag. 177 p.

Nienstaedt, H. & Kang, H. 1987. Establishing a *Picea glauca* (Moench) Voss base breeding population for the Lake States region of the United States. Silvae Genetica 36(1):21-30.

Nikkanen, T. 1982. Pohjois-Suomen mäntyjen nuorissa siemenviljelyksissä syntyneen siemenen käyttömahdollisuuksista Oulun läänin alueella. Summary: Survival and height growth of North Finland × South Finland hybrid progenies of Scots pine in intermediate areas. Folia Forestalia 527. 31 p.

Nikkanen, T. & Ruotsalainen, S. 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34(3): 205-222.

Oskarsson, O. 1972. Suomalaiset plusmännyt ja pluskuuset. Finnish plus trees of Scots pine and Norway spruce. Folia Forestalia 150. 138 p.

Oskarsson, O. 1995. Silmällä tehty savotta. Pluspuiden valinnnan historia ja arki. [Logging done by eye. The history and everyday life of plus tree selection] (in Finnish). Metsäntutkimuslaitoksen tiedonantoja 579. 67 p.

Oskarsson, O. & Nikkanen, T. 1999. Säregna former av skogsträd från guldgran till kvastbjörk. [Special forms of forest trees from golden spruce to whisk birch] (in Swedish) Skogsforskningsintitutets meddelanden 740. 54 p.

Ouborg, N. J., Biere, A. & Mudde, C. L. 2000. Inbreeding effects on resistance and transmission-related traits in the Silene-Microbotryum pathosystem. Ecology 81(2):520-531.

Palmer, H. E., Newton, A. C., Doyle, C. J., Thomson, S. & Stewart, L. E. D. 1998. An economic evaluation of alternative genetic improvement strategies for farm woodland trees. Forestry 71(4):333-347.

Park, Y.S. & Gerhold, H. D. 1986 Population hybridization in Scotch pine (*Pinus sylvestris* L.): Genetic variance components and heterosis. Silvae Genetica 35(4):159-165

Perala, D. A. 1990. Populus tremuloides Michx. Quaking Aspen. In: Burns R. M. & Honkala, B. (eds.) Silvics of North America. Volume 2, Hardwoods. USDA Agriculture Handbook 654. pp. 555-569.

Persson, T. 1999a. Finska plusträdskloner till förädlingspopulation Tpop 4. [Finnish plus tree clones to breeding population Tpop 4] (in Swedish) Avelsvärden 71. 33 p.

Persson, T. 1999b. Finska plusträdskloner till förädlingspopulation Tpop 7. [Finnish plus tree clones to breeding population Tpop 7] (in Swedish) Avelsvärden 69. 30 p.

Pitkäntähtäyksen metsänjalostusohjelma. 1989. Metsänjalostuksen tarkennustyöryhmä. Metsähallitus. Intern report. [Long-term forest tree breeding program. Working group for focusing forest tree breeding. Finnish Forest and Park Service] (in Finnish) 127+8 p.

Plomion, C., Durel, C. É. & Verhaegen, D. 1996. Utilisation des marqueurs moléculaires dans les programmes d'amélioration génétique des arbres forestiers: exemple du pin maritime et de l'eucalyptus. Summary: Marker-assisted selection in forest tree breeding programs as illustrated by two examples: maritime pine and eucalyptus. Annales des Sciences Forestières 53:819-848.

Porterfield, R. L., Zobel, B. J. & Ledig, F. T. 1975. Evaluating the efficiency of tree improvement programs. Silvae Genetica 24:33-44.

Pöykkö, T. 1993. Selection criteria in Scots pine breeding with special reference to ideotype. (Doctoral thesis) Reports from the Foundation for Forest Tree Breeding. No. 6. 66 p.

Prakash, C. S. & Thielges, B. A. 1986. Genetics and breeding for durable resistance to leaf rust in poplars. Proceedings of a joint meeting of IUFRO WPs on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, October 13-17. 1986. pp. 342-350.

Pulkkinen, P. 1991. The pendulous form of Norway spruce as an option for crop tree breeding. (Doctoral thesis) Reports from the Foundation for Forest Tree Breeding. No. 2. 30 p. +app.

Pulkkinen, P., Haapanen, M. & Mikola, J. 1995. Effect of southern pollination on the survival and growth of seed orchard progenies of northern Scots pine (Pinus sylvestris) clones. Forest Ecology and Management 73: 75-84.

Puri, S. 1998. Plus-tree selection as a tool in tree improvement. In: Puri, S. (ed.) Tree Improvement: Applied Research and Technology Transfer. Science Publishers Inc. U.S.A. pp 97-105.

Quinton, M., Smith, C. & Goddard, M. E. 1992. Comparison of selection methods at the same level of inbreeding. Journal of Animal Science 70:1060-1067.

Rajora, O. P. 1999. Genetic biodiversity impacts of silvicultural practices and phenotypic selection in white spruce. Theoretical and Applied Genetics 99:954-961.

Rosvall, O. 1999. Enhancing gain from long-term forest tree breeding while conserving genetic diversity. (Doctoral thesis) Acta Universitatis Agriculturae Sueciae. Silvestria 109. 65 pp. + app.

Rosvall, O. & Andersson, E. 1999. Group-merit selection compared to conventional restricted selection for trade-offs between genetic gain and diversity. Forest Genetics 6:11-24.

Rosvall, O., Lindgren, D. & Mullin, T. J. 1998. Sustainability robustness and efficiency of a multigeneration breeding strategy based on within-family clonal selection. Silvae Genetica 47:307-321.

Rosvall, O., Jansson, G., Andersson, B., Ericsson, T., Karlsson, B., Sonesson, J. & Stener, L.-G. 2001. Genetiska vinster i nuvarande och framtida fröplantager och klonblandningar. Summary: Genetic gain from present and future seed orchards and clone mixtures. SkogForsk. Redogörelse No. 1, 2001 41 p.

Rousi, M. 1983. Pohjois-Suomen siemenviljelysjälkeläisten menestymisestä Kittilässä. Summary: The thriving of the seed orchard progenies of northern Finland at Kittilä. Folia Forestalia. 547. 14 p.

Rudolf, P. O., Dorman, K. W., Hitt, R. G. & Plummer, A. P. 1974. Production of genetically improved seed. In: Seeds of woody plants in the United States. USDA Agriculture Handbook 450. pp. 53-74.

Ruotsalainen, S. 1999. Metsänjalostuksen populaatiokäsitteet. [Population concepts in forest tree breeding] (in Finnish) Metsätieteen aikakauskirja 1/1999: 79-91.

Sarvas, R. 1953. Ohjeita pluspuiden valitsemista ja ilmoittamista varten. Summary: Instruction for the selection and registration of plus trees. Silva Fennica 80:93-101

Sarvas, R. 1970. Establishment and registration of seed orchards. Folia Forestalia 89. 24 p.

Sanchez, L., Toro, M. A. & Garcia, C. 1999. Improving the efficiency of artificial selection: more selection pressure with less inbreeding. Genetics 151: 1103-1114.

SAS Institute Inc. 1989 SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC. USA, SAS Institute Inc. 846 p.

SAS Institute Inc. 1996 SAS/STAT® Software: Changes and Enhancements through Release 6.11, Cary, NC. USA, SAS Institute Inc. 1104 p.

Savolainen, O. & Hedrick, P. 1995. Heterozygosity and fitness: no association in Scots pine. Genetics 140:755-766.

Savolainen, O. & Kärkkäinen, K. 1992. Effect of forest management on gene pools. New Forests 6:329-345.

Schmidtling, R. C., Carroll, E. & LaFarge, T. 1999. Allozyme diversity of selected and natural loblolly pine populations. Silvae Genetica 48:35-45.

Shelbourne C. J. A. 1986. New approaches in breeding radiata pine. Proceedings of a joint meeting of IUFRO WPs on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, October 13-17. 1986. pp. 269-284.

Shelbourne C. J. A., Carson, M. J. & Wilcox, M. D. 1989. New techniques in the genetic improvement of radiata pine. Commonwealth Forestry Review 68(3): 191-201.

Shepherd, R. K. and Kinghorn, B. P. 1994. A deterministic multi-tier model of assortative mating following selection. Genetics Selection Evolution 26: 495-516.

Simpson, D. 1998. Selection of superior trees. In: Puri, S. (ed.) Tree Improvement: Applied Research and Technology Transfer. Science Publishers Inc. U.S.A. pp. 107-124

Skrøppa, T. 1982. Breeding strategies with Norway spruce in south-eastern Norway. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including Multiclonal Varieties, Sensenstein, September 6-10 1982. Lower Saxony Forest Research Institute, Department of Forest Tree Breeding, Staufenberg-Escherode, Germany, pp. 1-9.

Sonesson, J., Bradshaw, R., Lindgren, D. & Ståhl, P. 2001. Ecolocical evaluation of clonal forestry with cutting-propagated Norway spruce. SkogForsk Report No. 1, 2001 59 p.

Sorensen, F. C. & Miles, R. S. 1974. Self-pollination effects on Douglas-fir and ponderosa pine seeds and seedlings. Silvae Genetica 23:135-138.

SPSS® Base 10.0 User's Guide. 1999. SPSS Inc. 537 p.

Ståhl, P. H. & Jansson, G. 2002. Tree breeding in Sweden. In: Haapanen, M. & Mikola, J. (eds.) Integrating Tree Breeding and Forestry. Proceedings of the Nordic Group for Management of Genetic Resources of Trees, meeting at Mekrijärvi, Finland, March 23-27, 2001. Finnish Forest Research Institute, Research Papers 842: 14-20.

Stern, K. & Roche, L. 1974. Genetics of forest ecosystems. Springer-Verlag. 330 p.

Stonecypher, R. W. 1969. Recurrent selection in forest tree breeding. Proceedings of Southern Conference on Forest Tree Improvement. Houston, Texas, June 17-19, 1969. pp. 7-16.

Stonecypher, R. & Arbez, M. 1976. In: Methods of selection. Proceedings of the IUFRO Joint meeting. Advanced generation breeding. Bordeaux. June 14-18 1976. pp. 31-46.

Strategi för framtida skogsträdsförädling och framställning av förädlat skogsodlingsmaterial i Sverige. 1995. Intern report. [Strategy for future forest tree breeding and production of improved material for forest cultivation in Sweden.] (in Swedish) Skogforsk. Uppsala, Sweden. 258 p.

Strauss, S. H. & Libby, W. J. 1987. Allozyme heterosis in radiata pine is poorly explained by overdominance. The American Naturalist 130:879-890.

Strauss, S. H., Campbell, M. M., Pryor, S. N., Coventry, P. & Burley, J. 2001. FSC's ban on research is counterproductive. Journal of Forestry. December 2001: 4-7.

Taira, H., Tsumura, Y., Tomaru, N. & Ohba, K. 1997. Regeneration system and genetic diversity of *Cryptomeria japonica* growing at different altitudes. Canadian Journal of Forest Research 27: 447-452.

Tigerstedt, P. M. A. & Malmivaara, E. 1970. Metsänjalostuksen mahdollisuudet. I. Pluspuiden valintaero ja siemenviljelysten valintahyöty. Summary: The possibilities in forest tree breeding. I. Selection differential of plus trees and genetic gain in seed orchards. Silva Fennica 4:101-118.

Toda, R. 1974. Vegetative propagation in relation to Japanese forest tree improvement. New Zealand Journal of Forestry Science 4:410-417.

Toro, M. A. & Nieto, B. M. 1984: A simple method for increasing the response to artificial selection. Genetical Research, Cambridge 44:347-349.

Toro, M. A., Nieto, B. & Salgado, C. 1988. A note on minimization of inbreeding in small-scale selection programmes. Livestock Production Science 20:317-323.

Tynkkynen, H. 1998. Männyn fenotyyppisen pluspuuvalinnan tehokkuus Etelä-Suomessa. [The efficiency of phenotypic plus tree selection of

Scots pine in southern Finland] (in Finnish). Kymenlaakson va. ammattikorkeakoulu. Metsäosasto. Insinöörityö. [Kymenlaakso prov. Polytechnic. Forestry department. Ingenieur work] 32 p + app.

van Buijtenen, J. P. 1975. The planning and strategy of seed orchard programmes, including economics. In: Faulkner, R. (ed.) Seed Orchards. Forestry Commission. Bulletin 54. London. pp. 9-24.

van Buijtenen, J. P. 1984. Strategies for maintaining the genetic base. How will advanced generation tests differ from first? In: Proceedings of Servicewide Genetics Workshop: Progeny Testing. Charleston, SC, 5-9 December 1983. USDA Forest Service, Washington, D.C., USA. pp. 566-572.

van Raemdonck, D., Jaziri, M., Boerjan, W. & Baucher, M. 2001. Advances in the improvement of forest trees through biotechnology. Belgian Journal of Botany 134:64-78.

Vargas, C. F., López, A., Sánchez, H. & Rodríguez B. 2002. Allozyme analysis of host selection by bark beetles in central Mexico. Canadian Journal of Forest Research 32:24-30.

Velling, P. & Nepveu, G. 1986. Männyn puuaineen laadun ja tuotoksen vaihtelu suomalaisessa provenienssikoesarjassa. Summary: Variation of wood quality and yield in a Finnish series of provenance trials on Scots pine. Silva Fennica 20:211-231.

Venäläinen, M. 1992. The Finnish regulations concerning the sale of vegetatively propagated forestation material. In: Nather, J. (ed.). Proceedings of the meeting of IUFRO - WP S2.02-21 on Actual Problems of the Legislation of Forest Reproductive Material and the Need for Harmonization of Rules at an International Level. Gmunden/Vienna - Austria, June 10.-14. 1991. FBVA-Berichte 65: 61-67.

Venäläinen, M. 1993. The combined results of 190 progeny tests of Scots pine in southern and central Finland. In: Lee, S. J. (ed.). Progeny Testing and Breeding Strategies. Proceedings of the Nordic Group for Tree Breeding, Edinburgh, Scotland, 6-10 October, 1993. Forestry Commission, Roslin. p. 36-42.

Venäläinen, M. & Ruotsalainen, S. 2002. Procedure for Managing Large-scale Progeny Test Data. The Case of Scots Pine in Finland. Silva Fennica 36:475-487.

Venäläinen, M., Annala, M-L., Kosonen, E., Rantanen, H. & Tynkkynen, H. 1994. Plusmäntyjen testaustulosrekisteri ja jalostushyöty. [Register of test results and genetic gain of Scots pine plus trees] (in Finnish) Metsäntutkimuslaitoksen tiedonantoja 497. 89 p.

Villanueva, B. & Woolliams, J. A. 1997. Optimization of breeding programmes under index selection and constrained inbreeding. Genetical Research, Cambridge 69:145-158.

von Wuehlisch, G., Muhs, H.-J. & Geburek, T. 1990. Competitive behaviour of clones of *Picea abies* in monoclonal mosaics vs. intimate clonal mixtures. A pilot study. Scandinavian Journal of Forest Research 5:397-401.

Wallace, B. 1981. Basic population genetics. Columbia University Press. 688 p.

Wei, R.-P. 1995. Predicting genetic diversity and optimizing selection in breeding programmes. (Doctoral thesis) Swedish University of Agricultural Sciences. Department of Forest Genetics and Plant Physiology. 59 p. + app.

Wei, R.-P. & Lindgren, D. 2001. Optimum breeding generation interval considering buildup of relatedness. Canadian Journal of Forest Research 31: 722-729.

Weir, R. J. 1974. A breeding and selection program for second-generation improvement. Proceedings of Joint IUFRO Meeting, S.02.04.1-3, Stockholm, 1974, Session II. pp.69-78.

Weir, R. J. & Zobel, B. J. 1975. Advanced-generation seed orchards. In: Faulkner, R. (ed.) Seed Orchards. Forestry Commission. Bulletin 54. London. pp. 118-127

Wellendorf, H. 1986. Danish spruce breeding. Proceedings of a joint meeting of IUFRO WPs on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, October 13-17. 1986. pp. 239-249.

Werner, M., Hadders, G. & Rosvall, O. 1981. Förädlingsprogram för tall, gran och contortatall. Summary: Breeding programs for Scots pine, Norway spruce and lodgepole pine. Föreningen skogsträdsförädling. Institutet för skogsträdsförädling. 1980 Årsbok. pp. 59 – 111.

White, T. 1992. Advanced generation breeding populations: size and structure. In: Resolving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species. Proceedings of IUFRO conference S2.02-08, Breeding tropical trees, 9-18 October 1992. Cartagena and Cali, Colombia. pp. 208-222.

White, T. L. 1987. A conceptual framework for tree improvement programs. New Forests 4:325-342.

White, T. L., Hodge, G.R. & Powell, G.L. 1993. An advanced-generation tree improvement plan for slash pine in south-eastern United States. Silvae Genetica 42(6):359-371.

Wilhelmsson, L., Eriksson, U., Danell, Ö. 1993. Produktion av förädlat frö. Summary: Production of genetically improved seed, SkogForsk Redogörelse nr 3 1993, 52 p.

Williams, C. G. & Hamrick, J. L. 1996. Elite populations for conifer breeding and gene conservation. Canadian Journal of Forest Research 26:453-461.

Williams, C. G. & Savolainen, O. 1996. Inbreeding depression in conifers: implications for breeding strategy. Forest Science 42: 102-117.

Williams, C. G., Hamrick, J. L. & Lewis, P. O. 1995. Multiple-population versus hierarchical conifer breeding programs: a comparison of genetic diversity levels. Theoretical and Applied Genetics. 90:584-594.

Wray, N. R. & Goddard, M. E. 1994. Increasing long-term response to selection. Genetics Selection Evolution 26:431-451.

Yanchuk, A. D. 2001. A quantitative framework for breeding and conservation of forest genetic resources in British Columbia. Canadian Journal of Forest Research 31:566-576.

Yrjänä, L., Karvinen, K. & Napola, J. 2000. Suomen metsänjalostuksen yleistilastoa 2000. General statistics on forest tree breeding in Finland 2000. Finnish Forest Research Institute, Research Papers 783. 45 p.

Zheng, Y.-Q., Andersson, E. W. and Lindgren, D. 1998. A model for infusion of unrelated material into a breeding population. Silvae Genetica 47:94-101.

Zhong, G-Y. 2001. Genetic issues and pitfalls in transgenic plant breeding. Euphytica 118:137-144.

Ziehe, M. & Hattemer, H. 1998. The significance of heterozygosity in tree breeding and gene concervation. Forest Tree Improvement 26: 3-9.

Zobel, B. J. & Talbert, J. 1984. Applied forest tree improvement. John Wiley & Sons, New York. 505 p.

Zobel, B. J., Weir, R. J. & Jett, J. B. 1972. Breeding methods to produce progeny for advanced generation selection and to evaluate parent trees. Canadian Journal of Forest Research 2: 339-345.

Zsuffa, L., Sennerby-Forsse, L. & Weisgerber, H. 1993. Strategies for clonal forestry with poplars, aspens and willows. In: Ahuja, M. R. & Libby, W. J. (eds.) Clonal Forestry II. Springer-Verlag. pp. 91-119.

Total of 208 references

I

The effect of plus tree selection intensity and selection conditions on the growth of *Pinus sylvestris* progeny

Seppo Ruotsalainen[1)], Bengt Andersson[2)] & Torgny Persson[2)]

[1)] Finnish Forest Research Institute, Punkaharju Research Station, FIN-58450 Punkaharju, Finland
[2)] SkogForsk, Box 3, SE-91821 Sävar, Sweden

Abstract

On the average, plus trees in two breeding populations of *Pinus sylvestris* accounted for over 80 % of the additive genetic variation in height growth in 11- to 13-year-old progeny tests. This was 3 to 8 times more than the additive genetic variation between the stands from which the plus trees originated. Phenotypic selection in the forest yielded only low genetic gain. Plus trees selected from special seed collection stands did not yield progeny with better height growth than the plus trees from other stands. In progeny tests, the height of progenies of plus trees with an average selection intensity was 1 to 2 percent higher than the height of progenies from trees with zero selection intensity. Slight positive correlation was observed between the parental selection intensity and offspring height growth (r = 0.143 and 0.247 for different breeding populations). Conclusion about the selection effect was obscured by the confoundedness between selection intensity, latitude and progeny height growth.

Keywords: genetic variation, offspring-parent correlation, plus tree, selection intensity

1. Introduction

Selection is the basic element in genetic improvement of any species or population. It has been practised, more or less intentionally, with many crop plants and domestic animals for thousands of years (Mayo 1987), and it has also been the first step in the initiation of tree breeding activities in the 20th century (Zobel and Talbert 1984). The selected plus trees have to be phenotypically superior to their neighbours in respect to one or several characters. In the early days of plus tree selection very high criteria were set on the plus trees, which led to a small number of selections per unit cost and time used. Later on opinions changed when plus trees were understood to be more the starting material for a breeding program than the final product as such (Oskarsson 1995, Simpson 1998, Lee 1999). Consequently, a less intensive selection method was adopted, leading to higher productivity of the selection work.

Plus trees can be selected either on the basis of comprehensive measurements and comparison to a standard, or more intuitively by ocular evaluation. Relatedness of the selected trees is not desired as this will cause inbreeding in the regeneration material and diminish the future gains by decreasing genetic variation. This is controlled by setting limitations on the number of trees selected from a stand or on the distance between selected plus trees (Lindgren and Gregorius 1976, Zobel and Talbert 1984, Puri 1998).

The degree of selection in the population is measured by the selection differential, which is the difference between the selected individual (or group) and the population mean in the measured character. A standardised selection differential, the selection intensity (Eq. 1), is used when selections from different populations are compared (Falconer and Mackay 1996). As standardisation is done within stands, the means of the stands are assumed to be genetically at the same level. The selection intensities for a selected group of trees can also be calculated from the proportion of the selected trees in the population (selection ratio), if the character is normally distributed and truncating selection has been utilised (Lindgren and Nilsson 1985, Falconer and Mackay 1996).

It can be expected, on the basis of high migration rates in most forest tree species, that the largest proportion of the genetic variation within populations is at the within-stand level. The distribution of variation between and within stands has been largely neglected in research in this field, but the few results published are in line with these expectations (Park and Gerhold 1986, Eriksson et al. 1987, Simpson 1998). In spite of the low between-stand variation, special seed collection stands have been selected for tree breeding purposes. This was common especially in the early phases of tree breeding, when the genetic structures of tree populations were not so well known, and when there was an attempt to rapidly fulfil the need of good quality seed for forest regeneration (Matthews and McLean 1957, Oskarsson 1971, 1995, Pihelgas 1991, Pajamäki and Karvinen 1997). Selection of seed collection stands was also motivated by the need to avoid the negative effects of selective cuttings, commonly practised in the beginning of large-scale forestry, on the genetic value of reforestation material. For the same reason plus tree selection was also concentrated on stands that had not been treated by selective cuttings. In addition, some further criteria were set on the structure of the stands in which plus trees were selected. The most important requirement has been that they should be middle aged, with little age variation between the trees (Sarvas 1953, Zobel and Talbert 1984, Simpson 1998).

The effect of plus tree selection on growth and other characters has usually been studied by comparing the progenies of plus trees to certain standard entries (Cornelius 1994, Venäläinen et al. 1994, Ruotsalainen and Nikkanen 1998), but there are only a few studies in which direct comparisons between plus tree characters and their progenies have been made. Some scattered observations and preliminary reports concerning the relationship between plus tree characters and the

growth of their progenies have been made (Eklundh Ehrenberg 1966, Pajamäki 1974, Tynkkynen 1998, Kurm et al. 2000), but more comprehensive studies are lacking. It is especially surprising that only a few studies have been carried out on offspring-parent regression for height growth in forest trees (Franklin and Squillace 1973), although this is one of the basic methods used to estimate heritability and success in selection in practical breeding (Casler 1982, Becker 1984, Anderson et al. 1991), in basic genetic studies (Gimelfarb and Willis 1994, Falconer and Mackay 1996, Lynch and Walsh 1998) and in ecological genetics (Hansen and Boonstra 2000). Although parent-offspring regression has several appealing properties in estimating heritabilities (Lynch and Walsh 1998), there are technical difficulties associated with it. The main obstacle is that both offspring and parent populations should have equal variances, and thus usually also equal means (Franklin 1974, Lynch and Walsh 1998). This condition is naturally rather difficult to fulfil with the long rotation time of forest trees. This question has therefore been omitted from the present study.

Our aim is to study the effectiveness of plus tree selection on the height growth of Scots pine in northern Finland. The main questions to be treated are: 1) which factors affect the selection efficiency, 2) how the variation in height growth is distributed within a population, and 3) how applicable is the regression of offspring height on parent selection intensity in describing the efficiency of selection.

2. Material and Methods

2.1. Plus tree material

The plus trees included in this study are part of the large body of Finnish Scots pine breeding stock that have mainly been selected from mature natural stands during a period of over twenty years (1940's to 1970's) (Oskarsson 1972). The trees belong to the material that was used in the beginning of the 1980's to complement Scots pine breeding populations in northern Sweden. The material was transferred as scions to Sweden from Finnish clone archives and seed orchards. Selection of the plus trees was done mainly on the basis of the plus tree origin in order to meet the requirements of climatic adaptation in the targeted breeding populations, and according to the availability of grafts for taking scions and making crosses. Within these restrictions, weak phenotypic selection was also performed on the basis of graft vigour.

The total number of plus trees in the study was 242. They were allocated to two different Swedish breeding populations: 137 trees to Tpop4, and 105 trees to the more southern Tpop7 (Table 2, Fig. 1).

Part of the plus trees originated from specially selected seed collection stands (45 % of the trees), and part from unselected stands. The requirements for a seed collection stand have varied slightly over the years, but the main criteria have been even age, even site conditions, large enough area (min. 3 ha), better than average

height growth and lack of selective cuttings (Oskarsson 1971, Pajamäki and Karvinen 1997). The very best trees in these seed collection stands were selected as plus trees. The plus tree selection did not, as such, differ in seed collection stands and other stands, and neither did the stand conditions deviate very much between the two stand categories. It was especially emphasised that stands treated with selective cuttings should be avoided in all plus tree selection (Sarvas 1953).

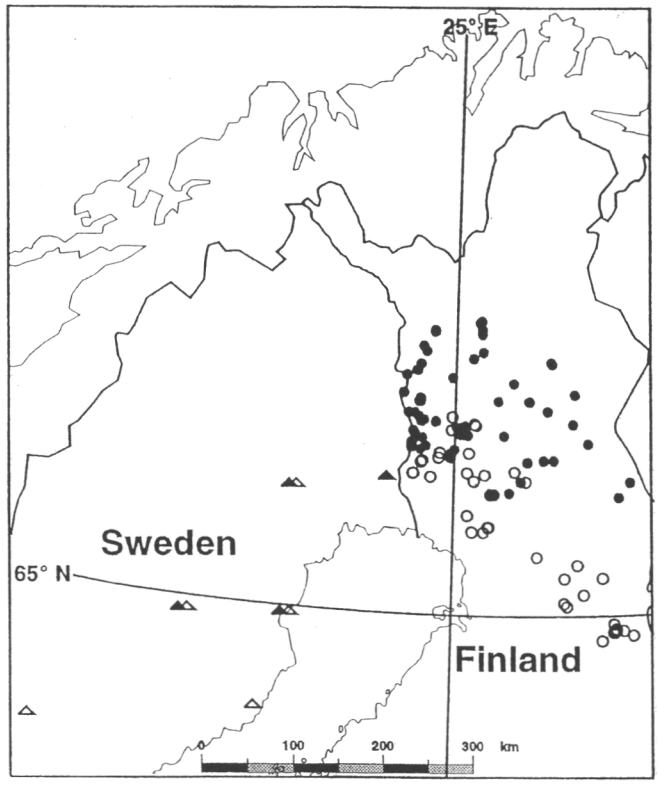


Figure 1. Location of the plus trees (● = Tpop4, ○ = Tpop7) and the progeny tests (▲ = Tpop4, △ = Tpop7).

In processing the material the plus trees were allocated in stands. In the case of plus trees selected from seed collection stands, this did not cause any problems, as the selection had already been performed standwise. However, in the case of other plus trees the allocation was sometimes slightly arbitrary, i.e. whether some plus trees should be denoted to the same or different stands. It was decided that trees located more than 1 km apart from each others belonged to different stands irrespective of their site characteristics. When this definition was applied rather many plus trees (23 %) remained as single exemplars of their stands. This was either because there really was only one selected plus tree in the stand, or because only one of several

plus trees in the stand had been selected further for the Swedish breeding populations.

2.2. Selection intensities

The plus trees have been selected using an overall visual criterion, consisting of growth, stem quality and general vigour (Sarvas 1953). In some special cases either growth or quality had been especially emphasised in the selection and this had been noted on the plus tree record. Height and several other characters of the plus trees and their neighbours have been measured to facilitate quantitative comparison. The difference in height between the plus tree and its neighbours has been standardised to obtain the selection intensity (i) (Eq. 1)

$$i = S / sd \qquad (1)$$

where S is the selection differential and sd is the standard deviation of height in the surrounding stand.

In principle, the plus tree selection method used can be considered to be individual or ocular selection (Oskarsson 1995) according to the definition of Simpson (1998). However, the method was modified so that, after selection, the trees were measured and the selection differential was calculated using one of two different methods. In the comparison-tree method the four thickest trees within a radius of 20 m around the plus tree were used as the basis of comparison. In stands with many selected plus trees a linear inventory was applied and comparison was made with the dominant trees in the whole stand (Oskarsson 1972). The standard deviation of height was calculated in the case of linear inventory from the measured dominant trees, but with the comparison-tree method the number of measured trees was considered too low to give a reliable estimate and the standard deviation was therefore derived from a larger material (Oskarsson 1972). Although the plus trees were measured and the selection differentials and intensities were calculated, the selection can be considered as pure ocular selection because the result of the measurement did not affect the selection of the tree; it only afterwards depicted the outcome of the selection.

The selection intensities, as well as other information describing the plus trees were obtained from the National Register of Forest Genetics of the Finnish Forest Research Institute. As some errors in the selection intensities were found in a preliminary analysis, the calculations for all the plus trees used were checked before the final analysis.

2.3. Progeny tests

Four progeny field trials testing material for Tpop4, and five trials with material for Tpop7, were studied (Table 1). The trials belong to the operational progeny test series of tree breeding, managed by the Forestry Research Institute of Sweden (SkogForsk). Originally there were five trials also in the Tpop4 series, but the northernmost trial had such a high mortality (98 %), that it could not be used in the analysis (Persson 1999a). Descriptive information on the test sites was obtained from internal reports of SkogForsk (Persson 1999a, b), except for the effective temperature sums which were estimated on the basis of latitude and altitude (Morén and Perttu 1994, eq. 12a, correcting for continentality). One-year-old, containerised seedlings were planted in 1986 according to a randomised, single-tree plot design. Each plus tree was represented by 15-16 progeny seedlings per trial at the establishment phase. The trials also included progenies of Swedish plus trees and standard entries, but they were not used in this study.

Table 1. Description of the progeny tests included in the study.

Location and population	Latitude, °N[1)]	Longitude, °E[1)]	Altitude, m a.s.l.[1)]	Site index[1)]	Temperature sum[2)]	No of progenies[3)]	Mean height, cm[4)]
Tpop4	66°30'[5)]				950[5)]		
Övertorneå	66°26'	23°19'	130	T18	853	135	291
Harads	66°17'	20°59'	165	T20	833	137	185
Lycksele	64°55'	18°45'	280	T22	819	132	291
Byske	64°59'	21°05'	40	T20	966	131	275
Tpop7	65°00'[5)]				975[5)]		
Harads	66°17'	20°59'	165	T20	833	72	165
Lycksele	64°55'	18°45'	280	T22	819	86	210
Byske	64°59'	21°05'	40	T20	966	105	232
Strömsund	63°38'	15°37'	315	T20	817	83	166
Sävar	64°01'	20°34'	65	T20	1003	98	253

[1)] from Persson (1999a, b), [2)] degree days, threshold temperature +5°C (Morén and Perttu 1994), [3)] = number of tested plus trees, [4)] 13 growing seasons (since sowing) in Tpop4, 11 growing seasons in Tpop7, [5)] target for the population

The plus tree progenies were produced by controlled crossing in grafts in Finnish and Swedish seed orchards using a pollen mix of Swedish plus trees (Andersson and Danell 1997). The pollen mix used for the crosses consisted of equal amount of pollen from each of 19 plus trees in Tpop4 (mean origin: 66°50'N, 280 m above sea level) and of 22 plus trees in Tpop7 (mean origin: 65°56'N, 110 m a.s.l.) (Persson 1999a, b). The progenies were thus considered as half-sibs. The estimated average latitude (average for both parents) for the progeny origins was 67 °N for Tpop4 and 66 °N for Tpop7. The corresponding average temperature sums for the estimated progeny origins were 713 and 837 d.d. for Tpop4 and Tpop7, respectively.

Height, vitality and several other variables were measured on each tree in autumn 1997 (13 growing seasons after sowing) for Tpop4, and in autumn 1995 (11 growing seasons) for Tpop7 (Persson 1999a, b). Only height measurements of trees with no severe damage (most frequently moose browsing) affecting height growth were utilised in this study.

2.4. Analysis of the progeny tests

Prior to the analyses, adjacent trees were clustered in blocks (9 to 13) in order to take into account the environmental variation in model fitting. The height growth of individual trees in the tests (y_{ijklmn}) for the whole breeding population was first analysed according to the following complete linear model:

$$y_{ijklmn} = \mu + \alpha_i + s_{j(i)} + p_{k(ij)} + t_l + b_{m(l)} + (st)_{ijl} + (pt)_{kijl} + e_{ijklmn}, \qquad (2)$$

where μ is a fixed overall mean and α_i is the fixed effect of stand type (i = 1, 2; 1 = seed collection stand, 2 = other stand). The other factors were random effects with zero expectation and normal, independent distribution. The random effects are as follows: $s_{j(i)}$ is the effect of the jth stand within the ith stand type (with variance σ_s^2); $p_{k(ij)}$ is the effect of the kth plus tree within the ijth stand (σ_p^2); t_l is the effect of the lth test (σ_t^2); $b_{m(l)}$ is the effect of the mth block within the lth test (σ_b^2); $(st)_{ijl}$ is the interaction of the ijth stand with the lth test (σ_{st}^2); $(pt)_{kijl}$ is the interaction of the kijth plus tree with the lth test (σ_{pt}^2), and e_{ijklmn} is the random residual (σ_e^2).

Analysis of variance was first made according to model (2) using the MIXED procedure of the SAS statistical software package (SAS Institute Inc. 1996). Thereafter a second analysis was performed with a model without the fixed stand type effect.

2.5. Correlation and regression

Plus tree level data files were constructed with variables describing the plus tree (e.g. location of origin, age, selection intensity) and the height growth of its progeny in different tests separately and as an average over the tests. Height growth of the progenies was estimated with the LSMEANS option in SAS GLM procedure (SAS Institute Inc. 1989),while adjusting for blocks in the single tests, and for tests and blocks in the average over tests. The plus tree level data were used in correlation and regression analyses to study the dependence of progeny height growth on the plus tree characters. The progeny means over tests were also subjected to one-way analysis of variance to test the effect of selection criteria and conditions on the progeny height growth. The most proper way of analysing the effect of parental selection intensity on offspring height growth would require use of Dummy variables to remove the stand effect from the regression. However, as there were on the average only a few trees in each stand, this was considered unsuitable and the

analyses were made using marginal progeny means to describe the plus tree progeny performance.

3. Results

3.1. Description of the plus tree characters

The Finnish plus tree material in breeding population Tpop4 was, on the average, one degree of latitude more northerly than material in Tpop7. The temperature sum was correspondingly lower for Tpop4 and, in spite of almost equal age at the time of selection, the average height of the plus trees was more than one meter less in Tpop4. The selection intensity for height was slightly higher in Tpop4 than in Tpop7 (Table 2). The variation between the temperature sum of the plus trees was greater in Tpop4 than in Tpop7, although the opposite was true for the variation in latitude. In both breeding populations selection intensity increased with latitude, selection age and tree height (Table 3).

Table 2. Mean values of Finnish Scots pine plus trees used for Swedish breeding populations Tpop4 (n = 137) and Tpop7 (n = 105). Data from the National Register of Forest Genetics of the Finnish Forest Research Institute.

		Mean	sd
Latitude, °N	Tpop4	67.1	0.44
	Tpop7	66.1	0.68
Temperature sum, d.d., (>+5 °C)	Tpop4	813.0	65.4
	Tpop7	891.0	26.7
Selection age ($d_{1.3}$), yrs	Tpop4	99	38.4
	Tpop7	97	33.0
Height, m	Tpop4	21.0	2.8
	Tpop7	22.3	2.5
Selection intensity for height (*i*)	Tpop4	2.06	1.02
	Tpop7	1.93	1.11

Table 3. Correlations between selection intensity and some characters of the Scots pine plus trees in breeding populations Tpop4 and Tpop7 (p-values in parentheses).

	Tpop4	Tpop7
Latitude,°N	0.252 (0.0030)	0.360 (0.0002)
Temperature sum, d.d.	-0.315 (0.0002)	-0.225 (0.0224)
Selection age (d1.3), yrs	0.287 (0.0014)	0.159 (0.1568)
Height, m	0.245 (0.0038)	0.285 (0.0035)

3.2. Distribution of the variation in progeny height growth within population

The height growth of the progenies of plus trees selected from special seed collection stands did not differ from the growth of progenies from other stands ($p = 0.5419$ for Tpop4 and $p = 0.9969$ for Tpop7). Greatest variances in the material were ascribed to residual and test site. Residual, block and tree had variances that differed statistically significantly from zero in the material of both breeding populations, whereas stand effect and interactions with test site were nonsignificant (Table 4). 89 % of the additive genetic variation in Tpop4 and 77% in Tpop7 was within stands, the remaining variation being between the stands.

Table 4. Variances (REML estimates) in the height growth of Scots pine plus tree progenies caused by different sources, and their significance (model 2 without stand type effect).

Source	Tpop4 Variance	Tpop4 p	Tpop7 Variance	Tpop7 p
Test site (σ_t^2)	2647.25	0.2262	1497.20	0.1618
Block in site (σ_b^2);	354.33	0.0001	244.57	0.0001
Stand (σ_s^2)	7.68	0.5624	14.75	0.2353
Tree in stand (σ_p^2)	62.00	0.0137	50.15	0.0030
Site × stand (σ_{st}^2);	8.42	0.6930	11.47	0.5056
Site × tree (σ_{pt}^2)	22.07	0.5271	1.60	0.9395
Residual (σ_e^2)	3640.64	0.0001	2135.98	0.0001

3.3. Dependence between progeny height growth and selection intensity

The correlation between selection intensity and height growth of the progenies was usually positive, but statistically significant only in a few cases (Table 5). Correlation coefficients were higher in Tpop7 than in Tpop4. The progeny mean over all the tests usually had higher correlation with selection intensity (0.14 in Tpop4 and 0.25 in Tpop7) than the individual tests. Adjustment of the selection intensity by the age difference between a plus tree and its neighbours did not influence the correlation with progeny height growth (data not shown).

In regression analysis of the progeny height on selection intensity, an increase in the selection intensity by one unit increased the progeny height by 1.9 cm in Tpop4, and by 2.5 cm in Tpop7 (Fig. 2). In the model with both selection intensity and latitude, the response to selection intensity was smaller because latitude explained part of the variation in progeny height growth (Table 6). The coefficients of determination were low, at the maximum slightly below 10 %, and the individual variables were seldom statistically significant. The response of progeny height growth to selection intensity and latitude varied greatly between the individual tests

(Table 6). The variation in regression coefficients approximately followed variation in corresponding correlation coefficients (Tables 5 and 6).

Table 5. Correlations (p-values in parentheses) between plus tree selection intensity and the height growth of their progenies in different tests separately, and for the breeding populations as a whole, in breeding populations Tpop4 and Tpop7.

Test site	Tpop4	Tpop7
Övertorneå	0.108 (0.2116)	-
Harads	0.138 (0.1065)	0.218 (0.0674)
Lycksele	-0.043 (0.6207)	0.133 (0.2245)
Byske	0.036 (0.6870)	0.056 (0.5713)
Strömsund	-	0.274 (0.0126)
Sävar	-	0.160 (0.1197)
Average over sites	0.143 (0.0951)	0.247 (0.0120)

Table 6. Regression coefficients (b), their significance (p) and coefficients of determination (R^2) of progeny height growth (in cm) on the selection intensity and latitude of origin of the Scots pine plus trees.

Breeding population and test site	Selection intensity b	p	Latitude b	p	R^2
Tpop4					
Övertorneå	3.10	0.2577	1.90	0.7656	0.012
Harads	1.34	0.4057	11.70	0.0021	0.086
Lycksele	-1.20	0.4597	4.33	0.2507	0.012
Byske	2.45	0.3316	-15.34	0.0101	0.052
Tpop4, average	1.37	0.2467	5.22	0.0589	0.046
Tpop7					
Harads	3.12	0.1066	1.02	0.7127	0.050
Lycksele	0.77	0.6249	4.10	0.0647	0.058
Byske	0.80	0.6257	0.28	0.9164	0.003
Strömsund	5.52	0.0648	5.98	0.1587	0.0984
Sävar	1.44	0.3467	3.22	0.1940	0.043
Tpop7, average	1.88	0.0721	2.76	0.1047	0.0854

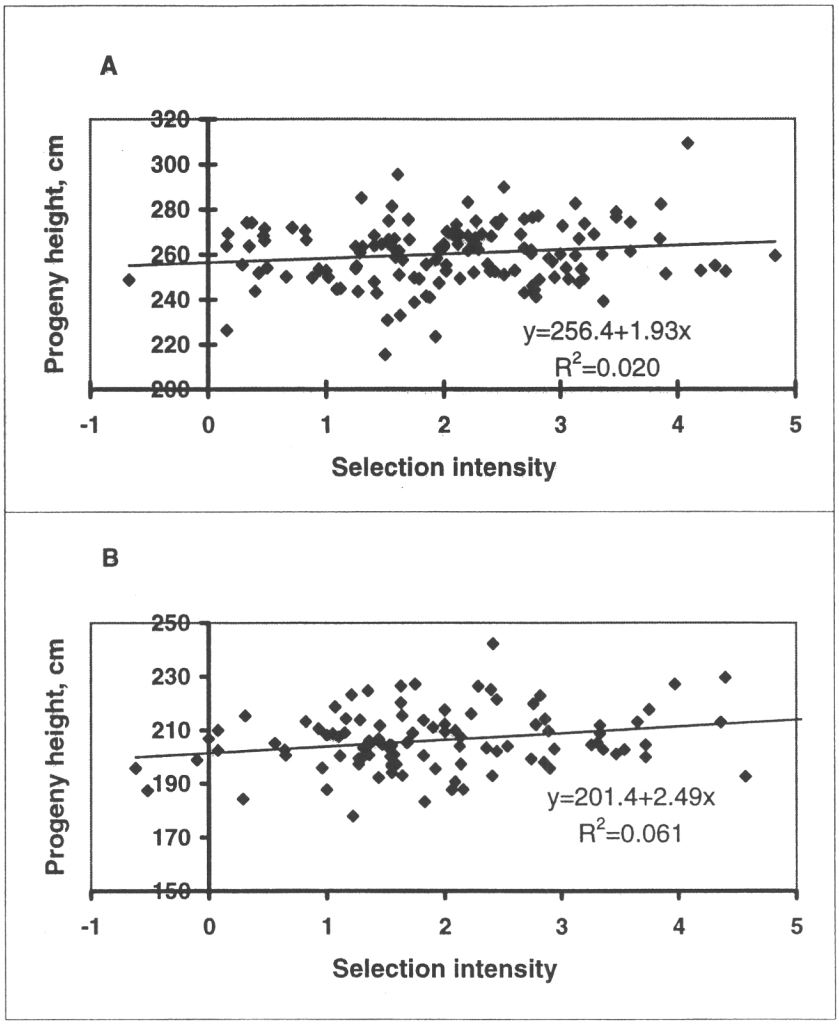


Figure 2. Dependence of mean progeny height in Tpop4 (A) and in Tpop7 (B) on the selection intensity of the plus tree.

3.4. Dependence of height growth on other characters of the plus trees

The height growth of the plus tree progenies was dependent on the characters emphasised in connection with selection in Tpop7 ($p = 0.0248$) but not in Tpop4 ($p = 0.9049$). In Tpop7 the growth of the progeny of the plus trees selected on the basis of both growth and quality was 211 cm compared to 198 cm for the progeny of plus trees selected with the main emphasis on quality.

On the average, the progeny means were the higher, the more northern was the origin of the plus trees (Table 7). This dependence was stronger in Tpop7 than in

Tpop4. The progeny tests located in Byske were exceptions to this trend in having close to zero (Tpop7) or negative correlation (Tpop4) between latitude of origin and progeny height. The model failed to show any significant dependencies between progeny height growth and plus tree height or age (Table 7).

Table 7. Correlation (p-values in parentheses) between plus tree progeny height growth and latitude of the plus tree origin, plus tree height and selection age.

Breeding population and test site	Latitude	Height	Selection age ($d_{1.3}$)
Tpop4			
Övertorneå	0.052 (0.5491)	0.102 (0.2409)	0.096 (0.2965)
Harads	0.286 (0.0007)	0.118 (0.1699)	0.074 (0.4156)
Lycksele	0.089 (0.3117)	-0.018 (0.8351)	0.051 (0.5859)
Byske	-0.211 (0.0154)	0.037 (0.6770)	-0.032 (0.7332)
Tpop4, average	0.192 (0.0249)	0.084 (0.3282)	0.074 (0.4154)
Tpop7			
Harads	0.107 (0.3699)	0.231 (0.0512)	-0.048 (0.7359)
Lycksele	0.234 (0.0305)	-0.076 (0.4871)	-0.158 (0.2063)
Byske	0.053 (0.5922)	0.009 (0.9313)	-0.138 (0.2195)
Strömsund	0.242 (0.0273)	0.065 (0.5564)	-0.045 (0.7245)
Sävar	0.218 (0.0308)	-0.042 (0.6805)	-0.175 (0.1343)
Tpop7, average	0.263 (0.0067)	-0.044 (0.6523)	-0.161 (0.1514)

4. Discussion

4.1. Variation in selection intensity

The average selection intensity in the material (close to 2) was slightly higher than that in the Scots pine plus trees from northern Finland on the average (1.63; Oskarsson 1972). It is possible that the weak selection, practised among Finnish plus trees when the trees were selected for the Swedish breeding populations, has caused some increase in the average selection intensity. The average selection intensity was rather close to that considered possible in practical selection work in natural forests (Wright 1976), but the maximum values were unrealistically high, corresponding to selection ratios of one to several million (Lindgren and Nilsson 1985). It is difficult to imagine achieving such uniform selection conditions that an equitable comparison between thousands of trees could be possible, even though even higher nominal selection intensities have been reported elsewhere, as reviewed by Cornelius (1994).

According to Oskarsson (1970), the average selection intensity of Scots pine plus trees in southern Finland is lower than would be expected on the basis of the selection ratio. This is caused by the fact that plus tree selection is based on several characters, so that trees with modest selection intensity for height are also selected if they are superior in other respects. This was also clearly visible in this study, as the minimum selection intensities for height were in fact negative. The extremely high selection intensities are probably caused by violations of certain basic assumptions, e.g. even-agedness or site homogeneity. An indication of the former factor was the positive correlation between selection intensity and age difference of the plus trees and the neighbouring trees (data not shown). In some cases great variation in the ages of the comparison trees of a single plus tree was also found.

Large standard errors, typically associated with the estimation of variances or standard deviations, could also explain some of the extreme selection intensity values observed. However, for several reasons this cannot be the main cause of the observed high selection intensity values. With small sample sizes the standard deviations are more biased upwards, thus tending to give downwards, rather than upwards deviating selection intensities. The unreliability of small samples for estimating standard deviation was recognised already during compilation of the selection intensity data. Therefore, in connection with the comparison-tree method, the standard deviation was derived from a larger regional material than merely from the four comparison trees (Oskarsson 1972). It was also seen that the maximum selection intensities were connected to the maximum selection differentials, indicating that variations in the standard deviations were of minor importance.

The increase in selection intensity with increasing plus tree height and age was probably caused by higher variability in older stands, which was not fully compensated by division of the selection differential by the standard deviation when calculating the selection intensity. The increase in selection intensity with increasing latitude and decreasing temperature sum of origin is more difficult to explain. At least this change was independent of the change in plus tree age or size. It might reflect increasing within-stand variation in natural stands with increasing latitude. Alternatively, it could be a pure artefact originating from the selection process in which the plus trees were selected for the Swedish breeding populations.

4.2. Variation in offspring height growth

On the average, more than 80 % of the additive genetic variation in height growth was between trees within a stand. The within-stand variation was 8 times greater than the between-stand variation in breeding population Tpop4, and 3 times greater in Tpop7. In other studies on the distribution of variation in the height growth of Scots pine, the within-population component has been about two to three times greater than the between-population component (Park and Gerhold 1986, Eriksson et al. 1987). The proportionally larger within-stand component in our study was probably caused by the smaller geographic range of the material. The lack of differences between the stands in progeny height growth observed in this study was

similar to that reported in southern Finland (Tynkkynen 1998). These results are in agreement with the general picture of the distribution of genetic variation in forest trees, which is mainly based on isozyme studies, i.e. on characters which are regarded as selectively neutral (Zobel and Talbert 1984, Mitton 1995, Simpson 1998), or on our knowledge of the effective pollen flow of conifers over long distances (Koski 1970).

The lack of difference in height growth between the progenies of plus trees from seed collection stands and other stands is to be expected as the between-stand variation was so small. Thus it can be concluded that only very limited, if any, genetic gain can be expected through the selection of special seed collection stands of Scots pine in northern Finland. This conclusion is slightly weakened by the fact that only a few plus trees from each stand, not a representative sample of all the trees, was progeny tested. Neither did the other stands from which plus trees were selected constitute a random sample of all pine stands in northern Finland, but a certain degree of selection was practised also on these e.g. to discard stands treated with selective cuttings (Sarvas 1953). The genetic gain of selection of seed collection stands has not been very well studied in general, but for native species it can be expected to be low (Zobel and Talbert 1984). Seed lot tests of loblolly and slash pine seed production areas revealed only slight or no improvement in growth rate, but a clear improvement in form and pest resistance (Rudolf et al. 1974). The studied seed production areas represented, however, a slightly higher degree of genetic improvement than the seed collection stands, as they had been upgraded by leaving only the best trees in the stands. In fact the main emphasis in selecting seed collection stands has been to guarantee the availability of forest regeneration material of known origin, rather than genetic improvement, although even opposite views have been expressed (Simpson 1998).

4.3. Selection intensity and offspring height growth

Comparison of parent and offspring phenotypes has seldom been utilised in studying the efficiency of phenotypic selection in forest trees. One natural reason for this is the longevity of trees, which leads to difficulties in carrying out meaningful comparisons between generations. This is why direct comparisons between parents and offspring have usually dealt with certain special growth forms of trees (e.g. Arnborg 1948, Kärki 1985) or anatomical characters of wood (Arbez et al. 1978, Loo et al. 1984, Williams and Megraw 1994, Harju and Venäläinen 2002), where equal dimensional stages are reached at an earlier age than in growth characters. Studies of this kind on height growth have been more descriptive or of preliminary character (Eklund Ehrenberg 1966, Pajamäki 1974, Tynkkynen 1998). The estimation of genetic gain by means of comparative studies using seed from selected trees and some control stands is also more straightforward and easier to apply to practical needs.

As a whole our results indicated a weak positive correlation between the selection intensity of the plus trees and the progeny height growth ($r = 0.143$ and 0.247 for

the test averages in Tpop4 and Tpop7, respectively), which is in line with the preliminary results published earlier (Eklund Ehrenberg 1966, Pajamäki 1974, Tynkkynen 1998). In a large material (n = 1205) of southern Finnish Scots pine plus trees, the correlation between the selection intensity of height and standardised height growth of their progeny was 0.139 (p = 0.000) (Tynkkynen 1998). Numerically this value is in good agreement with our results but, when we consider the larger number of plants from which the progeny means were derived in the above study, higher correlations would be expected. However, the many differences in the origin of the material, experimental design and analysis of progeny tests renders further speculation unfruitful. The two other studies (Eklund Ehrenberg 1966, Pajamäki 1974) were of a more descriptive character, but indicated a positive association between offspring and parent height growth. Kurm et al. (2000) obtained rather high correlation (r = 0.395, p = 0.0072) between plus tree height deviation and progeny height growth.

The method used for explaining progeny height growth on the basis of the selection intensity of their parents can be criticised as it does not properly consider the between-stand genetic differences. This difficulty could be avoided by using a sufficiently large material originating from a single stand. However, as shown in this study, the between-stand genetic differences are rather small, so this has probably not affected the results very much. From the practical point of view, too, this kind of correlation is the most applicable, as the genetic level of the stand is not known at the time of plus tree selection.

The correlations between selection intensity and offspring height growth differed in the progeny tests in a manner that is difficult to explain. Low or even negative correlations can be expected if the environmental conditions of parent trees and their progenies differ greatly, and different genotypes react differently to the environmental factors (i.e. if there is genotype-environment interaction). In progeny tests of the breeding population Tpop4, the correlations were lowest in the southernmost test sites at Lycksele and Byske, which could be explained as being a sign of unadaptedness, because the calculated average latitude of the tested material was about two degrees of latitude more to the north. However, in the Tpop7 material the highest correlation was obtained in the southernmost test site, Strömsund, which was even more deviating from the average latitude of the material.

According to the regression equations, a selection intensity that was one standard deviation higher increased offspring height at 11 to 13 years of age by about 2 cm. This means a 10 cm difference over the whole range of selection intensities studied. The genetic gain for the whole plus tree material with the average selection intensity was 4 cm (1 to 2 %). This value is low compared to the estimated genetic gains obtained by comparing plus tree and natural stand offspring in field trials (Wilhelmsson et al. 1993, Venäläinen et al. 1994, Andersson et al. 2002). There are many possible explanations for this difference. When comparison is based only on within-stand differences, as in this study, the between-stand selection effect is

neglected. However, according to this and several other studies, it cannot be of any major importance. In a comparison between plus tree and stand offspring better physiological condition (e.g. heavier seed) and a lower inbreeding level of seed orchard seed can also have some effect on the height growth of the seedlings. These effects are estimated to be about 4 % in Swedish Scots pine progeny tests (Wilhelmsson et al. 1993), but they are excluded from the comparison between plus trees with different selection intensities. One can also speculate that selection for general vigour, which has been included in the criteria of plus tree selection (Sarvas 1953), also increases the genetic gain of the selected plus trees compared to unselected controls, but is not seen in the selection intensity for height.

4.4. Factors affecting the efficiency of plus tree selection

The character that was specially emphasised in connection with selection did not have any clear effect on the progeny height. This was to be expected as the differences between the trees selected according to different selection criteria were not so great. All the plus trees had to fulfil certain basic requirements, and some of them also possessed either especially good quality, growth or both.

The age of the plus trees at the time of selection did not have any clear effect on the offspring performance although, in many plus tree selection guidelines, it is emphasised that selections should be made in stands at the second half of the economical rotation age, while avoiding over-aged stands (Sarvas 1953, Brown and Goddard 1961, Simpson 1998). In our material, although many of the plus trees originated from over-aged stands, there was only a weak, statistically non-significant tendency in Tpop7 for a lower height growth of offspring from older plus trees. In southern Finnish Scots pine plus trees there is a slight, statistically significant negative correlation between plus tree age and offspring height growth (Tynkkynen 1998), whereas no such dependence has been observed in Estonian Scots pine material (Kurm et al. 2000).

A somewhat surprising result was that, in almost all the progeny tests, the offspring from northern plus trees had higher height growth than the offspring from southern plus trees. This was contrary to what is known about the dependence of geographic origin and height growth in provenance experiments (Persson and Ståhl 1993). One explanation could be that, in such a geographically limited area, the observed higher selection intensity at northern latitudes overrides the opposite provenance trend, resulting in an apparent positive dependence between latitude and height growth. However, this would mean a rather high correlation between selection intensity and offspring height, without the counteracting provenance effect.

4.6. Conclusions

The present study has shown that most of the genetic variation at the population level in the height growth of Scots pine is between the individual trees within a

stand, and that there are no differences in breeding value between plus trees from specially selected seed collection stands and from other stands. More intensive selection yielded a slightly better height growth of the offspring. The improvement in height growth was, however, so small that the pursuit of very high selection intensities is not likely to be economically viable. Furthermore, the expected low heritability values for selection in the forest supports the use of rather a modest selection intensity and the advice about putting more weight on progeny testing (Namkoong 1969).

Further studies are needed with material derived from a geographically more limited area, preferably from one or a few stands, in order to avoid the confounding geographic trends observed in this material. However, the most relevant study material would be younger plantations and progeny trials, which are the main selection fields both at the present time and in the future.

Acknowledgement

The authors thank Dr. Anni Harju and Prof. Dag Lindgren for their constructive criticism to an earlier draft of the paper. They helped to considerably improve the article. The language of the article was checked by John Derome.

Literature

Anderson, W.F., Holbrook, C.C. and Wynne, J.C.: Heritability and early-generation selection for resistance to early and late leafspot in peanut. Crop Science 31:588-593 (1991).

Andersson, B. and Danell, Ö.: Is Pinus sylvestris resistance to pine twist rust associated with fitness costs or benefits? Evolution 51(6):1808-1814 (1997).

Andersson, B., Elfving, B., Ericsson, T., Persson, T. and Gregorsson, B.: Improved Pinus sylvestris performance in northern Sweden. (manuscript) (2002).

Arbez, M., Baradat, P., Birot, Y., Azoeuf, P. and Hoslin, R.: Variabilité et hérédité de l'angle du fil du bois mesuré à l'aide d'un traceur radioactif chez le pin maritime et le pin laricio de Calabre. Canadian Journal of Forest Research 8:280-289 (1978).

Arnborg, T.: Study of a pine (in Swedish with English summary) Lustgården 1947-48, pp 93-104. (1948).

Becker, W.A.: Manual of procedures in quantitative genetics. 4th ed. Academic Enterprises, Pullman, 196 p. (1984).

Brown, C.L. and Goddard, R.E.: Silvical considerations in the selection of plus phenotypes. Journal of Forestry 59:420-426 (1961).

Casler, M.D.: Parent-offspring regression in reed canarygrass: methods for parent and offspring evaluation and their effect on heritability estimates. Canadian Journal of Genetics and Cytology 24:467-473 (1982).

Cornelius, J.: The effectiveness of plus-tree selection for yield. Forest Ecology and Management 67:23-34(1994).

Eklundh Ehrenberg, C.: Parent-progeny relationship in Scots pine (Pinus sylvestris L.). Studia Forestalia Suecica 40. 54 p. (1966).

Eriksson, G., Ilstedt, B., Nilsson, C. and Ryttman, H.: Within and between-population variation of growth and stem quality in a 30-year-old Pinus sylvestris trial. Scandinavian Journal of Forest Research 2:301-314 (1987).

Falconer, D. S. and Mackay, T. F. C.: Introduction to Quantitative Genetics. 4th ed. Longman Group. London and New York. 464 p. (1996).

Franklin, E.C.: Adjustment of heritabilities estimated by correlation coefficients for typical forest tree breeding experiments. Silvae Genetica 23(6):176-180 (1974).

Franklin, E.C. and Squillace, A.E.: Short-term progeny tests and second generation breeding in slash pine. Canadian Journal of Forest Research 3:165-169 (1973).

Gimelfarb, A. and Willis, J.H.: Linearity versus nonlinearity of offspring-parent regression: an experimental study of Drosophila melanogaster. Genetics 138:343-352 (1994).

Hansen, T.F. and Boonstra, R.: The best in all possible worlds? A quantitative genetic study of geographic variation in the meadow vole, Microtus pennsylvanicus. Oikos 89:81-94. (2000).

Harju, A.M. and Venäläinen, M.: Genetic parameters regarding the decay resistance of *Pinus sylvestris* heartwood to decay caused by *Coniophora puteana*. Scandinavian Journal of Forest Research 17:199-205. (2002).

Kärki, L.: Genetically narrow-crowned trees combine high timber quality and high stem wood production at low cost. In: Tigerstedt PMA, Puttonen P, Koski V (eds) Crop Physiology of Forest Trees. Helsinki University Press, Helsinki, pp 245-256 (1985).

Koski, V.: A study of pollen dispersal as a mechanism of gene flow in conifers. Communicationes Instituti Forestalis Fenniae 70.4. 78 p. (1970).

Kurm, M., Möls, T., Tamm, Ü. and Jõepera, E.: Kõrguse ja rinnasdiameetri päritavuse uurimine harilikul männil. Abstract: Heritability of stem height and BHD in Pinus sylvestris L. Metsanduslikud uurimused XXXII:32-42. (2000).

Lee, S. J.: Improving the timber quality of Sitka spruce through selection and breeding. Forestry 72:123-133. (1999).

Lindgren, D. and Gregorius, H. R.: Inbreeding and coancestry. Proceedings of the IUFRO Joint meeting. Advanced generation breeding. Bordeaux. June 14-18 1976. pp. 49-69. (1976).

Lindgren, D. and Nilsson, J.E.: Calculations concerning selection intensity. Swedish University of Agricultural Sciences. Department of Forest Genetics and Plant Physiology. Report 5. 28 p. (1985).

Loo, J.A., Tauer, C.G. and van Buijtenen, J.P.: Juvenile-mature relationships and heritability estimates of several traits in loblolly pine (*Pinus taeda*). Canadian Journal of Forest Research 14:822-825 (1984).

Lynch, M. and Walsh, B.: Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc. Sunderland, 980 p. (1998).

Matthews, J.D. and McLean, C.: Improvement of Scots pine in Britain by selection and breeding. Seventh British Commonwealth Forestry Conference. Forestry Commission, London. 14 p. (1957).

Mayo, O.: The theory of plant breeding. 2nd edition. Clarendon Press. Oxford. 334 p. (1987).

Mitton, J. B.: Genetics and the physiological ecology of conifers. In: Smith, W. K. & Hinckley, T. M. (eds.) Ecophysiology of coniferous forests. Academic Press. pp. 1-36. (1995).

Morén, A.S. and Perttu, K.L.: Regional temperature and radiation indices and their adjustment to horizontal and inclined forest land. Studia Forestalia Suecica 194. 19 p. (1994).

Namkoong, G.: Choosing selection intensities for seedling seed orchards. Silvae Genetica 18:174-176 (1969).

Oskarsson, O.: Pluspuiden fenotyyppisessä valinnassa sovellettuja valinnan asteita. [Selection degrees applied in the phenotypic selection of plus trees] (in Finnish) Folia Forestalia 83. 14 p. (1970).

Oskarsson, O.: Plusmetsiköiden valintaero ja jalostusvoiton ennuste. Summary: Selection differential and the estimation of genetic gain in plus stands. 10 p. (1971).

Oskarsson, O.: Suomalaiset plusmännyt ja pluskuuset. Finnish plus trees of Scots pine and Norway spruce. Folia Forestalia 150. 138 p. (1972).

Oskarsson, O.: Silmällä tehty savotta. Pluspuiden valinnan historia ja arki. [Logging done by eye. The history and everyday life of plus tree selection] (in Finnish). Metsäntutkimuslaitoksen tiedonantoja 579. 67 p. (1995).

Pajamäki, J.: Jälkeläiskokeet varmistavat valintahyödyn metsänjalostuksessa. [Progeny tests assure selection gain in forest tree breeding] (in Finnish). Metsä ja puu 8/1974: 23-24 (1974).

Pajamäki, J. and Karvinen, K.: Siemenkeräysmetsiköt metsänjalostuksen vankkana perustana. [Seed collection stands as a sound basis for forest tree breeding] (in Finnish). Metsäntutkimuslaitoksen tiedonantoja 627. 60 p. (1997).

Park, Y.S. and Gerhold, H. D.: Population hybridization in Scotch pine (Pinus sylvestris L.): Genetic variance components and heterosis. Silvae Genetica 35(4):159-165 (1986).

Persson, B. and Ståhl, E. G.: Effects of provenance transfer and spacing in an experimental series of Scots pine (Pinus sylvestris L.) in northern Sweden. (in Swedish with English summary) Swedish University of Agricultural Sciences. Department of Forest Yield Research. Report No. 35, 92 p. (1993).

Persson, T.: Finska plusträdskloner till förädlingspopulation Tpop 4. [Finnish plus tree clones to the breeding population Tpop 4] (in Swedish) Avelsvärden 71. 33 p. (1999a).

Persson, T.: Finska plusträdskloner till förädlingspopulation Tpop 7. [Finnish plus tree clones to the breeding population Tpop 7] (in Swedish) Avelsvärden 69. 30 p. (1999b).

Pihelgas, E.: Seed stands and plus trees. In: Giertych M and Matyas C (ed) Genetics of Scots Pine. Akadémiai Kiadó, Budapest, pp. 117-123. (1991).

Puri, S.: Plus-tree selection as a tool in tree improvement. In: Puri S (ed) Tree Improvement: Applied Research and Technology Transfer. Science Publishers Inc. U.S.A. pp 97-105. (1998).

Rudolf, P. O., Dorman, K. W., Hitt, R. G. and Plummer, A. P.: Production of genetically improved seed. In: Seeds of woody plants in the United States. USDA Agriculture Handbook 450. pp. 53-74. (1974).

Ruotsalainen, S. and Nikkanen, T.: Kuusen siemenviljelysaineiston menestyminen Pohjois-Suomessa. Summary: Survival and growth of Norway spruce seed orchard material in northern Finland. Metsäntutkimuslaitoksen tiedonantoja 709. 33 p. (1998).

Sarvas, R.: Ohjeita pluspuiden valitsemista ja ilmoittamista varten. Summary: Instruction for the selection and registration of plus trees. Silva Fennica 80:93-101 (1953).

SAS Institute Inc. SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC. USA, SAS Institute Inc. 846 p. (1989).

SAS Institute Inc. SAS/STAT® Software: Changes and Enhancements through Release 6.11, Cary, NC. USA, SAS Institute Inc. 1104 p. (1996).

Simpson, D.: Selection of superior trees. In: Puri S (ed) Tree Improvement: Applied Research and Technology Transfer. Science Publishers Inc. U.S.A. pp 107-124 (1998).

Tynkkynen, H.: Männyn fenotyyppisen pluspuuvalinnan tehokkuus Etelä-Suomessa. [The efficiency of phenotypic plus tree selection of Scots pine in southern Finland] (in Finnish). Kymenlaakson va. ammattikorkeakoulu. Metsäosasto. Insinöörityö. [Kymenlaakso prov. Polytechnic. Forestry department. Ingenieur work] 32 p + app. (1998).

Venäläinen, M., Annala, M. L., Kosonen, E., Rantanen, H. and Tynkkynen, H.: Plusmäntyjen testaustulosrekisteri ja jalostushyöty [Register of testing results and genetic gain of plus Scots pines] (in Finnish). Metsäntutkimuslaitoksen tiedonantoja 497.89 p (1994).

Wilhelmsson, L., Eriksson, U. and Danell, Ö.: Production of genetically improved seed, (in Swedish with English summary) SkogForsk Redogörelse nr 3 1993, 52 p. (1993).

Williams, C. G. and Megraw, R. A.: Juvenile-mature relationships for wood density in *Pinus taeda*. Canadian Journal of Forest Research 24:714-722 (1994).

Wright, J. W.: Introduction to forest genetics. Academic Press, New York, 463 p. (1976).

Zobel, B. and Talbert, J.: Applied Forest Tree Improvement. John Wiley & Sons. New York, 505 p. (1984).

II

Predicting Genetic Gain of Backward and Forward Selection in Forest Tree Breeding

By S. RUOTSALAINEN[1]) and D. LINDGREN[2])

(Received 17th November 1997)

Abstract

In several forest tree breeding operations either backward or forward selection is used, although a proper combination of both might be more advantageous. Using calculations based on quantitative genetic theory, comparisons were made between the relative merits of backward and forward selection for individual families. In backward selection the mother was chosen based on her offspring and in forward selection the best offspring was selected from the family consisting of the mother and her children. A range of heritabilities and selection intensities in natural forest and progeny test (the latter is a function of progeny size) were compared.

It is the more favourable to select backward the higher the mother ranks. Depending on the combination of parameter values, backward selection was superior to forward selection for open pollinated progeny in 1% to 57% of the top ranking families. High intensity of selection in the forest, low heritability and small progeny size favoured backward selections. Backward selection for families with plus tree fathers was usually not favourable for more than a few percent of the families. Only when heritability was low it could be favourable to select 10% of top-ranking mothers.

The calculations show that it can often be optimal to apply backward selection for the best families and forward selection for the lower ranking families side by side. However, the difference is often small and then other considerations can be more important.

Key words: backward selection, forward selection, breeding strategy, intensity of selection, progeny size, heritability, genetic gain.

FDC: 165.3; 165.6.

1. Introduction

Progeny testing aimed at parental ranking is a commonly used method in both animal and plant breeding. Progeny testing can be used for several purposes and the term can have different meanings. In this study it is used as a synonym of parental ranking (LINDGREN, 1991). In forest tree breeding, progeny testing is often viewed as a fundamental part of the whole procedure (ZOBEL and TALBERT, 1984). It is used to obtain additional genetic gain through roguing seed orchards and establishing seed orchards with the top ranking clones. Progeny testing is also considered essential for advanced generation breeding, because the progenies of the best plus trees form the base material for the next generation breeding (forward selection). Ranking parents according to the performance of their progeny (backward selection) is especially important with characters which have a low heritability (FALCONER and MACKEY, 1996), as is the case with many important characters in forest tree breeding (WRIGHT, 1976, CORNELIUS, 1994). Forest tree breeders have been aware of the choice between backward and forward selection for a long time; the gain may differ considerably for the different alternatives (FALCONER and MACKEY, 1996). However, breeders have considered it to be an "either/or" decision, and not realised that they can use different options for different families.

The original idea in many tree breeding programs was to use the progeny tests of the phenotypically selected plus trees only for parental ranking. The base material for second generation breeding was to be created by crossing the best ranking plus trees (WERNER *et al.*, 1981; Pitkäntähtäyksen metsänjalostusohjelma, 1989). This plan has also been put into practice for example with Sitka spruce in Britain (LEE, 1993). However, the question that arises is whether to use the progeny tested plus trees or their offspring in long term breeding. Phenotypic selections among the progeny from plus trees may actually be a favourable option under certain circumstances (SPANOS *et al.*, 1997).

LINDGREN (1986) stated, as a general principle, that the best progeny tested genotypes should be selected backward and others forward. This is caused by the fact that the best genotypes in most forest tree breeding cases are mated with individuals which can be assumed to have a considerably lower breeding value. Thus the progeny have, on the average, a lower breeding value than the best parents themselves. The differential increases with increasing genetic value of the parent, whereas the within-family selection gain is independent of the parental values. Thus the gain achieved by selecting the best individual within the progeny becomes more and more difficult to balance against the difference which increases consistently with the breeding value of the parent. Therefore backward selection is the most favourable method for the best ranked parents. One contributing reason is that selection backwards does not mean the introduction of new genetic relationships but instead keeps the gene mass of the best founders intact.

The aim of this study was to develop a method for comparing backward selection and forward selection for individual genotypes as a function of their rank, and to create a firmer theoretical basis for a comparison between these selection schemes. The study also evaluates the magnitude of the difference between backward and forward selection gain, and determines which factors influence the balance between backward and forward selection.

2. Methods

2.1. Selection alternatives to be compared

2.1.1. Backward selection (BACKW)

Backward selection (*i.e.* reselection) means the acceptance of the best plus trees to be used as parents in the next multiplication population (ramets in seed orchards) or for breeding population (so that a new progeny is created to serve as a resource for further selections), based on the results of progeny tests. The kind of material or experimental lay-out which has been used in progeny testing is of no significance as long as the method is sound and yields accurate predictions for the breeding values of the plus trees. The offspring used to rank the plus trees are not used in creating the next generation of

[1]) Finnish Forest Research Institute, Kolari Research Station, FIN-95900 Kolari, Finland

[2]) Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, S-901 83 Umeå, Sweden

the breeding population, but it is instead based on progenies that are crosses between the highest ranking plus trees.

2.1.2. Forward selection from open pollinated progeny (FORW0)

Open pollinated seed is widely used, cheap and often rapidly available material for the progeny tests of forest trees. It can be collected either from the original plus trees or from their grafts (ZOBEL and TALBERT, 1984). Members of an open pollinated family are assumed to be true half-sibs. However, slight deviations from this assumption will probably not have any marked influence on the model. Instead of crossing the best ranked plus trees, the best individuals from their open pollinated progenies are selected. Unimproved pollen which reduces the obtained genetic gain is the main drawback in using open pollinated material for forward selection. For a wind pollinated common tree species like Scots pine, high levels of background pollination seem inevitable even in mature seed orchards (HARJU, 1995).

2.1.3. Forward selection from polycross progeny (FORW+)

In some cases progeny testing is carried out with material created using a mixture of pollen collected from the plus trees (polycross). In this case the pollen parent is assumed to have, on the average, as high a genetic value as the seed parent. Thus, forward selection within polycross progeny will lead to a higher genetic gain than selection within open pollinated progeny. Open pollinated material from a seed orchard can sometimes be genetically at the same level as polycross material. This is true if the species in question is an exotic or of otherwise such low abundance that background pollination is negligible (*e.g.* SHELBOURNE, 1992). Background pollination can sometimes be prevented by using seed orchards located in greenhouses. Controlled polycross is still more reliable for progeny test as selfing is excluded and the different pollens are mixed to obtain equal contributions. However, the father is unknown, so several selections may have the same father, and the phenotype of relatives to the unknown father (e.g. half-sibs to the selection) cannot be used to increase the accuracy of the breeding value of the selection. The genetic gain of selections from a polycross progeny gives a considerable indication of the gain which would be achieved if there were controlled crosses with known parents.

2.2. General features of the procedure

Methods are available for comparing genetic gains from different breeding alternatives (e.g. NAMKOONG *et al.*, 1966; LINDGREN and WERNER, 1989; SHELBOURNE, 1992). A common feature in these methods is that the response to selection is predicted for the average of a population, using the formulae for genetic gain. Formulae predicting the selection response can be applied to any data, but a standardized normal distribution is usually used for non-experimental studies, resulting in a standardized selection response (intensity of selection). One development in this method is that predictions are made for individual genotypes rather than for the average of all selections (LINDGREN, 1986). Predictions of individual values are made according to their rank, which is a conventional statistical quantity called order statistics. Breeding values are predicted for each plus tree and the best of its offspring. A plus tree which is acting as a mother forms a family together with its progeny. Either the plus tree or its best offspring is selected from each family. HODGE and WHITE (1993) used a rather similar method for predicting genetic gain, although it differed in some details. Their comparison did not include forward selection in open pollinated progeny.

2.3. Formulae for breeding value and genetic gain

In the following calculations breeding values and genetic gains are expressed with additive genetic standard deviation (σ_A) as a unit. The calculation procedure includes several successive steps, which are presented in the following. The formulae are adopted from FALCONER and MACKEY (1996).

The genetic gain in phenotypic selection in the forest (wild population) can be generally expressed as

$G_1 = i_1 h^2 \sigma_P = i_1 h \sigma_A$

where

i_1 = intensity of selection in the forest
h^2 = narrow sense individual tree heritability
σ_P = phenotypic standard deviation
As $\sigma_A = 1$ by definition, this reduces to

(1) $G_1 = i_1 h$,

The following formulae (2 to 7) are applied to each family separately.

The formula for selecting a mother according to the results of an open pollinated progeny test is obtained as follows. The basic formula for sib selection is

$$R_s = i_2 \sigma_P h^2 \frac{nr}{\sqrt{n(1+(n-1)t)}}$$

where,

i_2 = intensity of selection for the particular parent
n = progeny size
r = correlation between breeding values
t = correlation between phenotypic values

By noting that $h^2 = \sigma_A^2/\sigma_P^2$, $t = rh^2$ and that for half sibs $r = 0.25$, the formula is reduced to

$$R_s = i_2 \sigma_A \frac{0.5}{\sqrt{1+(4/h^2-1)/n}}$$

Because it is question of a population with finite size, the standard deviation must be corrected by a factor $\sqrt{1-1/m}$, where m is number of plus trees (families). However, $\sqrt{1-1/m}$ σ_A becomes $\sqrt{1-1/m}$, because $\sigma_A = 1$. Finally, by noting that the formula for sib selection from open pollinated progeny gives only half the breeding value of the parent, the result must be multiplied by two.

So the formula for genetic gain when selecting a mother according to the results of open pollinated progeny test is obtained as

$$(2) \quad G_2 = 2R_S\sqrt{1-1/m} = 2i_2\sqrt{1-1/m}\frac{0.5}{\sqrt{1+(4/h^2-1)/n}} = i_2\frac{\sqrt{1-1/m}}{\sqrt{1+(4/h^2-1)/n}}$$

Analogously the genetic gain when selecting a family is

$$(3) \quad R_f = i_3\frac{\left(\sqrt{1-1/m}\right)(1.5/n+0.5)}{\sqrt{1+(4/h^2-1)/n}}$$

where

i_3 = intensity of selection for the family.

The genetic gain when selecting the best offspring within a family (R_w) is

$$(4)\ R_w = i_4 0.75\sqrt{\frac{(1-1/n)}{(1/h^2-0.25)}}$$

where

i_4 = intensity of selection in the test when selecting the best offspring within a family (selecting the best out of n values).

The breeding value for a parent (G_3 = BACKW) is obtained as the sum of genetic gain in phenotypic selection in the forest and selection of a mother according to the results of a progeny test (operational definition for breeding value)

$$(5)\ G_3 = G_1 + G_2$$

The breeding value for the best individual within an open pollinated progeny in forward selection (G_5 = FORW0) is predicted as:

$$(6)\ G_5 = G_1/2 + R_f + R_w$$

The breeding value for the best polycross offspring in forward selection (G_6 = FORW+) includes the genetic gain in phenotypic selection in the forest as a whole

$$(7)\ G_6 = G_1 + R_f + R_w$$

The effects of the parameters on the relative order of the selection alternatives were studied in more detail by setting the formulae for backward (5) and forward selection (6) equal, and then solving the value for intensity of selection at which the alternatives give equal breeding values (formula 8).

$$(8)\ i_{lim} = \frac{(i_4 0.75\sqrt{(1-1/n)/(1/h^2-0.25)} - i_1 h/2)\sqrt{1+(4/h^2-1)/n}}{\sqrt{(1-1/m)(0.5-1.5/n)}}$$

where

i_{lim} = limit value for intensity of selection (i_2 and i_3) at which $G_3 = G_5$.

By varying the values of one parameter and fixing the others, the effects of the parameters could be studied separately. When the i_{lim} values had been solved, the percentage of families in which backward selection was superior could be obtained from the distribution of the order statistics. A modification using fixed genetic gain in selection in the forest (G_1) instead of $i_1 h/2$ was also studied.

2.4. Calculation of genetic gain

The breeding values used for a population of 100 plus trees were generated by the SELEINT2 program as a function of their rank using order statistics for the normal distribution. The normal order statistics for n = 100 could also have been taken from *e.g.* the tables by HARTER (1970). SELEINT2 [3]) has the advantage over published tables in that it can be used for any sample size. These calculations would have been simplified if a large number of plus trees had been assumed, because in such a case the expected percentiles from the normal distribution could have been used. However, as breeders work with a finite number of plus trees we preferred the method used here, which demonstrates the handling of small numbers. More information about the statistics connected to selection from normal distributions is given by LINDGREN and NILSSON (1985). These order statistics are the predicted standardized intensities of selection for the plus trees of the ranking in question, but they can also be regarded as the breeding value of a plus tree with this ranking. Breeding values for each family were predicted for the plus tree itself (BACKW) and the best individual in open pollinated (FORW0) and polycross progeny (FORW+) (formulae (5), (6) and (7), respectively).

For each selection alternative (BACKW, FORW0, FORW+) genetic gain was calculated as a mean of the breeding values of the individual plus trees or their best offspring. This was done both for the whole population and for the top 50% of it. In addition to these three pure selection alternatives, two cases with combined backward and forward selection were studied. In BESTBAF0 the selection within each family was either the plus tree or its best open pollinated offspring, depending on which had the higher breeding value. The genetic gain for this alternative was obtained as a mean of this mixed population of breeding values. BESTBAF+ was the corresponding combination of backward selection and forward selection from polycross progeny. Backward selection was also compared with both alternatives of forward selection (FORW0, FORW+) on the family level in order to determine in which families backward selection was the preferred choice.

Three parameters affect the genetic gain in our model: intensity of selection in the forest (i_1), individual heritability (h^2) and progeny size (n). Calculations were performed with all combinations (27 cases) of three parameter value levels: low, medium and high (*Table 1*). It was assumed that the individual heritability for plus tree selection in the forest and for progeny tests was the same.

Table 1. – The parameter values used in the calculations. The parameters and values given in parentheses are secondary ones derived from the original parameters.

Parameter	Parameter value		
	low	medium	high
intensity of selection in forest, i_1 (selection ratio)	1.8675 (1/20)	2.5076 (1/100)	3.2414 (1/1000)
heritability, h^2	0.05	0.2	0.5
progeny size, n (intensity of selection in test, i_4)	25 (1.9653)	100 (2.5076)	500 (3.0367)

3. Results

3.1. Genetic gain for different alternatives

(a) No family selection

Forward selection in the open pollinated progeny test was usually superior to the phenotypic plus tree selection in the forest (FORW0 > BACKW) when the whole population was included (no family selection) (*Table 2*). When there is no family selection it is not strictly justified to call BACKW backward selection, since this alternative merely describes the effect of phenotypic plus tree selection. Forward selection from the open pollinated progeny (FORW0) was unfavourable at the

[3]) SELEINT2 is authored by DAG LINDGREN in 1990 and is available on the internet via http://linne.genfys.slu.se/breed/breed.htm

Table 2. – Genetic gain (as per cent of the gain in BACKW) for forward selection or combination of forward and backward selection with two population compositions and sizes (all phenotypically selected 100 or top ranking 50 plus trees)[1]).

i_1 (selection ratio)	i_4 - n	h^2	The population of all selected 100 plus trees or their progeny ($\bar{i}_2 = \bar{i}_3 = 0$)				The population of best ranking 50 plus trees or their progeny ($\bar{i}_2 = \bar{i}_3 = 1.844$)			
			FORW0	BEST BAF0	FORW+	BEST BAF+	FORW0	BEST BAF0	FORW+	BEST BAF+
1.8675 (1/20)	1.9653 - 25	0.05	128	137	178	179	93	103	119	121
		0.20	129	134	179	180	99	105	128	128
		0.50	133	135	183	183	106	109	139	139
	2.5076 - 100	0.05	151	165	201	206	93	105	113	118
		0.20	153	157	203	203	106	110	133	133
		0.50	157	158	207	207	118	119	150	150
	3.0367 - 500	0.05	173	189	223	230	95	107	113	118
		0.20	175	178	225	225	115	118	141	141
		0.50	180	180	230	230	132	132	163	163
2.5076 (1/100)	1.9653 - 25	0.05	108	119	158	159	87	100	116	118
		0.20	109	117	159	159	91	101	123	124
		0.50	112	116	162	162	96	102	132	132
	2.5076 - 100	0.05	125	140	175	179	87	102	112	115
		0.20	127	132	177	177	97	104	128	128
		0.50	130	131	180	180	106	109	141	141
	3.0367 - 500	0.05	141	157	191	197	90	104	111	116
		0.20	143	147	193	193	105	109	135	135
		0.50	147	147	197	197	117	118	152	152
3.2414 (1/1000)	1.9653 - 25	0.05	95	109	145	146	81	100	114	115
		0.20	96	107	146	146	84	100	120	120
		0.50	98	106	148	148	88	100	126	126
	2.5076 - 100	0.05	108	124	158	161	83	100	110	113
		0.20	109	117	159	159	90	101	123	124
		0.50	112	115	162	162	97	102	134	134
	3.0367 - 500	0.05	121	137	171	175	85	101	110	114
		0.20	122	127	172	172	97	104	130	130
		0.50	125	126	175	175	106	108	143	143

[1]) Four selection alternatives are compared as follows: FORW0 = forward selection of the best tree from each open pollinated progeny; BESTBAF0 = in each family either the plus tree or its best open pollinated progeny is selected, depending on their breeding values; FORW+ = the best selection from each polycross progeny; BESTBAF+ = as BESTBAF0, but comparison between the plus tree and its best polycross progeny. i_1 = intensity of selection in the forest, i_4 = intensity of selection in the test when selecting the best offspring within a progeny, n = size of progeny, h^2 = individual narrow sense heritability. $\bar{i}_2$ = average intensity of selection for the plus trees, $\bar{i}_3$ = average intensity of selection for the families

whole population level only in the case of the highest intensity of selection in the plus tree selection and the lowest intensity of selection in the test. FORW0 gave a maximum of 80% higher genetic gain than the plus tree selection (BACKW). Forward selection was most advantageous in cases where the intensity of selection was low in the forest and high in the experiment and when the heritability was high.

Forward selection from the polycross material (FORW+) increased the genetic gain by 45% to 130% over the phenotypic plus tree selection (BACKW). The parameter values (i_1, h^2, n) influenced the relative genetic gains in the same way as with open pollinated progeny, except that intensity of selection in the forest had only a slight effect on the genetic gain. In absolute values intensity of selection in the forest did not influence the amount of genetic gain with which FORW+ exceeded phenotypic plus tree selection. However, because the level of genetic gain was higher with a higher intensity of selection in the forest, the relative values for FORW+ correspondingly decreased.

A combination of backward and forward selection (BESTBAF0, BESTBAF+) increased the genetic gain in some cases by more than 10% compared to the best single form of selection. The greatest additional gains through combination were obtained at those parameter values when neither of the pure alternatives was excessively superior, *i.e.* cases in which backward selection was used in about one half of the families utilized in breeding. When the material was of polycross origin, the possibilities for additional genetic gain were limited due to the superiority of forward selection. Heritability made a surprisingly small contribution to the effect of combining backward and forward selection, and did not follow any clear general pattern.

(b) Selecting the top 50% of families

In the case of a stronger selection comprising half as many selections as the initial number of plus trees, forward selection was less favourable than when no family selection was used (*Table 2*). Only when selection in the forest was less intense, heritability high and the families large did forward selection in open pollinated families show any overall superiority over backward selection. Combination of both selection alternatives gave a maximum increase of 7% in genetic gain. In the best 50% subpopulation too, forward selection in polycross material (FORW+) was always better than backward selection, but its superiority was considerably diminished compared to the calculation at the whole population level.

3.2. Backward vs. forward selection at the family level

The proportion of top ranking families in which backward selection (BACKW) was better than forward selection from open pollinated material (FORW0) ranged from 1% to 57% (*Table 3*). The effect of the parameter values parallelled those on genetic gain. Thus, backward selection was most favourable when the intensity of selection was high in the forest, but low in the test. Low heritability also favoured backward selection.

Table 3. – Percentage of top ranking families in which the plus tree has a higher breeding value than its best offspring (*i. e.* backward selection better than forward selection). Plus trees are compared both to open pollinated (OP) (alternative FORW0) and polycross (PC) progeny (alternative FORW+). Altogether 27 combinations of parameter values are presented. The parameters are as in *table 2.*

i_1 (selection ratio)	i_4 - n	h^2	plus tree better than best offspring in	
			OP progeny	PC progeny
			% of families	
1.8675	1.9653 - 25	0.05	29	6
(1/20)		0.20	23	2
		0.50	13	0
	2.5076 - 100	0.05	28	12
		0.20	16	3
		0.50	5	0
	3.0367 - 500	0.05	25	13
		0.20	10	1
		0.50	1	0
2.5076	1.9653 - 25	0.05	42	6
(1/100)		0.20	38	2
		0.50	30	0
	2.5076 - 100	0.05	35	12
		0.20	25	3
		0.50	13	0
	3.0367 - 500	0.05	31	13
		0.20	16	1
		0.50	4	0
3.2414	1.9653 - 25	0.05	57	6
(1/1000)		0.20	57	2
		0.50	56	0
	2.5076 - 100	0.05	44	12
		0.20	38	3
		0.50	28	0
	3.0367 - 500	0.05	37	13
		0.20	25	1
		0.50	12	0

If polycross progeny is available, backward selection will rarely produce a higher predicted gain than FORW+. Only in the cases of the lowest heritability and medium or large families, can slightly over 10% of the top ranking plus trees be selected backward. As would be theoretically expected, the relationship between BACKW and FORW+ was not affected by the genetic gain in phenotypic selection in (*Table 3*). A typical situation describing the three selection alternatives is shown in *figure 1.* In the case of medium parameter values backward selection was better than forward selection from open pollinated progeny in 25% of the top ranking families (BACKW and FORW0 crossing at this point). On the other hand, backward selection was superior to FORW+ in only the best 3% of families. As can be seen from the curves for BACKW and FORW0 close to the crossing point, the difference between these alternatives is small with a large number of families.

3.3. Effects of parameters

The intensity of selection in the forest (*i.e.* genetic gain in phenotypic selection) had a pronounced effect on the proportion of families in which backward selection was to be preferred (*Figure 2*). When the intensity of selection in the forest was low, the proportion of families where BACKW is superior to FORW0 was low, but with intensities of selection above 2 their proportion rapidly increased especially with a smaller progeny size.

In our main model we considered that the gain of plus tree selection in the forest (G_1) is a function of the heritability. An alternative method is to consider the average genetic gain when selecting the plus trees as fixed, and letting the heritability affect only the gain through selection based on a test. In both alternatives the proportion of families in which backward selection was a better method decreased with increasing heritability (*Fig. 3*). Compared to the situation where genetic gain was expressed as a function of heritability and intensity of selection, fixed genetic gain gave a higher proportion of backward selection at low heritabilities and a lower proportion at high heritabilities. This was caused by the fact that when genetic gain is given as a function of heritability, low heritability decreases genetic gain in the forest (G_1) and thus also decreases the genetic gain in backward selection.

Progeny size had the greatest effect on the superiority of the selection methods at the s#mallest (below 50) progeny sizes (*Figure 4*). When progeny size increased from 10 to 50 the proportion of families in which backward selection was better dropped from a maximum of 60% to 20% to 30%. When progeny size was over 100, its changes had only a weak effect on the proportion of families selected backward.

The number of families (plus trees) had practically no effect on the superiority of the selection alternatives when the proportion of families was used as the criterion.

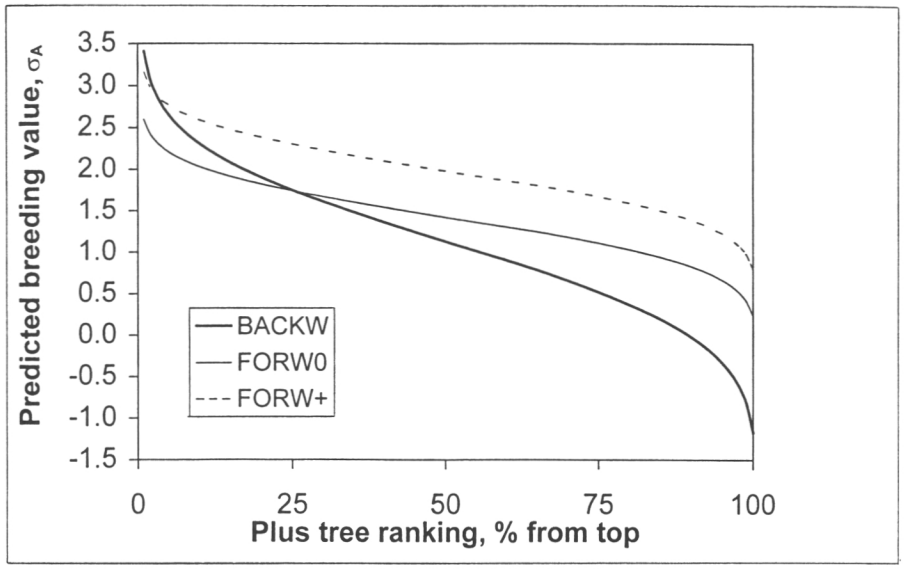


Figure 1. – Predicted breeding values for the individual progeny tested plus trees (BACKW) compared with the best offspring. FORW0 = best offspring in an open pollinated progeny of the plus tree, FORW+ = best offspring in a polycross progeny of the plus tree. This figure describes an intermediate situation in which $i_1 = i_4 = 2.5076$ and $h^2 = 0.2$.

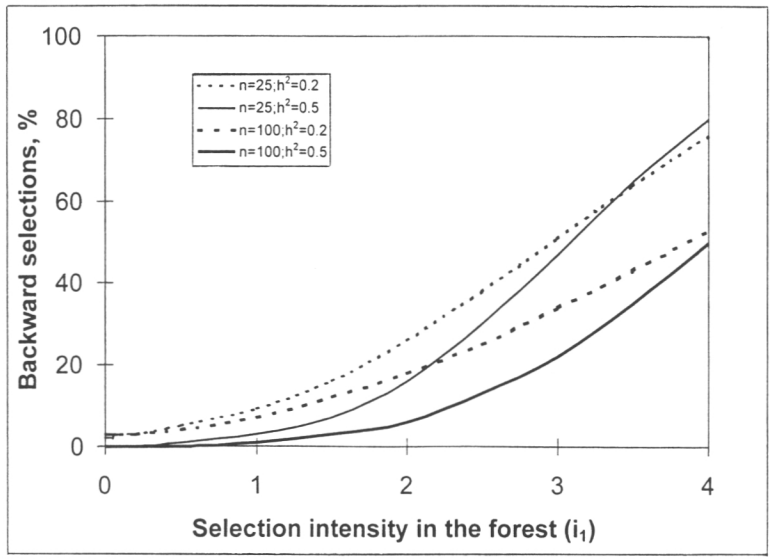


Figure 2. – Percentage of families in which backward selection (BACKW) is a better alternative than forward selection (best individual from n) from open pollinated progeny (FORW0) as a function of intensity of selection in the forest. Four different combinations of parameters n and h^2 are shown.

4. Discussion

4.1. General discussion

The proportion of families in which backward selection (BACKW) was the preferred alternative varied greatly depending on the values of the parameters (i_1, h^2, n). When BACKW was compared to forward selection from open pollinated families (FORW0) at medium parameter values, backward selection was the better alternative in some 25% of the top ranking families. A comparison with polycross families (FORW+) with the same parameter values indicated that backward selection was better in only 3% of the families. The results obtained using the same method by LINDGREN (1986) are in fairly good agreement with those presented here.

In a simulation study with full sib families (both parents plus trees) HODGE and WHITE (1993) found that the highest ranking parents were superior to the best offspring selections. They also observed that backward selection was favoured when a decreasing proportion of the original population was used. They did not allow related individuals in the offspring, and this greatly reduced the gain of the lower ranking offspring selec-

tions. According to LINDGREN and WERNER (1989), in the establishment of Norway spruce seed orchards, forward selection from full sib families gave higher general genetic gain than backward selection.

The observed regularities are rather evident. The intensity of selection in the forest influenced the balance between backward and forward selection by affecting the relative breeding value of the pollen pool. When selection in the forest has been ineffective, the unselected pollen cloud has almost as high a genetic value as the plus trees themselves, thus making forward selection from open pollinated progeny a useful option. Especially in cases where genetic gain in phenotypic selection in the forest is zero ($G_1 = 0$), forward selection in open pollinated progeny gives the same result as forward selection in polycross progeny ($G_5 = G_6$, formulae 6 and 7). On the other hand, with a higher intensity of selection in the forest the breeding value of the background pollen pool is decreased in relative terms, thus favouring backward selection. LINDGREN and

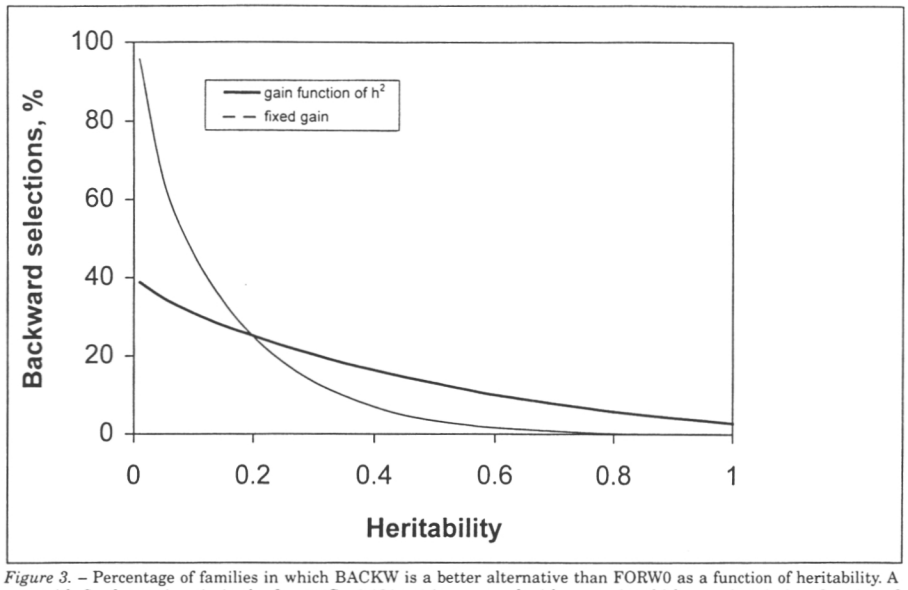


Figure 3. – Percentage of families in which BACKW is a better alternative than FORW0 as a function of heritability. A case with fixed genetic gain in the forest (G_1=1.121 σ_A) is compared with a case in which genetic gain is a function of fixed intensity of selection (i_1 = 2.5) and heritability. Size of progeny (n) is 100 in both cases.

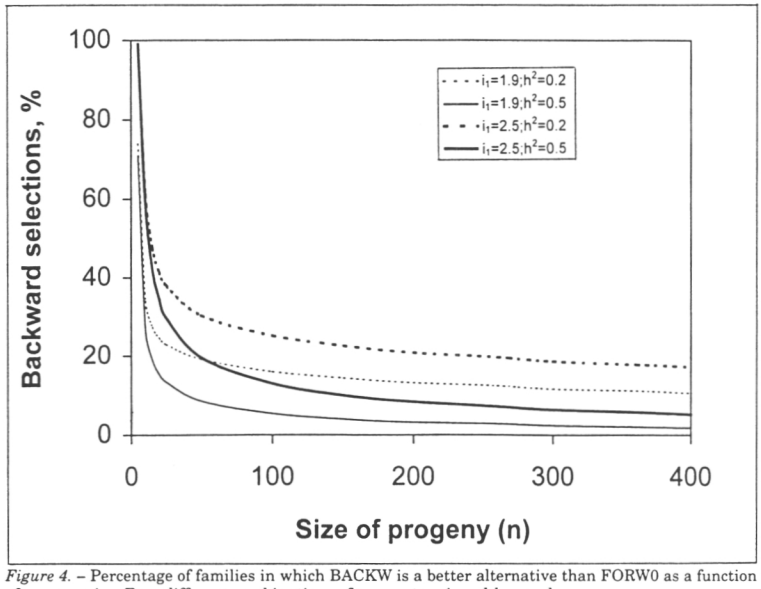


Figure 4. – Percentage of families in which BACKW is a better alternative than FORW0 as a function of progeny size. Four different combinations of parameters i_1 and h_2 are shown.

WERNER (1989) also pointed out in a study on Norway spruce that open pollinated material is worth considering when genetic gain in phenotypic selection in the forest is low.

The relationship between backward selection and forward selection in polycross progenies is not affected by the intensity of selection in the forest, because both these alternatives deal only with selected material. This can also be verified analytically by setting G_3 and G_6 equal (formulae 5 and 7). As a result, the phenotypic genetic gain in the forest (G_1) is reduced from the equation.

Low heritability favours backward selection, because in such cases forward selection is not effective. This is true both with a situation in which genetic gain in the forest is expressed as a function of heritability and intensity of selection, and especially with fixed genetic gain. For practical applications it is better to study the effect of heritability with a fixed genetic gain, because in real situations one cannot change it at the time when the selections for next generation seed orchards or breeding populations are actual. On the other hand, heritability in the experiments can be influenced by experimental lay-out, tending or finally by selecting experiments according to heritability. The same relationship with heritability is also observed between backward selection and forward selection from polycross progeny. Low heritability is one of the main factors favouring progeny testing and thus backward selection (FALCONER and MACKEY, 1996).

Both selection backwards and forwards are affected by heritability, but the relationships are different. These combined effects can give the sort of complex dependences which are sometimes observed for BESTBAF0 and BESTBAF+ in *table 2*, although in the major comparisons forward selection became slightly better with increasing heritability. The overall impression from *table 2* is that heritability has only a slight effect, while intensities of selection are considerably more important. A parallel could be made with the studies of LINDGREN (1986) and LINDGREN *et al.* (1997), who conclude that heritability is of little importance for the breeding decisions studied.

Small families have the consequence that intensity of selection is reduced when selecting the best, and they thus reduce gain by forward selection. They also have the drawback that the average of the family will reflect less precisely the true family average, and thus the breeding value of the parent. This will make selection backwards less efficient.

4.2. The model and the parameters

The lowest values used for intensity of selection in the forest (i = 1.8675) may seem too low. However the intensities of selection achieved in practical plus tree selection are not necessarily very high. In Finnish plus tree selections of Scots pine and Norway spruce, the average intensities of selection for height were in the range of 1.5 to 2.0 depending on the species and area (OSKARSSON, 1972). In practical selection work the intensity of selection for a single trait is lowered by selection for several traits. As a result of varying environmental conditions, reliable comparisons can only be made with a limited number of trees. Intensities of selection of 2 to 3 are realistic in the case of mass selection in uniform forest or unimproved plantation (WRIGHT, 1976). As a consequence of this reasoning rather low intensities of selection (below 2.5) seem to be most relevant. SHELBOURNE (1992), for instance, used a selection ratio 1:60 (i = 2.32) in a simulation study for recurrent mass selection.

Although in some breeding programs plus trees are tested with several hundred offspring, the potential for within-family selection is limited to a much lower number of trees due to for example mortality, unsuitability of tests for selection and difficulties in making reliable comparisons between tests. WRIGHT (1976) stated that the intensity of selection in most cases of within-family selection does not exceed 2.5 (responding selection ratio 1:100). According to LINDGREN (1991), not more than 30 to 100 offspring are justified for the ranking of parents. In other simulation studies selection intensities of between 2.04 and 2.51 have been used (SHELBOURNE, 1992; HODGE and WHITE, 1993). All this leads to the conclusion that the maximum intensity of selection for within family selection that can be achieved in a test is 2.5.

In a review covering several species and traits, CORNELIUS (1994) found that for growth traits the average heritabilities were between 0.2 and 0.3, and more than 75% of the heritability values were less than 0.4. According to HAAPANEN *et al.* (1997), the mean weighted heritability value for height was 0.24 in 16 Scots pine progeny trials in Finland. In individual trials heritability values of as high as 0.77 were observed. In a similar simulation study, SHELBOURNE (1992) used heritability values of between 0.1 and 0.4, and HODGE and WHITE (1993) 0.2. It can be stated that heritability values of between 0.2 and 0.4 are realistic for this kind of calculation. Actually, we have made a simplification which is probably not realistic by assuming that the heritability in the plus tree selection is the same as in the progeny test. A more realistic but also more complex model which uses G_1 (the genetic gain in phenotypic plus tree selection in the forest) as an independent entry could be considered.

The fixed values for phenotypic genetic gain in the forest (G_1) cannot easily be compared to real values, because not many published genetic gains have been expressed in terms of additive genetic standard deviation (σA). However, some information can be obtained by analysing the values of heritability and intensity of selection yielding the used value. The value used for genetic gain (1.121) can be regarded as realistic, because it is a result of *e.g.* rather typical parameter values of $h^2 = 0.2$ and $i = 2.507$.

4.3. Combination of the generations

When the whole population was considered, a combination of generations was in many cases favourable. However, because some degree of family selection is almost always utilized in advanced generation breeding and the establishment of seed orchards, results dealing only with the better half of the population are more relevant. In this case the additional genetic gain obtained with a combination of generations was small. The smaller the proportion of families needed for the next generation or seed orchard, the less use there is for a combination of the generations, because selection is then performed backwards in a higher proportion of families. This is in accordance with the results of a simulation study with full sib families (HODGE and WHITE, 1993). A seed orchard which combined the best material from parent and offspring generation was about one percent better in a small breeding population than corresponding orchards which utilized only one generation. In a large breeding population the combination of generations did not increase the genetic gain, because there were more than enough superior parental selections.

4.4. Application to real situations

The limit between backward and forward selection can be solved for each situation if the essential parameter values are known. Using the most realistic set of parameters ($i_1 = 2-2.5$, $n = 50-100$ and $h^2 = 0.2-0.4$) it can be estimated that some 20% of the originally selected plus trees are generally superior to their best open pollinated offspring. If polycross progeny is

available, only a few per cent of the best plus trees will outperform their best offspring. However, this information only has practical value if the relatedness can be sufficiently controlled after selection in polycross progenies. The practical consequences of this result depend greatly on the size of the breeding population compared to the number of originally selected plus trees. If the size of the breeding population is only 20% or less of the total number of plus trees, then all selections can be made backward. On the other hand, if considerably more than 20% of the phenotypic plus trees are needed, then the use of the best open pollinated offspring instead of the lower ranking plus tree itself is a good alternative. The difference in the breeding values of the plus tree and its best offspring is rather small around the critical value, so there will be a rather large number of families where the question whether to select backward or forward can be solved on practical grounds.

It should be noted that the variation among the forward selections will be greater than that of the backward selections, and in some cases there may be some additional gain to harvest. HODGE and WHITE (1993) pointed out that the precision of the breeding value estimates of the offspring of tested plus trees is lower than that of the parents themselves, but higher than untested phenotypically selected plus trees. This higher uncertainty in using forward selections can limit their use in seed orchards. On the other hand, it makes it rewarding to progeny test such selections, because the breeding value at selection is uncertain. The results presented here can most straightforwardly be applied to seed orchard situations. In breeding populations more intricate developments are probable; for instance, selecting several offspring from one family or selecting a plus tree and its offspring.

In addition to the genetic gain several other factors also affect the feasibility of the selection alternatives. Parent trees are often grafted in seed orchards or clone archives and thus are ready for crosses for the next generation, whereas forward selected offspring often need to be grafted. This causes extra costs and a time lag, which can cancel out the small additional genetic gains predicted by theoretical calculations. Biological characteristics of the species in question and other practical restrictions highly influence the optimal solution. In creating breeding populations of radiata pine in South Australia slight family selection was followed by forward selection within families (COTTERILL, 1984). On the other hand, forward selection within a late flowering species like Sitka spruce in Britain was not a justified option in composing breeding populations (LEE, 1993).

The handling of parental and offspring material as a basis for advanced generation breeding can cause practical difficulties beyond those associated with using only one generation. However, according to HODGE and WHITE (1993), the combined parent and offspring seed orchards for pine in southeastern USA are a flexible and straightforward option. Combinations can be made in such a way that the backward selections are grafted clones which can be used as seed parents, while the forward selections for long term breeding are young trees in progeny tests, that are harvested for pollen only and used as pollen parents.

The use of open pollinated offspring as a base material for advanced generation breeding has the disadvantage that it is impossible to keep record of the pedigree of the trees. However, if the pollination is mainly derived from outside the seed orchard, it can be presumed that the risk of a high proportion of common ancestors in the offspring is low. Also if the seed for open pollinated progeny tests is collected from original plus trees that are widely separated from each other there is practically no risk that the selections will be related through a common male parent. Background pollination can even be regarded as an advantage because it increases genetic diversity.

Breeding is not only a question of producing gain, but also controlling diversity. Less related individuals can be considered as more valuable for long term breeding. For this reason reliance on polycross selection is questionable. Selection should take into account not only breeding, but also relatedness (ZHENG *et al.*, 1997).

5. Acknowledgement

The authors express their sincere thanks to Mr. MATTI HAAPANEN and an anonymous referee who made valuable comments on the manuscript and Mr. JOHN DEROME, who checked the English language of the article.

6. References

CORNELIUS, J.: Heritabilities and additive genetic coefficients of variation in forest trees. Canadian Journal of Forest Research **24**:372–379 (1994). — COTTERILL, P. P.: A plan for breeding radiata pine. Silvae Genetica **33**(2–3):84–90 (1984). — FALCONER, D. S. and MACKEY, T. F. C.: Introduction to quantitative genetics. 4th ed. Longman Group, London, UK. 463 pp. (1996). — HAAPANEN, M., VELLING, P. and ANNALA, M.-L.: Progeny trial estimates of genetic parameters for growth and quality traits in Scots pine. Silva Fennica **31**(1):3–12 (1997). — HARJU, A.: Genetic functioning of Scots pine seed orchards. Acta Universitatis Ouluensis. A. Scientiae Rerum Naturalium 271. 39 pp. (1995). — HARTER, H. L.: Order statistics and their use in testing and estimation. –2v. U.S. Air Force Aerospace Research Laboratories, Wright Patterson Air Base, Ohio (1970). — HODGE, G. R. and WHITE, T. L.: Advanced generation wind-pollinated seed orchard design. New Forests **7**:213–236 (1993). — LEE, S. J.: Breeding strategy for Sitka spruce in Britain. *In*: LEE, S. J. (Ed.). Progeny testing and breeding strategies. Proceedings of the Nordic Group for Tree Breeding, Edinburgh, Scotland. 6 to 10 October 1993, 95–107 (1993). — LINDGREN, D.: How should breeders respond to breeding values? – Conference Proceedings: A joint meeting of IUFRO WP on Breeding Theory, Progeny Testing and Seed Orchards. Virginia, October 13 to 17 1986. 361-372 (1986). — LINDGREN, D.: Progeny testing. *In*: Genetics of Scots pine. GIERTYCH, M. and MATYÁS, Cs. (eds.) Developments in Plant Genetics and Breeding **3**, 191–203 (1991). — LINDGREN, D. and NILSSON, J.-E.: Calculations concerning selection intensity. Sveriges Lantbruksuniversitet, Institutionen för skoglig genetik och växtfysiologi. Rapport 5. 28 pp. (1985). — LINDGREN, D., WEI, R.-P. and LEE, S.: How to calculate optimum family number when starting a breeding program. For. Sci. **43**(2): 206–212 (1997). — LINDGREN, D. and WERNER, M.: Gain generating efficiency of different Norway spruce seed orchard designs. *In*: STENER, L.-G. and WERNER, M. (Eds.). Norway Spruce; Provenances, Breeding and Genetic Conservation. Proceedings of the IUFRO working party meeting, S2.02-11, in Sweden 1988. Institutet för skogsförbättring. Rapport nr. 11:189–206 (1989). — NAMKOONG, G., SNYDER, E. B. and STONECYPHER, R. W.: Heritability and Gain Concepts for Evaluating Breeding Systems such as Seedling Orchards. Silvae Genetica **15**:76–84 (1966). — OSKARSSON, O.: Finnish plus trees of Scots pine and Norway spruce. Folia Forestalia **150,** 138 pp. (1972). — Pitkäntähtäyksen metsänjalostusohjelma: [Long term forest tree breeding program, only in Finnish]. Helsinki.127 + 8 pp. (1989). — SHELBOURNE, C. J. A.: Genetic Gains from Different Kinds of Breeding Population and Seed or Plant Production Population. South African Forestry Journal, No. 160: 49–65 (1992). — SPANOS, K., ANDERSSON, E. W. and LINDGREN, D.: Multi-generation comparison between restricted phenotypic and combined index selection for gain and diversity. *In*: KURM, M. and TAMM, Ü. (Eds.). Conservation of Forest Genetic Resources. Nordic Group for Forest Genetics and Tree Breeding Meeting in Estonia, June 3 to 7, 1996. — Estonian Agricultural University, Tartu. ISBN 9985-830-11-3. Forestry studies **28**:86–100 (1997). — WERNER, M., HADDERS, G. and ROSVALL, O.: Förädlingsprogram för tall, gran och contortatall. Summary: Breeding programs for Scots pine, Norway spruce and lodgepole pine. Föreningen skogsträdsförädling. Institutet för skogsträdsförädling. 1980 Årsbok. pp. 59–111. (1981). — WRIGHT, J. W.: Introduction to Forest Genetics. Academic Press. 463 pp. (1976). — ZHENG, Y. Q., LINDGREN, D., ROSVALL, O. and WESTIN, J.: Combining genetic gain and diversity by considering average coancestry in clonal selection of Norway spruce. Theoretical and Applied Genetics, in press (Accepted June 97). — ZOBEL, B. and TALBERT, J.: Applied Forest Tree Improvement. John Wiley & Sons. 505 pp. (1984).

III

NUMBER OF FOUNDERS FOR A BREEDING POPULATION USING VARIABLE PARENTAL CONTRIBUTION

Seppo Ruotsalainen[1] & Dag Lindgren[2]

[1)] Forest Research Institute, Punkaharju Research Station, FIN–58450 Punkaharju, Finland
[2)] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE–90183 Umeå, Sweden

Received August 17, 1999; revised January 3,2001; accepted January 10, 2001

ABSTRACT

This study considers a situation in which there is access to a high number of progeny tested plus trees, of which the best ranking ones are used as founders of the next generation breeding population. The question was how to obtain maximum genetic gain with a given level of genetic diversity. The level of genetic diversity was fixed to the level obtained with equally contributing within-family selection, and the resources were fixed by keeping the number and size of full sib families constant. The between-family genetic gain in establishing a breeding population was maximised by means of deterministic modelling. This was performed by selecting more founders than in the case with equal contribution and allowing them to be represented in the next generation by one, two or three offspring depending on their breeding value. The effect of variable contribution was studied by altering two parameters: selection intensity (number of tested plus trees) and genetic diversity in the resulting second generation breeding population (status number).

An increase of 20 to 23 % in the number of founders as compared to equal contribution gave the maximum genetic gain when the genetic diversity was kept constant. The optimum founder number increased with increasing number of tested plus trees and status number. The size of the highest contributing fraction of founders increased with increasing number of tested plus trees and decreasing status number. The amount of additional genetic gain obtained by variable contribution was greater with a smaller selection intensity and lower status number. In a typical case the between-family genetic gain could be increased by 7 % by increasing to the number of founders by 22 % and allowing them to contribute unequally to the next generation. Most of the additional genetic gain was obtained after the first few founders had been added.

Key words: breeding population, founder number, selection limit, genetic gain, status number, genetic diversity

INTRODUCTION

A forest tree breeding program is often initiated by choosing a number of parents – plus trees – from the forest, testing and mating them and then selecting from among their progeny. The number of founders can be based on predictions in order to keep the inbreeding and relatedness at an acceptable low level in the future, and genetic variation at so high a level that the future gains are not severely reduced (WHITE 1992). Economic compromises and other considerations also apply (LINDGREN 1991). The importance of retaining large genetic diversity depends on the time horizon of the breeding plan (WRAY & GODDARD 1994). A breeding plan aiming at continuous improvement during several generations must consider genetic diversity more than a short time plan.

One way of utilising a predetermined number of founders in a way that maximises the genetic diversity is to use within-family selection and allow each founder to contribute equally to the next generation. This balanced within-family selection procedure is likely to maximise the long term limit of selection (DEMPFLE 1975). However, this conclusion has recently been challenged (VILLANUEVA & WOOLLIAMS 1997). The current Swedish forest tree breeding program can be described as a (mainly) balanced within-family selection procedure (DANELL 1995, ROSVALL *et al.* 1998). It is therefore interesting to have a balanced program based on within-family selection as a reference.

It has been shown that, in order to make an optimal compromise between genetic diversity and genetic gain, it is preferable to let the best selected genotypes contribute more to the next generation than the lower ranking ones (LINDGREN 1974, TORO & NIETO 1984, LINDGREN 1986, LINDGREN & MATHESON 1986, TORO & PÉREZ-ENCISO 1990). There are several possible ways of handling the trade-off between genetic gain and diversity. Some methods set a cost in terms of genetic gain for the relatedness and thus transform both quantities to the same scale (LINDGREN 1986, LINDGREN

© ARBORA PUBLISHERS

& MATHESON 1986, WRAY & GODDARD 1994). The problem is to find the correct cost factor needed to make the transformation. TORO and NIETO (1984) presented a method for selecting the number of founders and their contributions so that the effective population size (and thus genetic diversity) is maximised with a given genetic gain. An alternative solution is to maximise the genetic gain with a given genetic diversity (LINDGREN *et al.* 1989, MEUWISSEN 1997).

The optimal proportion of a clone in a seed orchard is linearly dependent on its breeding value (LINDGREN & MATHESON 1986). The same relationship also holds approximately with a breeding population: the top ranking phenotypes should be selected from each family in linear proportion to the family average in order to obtain the maximum gain at a given effective population size (LINDGREN 1986, WEI & LINDGREN 1995). In some forest tree breeding plans the breeding population is divided into several genetically different fractions which contribute the more to the next generation the higher is their genetic value (*e.g.* WHITE *et al.* 1993). One example is the open nucleus breeding strategy, in which higher ranking trees form a nucleus that is bred more intensively than the main population (COTTERILL *et al.* 1989).

This study focuses on a situation in which a number of tested plus trees are available for mating, and the desirable next generation breeding population size and diversity have been fixed. The purpose of this study is to develop and analyse an explicit method for deciding which contributions the tested plus trees should make when generating the recruitment population for the second generation selections. In this variable contribution method such number of founders and combination of their contributions is searched for which maximises genetic gain with the given genetic diversity.

METHODS

Model formulations

We considered a situation with a number of tested plus trees in which we wanted to generate a recruitment population for the next round of selections. We assumed that this would be done by controlled crossings of the best plus trees. We will call these plus trees, which are involved as crossing partners, as founders, because they are the genotypes that can transmit genes to the following generations in long-term breeding. The number of crosses (0–3), in which a plus tree was involved in was related to its breeding value. Only one selection was subsequently made from each family (from this point of view the system can be described as within-family selection). The number of crosses and number of selected offspring were therefore equal. This formulation was rather easy to use as a basis for the calculations.

When determining an efficient solution to a genetic problem the most important factors are genetic gain, genetic diversity, time and cost. Genetic gain was regarded as proportional to the selection intensity related to the choice of plus tree parents. Time was assumed to be constant. Cost was kept constant by fixing the number and size of families. The genetic diversity was described using group coancestry (θ) (COCKERHAM 1967) or, equivalently, by a measure called status number (N_S) derived from it ($N_S = 0.5/\theta$) (LINDGREN *et al.* 1997, LINDGREN & KANG 1997).

In the model case we considered a recruitment population of size N_{R1}, consisting of unrelated non-inbred progeny-tested plus trees. N_1 were selected from these N_{R1} plus trees for the first generation breeding population (the founders) according to their breeding values. These founders were then mated randomly (selfing excluded) with each other to create the second generation recruitment population. Mating design as such does not matter for the results of this study, as we are only concerned with the gene pool of the selected progeny, not with the recombination of its genes. According to the definitions given by WHITE (1987), the first generation recruitment population corresponds to the selected population, and subsequent recruitment populations to the base population. The breeding population is used in the same meaning as in WHITE (1987).

The contribution of the first generation plus trees to the next generation could be varied so that they were used in zero (rejected tested plus trees with a low breeding value), one (the plus trees with the lowest accepted breeding values), two (intermediate plus trees) or three crosses (fraction with the highest breeding values) (Fig. 1). This method of allocating parent contributions is rather similar to the unbalanced 3:2:1 mating system used by ROSVALL (1999), except that we allowed the first generation breeding population size (N_1) to vary (see details later). One offspring was selected from each cross (full-sib family) for the second generation breeding population, which had the size N_2. The family size was the same in all the crosses. This design had the great advantage that the gain through within-family selection was kept constant, and thus did not have to be considered when searching for the optimum contribution of the founders.

The size of the second generation breeding population (N_2) can be expressed as a function of the sizes of the individual fractions of first generation

breeding population ($N_1 = n_3 + n_2 + n_1$),

$$N_2 = \frac{3n_3 + 2n_2 + n_1}{2} \quad [1]$$

where n_1, n_2 and n_3 are the sizes of the individual fractions of first generation breeding population, with the subscript showing their contribution to the next generation (Fig. 1 and Table 1).

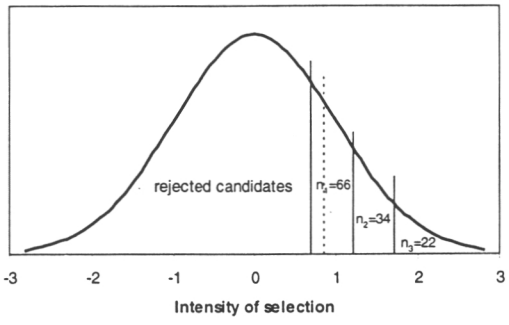


Figure 1. The first generation recruitment population (the tested plus trees = N_{R1}) arranged according to the breeding values of the trees, and divided into fractions according to the number of families for which they will be parents (0, 1, 2 or 3). The dotted line marks the limit of the breeding population in the base option, in which 100 founders have 2 families and thus 2 selected offspring each. N_{R1}=500, N_2=100, N_{S2}=66.7

The average between-family selection intensity for the first generation breeding population is

$$i_f = \frac{3n_3 i_3 + 2n_2 i_2 + n_1 i_1}{3n_3 + 2n_2 + n_1} \quad [2]$$

with terms as described in Table 1.

The group coancestry in the second generation breeding population (θ_2) is

$$\Theta_2 = \frac{0.5N_2 + 0.75n_3 + 0.25n_2}{N_2^2} \quad [3]$$

This formulation can be derived as follows. Because the selected plus trees are unrelated and only one second generation tree is selected from each family, only selfing and half-sib relationships need to be considered. The first term in the numerator gives the contribution of self coancestry to group coancestry. The following terms represent coancestries caused by half sibs in the highest and intermediate fractions, respectively. The coancestry between half sibs is 0.125, and there are three half sib combinations within the progeny of each founder tree in the highest fraction. When the reciprocal combinations are included we obtain the coefficient 0.75 (= 0.125 × 3 × 2). In the intermediate fraction each tree has two offspring, so there is only one half sib combination for each tree. When this is also taken reciprocally, we arrive at the coefficient 0.25. In the lowest fraction with only one offspring from each family there are no half sibs, and thus this fraction does not contribute to group coancestry through the half sibs (coefficient is 0).

Using formulae (1) and (3), we can express n_1 and n_2 as a function of the fixed parameters (N_2 and Θ_2) and n_3.

$$n_1 = 6N_2 + 3n_3 - 8\Theta_2 N_2^2 \quad [4]$$

and

$$n_2 = 4\Theta_2 N_2^2 - 2N_2 - 3n_3 \quad [5]$$

The size of the first generation breeding population (N_1) is the sum of the three fractions n_1, n_2 and n_3. It can also be expressed as a function of n_3 and the fixed parameters Θ_2 and N_2 by substituting n_1 and n_2 with the corresponding formulae (4) and (5). This gives us the following expression for N_1

$$N_1 = 4N_2 (1 - \Theta_2 N_2) + n_3 \quad [6]$$

Simulation

We fixed the number of selections and thus the number of crosses at 100 (N_2=100).

If there are two crosses per accepted plus tree (N_1 = 100), with one selected offspring from each family (double pair mating), the status number for these selections is 66.66... and the group coancestry 0.0075. This group coancestry is for equal parental contribution, but it can also be called the group coancestry for within-family selection. This selection scheme was used as the base option to which the alternative selection procedures were compared. It follows the guidelines for current Swedish tree breeding strategies (DANELL 1995, WILHELMSSON & ANDERSSON 1995), except that the breeding population size is twice as large (100 *vs.* 50). Second generation breeding population size (N_2) was set at 100 in the calculations, and the other parameters were expressed in relation to this (Table 1). A breeding population size of 100 made it possible to interpret the results

© ARBORA PUBLISHERS

Table 1. Parameters and parameter values used in calculations for maximising genetic gain for the 2nd generation breeding population by altering contributions of the founders according to their breeding values. The parameter values which define the main alternative are given in bold.

Parameter	Description	Value	Remarks
Value fixed			
N_{R1}	Size of first generation recruitment population	N_2, $2N_2$, **$5N_2$**, $100N_2$	
N_2	Size of 2nd generation breeding population	**100**	
Θ_2	Group coancestry of 2nd generation breeding population	0.0083, **0.0075**, 0.0067, 0.0059	
N_{S2}	Status number of 2nd generation breeding population	$0.6N_2$, **$0.66...N_2$**, $0.75N_2$, $0.85N_2$	$N_{S2} = 0.5/\Theta_2$
Value allowed to vary			
N_1	Size of 1st generation breeding population (= number of founders)	Varies	Result of the calculation
n_3, n_2, n_1	Numbers of trees in high, intermediate and low fractions of N_1, respectively	Varied	The numbers are actively altered.
i_3, i_2, i_1	Intensities of family selection applied for 2nd generation in high, intermediate and low fractions of N_1, respectively	Varies	Defined by proportions selected
– i_f	Average between family selection intensity for the 2nd generation breeding population	Varies	Result of the calculation

concerning N_1 and n_3 as a percentage of N_2.

The alternative procedures used a breeding population that was divided into three differently contributing fractions according to the breeding values of their trees, as described earlier (Fig. 1). The costs were maintained at the same level as in the base option by keeping the number of crosses and family size the same. It was assumed here that the number of progeny tested first generation plus trees (N_{R1}) was considerably higher than the number of founders needed (N_1). Often such plus trees are stored in clone archives during progeny testing and are thus available with little or no extra costs as additional founders, if needed. The main alternative studied was defined by the parameter values N_{S2} = 66.7 (Q_2 = 0.0075) and $N_{R1} = 5N_2$. Some other alternatives with different status number (N_{S2}) and recruitment population size (N_{R1}) were also studied, but less intensively.

In the search for the greatest genetic gain with a fixed diversity and costs, the borderline between the rejected candidates and the lowest fraction belonging to the breeding population could be changed and more trees included in the breeding population. Also the fraction division within the breeding population could be altered in order to maximise the genetic gain. In the following this selection procedure is called *variable contribution*, and it was compared with the *base option* in which all the trees contributed equally to the next generation and the breeding population size was constant.

Starting with the values of the fixed parameters (Table 1) and varying the values of n_3 within the desired range, the values for n_1 and n_2 were calculated using formulae (4) and (5), respectively. The average between-family selection intensity was then calculated for the different alternatives using formula (2). The n_3 value giving the highest genetic gain was noted and the corresponding N_1 value was calculated. With the given restrictions n_1 and n_2 are functions of n_3, and thus only n_3 needs to be varied. The within-family selection intensity was not considered in these calculations. Due to the constant family size and one selection per family its expectation is the same in all the families and selection alternatives so that it does not affect the ranking of different solutions. The selection intensities for the different fractions were calculated using function for infinite population size (LINDGREN & NILSSON 1985). This function was easier to program, and as the differences between the true selection intensities and those obtained by the infinite function

Table 2. Founder number (N_1) and the size of the highest contributing fraction (n_3) when the selection intensity reached its maximum at different levels of genetic diversity (N_{S2} and θ_2) and recruitment population size (N_{R1}).

		Founder number (N_1)				Size of the highest fraction (n_3)			
	N_2	100[a]				100[a]			
	N_{S2}	60	66.7	75	85	60	66.7	75	85
N_{R1}	Θ_2	0.0083	0.0075	0.0067	0.0059	0.0083	0.0075	0.0067	0.0059
100	$=N_2$	97	100	-	-	30	0	-	-
200	$=2N_2$	102	120	140	166	35	20	7	1
500	$=5N_2$	102	122	143	167	35	22	10	2
10000	$=100N_2$	103	123	144	168	36	23	11	3

[a] The breeding population size (N_2) 100 allows the values for N_1 and n_3 to be interpreted as per cent of N_2.

were smaller than 0.01, the function could be safely used.

RESULTS

Number of founders and size of the highest fraction

The size of the highest contributing fraction (n_3) which gave the highest selection intensity varied depending on the size of the recruitment population and especially on the group coancestry (status number) in the second generation progeny (Table 2). When the size of the recruitment population increased, the maximum genetic gain was reached with a larger founder number and larger number of trees in the highest contributing fraction. This effect was, however, trivial when the recruitment population size was more than 1.5 times N_2 (Fig. 2).

When the status number in the second generation was allowed to differ from that in the main alternative (N_{S2} = 66.7), the founder number and the sizes of the individual fractions were greatly influenced (Table 2, Fig. 3). The increase in status number (decrease in group coancestry) in the second generation greatly increased the total number of founders. At the same time, the size of the highest contributing fraction decreased and the lowest increased, until, finally all trees contributed only one offspring to the next generation (Fig. 3). In contrast, when the status number was decreased from that in the main alternative (more coancestry allowed), the total founder number decreased and an increasing number of trees belonged to the fraction which contributed 3 offspring to the second generation.

The largest founder number (68 % greater than N_2) in the studied alternatives was obtained when both the status number and the recruitment population size were the greatest (Table 2). Correspondingly, the smallest founder number (3 % smaller than N_2) occurred with the opposite parameter combination. The size of the highest contributing fraction (n_3) could not be deduced directly from the size of the founder population. Its greatest size (36 % of N_2) was obtained with the lowest

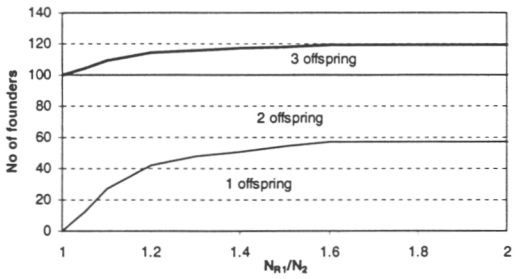


Figure 2. Optimal founder number (size of first generation breeding population) and the sizes of different fractions of the breeding population as a function of relative recruitment population size. N_2 = 100, N_{S2} = 66.7.

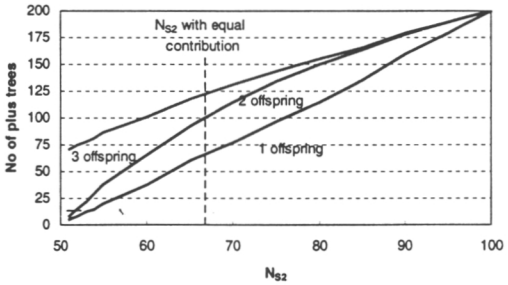


Figure 3. Optimal founder number and optimal allocation of founders to differently contributing fractions as a function of the desired status number in the new breeding population. N_2 = 100, N_{R1}=500.

Table 3. Maximum between-family selection intensity achieved with different recruitment population sizes (N_{R1}) and second generation status number (N_{S2}), when second generation breeding population size (N_2) is 100.

		Maximum selection intensity					Maximum selection intensity in %[a]			
		Base option		Variable contribution			Variable contribution			
N_{R1}	N_{S2}	66.7	60	66.7	75	85	60	66.7	75	85
	Θ_2	0.0075	0.0083	0.0075	0.0067	0.0059	0.0083	0.0075	0.0067	0.0059
100	$=N_2$	0	0.3753	0	-	-	-	-	-	-
200	$=2N_2$	0.7979	1.0127	0.9095	0.7506	0.5186	127	114	94	65
500	$=5N_2$	1.3998	1.5619	1.4931	1.3892	1.2465	112	107	99	89
10000	$=100N_2$	2.6652	2.7694	2.7296	2.6693	2.5867	104	102	100	97

[a] The relative values are calculated from the base option ($N_1 = N_2 = 100$, $N_{S2} = 66.7$, two crosses per tree, one selection per family).

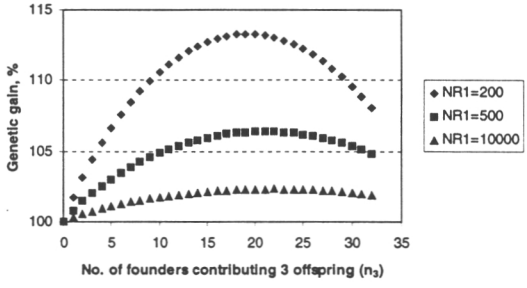


Figure 4. Genetic gain of family selection (in relation to base option) as a function of the size of the highest contributing fraction (n_3) of the breeding population at three different recruitment population sizes. N_2=100, N_{S2}=66.7.

status number and the greatest recruitment population size. The highest contributing fraction disappeared completely with the opposite combination: high status number and small recruitment population size.

Amount of maximum gain

The effect of the founder number and variable contribution on selection intensity was influenced both by the size of the recruitment population and by coancestry (status number). With the larger recruitment population the effect of a variable contribution of founders on selection intensity was smaller both in absolute and relative terms (Table 3, Fig. 4). However, there was a different trend in the relative selection intensity with a low and high status number. With a low status number the obtained selection intensity in relation to base option decreased with increasing recruitment population size. With a high status number this relative selection intensity increased in the same direction (Table 3).

An increase in status number greatly decreased both the absolute and relative selection intensities (Table 3, Fig. 5). The decrease was more pronounced with a lower recruitment population size. The greatest relative increase in selection intensity (27 % compared to the

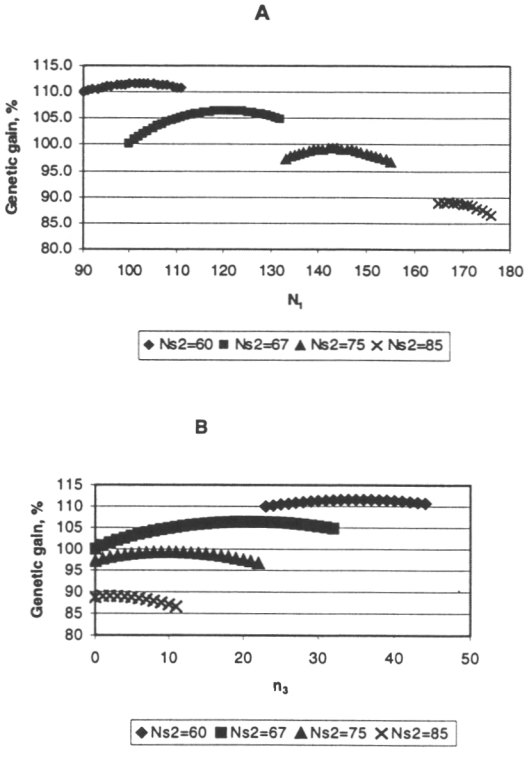


Figure 5. Genetic gain of family selection (in relation to base option) as a function of total founder number (A) and the size of the highest contributing fraction of the founders (B) at four different levels of diversity (status number). N_{R1}=500, N_2=100.

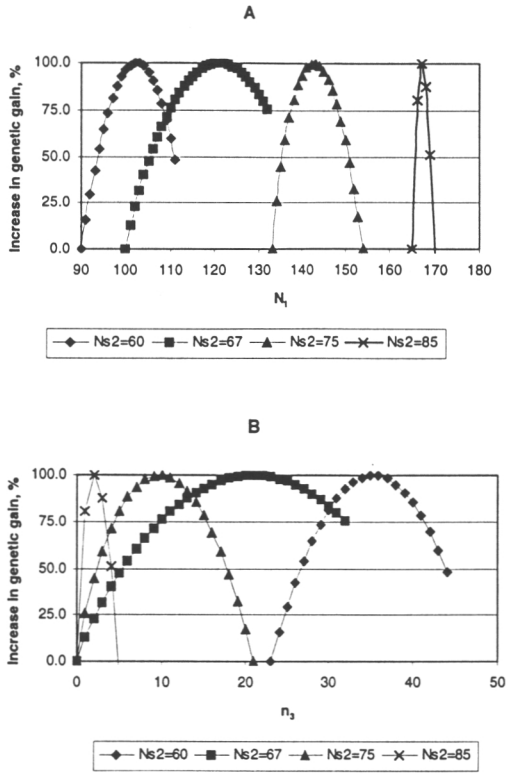


Figure 6. Relative increase in genetic gain as a function of total founder number (A) and the size of the highest contributing fraction (B) at four different levels of diversity (status number). The values are relative to the total increase between the lowest possible value of n_3 and that giving the maximum gain.

base option) was obtained when both the status number and recruitment population size were smallest. The smallest increase (in fact a decrease of 35 %) occurred with the smallest recruitment population size and greatest status number.

When the status number was high the total number of founders needed was also high, but only a few of them could contribute three offspring to achieve the greatest genetic gain. However, when the number of founders was further increased a situation arouse in which genetic gain was lower than with equal contribution (Fig. 6).

The greatest part of the total increase in genetic gain obtained by increasing the size of the highest contributing fraction of founders was achieved already after adding the first few trees (Fig. 6). When the status number was 66.7, 50 % of the maximum increase was obtained after adding only six trees (or 6 % of N_2) to the highest contributing fraction, even though 21 trees (21 % of N_2) had to be added to that fraction in order to reach the maximum genetic gain. With a status number of 75, the corresponding level (50 %) was achieved already after the addition of two trees (%), whereas the maximum was reached at 10 trees (%) in the highest contributing fraction.

DISCUSSION

The obtained results show that, by using a simple method to control the contribution of the founders, genetic gain can be increased without sacrificing any genetic diversity compared to the situation with equal contribution. This was achieved by allowing the best founders to contribute more to the next generation than those with a lower breeding value, as suggested by LINDGREN (1986) and similar to the finding of LINDGREN and MATHESON (1986). Our method divided the breeding population into three fractions which contributed to the next generation in the ratio 3:2:1 beginning from the fraction with the highest breeding value.

The contributions of the founders were approximately the same, as suggested by the model of linear deployment (LINDGREN *et al.* 1989). However, linear deployment assumes that genotypes can be deployed in any rational numbers. When we are discussing founder contributions through offspring, we are dealing with small integer numbers and, from that point of view, the current analysis is more accurate.

In a case with genetic diversity at the base option level, the number of founders should be increased by around 20 % from the original number in order to obtain the maximum genetic gain. The increase in the number of founders was, at the same time, the number of founders belonging to the highest contributing fraction. Using the model for linear deployment the increase in the number of founders was more than double (LINDGREN *et al.* 1989, Table 2). However, this model resulted in a large number of low ranking founders with very small contributions that could not be utilised when using trees as parents for a new generation. TORO and NIETO (1984) also observed that, although theoretically it would be advisable to use all the potential founders, the founders with the lowest ranking made such a small contribution that they had no practical significance.

It can be argued that it is not recommendable to use, as founders, those candidate trees from the recruitment population which are below the average, because the expected breeding value of an untested new selection will be higher (TORO & NIETO 1984). Such a situation is inevitable if the recruitment population is, at its highest, double the size of the breeding population. However, in practice the costs, practical difficulties and

time delays in selecting new plus trees may counteract the obtainable genetic gain. If the distribution of the breeding values in a real population differs markedly from the assumed normal distribution of this study, then the founder contributions could also be affected.

When the level of genetic diversity was increased from that of equal contribution after double pair mating, the number of founders needed increased, whereas the contributions of the intermediary founders decreased. On the other hand, when the genetic diversity was allowed to decrease, a lower number of founders was needed and their contributions increased. The same relationship between genetic diversity and number of utilised families is also observed with optimal contribution from families (LINDGREN *et al.* 1993).

The obtained increase in genetic gain when using vriable contribution was close to 10 % of the between-family gain with the most relevant recruitment population sizes. This is in accordance with the increase in genetic gain obtained by optimal deployment in plantations (LINDGREN *et al.* 1989). However, it must be noted that within-family selection gain is not considered here. Thus the obtained relative increase is smaller in relation to the total genetic gain. Because the genetic gain in within-family selection is affected by *e.g.* heritability and family size, no general rules can be derived for the increase in total genetic gain.

The increase in genetic gain obtained by using the variable contribution method was larger with a smaller recruitment population size and smaller status number. This is in accordance with results concerning linear deployment (LINDGREN *et al.* 1989, LINDGREN 1991, 1993). When the recruitment population size is small, the between-family selection intensity is also usually low, and there are great differences in selection intensities between the founders. Thus it is more advantageous to manipulate the contributions of different fractions of founders than in the case with a high selection intensity when the differences between founders are smaller. In relative terms this difference is still exaggerated, because comparison in the case of a small recruitment population size is made to a smaller value than with a large recruitment population. In establishing long-term breeding populations the selection intensity from the progeny-tested recruitment population of plus trees is usually not very high. Thus there are good possibilities for increasing the genetic gain by means of variable contribution.

The influence of genetic diversity on the increase in genetic gain can be interpreted in the same way as the effect of recruitment population size. When the genetic diversity was increased by allowing more trees to contribute to the next generation, it inevitably meant a decrease in genetic gain compared to equal contribution after double pair mating and a fixed breeding population size. This appears to be contrary to the results of MEUWISSEN (1997), whose rather similar optimal contribution method was more superior at lower levels of inbreeding. This contradiction can be explained by the fact that MEUWISSEN (1997) did not restrict breeding population size, but allowed it to vary. If this restriction is released, according to our model variable contribution will also be relatively more advantageous at greater breeding population sizes (lower inbreeding).

Most of the additional gain was obtained after adding the first few extra trees. However, because of the flat response curve of genetic gain close to the optimum, even a considerably larger number of founders than the optimum could be included without losing much of the additional genetic gain. We can see reasons to keep offspring from more founders than was found to be optimal in this study, because our definition of diversity does not fully consider the arguments of keeping offspring from many founders. One may want to include founders in the breeding population that are good in aspects other than the one (or the index) used to rank the trees. It may also be desirable to obtain a more balanced geographic representation in the breeding population. If offspring from more founders are transmitted this will also mean that more options are kept open to offer a high number of unrelated clones for future seed orchards. In all biological activities there is a risk of loss; founders may be lost *e.g.* because grafting does not work. Keeping the number of founders on the upper side of the optimum is cheap insurance against such unforeseeable losses. Thus we recommend choosing a number of founders that is slightly above the optimal found in this study.

In this study the contribution of founders to the next generation was varied by altering the number of crosses in which the trees participated, but selecting only one offspring from each family. It is also possible to alter the family size and make it dependent on the breeding value of the parents. At selection it would be more favourable to allow a different number of selected offspring from different families (*e.g.* using population merit selection, LINDGREN & MULLIN 1997) depending on the breeding value of the parents. However, genetic gain of an unbalanced mating system was equal to unbalanced selection system in breeding population, and superior in multiplication population (seed orchard) in a simulation study covering five generations (ROSVALL 1999).

The question of the best possible contribution of the founders to the next generation has also been studied earlier in both animal (MEUWISSEN 1997) and tree breeding (KERR *et al.* 1998, ANDERSSON *et al.* 1999,

ROSVALL 1999). All these studies differ from the present one in the respect that they are not searching for an analytical solution, but use either simulation or linear programming. There are also differences concerning the restrictions applied in the methods. The model described by KERR *et al.* (1998) does not have such rigorous requirements for group coancestry and costs as in our study, so closer comparison is useless. The method of ANDERSSON *et al.* (1999) is in fact optimising the contribution of families, not single founders, because it allows several selections from a family. It has options to control group coancestry and to some extent also costs (number of selections). However, the number of families is not restricted, which can result in slightly enhanced costs. The most restrictive alternative in ANDERSSON *et al.* (1999) gives both family contributions and gain increment that are in agreement with our results.

In this study the maximum number of crosses in which a plus tree participated was limited to three. Again, this is a constraint which was made to facilitate the calculations. Theoretically it would be better not to set such upper limits, but in practice this is of little importance if the size of the breeding population is not greater than 100. In a breeding populations of this size there is very little space for founders that are represented by more than three offspring if its size and status number are kept constant. For example, for a case with N_{R1}=200, N_2=100 and N_{S2}=66.7 it was possible to find a solution in which the highest ranking founder contributes four offspring. This solution more closely followed the optimal deployment function (LINDGREN *et al.* 1989) than the contributions with a restriction of at highest three offspring per founder, but its genetic gain was not higher.

If the breeding population size is considerably larger than 100, the solutions obtained by this method would deviate from the optimum one obtained by not limiting the contribution of the best trees to any fixed number. However, in such a case the optimum solution would be difficult to calculate using the methodology presented here, but it could be approximated by using linear deployment (LINDGREN *et al.* 1989).

For long-term breeding it is important to consider the genetic contributions from the founders to the breeding population some generations ahead. Gain can be expected to be proportional to the contributions, and diversity to the square of the contributions (WOOLLIAMS 1998). It can thus be assumed that the optimal long-term impact of a founder on gain versus that on diversity is obtained by using linear deployment in analogy with the case for clonal deployment for the multiplication population (LINDGREN 1986). This can justify the mating of founders with a similar breeding value (positive assortative mating), as it will make it easier to obtain similar genetic contributions from these founders in the future. In the first rounds of breeding and selection it will probably be optimal to get founder contributions that are linearly related to their breeding values. In later generations favourable genes from different founders may be differently selected and are subject to drift in such a way that the genetic contributions are less informative as regards the distribution of valuable genes. At the same time information about the breeding values of later generation genotypes provides new bases for selection decisions. For this reason future generation breeding will most likely primarily focus on selection while keeping an approximate balance of the founder contributions. On the other hand, in the initial steps of breeding efforts should be made in the matings and selections to obtain a founder contribution that is linearly related to the breeding values of the founders.

Increased contribution of the best trees makes the best fraction of the breeding population to be more related to others than lower ranking trees. If repeated over many generations this leads to increased inbreeding. However, this did not abolish the additional genetic gain obtained by unbalanced mating system in a five-generation simulation study (ROSVALL 1999). Unbalanced mating is a better method than unbalanced selection for increasing contribution of best trees, as it creates less relatedness between selected trees, and gives thus better opportunities for future selections. For the same reason two crosses per tree are more recommendable than one (ROSVALL 1999).

The results presented here cover only the first round of selection, but the final goal is long-term improvement. However, even the first generation genetic gain is important as such, especially with such breeding objects as trees, since they have rather long generation intervals. It can also be argued that the gain obtained in the beginning of the breeding program will not be lost, irrespective of the decisions made in later generations. Several studies with stochastic simulations have shown that selection methods implementing the contribution of parents in relation to their breeding values also produce, after many generations of selection, higher genetic gain than equal contribution, with equal or lower inbreeding (TORO & NIETO 1984, WRAY & GODDARD 1994, LINDGREN & MULLIN 1997, MEUWISSEN 1997, ROSVALL 1999, ROSVALL & ANDERSSON 1999). Experimental results from a six-generation selection experiment with *Drosophila melanogaster* have shown that a method that optimises mate selection and contribution at the same inbreeding level gives a higher genetic gain than an equally contributing and random mating system (SANCHEZ *et al.*

© ARBORA PUBLISHERS

1999). Continuously improving genetic gain by optimising the genetic contributions of the parents thus seems to be possible.

Exactly which method is used to allocate the contributions of the founders to the next generation is not essential, but what is most important is that the contributions approximately increase along with breeding value (WEI 1995). Small deviations from the theoretically optimal contributions do not greatly impair the result. The contributions giving maximum genetic gain with retained diversity can be approximated by the following rule of thumb. Rank the recruitment population trees by their breeding values and select 20 % more founders than is the intended later generation breeding population size. Then divide the obtained breeding population into six sections of equal size. Let the highest section trees contribute three offspring to the next generation, the two following sections two offspring and the last three sections one offspring. This variable contribution method makes it possible to increase the genetic gain without losing genetic diversity and with essentially no extra costs. The number of crosses remains the same and, in most tree breeding situations, the additional founders needed in this method already exist *e.g.* in clone archives. It therefore only requires some more technical documenting when the number of founders is increased and all the founders are not contributing equally.

Variable contribution makes better use of the available genetic resources by saving more of the available gene pool and thus increasing the ultimate selection limit. This study confirms that the relationship between genetic diversity and gain is not fixed, but can be influenced by sound planning of the number of founders and their contributions.

ACKNOWLEDGEMENT

We acknowledge the financial support from Föreningen Skogsträdsförädling and the Nordic Academy for Advanced Study (NorFa) for this study. Erik D. Kjær has contributed valuable comments. The language was revised by John Derome whose help we greatly appreciate.

REFERENCES

ANDERSSON, E.W., SÁNCHES RODRIGUEZ, L. & ANDERSSON, B. 1999: Group coancestry controlled selection in a *Pinus sylvestris* L. breeding program. *Theoretical and Applied Genetics* **99**(1/2):73–80.

COCKERHAM, C. C. 1967: Group inbreeding and coancestry. *Genetics* **56**: 89–104.

COTTERILL, P. P., DEAN, C., CAMERON, J. & BRINDBERGS, M. 1989: Nucleus breeding: a new strategy for rapid improvement under clonal forestry. *In*: Breeding tropical trees: Population structure and genetic improvement strategies in clonal and seedling forestry. (eds. G. I. Gibson, A. R. Griffin, & A. C. Matheson). pp. 39–51. Proc. IUFRO Conf. Pattaya, Thailand. November 1988. Oxford Forestry Institute, Oxford, UK and Winrock International, Arlington, Virginia, USA.

DANELL, Ö. 1995: Breeding programs in Sweden: 1. General approach. SkogForsk. *Arbetsrapport* **302**: 1–4.

DEMPFLE, L. 1975: A note on increasing the limit of selection through selection within families. *Genet. Res., Camb.* **24**: 127–135.

KERR, R. J., GODDARD, M. E. & JARVIS, S. F. 1998: Maximising genetic response in tree breeding with constraints on group coancestry. *Silvae Genetica* **47**(2–3):165–173.

LINDGREN, D. 1974: Aspects on suitable number of clones in a seed orchard. Proceedings, Joint IUFRO meeting, S.02.04.1–3, Stockholm, 1974, Session V. pp. 293 –305.

LINDGREN, D. 1986: How should breeders respond to breeding values? *In*: Conference proceedings: A joint meeting on IUFRO WP on breeding theory, progeny testing and seed orchards. Virginia, October 13 to 17, 1986. pp. 361–372.

LINDGREN, D. 1991: Optimal utilization of genetic resources. *Forest Tree Improvement* **23**: 49–67.

LINDGREN, D. 1993: Quantitative comparison between truncation selection and a better procedure. *Hereditas* **118**:289–292.

LINDGREN, D. & KANG, K. S. 1997: Status number – a useful tool for tree breeding. *Res. Rep. For. Gen. Res. Inst. Korea* **33**:154–165.

LINDGREN, D. & MATHESON, A. C. 1986: An algorith for increasing the genetic quality of seed from seed orchards by using better clones in higher proportions. *Silvae Genetica* **35**(5–6):173–177.

LINDGREN, D. & MULLIN, T. J. 1997: Balancing gain and relatedness in selection. *Silvae Genetica* **46**(2–3): 124–129.

LINDGREN, D. & NILSSON, J.-E. 1985: Calculations concerning selection intensity. Sveriges Lantbruksuniversitet, Institutet för skoglig genetik och växtfysiologi. *Rapport* 5. 28 pp.

LINDGREN, D., LIBBY, W. S. & BONDESSON, F. L. 1989: Deployment to plantations of numbers and proportions of clones with special emphasis on maximizing gain at a constant diversity. *Theoretical and Applied Genetics* **77**: 825–831.

LINDGREN, D., WEI, R. P. & BONDESSON, L. 1993: Optimal selection from families. *Heredity* **70**:619–621.

LINDGREN, D., WEI, R.-P. & LEE, S. J. 1997: How to calculate optimum family number when starting a breeding program. *Forest Science* **43**(2):206–212.

MEUWISSEN, T. H. E. 1997: Maximizing the response of selection with a predefined rate of inbreeding. *J. Anim. Sci.* **75**:934–940.

ROSVALL, O. 1999. Enhancing gain from long-term forest tree breeding while conserving genetic diversity. *Acta Universitatis Agriculturae Sueciae. Silvestria* **109**, 65 pp. + app.

ROSVALL, O. & ANDERSSON, E. 1999: Group-merit selection compared to conventional restricted selection for trade-offs between genetic gain and diversity. *Forest Genetics*

6:11–24.

ROSVALL, O., LINDGREN, D. & MULLIN, T. J. 1998: Sustainability robustness and efficiency of a multigeneration breeding strategy based on within-family clonal selection. *Silvae Genetica* **47**:307–321.

SANCHEZ, L., TORO, M. A. & GARCIA, C. 1999: Improving the efficiency of artificial selection: more selection pressure with less inbreeding. *Genetics* **151**: 1103–1114.

TORO, M. A. & NIETO, B. M. 1984: A simple method for increasing the response to artificial selection. *Genet. Res., Camb.* **44**:347–349.

TORO, M. & PÉREZ-ENCISO, M. 1990: Optimization of selection response under restricted inbreeding. *Genetics Selection Evolution* **22**: 93–107.

VILLANUEVA, B. & WOOLLIAMS, J. A. 1997: Optimization of breeding programmes under index selection and constrained inbreeding. *Genet. Res., Camb.* **69**:145–158.

WEI, R.-P. 1995: Predicting genetic diversity and optimizing selection in breeding programmes. Swedish University of Agricultural Sciences. Department of Forest Genetics and Plant Physiology. Dissertation. pp. 64+app.

WEI, R.-P. & LINDGREN, D. 1995: Optimal family contributions and a linear approximation. *Theoretical Population Biology* **48**:318–332.

WHITE, T. L. 1987: A conceptual framework for tree improvement programs. *New Forests* **4**:325–342.

WHITE, T. 1992: Advanced-generation breeding populations: size and structure. *In*: Proceedings of IUFRO conference S2.02.–08 Breeding tropical trees. Cartagena and Cali, Colombia, October 9–18, 1992, pp. 208–222.

WHITE, T. L., HODGE, G. R. & POWELL, G. L. 1993: An advanced-generation tree improvement plan for slash pine in southeastern United States. *Silvae Genetica* **42**(6): 359 –371.

WILHELMSSON, L. & ANDERSSON, B. 1995: Breeding programs in Sweden. 2. Breeding of Scots pine (*Pinus sylvestris*) and lodgepole pine (*Pinus contorta* ssp. *latifolia*). SkogForsk. *Arbetsrapport* **302**: 5–15.

WOOLLIAMS, J. A. 1998: A recipe for the design of breeding schemes. *In*: Proceedings of the 6th world congress on genetics applied to livestock production. Armidale , NSW, Australia, January 11–16, 1998, p. 427–430.

WRAY, N. R. & GODDARD, M. E. 1994: Increasing long-term response to selection. *Genet. Sel. Evol.* **26**:431–451.

© ARBORA PUBLISHERS

IV

Stratified sublining: a new option for structuring breeding populations

Seppo Ruotsalainen and Dag Lindgren

Abstract: When structuring a breeding population into sublines, the conventional approach is to assign parents to sublines randomly, so that each subline has approximately the same genetic value. By using deterministic infinitesimal model we study an alternative, stratified sublining system, where sublines are initially formed by positive assortative grouping of parents according to their breeding values. Stratified and random allocation to sublines are compared by evaluating the genetic quality of the seed orchards that each approach can provide. The seed orchards were established by selecting first the best individual from each subline and then a given best proportion from them. The greater among-subline variance in stratified sublining led to higher genetic gain in resulting seed orchards than did random sublining. For the case studied, stratified sublining gave considerably more genetic gain than random sublining, over 15% more, making it an interesting alternative that deserves further consideration and study.

Résumé : L'approche conventionnelle de structuration d'une population d'élevage en sous-populations consiste à assigner des parents aléatoirement dans chaque sous-population, de façon à ce que chaque sous-population soit génétiquement équivalente. À l'aide d'un modèle déterministe infinitésimal, les auteurs ont étudié une approche alternative de structuration stratifiée en sous-populations, où ces dernières sont initialement constituées en regroupant les parents par assortiment positif selon leurs aptitudes à la combinaison. Les approches d'assignation stratifiée et aléatoire sont comparées en évaluant la qualité génétique des vergers à graines établis selon chaque méthode. La composition des vergers à graines fut déterminée en sélectionnant en premier lieu le meilleur individu de chaque sous-population, suivi d'une proportion donnée des meilleurs individus issus de ces sous-populations. Plus la variance entre sous-populations résultant d'une assignation stratifiée était grande, plus le gain génétique était important au sein des vergers à graines résultants, comparativement à la méthode d'assignation aléatoire. En fonction du cas étudié, la méthode d'assignation stratifiée a produit un gain génétique considérablement supérieur, plus de 15%, à celui obtenu avec la méthode d'assignation aléatoire. Il s'agit là d'une alternative intéressante qui mérite qu'on s'y attarde et qu'on l'étudie davantage.

[Traduit par la Rédaction]

Introduction

In advanced-generation tree breeding, relatedness among trees in the breeding population inevitably increases. This can lead to growth losses in plantations, if the planted trees suffer from inbreeding depression (Lundkvist et al. 1987; Williams and Savolainen 1996). Selfing is not usually a serious problem, because higher homozygosity of recessive lethal genes leads to zygote mortality; however, this same mechanism does not protect as effectively against inbreeding caused by less related matings. This has the potential to become a serious problem in future seed orchards. Dividing the breeding population (sensu lato) into reproductively isolated sublines is a method to avoid detrimental effects of inbreeding (e.g., McKeand and Beineke 1980; van Buijtenen 1984; Zobel and Talbert 1984; White 1992). All matings in the breeding population are made strictly within sublines, so that the sublines remain unrelated. This leads to more rapid accumulation of inbreeding in the breeding population than if it was maintained as a single intermating group.

Seed orchards established with a single (top-ranking) genotype from any one subline will produce totally non-inbred seed (in the absence of selfing), even if the orchard parents are themselves highly inbred. The number of sublines in a breeding population may therefore depend on the number of unrelated clones desired for a seed orchard. The number of sublines suggested is typically between 15 and 30, but as few as two may suffice, if seed production is done with controlled pollination (Burdon 1986; White 1992). The size of sublines depends on the magnitude of inbreeding depression and the time horizon of the breeding plan. Usually sublines consist of 15–40 genotypes (Burdon et al. 1977; White 1992); however, there are also suggestions for using much smaller sublines, e.g., McKeand and Bridgwater (1998) propose four.

Sublining for forest tree breeding targeted for a certain ecophysiological region, or some other specific breeding objective, is often done by assigning selected trees randomly to sublines in the breeding population. The aim has been that the different sublines are genetically at about the same level. In fact, sometimes this has been included in the definition of sublines (Burdon and Namkoong 1983). If the division to groups is intended to lead to different genetic values of the groups, it can be regarded as being more a question of hierarchical breeding population structures (Cotterill et al. 1989;

Received June 7, 1999. Accepted November 25, 1999.

S. Ruotsalainen.[1] Forest Research Institute, Punkaharju Research Station, FIN-58450 Punkaharju, Finland.
D. Lindgren. Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden.

[1]Corresponding author. e-mail: seppo.ruotsalainen@metla.fi

© 2000 NRC Canada

Cramer and Kannenberg 1992; White et al. 1993), or more generally, a fine-tuning of the population hierarchy in breeding cycle.

Positive assortative mating (PAM) can be used to increase variation in the population and thus make selection more effective (Falconer and Mackay 1996; Rosvall 1999). If done with single-pair mating, it can also be regarded as dividing a population into sublines of size two according to the breeding value of the trees. However, in continued PAM the isolation of the sublines is broken in each generation. An interesting question is "how is genetic gain affected when PAM and sublining are combined, so that sublining is done according to the genetic value and the sublines are kept isolated for several generations?" In this study, we evaluate the consequences of stratifying a breeding population according to breeding values, so that the sublines represent different genetic levels. In our stratified sublining method, the sublines remain strictly unrelated to each other, their main purpose being to prevent inbreeding in multiplication populations, by providing unrelated seed orchard parents. This is an essential feature of sublines (Burdon and Namkoong 1983; White 1992).

The purpose of this study is to suggest a breeding strategy based on stratified sublining and to compare it with a conventional parallel sublining strategy with respect to the genetic value of the seed orchards supported by them.

Methods

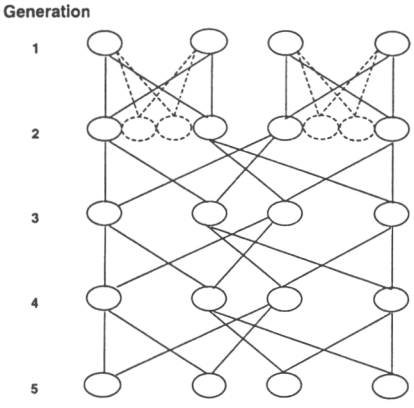
General description

The breeding population was presumed to be very large and the population structure to be regular, for analytical convenience in using a deterministic approach. A founder population of N unrelated, non-inbred genotypes with a normal distribution of breeding values was subdivided into $N/4$ sublines (i.e., each subline was composed of four founders). In stratified sublining, the assignment of founders to sublines was done according to ranking for breeding value, whereas in parallel sublining they were assigned to sublines randomly. Subsequent matings were made among individuals within sublines, and the sublines maintained at size four.

Generation turnover was by means of discrete breeding cycles (White 1987). In each cycle, the trees selected for crossing constitute the breeding population (BP). The offspring of the BP are referred to as the recruitment population (RP) from which the new BP is selected. Genetic advancement was assessed in terms of the genetic gain realized from hypothetical seed orchards, which are referred to as multiplication populations (MP). Conceptually, these definitions follow those of White (1987), although the names for populations are not exactly the same (i.e., recruitment population is used here instead of base population, and multiplication population for production population).

The MP was a clonal seed orchard, composed of $N/8$ clones representing the single best clone from each of $N/8$ best sublines (thus, after the initial generations, half of the sublines will contribute their best member to the MP, the probability of the sublines being represented in the MP decreasing together with their ranking). To calculate genetic gain for the MP, we need to consider both within- and among-subline variances. It is assumed that the founders (generation 1) are in genetic equilibrium, in that the additive genetic variance of the plus trees is the same as in the "wild forest." This assumption seems reasonable if the heritability for initial plus-tree selection is low and the wild forest homogenous.

Fig. 1. Balanced double first-cousin mating scheme used to reproduce the sublines. In the second generation, rejected RP members are shown by broken lines; in later generations, only those selected to BP are shown.



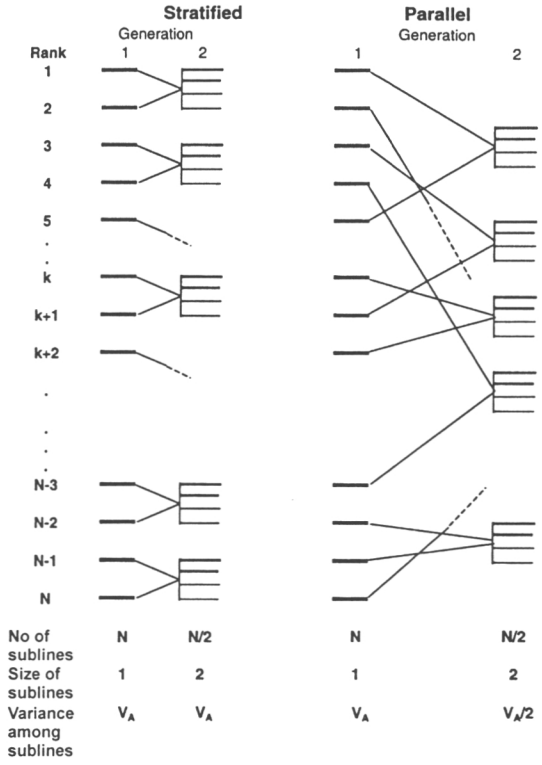
Constructing the sublines

The sublines of four trees were assembled over two generations. In the first generation, the founders were single-pair mated to produce families of size four (= subline size in the second-generation RP). The two best sibs (= subline in BP) from each family were selected and mated to two similarly selected full sibs from another family, giving two pair crosses with a total of eight individuals for a subline in the third generation RP. After repeating the selection process, the BP was composed of sublines of size four. The matings in later generations were made solely between the two families within the subline, so that the members of the two families were related to each other as double first cousins (Fig. 1) (cf. Wright 1921). This means that the subline size was stable from generation three on, alternating in size between eight in the RP phase and four in the BP phase. At the whole population level, this means alternation between $2N$ and N in size, respectively. All individuals in the same generation had the same inbreeding and pattern of relatedness. This made the development of coancestry within sublines balanced so that the problem could be formulated algebraically. The balance, symmetry, and simplicity of this breeding strategy seems appealing to us even if it may appear complicated at first sight.

The stratified sublines were formed in the following way. First, the founders were ranked for breeding value by error-free progeny testing. Founders with adjacent breeding values were then mated in a pairwise fashion to form the second generation (Fig. 2). The mating was thus done with PAM. The individuals within the full-sib families (= second-generation RP) were progeny tested in the same way as their parents. The results of the progeny testing were used to rank the sublines (equal to families in this generation) according to the average breeding value of the two top ranking members of the families (of the four tested). Then these two full-sibs were paired randomly with the similarly selected full-sibs of the family next in ranking to create two new full-sib families that were related as first cousins. For the parallel sublines, these two first-generation matings were done in random order (Fig. 2). In the following generations of both the stratified- and parallel-subline strategies, matings were made only between the members of the two families in a subline. Thus, the two sublining alternatives differed only by the method of pairing the parents in first and second generations. In later generations, after obtaining the final subline structure, the

© 2000 NRC Canada

Fig. 2. Schematic comparison of matings in creating stratified and parallel sublines. In the second generation the bold horizontal lines represent individuals selected to BP.



mating scheme was the same, as well as the selection phase in all generations.

There are three kinds of genetic relationships found within sublines: selfing, full sibs, and double first cousins (S, FS, and DC, respectively). The coancestries (f) of the respective relative types for generation t are

$$[1a] \quad f_{S(t)} = 0.5(1 + f_{DC(t-1)})$$

$$[1b] \quad f_{FS(t)} = 0.5(f_{S(t-1)} + f_{DC(t-1)})$$

$$[1c] \quad f_{DC(t)} = 0.5(f_{FS(t-1)} + f_{DC(t-1)})$$

The group coancestry for a subline in generation t (Θ_t) is obtained as the average of all coancestries within the subline:

$$[2] \quad \Theta_t = \frac{f_{S(t)} + f_{FS(t)} + 2f_{DC(t)}}{4}$$

Variances

We studied the effect of selection on a single character controlled by many unlinked loci with small additive effects (infinitesimal model), and with no dominance or epistatic effects. The breeding values were assumed to be known, i.e., available from error-free progeny testing. (Our scenario considers small RP families, as would be the case if progeny testing were used.) The variance of the breeding values in the first generation was $V_A = \sigma_A^2$, and the breeding values of the N plus trees were normally distributed ($N[0, \sigma_A]$). When the trees were allocated to sublines according to their breeding values, the means of the resulting $N/2$ sublines were still distributed $N[0, \sigma_A]$. After random parallel sublining, the distribution of the subline means was $N[0, \sigma_A/\sqrt{2}]$ (Fig. 2). The long-term effect of subdividing a breeding population is that sublines will drift apart because of sampling error and inbreeding will accumulate faster than in an undivided population. In the first generations when sublines are constructed the method of sublining affects the drifting process. This difference in among sublines variance between stratified and parallel sublining generated during the initial generations is the source for difference in genetic gain in multiplication populations derived from the studied sublining alternatives.

The variance of breeding values within a full-sib family in the RP in generation t is (following Falconer and Mackay 1996):

$$[3] \quad V_{WF(t)RP} = 0.5[1 - 0.5(F_A + F_B)]V_A = 0.5(1 - F_P)V_A$$

where F_A and F_B are inbreeding coefficients of the two parents ($F_A = F_B = F_P$). In the first three generations the inbreeding is zero, so that eq. 3 reduces to $V_{WF(t)RP} = V_A/2$ for these generations.

In the second generation, the within-family variance is, at the same time, the within-subline variance. In the third generation, the subline structure is complete. A subline is then composed of two families (each initially having four individuals, of which two are selected and bred) that are represented equally in the BP of the following generation. Thus, while the variance between these two families does not affect the variance among sublines in the next generation, it does influence the selection of the very best individual in the line, used as a parent in the seed orchard.

Within-subline variance ($V_{WS(t)RP}$) can be approximated as follows. Two individuals, one from each of the two families in a subline, are chosen to be the parents of a family, which consists of four offspring. The subline consists of two such families. The "true" family mean is the mean of the breeding values of its parents, and the variance within families around the true value is $V_{WF(t)RP}$. The genetic sampling variance of the sum of the two parent pairs, which will form the new families, is $2V_{2:4}V_{WF(t-1)RP}$. The entity $V_{2:4}$ is the variance of the two best values (individuals) from four normally distributed ones; for more details see later. The breeding value of the family will be the midparent breeding value, and thus the genetic sampling variance of the family mean is one quarter of the variance of the sum ($= V_{2:4}V_{WF(t-1)RP}/2$). This is the variance around the expected value of the subline for one family, but we are interested in the expected variance around the true expected value of the pair of families. This is half as large, thus between-family variance ($V_{BF(t)RP}$) is $V_{2:4}V_{WF(t-1)RP}/4$. Note that it does not matter how different are the families from which the parents were recruited, as both parent pairs are represented by one from each family. Finally, the within-subline variance is obtained as the sum of the within- and between-family variances

$$[4] \quad V_{WS(t)RP} = V_{WF(t)RP} + V_{BF(t)RP} = V_{WF(t)RP} + \frac{V_{2:4}V_{WF(t-1)RP}}{4}$$

Quite naturally, in the first two generations in both the stratified and parallel subline strategies, the question about between-family variance is irrelevant, as there is only one family within a subline.

The variance of the subline means in generation t is composed of two parts: the variance in the previous generation BP ($V_{BS(t-1)BP}$), and the new variance caused by the sampling process. The equations

© 2000 NRC Canada

Table 1. Values for the three components used in calculating among-subline variance (eqs. 5*a* and 5*b*) for breeding (BP) and multiplication populations (MP) in different generations.

	Random sublining factor (r)		Sampling variance of next generation (V_S)		No. of selected families in a subline (m)	
Generation	Stratified[a]	Parallel[b]	Stratified	Parallel	Stratified	Parallel
BP						
2	1	0.5	$V_{2;4}$	$V_{2;4}$	1	1
3	1	0.5	$V_{2;4}$	$V_{2;4}$	2	2
4+	1	1	$V_{2;4}$	$V_{2;4}$	2	2
MP						
2	1	0.5	$V_{1;4}$	$V_{1;4}$	—[c]	—
3	1	0.5	$V_{1;8}$	$V_{1;8}$	—	—
4+	1	1	$V_{1;8}$	$V_{1;8}$	—	—

[a]Stratified sublining for BP.
[b]Parallel sublining for BP.
[c]Not a relevant parameter.

for the variances are of the same general form both for the BP and the candidates for the MP, but they differ in some details. The variance of subline means in BP ($V_{BS(t)BP}$) is

$$[5a] \quad V_{BS(t)BP} = rV_{BS(t-1)BP} + \frac{V_S V_{WF(t)RP}}{m}$$

where r is a random sublining factor, V_S is the variance caused by sampling the next generation, and m is the number of families selected in a subline (Table 1).

Correspondingly, the variance among the best individuals in each subline (among the candidates for seed orchard; $V_{BS(t)MP}$) is

$$[5b] \quad V_{BS(t)MP} = rV_{BS(t-1)BP} + V_S V_{WS(t)RP}$$

It contains also the variance from previous generation BP, but the within-subline variance is added instead of the within-family variance, because selection is in this case done within the subline. The construction and the rationale of eqs. 5*a* and 5*b* are described in detail in the following. For stratified sublines, the first part of the variance in eq. 5 is the same as the total among-subline variance in the previous generation (r = 1). On the other hand, with parallel sublines, the random pairing of plus trees and families reduces the corresponding variance to half of that in the previous generation in generations two and three (r = 0.5) (Table 1, Fig. 2). When creating the sublines in generation four and later, there was no random pairing, but matings were solely within sublines. Thus, even in the BP with parallel sublines, the variation among sublines was kept intact and there was no more reduction from the variance of the previous generation (r = 1).

The increase in variance of family and thence of subline means caused by random variation in the mean of the selected trees (V_S) was obtained using tabulated values for variances (V_i) and covariances (Cov_{ij}) of $N[0,1]$ distributed normal-order statistics (Pearson and Hartley 1972, Table 10). The variance of the expected mean of the selected number (n) of highest values (referred by i and j; $i = 1 \ldots n$, $j = 1 \ldots n$) from a total of N values sampled at random from a normal distribution is according to the rules of linear transformations of variances:

$$[6] \quad V_{S(\overline{n;N})} = \frac{\sum_{i}^{n} V_i + 2 \sum_{i,j,i \neq j}^{n} \mathrm{Cov}_{ij}}{n^2}$$

In eq. 6, V_i is variance of the ith normal-order statistics of a sample of size N and Cov_{ij} correspondingly the covariance between ith and jth normal-order statistics.

V_S was used as a factor to scale the within-family variance ($V_{WF(t)RP}$) according to the number of selected trees and total number of trees in the group. In selecting trees for next generation BP, two trees were always selected from four in each family irrespective of the sublining alternative or generation. Thus, V_S always has the same value (Table 1) ($V_{2;4}$ = 0.3358). On the other hand, when trees were selected to MP, there were differences from selecting for BP and between generations in the MP. For the MP, trees were only selected among the trees which were the best in their subline, and among those trees only $N/8$ were actually selected. Because the subline size in the RP was four in generation two and eight in following generations, the corresponding factors were $V_{1;4}$ = 0.4917 and $V_{1;8}$ = 0.3729.

When the selections for second-generation BPs were made, there was only one full-sib family in each subline. In following generations, there were always two full-sib families within a subline and two individuals were selected from each family. Thus, the mean of the subline is the mean of two families and, correspondingly, the variances must be divided by two (m; Table 1). No reduction factor was needed for the MP (eq. 5*b*) where only one selection was made from each of the best sublines.

The general eq. 5 for among-subline variance described a specific population structure, depending on the sublining alternative, generation and population type. In all, eight different formulations were needed to cover all situations. These were constructed by inserting the proper values of the three factors given in Table 1. Some examples are presented below to clarify the calculation method.

The variance among the subline means of the second-generation BP, under the stratified subline alternative, is

$$V_{BS2BP} = V_{BS1BP} + V_{\overline{2;4}} V_{WF2RP}$$

and in the parallel case:

$$V_{BS2BP} = 0.5V_{BS1BP} + V_{\overline{2;4}} V_{WF2RP}$$

The difference between the equations is caused by the pairing method of the parents for second generation. Because sublines in the second generation consist only of one family of size four, from which two trees are selected, factor m has a value of one.

© 2000 NRC Canada

Table 2. Number and size of sublines, relative effective population sizes, and selection intensities applied to generate the hypothetical seed orchards (MP).

Generation	No. of sublines	Subline size	Relative effective subline number (N_r)	Selection intensity within sublines for MP candidates (i_w)	Selection intensity among sublines (i_b) for MP	
					Linear[a]	Equal[a]
1	N	1	0.125	0	1.7620	1.6468
2	$N/2$	2	0.25	1.0294	1.3984	1.2711
3+	$N/4$	4	0.5	1.4236	0.9407	0.7979

[a]Method used to allocate material to seed orchards.

A formulation for among-subline variance in the BP, which could be used in the stratified case from generation three onwards and in the parallel case from fourth generation, is

$$V_{BS(t)BP} = V_{BS(t-1)BP} + \frac{V_{\overline{2:4}}V_{WF(t)RP}}{2}$$

A difference in the variance among sublines in the third and subsequent generation BPs compared with the second generation was caused by the fact that sublines then each consisted of two families, and selections were made from both of them (thus, m = 2). Because there was no random mating among sublines in the parallel case when creating generations four and onwards, the variance from previous generation was not reduced (r = 1). The corresponding formulation for variance among the best individuals in sublines (when selecting the best from the RP subline of size eight for the MP) is

$$V_{BS(t)MP} = V_{BS(t-1)BP} + V_{\overline{1:8}}V_{WS(t)RP}$$

In this case, the sampling variance was different, because one tree was selected from eight in a subline. Further, because only the best individual in a subline was selected, the within-subline variance must be used, rather than the variance within a family.

Gain

The genetic gain in the BP can be calculated using the familiar equation $G = ih\sigma_A$, where i is the selection intensity, and h is the square root of heritability. The two best trees were selected from the four full sibs in a family ($i_{2:4}$ = 0.6632), based on breeding value. Since progeny testing was assumed to be error free, heritability is unity and can be dropped from the equation. Selection was done within families, so the variance needed in the calculation is the within-family variance ($V_{WF(t)RP}$). Selection was done in same way in all families, so that the breeding value was increased in the whole BP by the same amount as in one family. The genetic improvement in the BP is a continuous process, where the gain in the current generation is added to that accumulated previously. So the genetic value of a given generation BP ($G_{BP(t)}$) was obtained as

$$[7] \qquad G_{BP(t)} = G_{BP(t-1)} + i_{2:4}\sqrt{V_{WF(t)RP}}$$

The merits of the two breeding strategies were assessed by comparing the breeding values of their respective hypothetical seed orchards. The seed orchard had $N/8$ clones, so that the best individual from only part of the sublines was established as an orchard parent. Depending on the number of sublines, this leads to different relative effective subline number in the seed orchard over time (Table 2). In the first generation, when there were not yet any sublines assigned (or sublines were of size one) the $N/8$ highest ranking clones were selected for the seed orchard, leading to a relative effective subline number, defined here as $N_r = (N/8)/N$ = 0.125. When the sublines were established, half of them had to be used to get the desired number of clones for the seed orchard, thus giving $N_r = (N/8)/(N/4)$ = 0.5.

The genetic gain of the seed orchard ($G_{MP(t)}$) was a sum of three terms: the accumulated average gain in the BP by selection in previous generations; the expected gain by selecting the best in each subline as a seed orchard candidate, and the gain by selecting the best among the candidates to serve as seed orchard parents.

$$[8] \qquad G_{MP(t)} = G_{BP(t-1)} + i_w\sqrt{V_{WS(t)RP}} + i_b\sqrt{V_{BS(t)MP}}$$

where i_w is the selection intensity within subline and i_b is the selection intensity among sublines.

The selected clones were used in the seed orchards in two different ways. The first alternative was to use all clones in equal amounts (truncation selection). However, a more effective way to utilize clones in seed orchards is to let them be represented at frequencies relative to their breeding values (linear deployment; Lindgren et al. 1989; Lindgren 1991). These two alternatives could be described by the same equation, the only difference being that the selection intensity for among sublines (i_b) was higher in linear deployment (Table 2). The selection intensities for linear deployment were obtained with program SELENOR3 (developed by D. Lindgren) which is available at http://www.genfys.slu.se/staff/dagl/GeneticCalc/sele/.

Results

The group coancestry within a subline (in this case, the four founders or their descendants were considered as a subline also in generations 1 and 2) increased steadily through generations starting from 0.125 at the first generation (Table 3). Status number of the subline decreased rapidly from the original four, so that already in the third generation it was two and in the eighth generation slightly below one. Most of this decline is a reflection of the unavoidable accumulation of relatedness over time, even if this is kept at a low level when the number of progeny per parent is equal for all parents. Because selfing and sib mating were not allowed, the inbreeding coefficient of the trees used as parents was zero during the first three generations. Subsequently, however, it increased more rapidly than the group coancestry.

The variance among sublines increased in each generation in the BP with stratified sublines (Fig. 3). In the BP with parallel sublines, the variance among sublines decreased during the first two breeding cycles. In the third generation, the variance among the parallel sublines was only slightly above 50% of that in the founder generation. After third generation, the development of among-subline variance in the parallel sublines followed the same course as the variance among stratified sublines, but at a lower level (Fig. 3).

Within-family variance, which behaved the same way in both sublining alternatives, was half the additive variance in

© 2000 NRC Canada

Table 3. The development over generations of status number, group coancestry, inbreeding, and coancestry among different members of the breeding population in a subline of size four, which is propagated using the double first cousin mating system.

Generation	Coancestries				Status No. (N_S)	Inbreeding (F)
	Self	Full-sib	Cousins	Group		
1	0.5	—	—	0.125	4	0
2	0.5	0.25	—	0.1875	2.6667	0
3	0.5	0.25	0.125	0.25	2	0
4	0.5625	0.3125	0.1875	0.3125	1.6	0.125
5	0.5938	0.3750	0.25	0.3672	1.3617	0.1875
6	0.6250	0.4219	0.3125	0.4180	1.1963	0.25
7	0.6562	0.4688	0.3672	0.4648	1.0756	0.3125
8	0.6836	0.5117	0.4180	0.5078	0.9846	0.3672
9	0.7090	0.5508	0.4648	0.5474	0.9135	0.4180
10	0.7324	0.5869	0.5078	0.5837	0.8565	0.4648

Fig. 3. Expected variances among sublines and within families and sublines over successive generations.

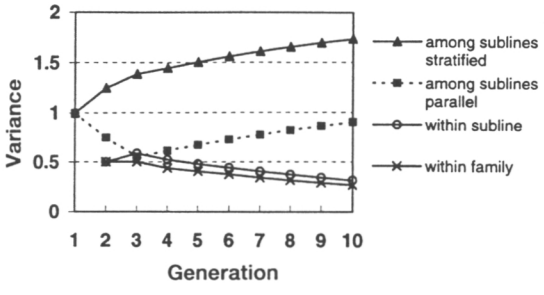
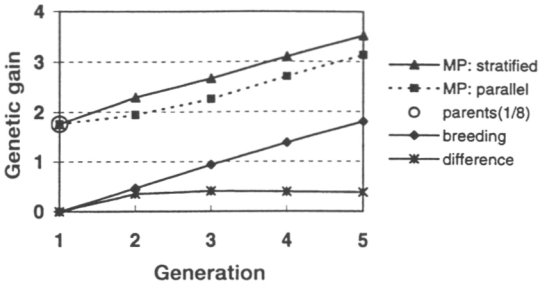
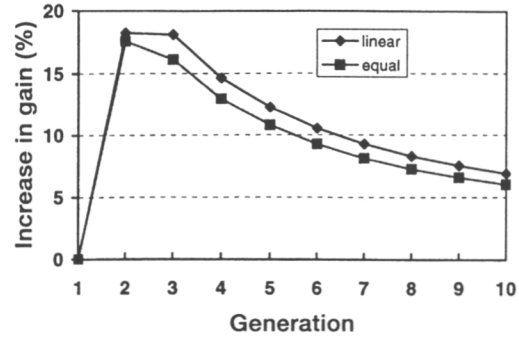


Fig. 4. Development of genetic gain through generations in the multiplication population of stratified and parallel sublining. Linear deployment was used. Genetic gain in the breeding population and the difference between stratified and parallel sublining are also shown.



the founder population in generations 2 and 3. When the parents became inbred, the within-family variance decreased (Fig. 3). Within-subline variance was slightly greater than within-family variance in generation three onwards. In the second generation, within-subline variance was equal to within-family variance (Fig. 3), because in that generation a subline consisted of a single family.

Fig. 5. Superiority of stratified over parallel sublining in terms of percent genetic gain through generations with equal and linear deployment in the multiplication population.



The efficiency of the sublining alternatives was compared in terms of the genetic value of the MPs which could be derived from them. The increase in genetic gain obtained by stratified sublining arose mainly in the second generation (Fig. 4). Genetic gain in the BP was not affected by the method of sublining.

The genetic gain was slightly lower with equal deployment than with linear deployment (Fig. 5), but otherwise, the development of genetic gain was the same in both methods of deploying the material. The increase obtained by stratified sublining was greater when clones were used in the seed orchard in proportions related linearly to their breeding value.

The maximum difference between stratified and parallel sublining was in third generation, when it was $0.35\sigma_A$ for equal deployment and $0.41\sigma_A$ for linear deployment. On an absolute scale, the superiority of stratified sublining remained at almost the same level for many generations. After 10 generations the difference was $0.29\sigma_A$ and $0.35\sigma_A$ for the equal and linear deployment, respectively. As the general genetic level increased in each generation, the superiority of stratified sublining in relative terms decreased each generation from a maximum in second generation (Fig. 5). The greatest relative increase in genetic gain obtained by stratified sublining was 18% for both methods of deployment. In

© 2000 NRC Canada

spite of a decrease in relative genetic gain over time, still at the tenth generation stratified sublining gave 6–7% greater genetic gain than parallel sublining.

Discussion

This study indicates that genetic gain from deployed material can be increased considerably (more than 15%) by a breeding program that uses stratified sublines instead of the more traditional method of parallel sublines. It can be argued that better systems can be found than the one used here as the basis for comparison. The strategy was, however, varied only in respect of the allocation of founders and second generation selections to sublines. The example thus seems well suited for an analysis of the possible advantages of the stratified sublines. One advantage in this respect is that it is simple. We do not claim that stratified sublining is the best breeding strategy, we just claim that it is worth analysing this strategy in more detail to learn from the principles that make it an interesting strategy.

The method of sublining does not affect the mean genetic value of the BP, but only that of the MPs derived from it. The greatest relative increase in MP gain was obtained in the second generation. Thereafter, the relative gain decreased because the absolute level of genetic gain increased, although the absolute superiority of stratified sublining method remained almost unchanged. The increase in genetic gain was roughly in the order of the genetic gain obtained in one generation. So, by using stratified sublining, it is possible to reach a given genetic level one generation sooner than by using conventional parallel sublining.

The superiority of stratified sublining results from the different behaviour of among-subline variance in the two sublining alternatives. In the two first generations, the among-subline variance decreased under parallel sublining, whereas it increased in stratified sublining. The variance among sublines of constant size is generally expected to rise between generations in small populations because of genetic drift, and this rise is expected to be the same for both stratified and parallel sublines. Thus, the difference in among-subline variance obtained in the first generations was maintained in all future generations, and was the reason for the difference in genetic gain between the two methods. The greater among-subline variance under stratified sublining made it possible to obtain greater genetic gain when only a selected portion of sublines was utilized in establishing seed orchards. We used an infinite population size in our study, but the results are probably applicable to all commonly used BP sizes.

The observed increase in genetic gain is related to the effect of PAM. In the second generation, it is in fact the same. However, in later generations, stratified sublines differed from continued PAM, because the isolation was maintained, and the ranking of individuals did not affect the mate selection once the sublines were fixed. Stratified sublining is likely to give a lower increase in theoretical genetic gain than might be obtained by continued PAM. On the other hand, the individuals selected for MP are totally unrelated, which cannot be achieved in PAM. In a simulation study comparing PAM to random mating, Shepherd and Kinghorn (1994) obtained an increase of 24% in genetic gain, which is comparable with our results.

The lack of gene flow among the sublines is a feature that distinguishes stratified sublining from many other breeding methods that use hierarchical population structures, e.g., nucleus breeding (as described by Cotterill et al. 1989), and hierarchical open-ended (HOPE) options (Cramer and Kannenberg 1992). Stratified sublining, however, can be questioned on the grounds that it does not utilize genetic material from the lower ranking sublines. Random accumulation of good genes can give an opportunity even for individuals from a lower ranking subline to enter the MP. However, because the sublines are genetically isolated from each other, a large proportion of the lower ranking sublines will probably, in practice, contribute little to production plantations and have no chance to develop into a higher ranking subline.

It could be argued that the lower ranking sublines should be totally discarded from breeding population, to make more efficient use of resources with practically no loss in genetic gain. However, the essence of breeding is to create options that may never be utilized; very few of the individuals a breeder creates will ever transmit their genes to production plantations. Most breeding programs carry more genetic material than may be needed from a strict production point of view. Descendants from many founders are kept just in case these founder genes might later be needed. Balanced within-family selection is thought to maximise the long-term gain even though that means some selection from the most inferior families (Dempfle 1975). The reason to keep some of the inferior lines is similar.

The requirements for numbers and diversity in future multiplication populations are uncertain. The stratified sublining system gives orchard managers options to get higher gain than would otherwise be possible if future demands are for small MPs with low relatedness. At the same time, options are retained to meet higher demands on unrelated MPs without losing any gain (compared to if the material was arranged in parallel lines).

There is uncertainty concerning what numbers are required to support long-term breeding. Breeders want to have sufficient census numbers in breeding populations, because inbreeding and relatedness may otherwise cause problems. Future knowledge may suggest that population sizes can be reduced, and if so, the gain will be higher if the best genotypes have not been arbitrarily mixed with lower ranking ones. On the other hand, if problems with inbreeding and relatedness in the best material become limiting, there is still improved material available in the lower ranking sublines to overcome the problems.

Noting that the greatest advantage of stratified sublining was obtained in the earliest generations, it is likely that, in later generations, the breeding population can be restructured in a way that facilitates use of more material from the currently low-ranking sublines in the MP. For example, new sublines could later be formed, using the very best material of the low-ranking lines. Stratified sublining could thus be seen, in the long term, as the first phase of a HOPE strategy (Cramer and Kannenberg 1992). The distinction from HOPE is that the hierarchic tiers are identically managed and reproductively isolated. Breeders would perhaps find it

© 2000 NRC Canada

worthwhile to relax these specifications after about four generations.

The breeding goal may change, and the low-ranking lines will contribute to the gene resource that can be used in such a case. They are as likely to contain previously unconsidered characters as the top-ranking lines, and they combine these characteristics with considerable improvement in the already considered characteristics.

It is an interesting possibility to breed the lower tiers among the stratified lines towards somewhat different goals.

Our model assumes equal within-family variance in both sublining alternatives, although theoretically it might be more correct to have in early generations slightly greater variance within families with parallel sublines than with stratified sublines. However, experimental results do not support this prediction from the infinitesimal model. Quite the contrary, the within-subline variation has been observed in many cases to increase (Williams and Savolainen 1996). On the other hand, increase of among-subline variation has been verified experimentally (Falconer and Mackay 1996); in this light it may be unimportant that the within-subline variance is slightly lower with stratified selection.

A heritability of one, thus a perfect genotype test, has been assumed in this study. Heritability as well as the correlation between the character measured and the goal character may matter. It is possible that the superiority of the stratified lines declines in situations with low heritability and low correlation.

Stratified sublining offers several possibilities to increase genetic gain even further. We suggest that the potentially most powerful refinement is to manage the sublines differently depending on their genetic value. When the best sublines are managed more intensively, the genetic gain obtained in the MP will be increased, with no increase in costs. For example, the best sublines could be based on the same number of founders, but the size of the RP and BP could be increased, so that the number of candidates and thus the seed orchard gain could be increased at the same time as the increment of inbreeding would be reduced. Also, the ratio of RP and BP sizes could be increased for the best lines increasing the gain from long-term breeding. With parallel sublines, more resources must be spent to get the same effect, as there are no good lines to concentrate efforts on; however, a part of the added resources would go to lines that are very unlikely to contribute to the seed orchard. This modification would make the strategy more similar to HOPE. Other subline sizes could also be studied, e.g., the quadruple second-cousin system suggested by Wright (1921), which leads to sublines of size eight with balanced inbreeding structure. This might lead to larger difference between stratified and parallel sublining, because larger sublines involve more generations with random pairing in parallel sublining, and thus a greater number of early generations when among-subline variance is decreased.

It must be noted that the effect of stratified sublining depends on the relative effective subline number (N_r). In our study we used after the initial generations half of the sublines in multiplication population ($N_r = 0.5$), but the superiority of stratified sublining would be increased by using less of the top-ranking sublines for MP. On the other hand, if more sublines were used for MP, the effect of stratifying would decrease, until the both sublining alternatives would give same genetic gain when all sublines are in use ($N_r = 1$).

This study has demonstrated the potential advantages offered by stratified sublining. We used a deterministic approach which forced us to emphasize simplicity. We suggest that a larger study should also employ stochastic simulations. There are approximations in this study, and some interesting cases and comparisons could not be addressed because the difficulty of handling them with the analytical equations entailed in the deterministic approach. The stratified subline concept could be studied over a wide range of conditions, and compared with alternatives other than parallel sublines. Future studies could include, for instance, different effective MP sizes and subline sizes, variable effective subline numbers, variable levels of culling of the founder material, acceptance of some relatives to the MP, and variable management intensity on different strata of the genetic material.

Acknowledgements

We acknowledge financial support from Philip Sörenssons Stiftelse and Nordic Academy for Advanced Study (NorFa) for this study. The language was revised and suggestions made on the manuscript by Tim Mullin, whose help we appreciate greatly. We also thank the two referees, of whom especially Rowland Burdon's comments were valuable in improving the manuscript.

References

Burdon, R.D. 1986. Clonal forestry and breeding strategies—a perspective. *In* Conference Proceedings: A Joint Meeting on IUFRO WP on Breeding Theory, Progeny Testing and Seed Orchards, 13–17 Oct. 1986, Williamsburg, Va. IUFRO, Vienna. pp.645–659.

Burdon, R.D., and Namkoong, G. 1983. Short note: multiple populations and sublines. Silvae Genet. **32**: 221–222.

Burdon, R.D., Shelbourne, C.J.A., and Wilcox, M.D. 1977. Advanced selection strategies. *In* Proceedings of Third World Consultation on Forest Tree Breeding, 21–26 Mar. 1977, Canberra, Australia. FAO, Rome, and IUFRO, Vienna. pp. 1134–1147.

Cotterill, P.P., Dean, C., Cameron, J., and Brindbergs, M. 1989. Nucleus breeding: a new strategy for rapid improvement under clonal forestry. *In* Proceedings of IUFRO Conference: Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry, Nov. 1988, Pattaya, Thailand. *Edited by* G.I. Gibson, A.R. Griffin, and A.C. Matheson. Oxford Forestry Institute, Oxford, U.K., and Winrock International, Arlington, Va. pp. 39–51.

Cramer, M.M., and Kannenberg, L.W. 1992. Five years of HOPE: the hierarchical open-ended corn breeding system. Crop Sci. **32**: 1163–1171.

Dempfle, L. 1975. A note on increasing the limit of selection through selection within families. Genet. Res. **24**: 127–135.

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to quantitative genetics. 4th ed. Longman Group, London and New York.

Lindgren, D. 1991. Optimal utilization of genetic resources. For. Tree Improv. **23**: 49–67.

Lindgren, D., Libby, W.S., and Bondesson, F.L. 1989. Deployment to plantations of numbers and proportions of clones with special emphasis on maximizing gain at a constant diversity. Theor. Appl. Genet. **77**: 825–831.

© 2000 NRC Canada

Lundkvist, K., Eriksson, G., Norell, L., and Ekberg, I. 1987. Inbreeding depression in two field trials of young *Pinus sylvestris* (L.). Scand. J. For. Res. **2**: 281–290.

McKeand, E., and Beineke, F. 1980. Sublining for half-sib breeding populations of forest trees. Silvae Genet. **29**: 14–17.

McKeand, S.E., and Bridgwater, F. 1998. A strategy for the third breeding cycle of loblolly pine in the southeastern U.S. Silvae Genet. **47**: 223–234.

Pearson, E.S., and Hartley, H.O. (*Editors*). 1972. Biometrical tables for statisticians. Vol. 2. Cambridge University Press, Cambridge, U.K.

Rosvall, O. 1999. Enhancing gain from long-term forest tree breeding while conserving genetic diversity. Acta Univ. Agric. Suec. Silvestria No. 109.

Shepherd, R.K., and Kinghorn, B.P. 1994. A deterministic multitier model of assortative mating following selection. Genet. Sel. Evol. **26**: 495–516.

van Buijtenen, J.P. 1984. Strategies for maintaining the genetic base. How will advanced generation tests differ from first? *In* Proceedings of Servicewide Genetics Workshop: Progeny Testing, 5–9 Dec. 1983, Charleston, S.C. USDA Forest Service, Washington, D.C. pp. 566–572.

White, T.L. 1987. A conceptual framework for tree improvement programs. New For. **4**: 325–342.

White, T. 1992. Advanced generation breeding populations: size and structure. *In* Resolving Tropical Forest Resource Concerns Through Tree Improvement, Gene Conservation and Domestication of New Species. Proceedings of IUFRO Conference S2.02–08, Breeding Tropical Trees, 9–18 Oct. 1992, Cartagena and Cali, Colombia. IUFRO, Vienna. pp. 208–222.

White, T.L., Hodge, G.R., and Powell, G.L. 1993. An advanced-generation tree improvement plan for slash pine in southeastern United States. Silvae Genet. **42**: 359–371.

Williams, C.G., and Savolainen, O. 1996. Inbreeding depression in conifers: implications for breeding strategy. For. Sci. **42**: 102–117.

Wright, S. 1921. Systems of mating. II. The effects of inbreeding on the genetic composition of a population. Genetics, **6**: 124–143.

Zobel, B.J., and Talbert, J. 1984. Applied forest tree improvement. John Wiley & Sons, New York.

© 2000 NRC Canada

ISBN 951-40-1860-5
ISSN 0358-4283
Hakapaino Oy 2002