

A close-up photograph of a pine branch, showing a central stem with numerous long, thin, green needles radiating outwards. The needles are vibrant green, with some showing slight yellowing or browning at the tips. The background is a soft, out-of-focus greyish-blue. The text 'Best Practice for Tree Breeding in Europe' is overlaid in white, bold, sans-serif font on the right side of the image.

# Best Practice for Tree Breeding in Europe

This research has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreement n° 211868 (Project Noveltree)

**BEST PRACTICE FOR  
TREE BREEDING  
IN EUROPE**  
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**PRINT**  
**GÄVLE OFFSET, 2013**  
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ISBN: 978-91-977649-6-4

# Preface

From 2007 to 2013, the European Community supported research under its Seventh Framework Programme (FP7/2007-2013) to elevate the state-of-the-art through novel approaches to tree breeding in Europe, under the grant agreement n° 211868, Project NovelTree. NovelTree brought together an interdisciplinary group of 15 public and private partners from 7 European countries with expertise in quantitative genetics and breeding, population genetics, genomics, pathology, tree physiology, forestry and economics. The ultimate goal of the project that kicked off in June 2008 was to enable significant genetic improvement of tree characteristics and forest products properties to satisfy the needs (quality, quantity, sustainability, vulnerability) of the forest-based sector and consumers while ensuring optimal long-term management of genetic resources.

Partners conducted collaborative research in several areas. Work Package 3 of Project NovelTree focussed on design and implementation of improved/novel tree breeding strategies. Research was conducted on development of new computer software tools, evaluating novel approaches to breeding, and documenting best practices for tree breeding in Europe. In this publication, we present what we believe are innovative approaches to tree breeding in general, and for a selection of model species that have particular importance in Europe.

Tim Mullin

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# Introduction to breeding strategies and evaluation of alternatives

1

Ola Rosvall and Tim Mullin

## Introduction

The climate and growth conditions of Europe vary from Mediterranean to boreal and include lowland as well as alpine areas. The natural forests comprise a variety of tree species, but the extent of forest cover and tree species composition has been much changed by human activities such as agriculture and tree harvesting, all since prehistoric time following the last glaciation. A variety of forestry systems have developed to satisfy human needs depending on the extent and composition of the natural forest resource, but also in many instances systems based on introduced tree species.

European forestry includes low intensity as well as intensive forest management. In addition to the choice of tree species and adapting silviculture methods, intensive forestry most often uses tree improvement to further adapt trees to environmental conditions as well as to develop economically important tree characteristics. Although forestry has always been a long-term endeavour, globalisation in terms of climate change and worldwide markets has generated concern about the adaptation of trees and forests, adding even more uncertainty over future conditions and needs. Since it is often suggested that a sustainable society has to rely on renewable resources, there is an expectation that forestry increase the supply of bioenergy, biomaterials and biochemicals. In that perspective, tree breeding will likely become even more important.

This introductory chapter on best practices for breeding strategies starts with a broad outline of tree breeding to show the space wherein any particular programme might find its place depending on tree species characteristics and breeding objectives. Since the main emphasis is on intensive breeding for plantation forests to supply wood-based industries, we focus in the second part on an evaluation of six alternative strategies representing intensive tree breeding. We demonstrate how comparisons can be made among the species-specific programmes detailed in the chapters that follow.

## Objectives of a breeding programme

The term ‘breeding strategy’ as used here relates to a plan for managing populations to meet the goals of a breeding programme. The goals set for breeding can be exceedingly diverse and can vary from simple to complex, for example:

### 1. Economic traits

Quite commonly traits such as productivity (growth, survival, resistance to pests and disease) or quality (stem shape, wood characteristics) are under some level of genetic control and respond to breeding and selection. While a programme may identify a principal target trait for improvement, it is probably more common that several objective traits with differing economic values and often adverse genetic correlations must be considered simultaneously.

### 2. Adaptability

Improvement is not always measured in terms of population mean for objective traits, but also in the resilience of tree populations under changing conditions, such as might occur under climate change or other environmental modification. Adaptability is under strong genetic control and often expressed as phenotypic plasticity and the level of genetic diversity exposed to genetic processes such as selection, migration and genetic drift.

### 3. Gene conservation

As forest crop species are usually long-lived and at a very early stage of domestication, there is commonly a desire to conserve the genetic diversity represented in existing populations, on which selection, be it natural or artificial, can act as fitness and economic factors change. Most programmes will have a stated objective for gene conservation, which may vary from maintenance of natural levels of diversity within or outside the main breeding population, to identification and preservation of rare alleles.

## Decision factors: considerations and constraints

While the specific conditions facing any given programme will vary, they can generally be considered under four headings: (1) Biological; (2) Economic; (3) Institutional; and (4) Socio-political. Many of these factors will impose serious constraints on what can be accomplished and its degree of difficulty. An effective breeding strategy is not possible without a good understanding and realistic assessment of these factors.

### 1. *Biological factors*

The biology of the target species and the ecological conditions under which it is deployed dictate much of what is possible and what constraints limit the methods that can be employed. Many boreal species have large natural distributions and there exists tremendous genetic variation available to the breeder, although the structure of this variation and the nature of genotype-environment interactions must first be understood. Other species in Europe have much smaller distributions that may have been already affected by human activity, leaving a more limited pool of genetic diversity. While trees in general are long-lived organisms, rotation lengths can still vary from as few as 10 years, to over 100. This has an obvious impact on testing times to obtain valid phenotypic data, as well as the length of the breeding cycle. Mating to generate a recruitment population requires male and female reproductive structures in sufficient numbers to complete crossing and produce sufficient seed for testing. The potential for vegetative propagation will create opportunities for clonal testing and deployment, but this varies tremendously among species. Improvement of traits requires sufficient heritability for accurate selection, a function of the genetic variance for the trait and the test environment where it is expressed. Understanding parameters such as heritability and genetic correlations can help ensure that testing and selection operations are effective.

### 2. *Economic factors*

Very rarely does the breeder target a single objective trait. More often, numerous traits are identified that contribute value, even if some of these have adverse genetic correlations. Maximising profit from tree improvement requires realistic economic values for target traits so that they can be weighted appropriately in a selection index. The economic reality of tree breeding can only be known through a disciplined economic evaluation to determine likely return on investment. This evaluation needs to be revisited periodically in the light of the conditions-of-the-day, to ensure that the breeding programme still makes sense alongside other

silvicultural investments. This will help to secure a level of stability for operating funds that are required long-term to achieve breeding outcomes.

### 3. *Institutional factors*

The task of planning and implementing a tree breeding programme usually falls on a key institution, often in cooperation with other academic and government institutions and industry players. While there may be a will to share costs and benefits, the programme will require a core of expertise and technical resources. The level of expertise depends on the methodology employed but must be appropriate if results are to be achieved. Beyond expertise in tree breeding, a programme will also require expertise in many other areas, including nursery propagation, plantation management, molecular markers, wood technology, etc. Whether these resources are found within the main institution or among collaborators, the breeding programme must be realistic in what resources are available to it.

### 4. *Socio-political factors*

All forest management activities exist within the context of legislative and social expectations. Many European countries have legal requirements for maintenance of biodiversity, genetic diversity of deployed material, and use of technology. Even where specific legislation does not exist on these issues, there are often market sensitivities and public values that must be respected. In the broadest terms, everything done in a European country will affect the forest landscape of several neighbouring states, so that tree breeding must be sensitive to public and political interests at home and in neighbouring jurisdictions.

## General strategies for deployment, breeding, testing and selection

The development of a breeding plan usually starts with an assessment of the regeneration systems anticipated for the species. The gains from tree breeding can only be realised when bred material is deployed to the forest, be it by planting or direct seeding. Propagation of the material can be from seed, in which case some kind of orchard population will be required to produce seed in sufficient quantities. An orchard can be established with seedlings raised from selected parents, or when a species can be grafted, orchards established by grafting will allow greater selection intensity and gain. For wind-pollinated species, orchards are commonly managed for collection of open-pollinated seed, but options also exist to establish orchards for production of control-pollinated seed. For species that can be propagated vegetatively, planting material can be produced by cloning,

either by bulking up small quantities of high-value seed, or for deployment as tested clones. The latter may require an archive of donor material in nursery stool beds or as stored tissue cultures.

Once the deployment system(s) is determined, the next task is assembly of a breeding population (BP). In its simplest form, this can simply be a series of planted blocks of selected material, which are then regenerated by collecting and planting open-pollinated seed. With such a lowtech approach, breeding can achieve some level of gain at very low cost. Where a larger expenditure is warranted, a closed breeding population can be assembled, usually from plus-trees selected in natural population, and managed to produce recruitment populations for future selection. The number of populations required, their distribution and size is a matter for careful consideration. Since species distributions often cross political boundaries, collaborative approaches may be worthwhile, and indeed the only likely way that lower-value species can be managed in populations of sufficient size to achieve meaningful gains, while conserving diversity.

There are many valid structures for breeding populations, depending on the nature of the programme and the distribution of deployment areas. Boreal species that typically span broad climate or environmental zones are often stratified into multiple breeding populations as structures for breeding, testing and deployment. Populations can also be subdivided in other ways to manage diversity, inbreeding, or elite lines (nucleus breeding). Breeding within populations can be carried out in discrete cycles, or managed in a continuous “rolling front”. The breeding of hybrids will require additional population management structures, particularly if parents are to be improved as separate species, while deploying hybrids. All of these population management structures will probably be linked to seed source management tools that attempt to optimise the deployment of available germplasm across the planting area.

There are several ways that a breeding population can be managed to generate recruitment populations. These include low-tech approaches involving open-pollinated seed, as well as traditional control-pollinated breeding, where the complete pedigree is recorded and tracked. Polycross breeding can be used to maintain incomplete pedigree information, while achieving strong estimates of breeding values for female parents. For polycross and OP systems, molecular marker methods can be used to reconstruct the unknown parts of the pedigree and thus improve estimates of breeding value and manage accumulation of relatedness.

Once recruitment populations are formed, the progeny must be assessed in some way to infer information about their genetic values and those of their relatives. This can be

as simple as visual assessment of phenotype in family blocks, but can be improved by employing stronger statistical tools such as Best Linear Unbiased Prediction (BLUP) and experimental designs to improve the accuracy of estimated breeding values. Genetic marker information can also be incorporated into the BLUP (marker-assisted BLUP) or associated at a genome level with phenotypic performance (genome-wide selection).

Whatever system of breeding, testing and selection is adopted, an important feature of any successful breeding programme is the flexibility to make changes as new information and technologies become available. The genetics of tree breeding is covered in several textbooks, one of the more recent being that by White et al. (2007).

## Comparing alternative strategies

After evaluating the basis for a breeding programme in terms of programme objectives and the options available considering biological, economic, institutional and socio-political factors, there will usually be a variety of possible strategies and tactics that could be used to reach programme objectives. There is seldom a simple “best” choice.

Alternative strategies can be evaluated and compared by computer simulation. One of the software tools enhanced through the Noveltree project for European conditions is POPSIM™ (Mullin et al. 2010; Mullin and Park 1995). POPSIM is a stochastic model mimicking the sampling process of alleles that takes place over a generation. It is based on the proven genetic theory of the infinitesimal model and is equipped to analyse operational breeding activities over many generations. For in-depth studies of the effects and interactions of individual genes, the allelic model simulation software METAGENE has also been developed within the Noveltree project (Sanchez 2010). METAGENE is particularly useful for testing hypotheses concerning gene action and interactions, and as a useful complement for research, whereas POPSIM makes broad but realistic assumptions about the behaviour of quantitative traits under selection for a small number of breeding cycles.

When comparing strategies, economic and operational considerations in terms of costs and required time are as important as the genetic effects in terms of gain achieved and genetic diversity maintained. A fair comparison should therefore compare genetic gain per unit time at the same level of genetic diversity and annual use of resources. Conserving the same level of genetic diversity is achieved by constraining on the same loss of genetic diversity and variance, i.e., the same rate of increase in relatedness in terms of group coancestry (or inbreeding under random mating). Costs can be based on cost functions relating to each

individual or grouped operation like controlled crosses, field experiments, genetic evaluation, breeding archives to a start-up cost and a marginal cost per additional unit. For valid comparisons, it is not necessary to consider costs common to all alternatives; rather the task is simplified by constraining only the principal cost-driving component(s) of the breeding programme at the same level of intensity. Quite often, the majority of operational costs can be simply described by the annual numbers of trees planted in field tests and this is sufficient to characterise the difference in use of resources for the breeding programmes to be compared.

## Scots pine in Sweden as an example case

In this section, as a demonstration, a number of alternative mating and selection systems are described and evaluated in terms of time and resources used, potential genetic gain achieved and genetic diversity maintained. To present a realistic example of biological constraints such as age-to-flowering, test time, etc., Scots pine (*Pinus sylvestris* L.) under central Swedish growth conditions is used for illustration, following Rosvall et al. (2011). The comparisons are simplified to highlight the principal differences.

We start by following briefly the steps of considerations presented earlier in this chapter. The biological decision factors for Scots pine are more thoroughly presented in a separate chapter of this publication (Ruotsalainen and Persson 2013).

### Programme objectives

The objective of the Swedish long-term breeding programme is to improve commercially important tree characteristics like adaptation, productivity and wood quality, to adapt to climate change and to maintain genetic diversity without compromising future selection response.

### Decision factors

#### 1. *Biological factors*

Scots pine has a wide distribution and comprises half of Swedish forests, dominating on low quality sites. Stands are thinned one or two times and harvested after 70-100 years. Scots pine easily regenerates naturally, but most stands are planted or direct seeded. In open-grown stands, Scots pine flowers by the age of 20. It is easily managed for seed production in seed orchards. Scots pine is difficult to root, but young seedlings can be used as donor plants for 10-20 cuttings that can be rooted and used successfully for clonal testing.

The genetic variation of Scots pine in Scandinavia is clinal by latitude as an adaptation to climate. The response is to photoperiod resulting in a large-scale GxE interaction

over a north-south gradient. Genetic variation is substantial for all characteristics and mainly additive, although heritabilities in general are low. Genetic correlations for commercial characteristics are favourable, but there are a complicated correlation patterns for growth and survival in harsh areas. The germplasm available in breeding archives and genetic field tests comprises millions of trees.

#### 2. *Economic factors*

Scots pine forestry has a multi-purpose objective for sawlogs, pulp and energy wood, which is considered in the economic weights for vitality, productivity and stem-wood quality. Annual logging is at a sustainably high level with national stocking rising slightly. Export of forest products makes up most of Sweden's foreign net trade exchange.

The current and future use of genetically improved planting stock will increase total growth of Sweden's forests and harvests by 10%. The corresponding increased economic net return motivates a much higher annual investment in tree breeding than is the case currently (Rosvall et al. 2011). The funding of tree breeding is based on an agreement between government and the forestry sector and has been continuous for more than 50 years. After a period of decreasing funding if measured in real terms, funding has more recently increased to compensate for inflation.

#### 3. *Institutional factors*

Sweden has a long history of forestry and forest tree breeding as well of forest genetics research. It has well-trained expertise and access to information technology and specialist support in many allied fields. Tree breeding in Sweden is not university-led, but rather managed on behalf of the industry and state by a separate institution.

#### 4. *Socio-political factors*

Most forests are privately owned. About 20% are managed by the government. Forestry has a strong position in the Swedish society. The forest policy emphasises an increased use of forests to develop a more sustainable society. Forests are seen a renewable resource for increased production of biomaterials, biochemicals and bioenergy, to substitute fossil-based products and energy. In addition, forests provide a number of environmental services like biodiversity, recreation, climate and carbon balance as well as cultural values for Sweden.

The forest policy supports the use of genetically improved planting stock, but regulates the extent of clonal forestry and exotic tree species. There is a debate on the intensity of forest operations and how best to balance productivity, environmental and social impacts, but all values are understood by most political parties and NGO groups. Sweden is an

important supplier of forest products to the growing world market and to the growing bioenergy market of Europe.

## General strategies for deployment, breeding, testing, and selection

Deployment of Scots pine is by seed orchards satisfying most demands for direct seeding and seedling production, although mass propagation of full-sib seed by controlled crosses might soon be an option. To cope with adaptation to current and future climates from north to south of Sweden, a multiple breeding population system (MBPS) is applied with 24 closed breeding populations of census size  $N=50$ , and totalling  $N=1200$  over the entire country. The original plus-trees were selected across all of Sweden. This census size of the population will conserve alleles at a level for sustainable gain over tens of generations, if effective population size is maintained at  $N_e$  greater than 1000 (Danell 1993a, b).

### Case example with 6 alternative strategies

#### *Assumptions and terminology*

Given the conditions described above for Scots pine in Sweden, we have defined 6 principally different mating and selection strategies for managing one individual closed breeding population. Although it is one of the subpopulations in an MPBS programme, it can be seen as a small breeding population, or a subline in a sublining strategy. The schedules of operational activities, use of materials and resources in terms of number of trees used for field testing and selection are approximately following Rosvall et al. (2011), as outlined in Table 1.1 and Figure 1.1.

A “recruitment population” comprises all trees in a new generation that can be considered for selection. “Candidate trees” make up a part of the recruitment population, having been selected or taken at random (unselected). Candidate trees are intended to be further evaluated before they are mated and entered in the breeding population. The term “progeny test” describes a separate test intended to estimate breeding values (BV) of the parents, to identify those that

should be crossed to generate a new generation. Progeny testing is used for backward selection. Forward selection is the selection of trees in the current recruitment population of either those trees to be crossed in the breeding population or candidate trees to be further tested by progeny testing before crossing in the breeding population. Clonal testing is used for forward selection to the breeding population by cloning seedlings of a new recruitment population before they are planted in field tests.

Breeding values are estimated by BLUP for all trees in the recruitment population using information from relatives and these BVs are used for backward as well as forward selection. Except when polycross is applied, the crossing scheme for the breeding population uses positive assortative mating (PAM) with avoidance of parents that are full- or half-sib relatives and two crosses per tree, referred to as double-pair mating (DPM). PAM will form a continuous rank distribution within a single population with the best trees in the top. Re-establishment of genetic variation in each generation and avoidance of mating close relatives will generate a successive upward movement of less-related trees (Rosvall & Mullin 2003).

Variation in founder contributions and effective population size are controlled by including genetic diversity in the breeding objective. The decrease in genetic diversity follows from the increase in group coancestry ( $\Theta$ ). Group coancestry is the average coancestry among all individuals in a population including reciprocals and “self” coancestries (Cockerham 1967). The loss of gene diversity (GD) is expressed by  $GD = 1 - \Theta$  (Lacy 1995). BLUP breeding values and coancestry are considered jointly by using group-merit selection (GMS) (Lindgren and Mullin 1997) or by means of a mathematical program optimisation, maximising gain subject to a constraint on diversity (Hallander and Waldmann 2009b; Kerr et al. 1998; Mullin et al. 2013). For GMS:  $B_\omega = \overline{BV}_\omega - c\Theta_\omega$  where the group merit ( $B_\omega$ ), is the average of the breeding values ( $\overline{BV}_\omega$ ) of the selected individuals, minus their group coancestry, ( $\Theta_\omega$ ) multiplied by a weighting factor, ( $c$ ), converting response and diversity to the same scale.

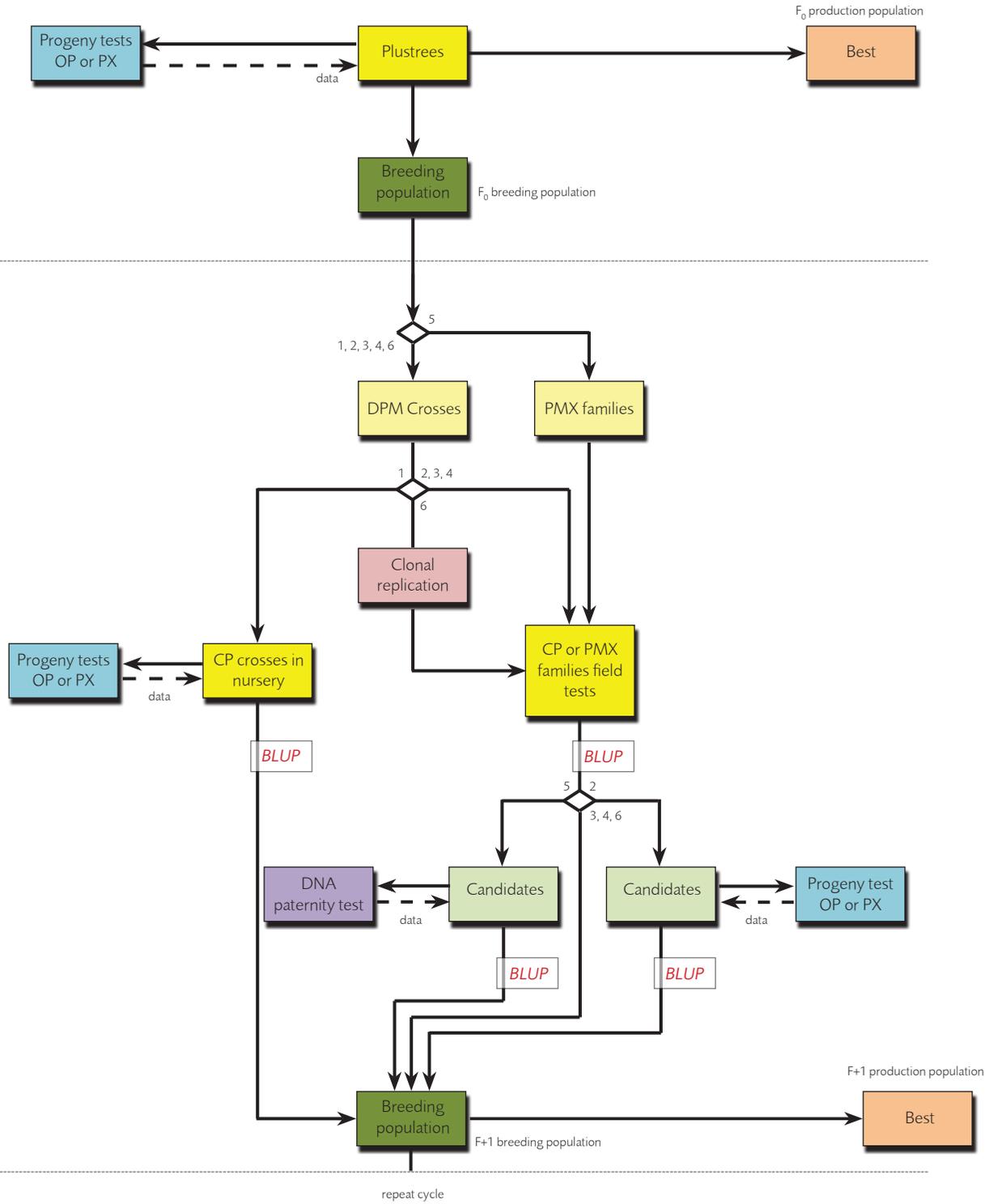


Fig. 1.1. Flowchart of activities required to implement the six breeding strategies.

**Table 1.1.** Summary of alternative mating and selection schemes (slightly modified from Rosvall et al. 2011). Breeding archive sizes assume top-grafts of both mothers and fathers. Note: bw and fw are backward and forward, respectively; op and px are open pollination and polymix crossing, respectively; and cp is controlled-pollination. The figures are chosen to give 25 000 trees in field experiments. This resource is not optimally portioned among types of field tests and number of candidate trees tested or bred, but can serve as a basis for comparing the use of resources.

Strategies	Cycle time years	Field tests				Crosses			Breeding archive	
		Candidates	Trees per family	Test sites	Trees in test	BP <sup>1)</sup> crosses (DPM)	Progeny test crosses	Trees	ha	
1. Seedling candidates backward	33	500	50 bw	4	25 000 bw	50 cp	500 px	2)	2)	
2. Field candidates backward	36	300	200 fw 50 bw	2 4	10 000 fw + 15 000 bw	50 cp	300 op	25	0.08	
3. Field forward	21		500 fw	4	25 000 fw	50 cp		25	0.08	
4. Field forward with expanded BP <sup>1)</sup>	21		250 fw	4	25 000 fw	100 cp		50	0.16	
5. Field candidates polymix forward with expanded BP <sup>1)</sup>	21	2500 <sup>4)</sup>	250 fw	4	25 000 fw	100 px		50	0.16	
6. Field forward clonal replicates	23	2500	10 fw	4	25 000 fw	50 cp		3)	3)	

<sup>1)</sup> BP is Breeding population

<sup>2)</sup> Breeding archives are planted with 500 seedlings per cycle

<sup>3)</sup> Breeding archive is a replicate of the field clone tests

<sup>4)</sup> Candidates selected for paternity analysis

In central Sweden, the average site index for Scots pine results in a dominant height of 26 m at age 100 years. Under these growth conditions, backward selection at a height of 3 m and forward selection at 4 m are expected to require 12 and 15 years, respectively.

To facilitate crosses, scions of trees selected in field tests are grafted into breeding archives. The archives consist of 333 grafted trees per ha used as inter-stocks with a capacity of 10 top-grafts each for crossing. Total archive size depends on population size and the number of crosses. Crosses will normally be repeated in two consecutive years to ensure a sufficient amount of seeds. It is also assumed it will take 2–3 years from top-grafting before collection of pollen and 2 additional years to pollinate all female flowers.

#### *Strategy 1, "Seedling candidates backward"*

Strategy 1 is characterised by progeny testing of unselected seedling candidates, followed by "backward" selection of breeding parents. We start by planting a breeding archive with full-sib  $F_{n+1}$  seedling progeny from the current  $F_n$  breeding population. These progeny comprise the candidates for selection, and may or may not themselves be the result of early selection. When the candidates reach a suitable size, they are stimulated to flower and crossed with a standard pollen mix (polymix), producing  $F_{n+2}$  progeny for a separate progeny test. After evaluating the progeny test, selection is then applied backward to identify the final  $F_{n+1}$  breeding population of census size 50. The final selected breeding parents are crossed by DPM in the original breeding archive to produce a new recruitment population of  $F_{n+2}$  full-sibs.

#### *Strategy 2, "Field candidates backward"*

Strategy 2 is characterised by selection of candidates in field tests, followed by progeny testing of the candidates to identify the final breeding parents by backward selection. We start with selecting a number of  $F_{n+1}$  candidates within-family from a full-sib field experiment, which are then tested by a separate progeny test (open pollination or controlled polymix pollination) in a second step. The progeny test results allow backward selection of the final  $F_{n+1}$  breeding population of 50 trees, which are crossed to produce a new recruitment population of  $F_{n+2}$  full-sibs.

#### *Strategy 3, "Field forward"*

In Strategy 3, we apply forward selection in the  $F_{n+1}$  full-sib field tests to directly identify the final breeding population of 50 trees, using BLUP BV estimates. The breeding parents are selected within family. The selected trees are mated to

produce a new  $F_{n+2}$  recruitment population to be planted in new field experiments.

#### *Strategy 4, "Field forward with expanded BP"*

Strategy 4 is similar to Strategy 3, in that forward selection is practiced in field tests, but differs in the number of families produced and the number of breeding parents selected. By selecting more than one parent within-family, we "expand" the breeding population, selecting multiple individuals and crossing these to produce additional families. In the example discussed here, we expand the breeding population, and the number of families in the recruitment population, by a factor of 2; thus the breeding population is expanded from 50 to 100 breeding parents. These 100 selections are mated to unrelated selections from different families under PAM to produce an  $F_{n+2}$  recruitment population of 100 families. With additional families, we can practice among-family selection, in addition to the within-family selection practiced in Strategy 3.

#### *Strategy 5, "Field candidates polymix forward with expanded BP"*

This strategy follows from the proposal by Lambeth et al. (2001) to use a polymix for both testing and forwarding the breeding population. We utilise the technique described in Strategy 4 to expand the breeding population from 50 to 100 parents. Rather than crossing these by DPM under PAM, we mate each selection with a polymix of the same selected candidates to produce  $F_{n+2}$  progeny for forward selection. A selected subset of 2500 candidates (the top 25 from each polycross family) from the resulting recruitment population are analysed for paternity using DNA markers. With pedigree information supplemented by this additional paternity information, the BLUP BVs are recalculated, before the final forward selection of 100  $F_{n+2}$  parents with similar restrictions on parent contributions to reach the target loss of gene diversity by using GMS. In each subsequent cycle, 100 parents in the expanded breeding population are selected forward and bred by polycross.

#### *Strategy 6, "Field forward clonal replicates"*

This strategy is an extension of Strategy 3, with the important difference that the recruitment population is established in field tests with cloned replicates, rather than with seedlings. The best clones within each family are selected forward to form a new BP of size 50. Copies of each clone are kept in a parallel breeding archive for making crosses, after selection.

### Comparison by computer simulation

The six strategies were compared by computer simulation using POPSIM. A base population was generated with standard genetic parameters for Scots pine assuming an index trait with mean=100,  $V_A=100$  and  $b^2=0.2$ . Three resource levels were applied, based on average annual planting requirements of 303, 606 and 1212 seedlings per year (Table 1.2). The size of progeny test families (40) in Strategies 1 and 2, and ramets per tested clone (12) in Strategy 6, were held constant, while the numbers of candidates to be tested as well as the recruitment population size was increased at higher levels of resources (Table 1.3).

For all strategies in cycle zero, 50 trees were selected from 300 progeny-tested founders to form the  $F_0$  breeding population. In general, Strategies 1 “Seedling candidates backward” and 6 “Field forward clonal replicates” start at the seedling stage of development with production of separate test material for progeny test or clone test the new  $F_{n+1}$

generation full-sib candidates. All other strategies start with planting out the new  $F_{n+1}$  generation full-sib seedlings in field experiments where forward selection of the final breeding population is made at age 12-15.

In this demonstration, the diversity target for the strategy with the fastest cycle time was set at the highest level possible to be conserved for a census size of 50 trees, which corresponds to  $N_e=100$  calculated on a per-generation basis (Falconer and Mackay 1996). In that case, the target is reached by balanced, within-family selection, i.e., selection among the full-sibs in all families from the selected parents. Under the strictly balanced within-family selection approach of Strategy 3 with a 21-year cycle, the lowest loss of gene diversity, i.e., increase in group coancestry is 0.005 per cycle. This corresponds to an annual increase in group coancestry of 0.00024, which was used as the common target for all strategies to be compared by relaxing restrictions on those strategies using more than 21 years.

**Table 1.2.** The level of resources in terms of field planted trees adjusted to an equal annual resource input in terms of the same number of trees used per year. Strategy 1 is the basis with 10 000, 20 000 and 40 000 seedlings used per cycle.

Strategy	Cycle length years	Resource level (numbers of seedlings)					
		Low		Medium		High	
		per cycle	per year	per cycle	per year	per cycle	per year
1. Seedling candidates backward <sup>1)</sup>	33	10 000 <sup>1)</sup>	303	20 000 <sup>1)</sup>	606	40 000 <sup>1)</sup>	1212
2. Field candidates backward <sup>1)</sup>	36	10 909 <sup>1)</sup>	303	21 818 <sup>1)</sup>	606	43 636 <sup>1)</sup>	1212
3. Field forward	21	6364	303	12 727	606	25 455	1212
4. Field forward with expanded BP <sup>2)</sup>	21	6364	303	12 727	606	25 455	1212
5. Field candidates polymix forward with expanded BP <sup>3)</sup>	21	6364	303	12 727	606	25 455	1212
6. Field forward clonal replicates	23	6970	303	13 939	606	27 879	1212

<sup>1)</sup> Total number of seedlings from polycross, open and pair crosses

<sup>2)</sup> BP is breeding population.

**Table 1.3.** Candidates to be tested for backward selection (bw), family size for forward selection (fw), size of progeny-test families and ramets per tested clone, at different levels of resources.

Breeding strategy Candidate partitioning	BP <sup>1)</sup> size	PT <sup>2)</sup> family size or number of ramets per clone	Resource level (numbers of seedlings)		
			Low	Medium	High
1. Seedling candidates backward	50	40			
Candidates preselected per family			5	10	20
Candidates preselected for bw selection			250	500	1000
2. Field candidates backward	50	40			
Family size for fw selection			138	276	553
Candidates preselected per family			2	4	8
Candidates preselected for bw selection			100	200	400
3. Field forward	50				
Family size for fw selection			127	255	509
4. Field forward with expanded BP <sup>1)</sup>	100				
Family size for fw selection			64	127	255
5. Field candidates polymix forward expanded BP <sup>1)</sup>	100				
Polycross family size for fw selection			64	127	255
Trees tested for paternity			2500	2500	2500
6. Field forward clonal replicates	50	12			
No clones per family			12	23	46
Total no of clones for fw selection			600	1150	2300

<sup>1)</sup> BP is breeding population

<sup>2)</sup> PT is progeny test

**Table 1.4.** Total and annual variable cost components for one Swedish pine breeding population with cycle time and dimensions from Table 1.1. according to (Almqvist et al. 2009), slightly modified from (Rosvall et al. 2011). Propagule costs include: cost of sowing and growing candidates, cost of flower stimulation and crossing, cost of sowing and maintenance of progenies, and cost of forming a new generation. Field trial costs include: cost of trial establishment and maintenance, cost of measurement, cost of data analysis and reporting. Grafting and archive costs include: cost of grafting on root stock, cost of top-grafting. DNA analysis cost includes: cost of DNA analyses for paternity determination.

Strategy	Cycle time years	Total variable costs per population (thousand €) <sup>1)</sup>				Annual variable costs for breeding programme (thousand €) <sup>1)</sup>					
		Propagule costs	Field trial costs	Archive costs	DNA analysis	Total cost	Propagule costs	Field trial costs	Archive costs	DNA analysis	Total cost
1. Seedling candidates backward	33	64	141	<sup>2)</sup>	<sup>2)</sup>	204	1.8	4.2			6.0
2. Field candidates backward	36	36	174	7.3		218	1.0	4.8	0.2		6.0
3. Field forward	21	33	143	7.3		183	1.6	6.8	0.3		8.7
4. Field forward with expanded BP	21	40	141	11		197	1.9	6.7	0.6		9.3
5. Field candidates polymix forward with expanded BP	21	46	141	29	22	248	2.2	6.7	1.3	1.6	12
6. Field forward clonal replicates	23	92	141	<sup>3)</sup>	<sup>3)</sup>	233	4.0	6.1			10

<sup>1)</sup> Variable costs for one breeding population with cycle time and dimension according to Table 1.1.

<sup>2)</sup> Breeding archives are planted with 500 candidate seedlings

<sup>3)</sup> Breeding archive is a replicate of the field clone tests

<sup>4)</sup> BP is breeding population

## Results

### Equal resources per breeding cycle

Based on the alternative mating and selection schemes described above and detailed in Rosvall et al. (2011), Table 1.4 summarises the use of time and resources when 25 000 trees per cycle are planted in field experiments under all strategies. This example with equal trees per generation does not represent the current simulations with equal resources per year and is reported for comparison. Tables 1.1 and 1.4 also show the need for breeding archives and crossing. To facilitate crosses in all strategies, except for Strategies 1 “Seedling candidates backward” and 6 “Seedling candidates clonal forward”, scions of selected trees are transferred for grafting from the field trials to a grafted breeding archive.

Strategies using forward selection, i.e., 3 “Field forward”, 4 “Field forward with expanded BP”, 5 “Field candidates polymix forward with expanded BP” and 6 “Field forward clonal replicates”, have the shortest cycle times, using between 21 to 23 years, while those requiring a separate progeny-testing step, i.e., 1 “Seedling candidates backward” and 2 “Field candidates backward”, have the longest, using 33 and 36 years, respectively.

From Table 1.4, it is seen that the total cost per population for all strategies is about 200 000 € per population and cycle. The total cost is dominated by costs associated with field experiments; however if the different cycle times are considered, the annual operational cost of strategies with long cycle times will be the lowest (Table 1.4).

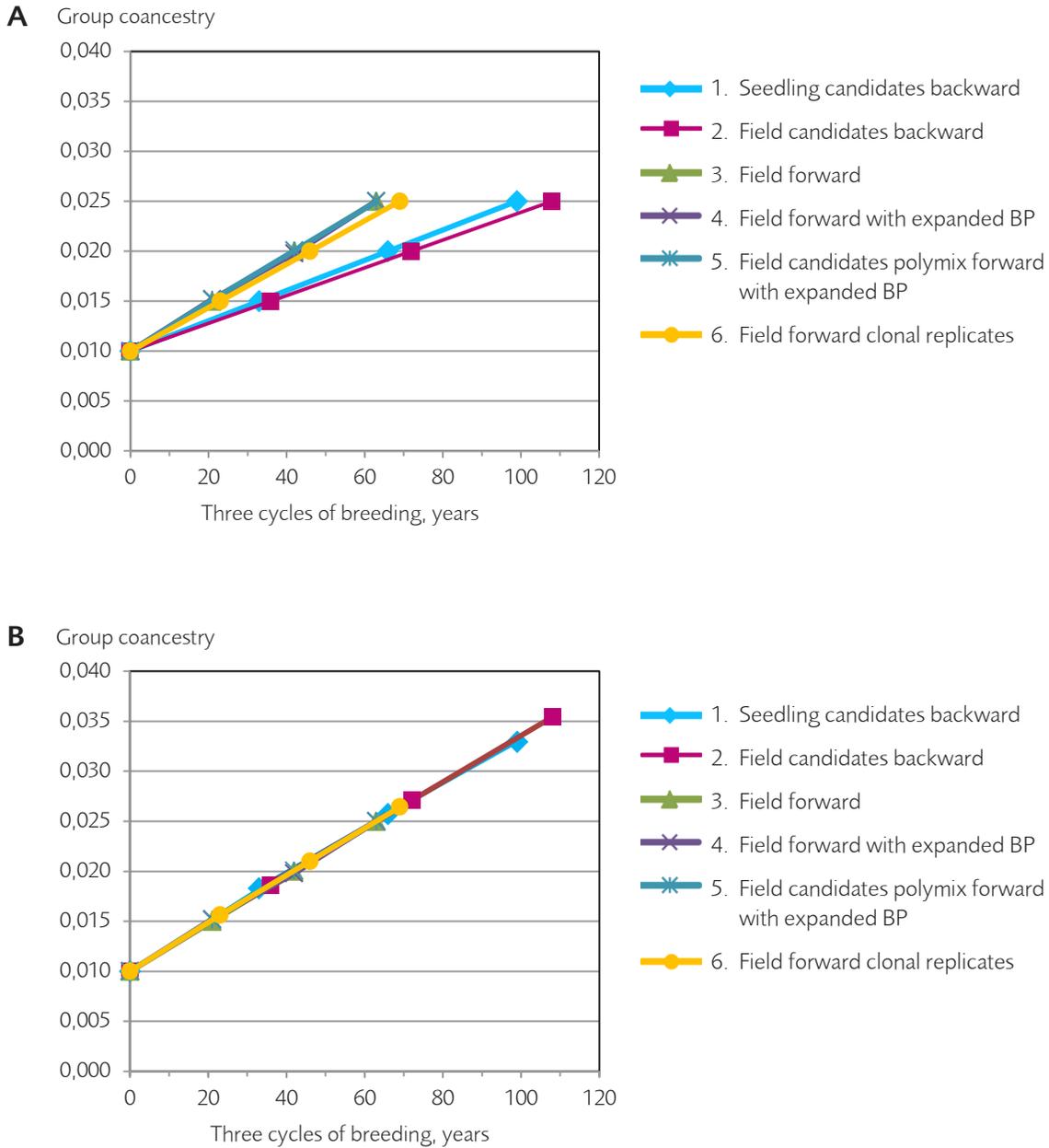
### Equal resources per year

Since the costs of field testing make up most of the total costs (Table 1.4), the same level of trees planted per year was used as an approximation for total costs in the simulations. The calculation of annual costs was based on the total effort of 10 000, 20 000 and 40 000 trees used per cycle in Strategy 1 “Seedling candidates backward”, which takes 33 years. Strategies with shorter cycle lengths will have less total input of trees per cycle (Table 1.2).

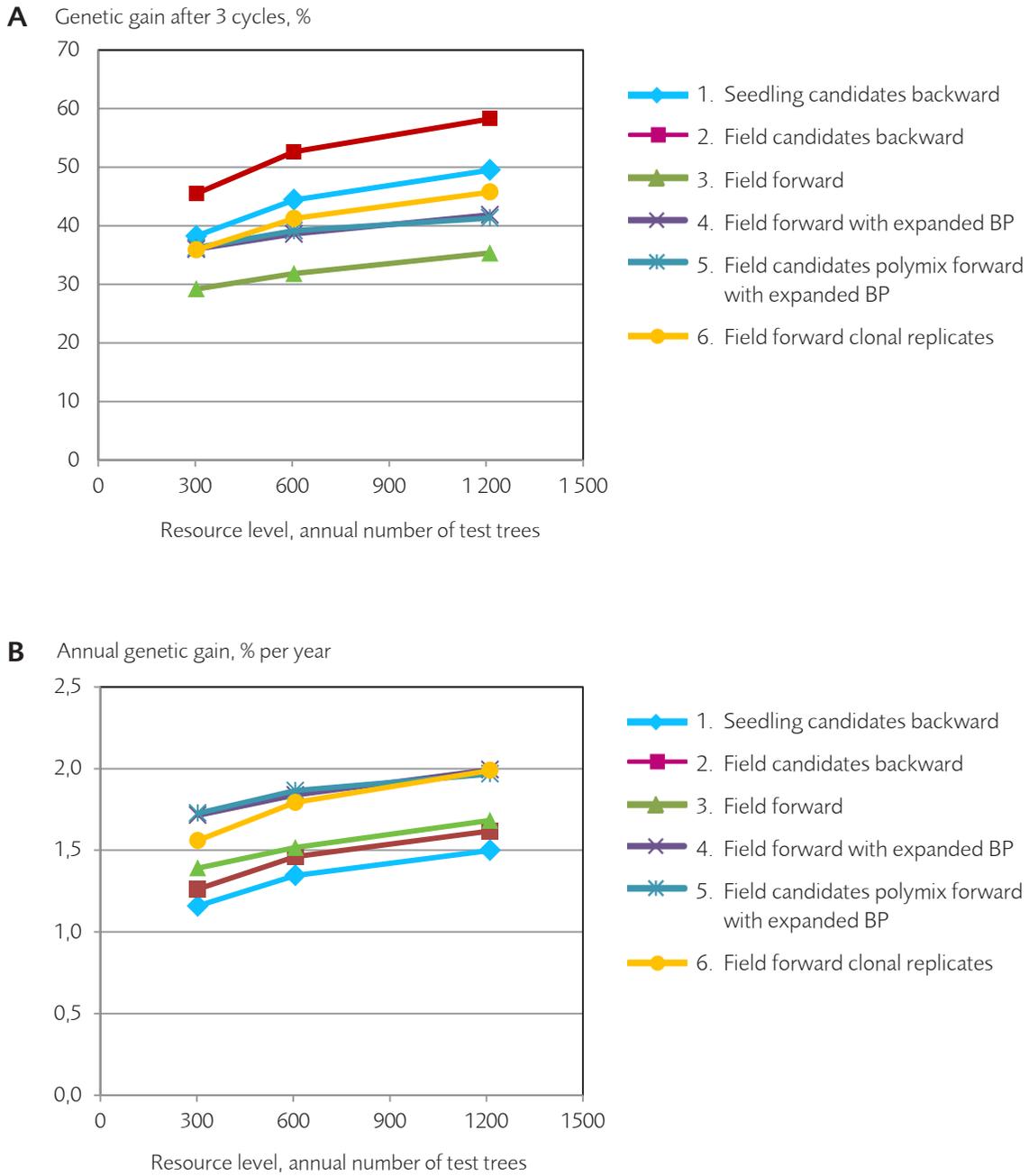
The increase in group coancestry over three cycles of breeding under maximally restricted selection reaching group coancestry 0.025 and after adapting selection restrictions to reach the same annual increase in group coancestry, are illustrated in Figure 1.2. In this way, strategies with long generation cycle time were allowed to increase more in group coancestry for each simulated generation. To apply maximally restricted selection for the PX strategy, 25 progeny per mother tree had to be analysed for paternity.

By adjusting the simulation parameters to use the same annual resource input and the same annual increase in group coancestry, the resulting genetic gains shown in Table 1.5 and Figure 1.3B make a more fair comparison than those shown in Figure 1.3A, which shows the gain after three cycles of breeding but over different periods of time.

The total gain after three cycles is greatest for Strategy 2 “Field candidates backward”, which use progeny tests to select the breeding population of 50 from a number of candidates selected forward in a field test (Figure 1.3A). Total gain is lowest for Strategy 3 “Field forward”, which directly selects the breeding population of 50 in the field test (Figure 1.3A). Because of the much shorter cycle time of the one-step Strategy 3, its annual genetic gain is larger than the annual gain of the two-step selection Strategy 2 (Table 1.5, Figure 1.3B). The greatest annual gain is achieved by Strategy 4 “Field forward with expanded BP” and 5 “Field candidates polymix forward with expanded BP” (Table 1.5, Figure 1.3B). They have the same cycle time as Strategy 3, but in the “expanded” breeding population 100 rather than 50 trees are selected and bred in each cycle. In the  $F_{n+1}$  generation, this expansion allows for combined selection among and within families. Selection among families implies that some of the 100 breeding individuals can be excluded without compromising the diversity target. Strategy 6 “Field forward clonal replicates” is as good a strategy at the medium and high resource levels; while its total gain is higher, using two more years to produce cuttings for field tests means that its annual gain is about equal to strategies using 21 years.



**Fig. 1.2** (A) Annual increase in group coancestry during 3 cycles of breeding, with maximal restricted selection. (B) Annual increase in group coancestry, with selection adjusted to have the same annual increase in group coancestry.



**Fig. 1.3.** Total (A) and annual (B) genetic gain for three cycles of breeding for 6 different breeding strategies, compared at three levels of annual use of resources and the same annual loss of gene diversity.

**Table 1.5.** Annual genetic gain for the 6 breeding strategies at the same level of annual use of resources (in terms of field planted trees) and same per cycle increase in group coancestry.

Strategy	Cycle length years	Annual resource planted trees per year (Total standard level) <sup>1)</sup>		
		303 (10 000) <sup>1)</sup>	606 (20 000) <sup>1)</sup>	1212 (40 000) <sup>1)</sup>
1. Seedling candidates backward	33	1.16	1.35	1.50
2. Field candidates backward	36	1.26	1.46	1.62
3. Field forward	21	1.39	1.52	1.68
4. Field forward with expanded BP <sup>2)</sup>	21	1.71	1.84	1.99
5. Field candidates polymix forward with expanded BP <sup>2)</sup>	21	1.73	1.87	1.97
6. Field forward clonal replicates	23	1.56	1.79	1.99

<sup>1)</sup> Total resource level is from Strategy 1 using 33 years (Table 1.2).

<sup>2)</sup> BP is breeding population

Progeny testing and clonal testing involve an initial cost in terms of a fixed number of seedlings or ramets per tree to be tested (Table 1.4), reducing the number of trees that can be generated for the recruitment population at a given resource level. Therefore, Strategies 1 “Seedling candidates backward”, 2 “Field candidates backward”, and 6 “Field forward clonal replicates” gain more from increased resources, especially up to the intermediate level than strategies that use one round of forward selection (Figure 1.3). Thus, Strategies 3 “Field forward”, 4 “Field forward with expanded BP”, and 5 “Field candidates polymix forward with expanded BP” that just rely on forward selection of untested trees are relatively more efficient at low resource levels (Figure 1.3).

The evaluation of a breeding strategy should also involve its ability to generate production populations to deploy gain to the forest. For example, a conventional seed orchard is selected as a subset of the best trees in the breeding population. High selection accuracy and unrestricted selection will make selected trees more similar reducing the additive variance among selected trees in the breeding population, the so-called “Bulmer effect” (Bulmer 1980), before it is re-established by recombination when the new recruitment generation is formed. By using PAM and crossing trees in rank order, the decrease in variance is counteracted.

Under our conditions, with an initial BP of 50 selected from 300 founders, the additive variance was reduced from 100 to about 45 at cycle  $F_0$ . In the following cycles, the BP variances were re-established at a fairly high rate under the totally or almost totally restricted selection in Strategies 3 “Field forward”, 4 “Field forward with expanded BP” and 6 “Field forward clonal replicates”, and will increase even more, while a lower BP variance developed under the more relaxed selection restrictions used for the long cycle-time Strategies 1 “Seedling candidates backward” and 2 “Field candidates backward” (Figure 1.4). The variance for Strategy 5 “Field candidates polymix forward with expanded BP” is the lowest, since PAM cannot be applied to a polymix. In general, the gain in the BP mean value by applying less restricted selection results in reduced variance, so that the superiority of the top-ranking trees over the breeding population mean is less.

Also important is the relatedness among the top trees selected for a seed orchard. In a seed orchard, the pair-wise coancestry among selected trees generates inbreeding in the progeny of the orchard, which can be expressed as inbreeding depression in the planted forest. Although breeding populations have been selected to have the same group coancestry, they can differ in the distribution of inbreeding for individual trees and pair-wise coancestry among trees.

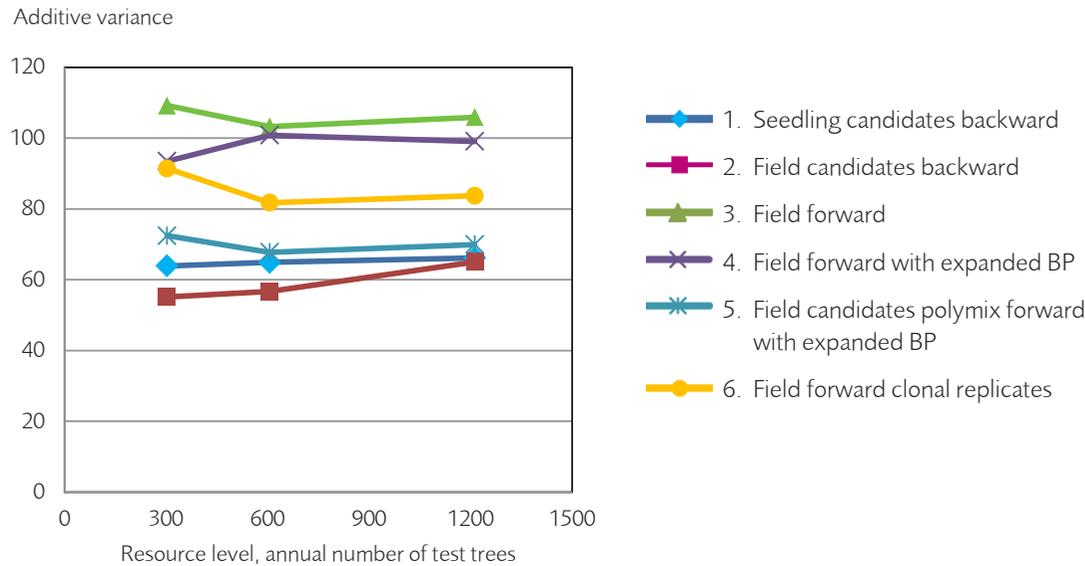


Fig 1.4. Additive variance among selected trees in the breeding population.

Using PAM will increase the chance of mating related trees, even though our crossing scheme avoids mating among the closest relatives (full and half sibs). At the population level, this will produce slightly more inbreeding, but also slightly less group coancestry, a “lining effect” (Rosvall and Mullin 2003; Rosvall et al. 2003). When the population size was increased, as in Strategy 4 “Field forward with expanded BP”, or when selection restrictions were relaxed to have the same annual increase in group coancestry, as in Strategies 1 and 2, relatedness among the top-ranking trees within the BP increased (Figure 1.5). Relatedness among top-ranking trees was lowest in Strategy 5. This was due to our simple way of selecting one tree from each of the best 6 families and Strategy 5 having very many small full-sib families.

#### Advantages and disadvantages with the alternative strategies

Based on these illustrative simulation results, experience and current literature, some general comments can be made on the alternative strategies. Given the constraint on gene diversity for long-term genetic resource management, the principal gain from a breeding programme must come from within-family selection. In a forward-selection programme, this is selection among the full-sibs within a family. Since

all sibs have the same family information, selection can be based solely on individual phenotype. On the other hand, selection among families can rely on differences in family means, and is further informed by information from other relatives in the same and earlier generations.

Our strategies represent various approaches to improve the quality of information within-family to increase selection accuracy when it comes to which of the full-sib candidates to select. For Strategies 1 and 2, this is done by a separate progeny testing for backward selection, i.e., a set of progeny is produced to test the sibs in a separate round of field tests either with polycross or open-pollinated progeny. In Strategy 6, accuracy is enhanced by cloning the sibs to have replications of each individual in the field test, without a need for a separate test. These two ways of individual-tree testing make it possible to test a large number of candidates before their entering the breeding population. In Strategy 5, the DNA test of candidates is just to control relatedness and to improve the estimates of breeding value by including paternity of the candidates.

A third approach to improving information quality is to “expand” the breeding population itself by selecting more sibs per family and crossing them with other sibs from other families. The family information in the following generation

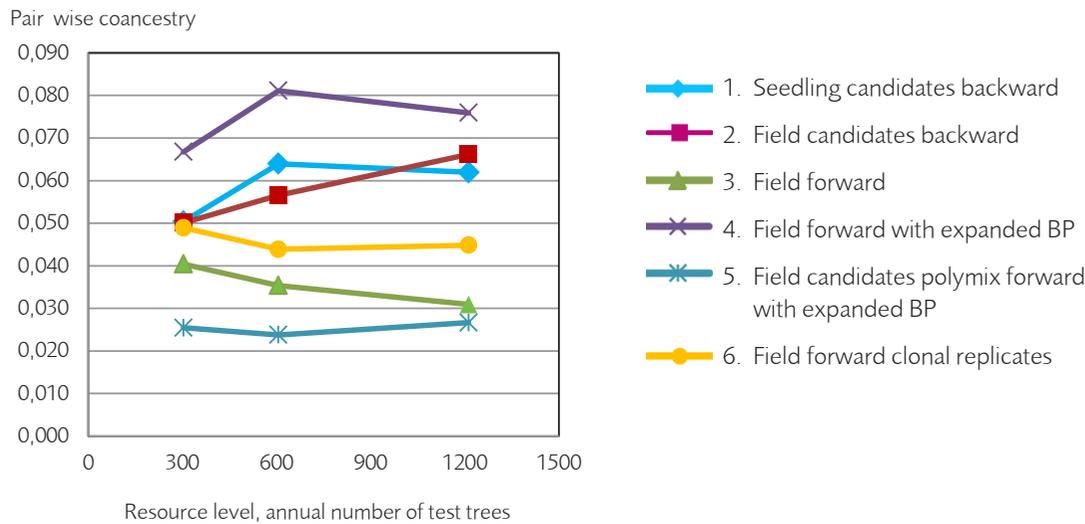


Fig. 1.5. Pair-wise coancestry (equivalent to inbreeding of progeny under random mating, excluding self-pollination or mortality of selfs) among the 6 best trees in the breeding population. Selection was simply restricted to one tree per full sib family.

is used to distinguish between the full sibs of the previous generation. In this way, within-family selection of additional trees becomes among-family selection one generation later. In practice, this is carried out as an integrated combined selection with restrictions on parental contributions to reach the diversity target. This approach is applied in Strategy 4 “Field forward with expanded breeding population” and 5 “Field candidates polymix forward with expanded BP”, where the breeding populations are expanded from 50 to 100, but the diversity target is kept the same as in other strategies. This is in contrast to Strategy 3 “Field forward”, where the final 50 breeding parents are selected purely within family, one per family under DPM.

Using PAM to create an elite part of the population will not increase coancestry and only slightly increase inbreeding, since mating among full- and half-sibs is avoided. Under the rather restricted selection conditions used in most strategies, the main effect of PAM is to maintain a high among-family variation in the breeding population. In this way, the decrease in variation by selection, the Bulmer effect (Bulmer 1980), is counteracted and the initial variation in the population is maintained or even increased. However, in strategies with more relaxed selection restrictions, the counteracting effect on the Bulmer effect by PAM is less. Increased

among-family variation increases selection accuracy both when selecting the breeding population from the recruitment population and when selecting a subset of the breeding population for a seed orchard (Rosvall and Mullin 2003). Using PAM is a more effective strategy than managing a nucleus (elite) population in combination with a distinct main-line breeding population (Lstibůrek et al. 2004a, b).

Important characteristics of the different strategies are summarised in Table 1.6. Genetic gain per unit time depends on selection intensity and accuracy, and the time required to complete the breeding cycle. Resources to increase selection accuracy in genetic tests will always compromise selection intensity. The greater the number of progeny or clonal ramets used for testing each selected tree, the fewer parent trees that can be tested. The optimal balance will depend on heritability. Testing also adds time to the cycle. The more the breeding population is expanded, the more families there are to select among, but the smaller is each family for forward selection. By “breeding archive complexity”, we consider that managing top-grafts of both sexes in a breeding archive is more complicated than simply planting seedlings or rooted cuttings at a specific site for breeding purposes, as in Strategies 1 or 6.

**Table 1.6.** Characteristics of the alternative breeding strategies. Realised selection intensity considers the influence of selection and mating restrictions. The complexity of the breeding archives considers managing top-grafts of both sexes.

Strategy	Selection intensity	Selection accuracy	Number of crosses	Breeding archive complexity	Field testing	Cycle time	Potential gain per year
1. Seedling candidates backward	Low	High	2 rounds many px <sup>1)</sup>	Low	1	Long	Moderate
2. Field candidates backward	High	High	2 rounds op <sup>2)</sup>	High	2	Long	Moderate
3. Field forward	High	Low	Few	High (few)	1	Short	Moderate
4. Field forward with expanded BP <sup>3)</sup>	High	Moderate	Moderate	High	1	Short	High
5. Field candidates polymix forward with expanded BP <sup>3)</sup>	High	Moderate	Moderate	High	1	Short	High
6. Field forward clonal replicates	Moderate	High	Few	Low (many)	1	Short	High

<sup>1)</sup> px is progeny testing with polymix

<sup>2)</sup> op is progeny testing with open pollination

<sup>3)</sup> BP is breeding population

Strategy 3 “Field forward”, as implemented here for restricted within-family selection, is an extreme in terms of high selection intensity and short cycle time, but it is the least accurate strategy because genotypes are never replicated or tested across environments. Strategy 1 “Seedling candidates backward” using separate progeny testing is the other extreme, characterised by low selection intensity and high accuracy, but requiring a long cycle time. The other alternatives are compromises in terms of selection intensity, accuracy and cycle time, by using combinations of forward selection and various ways to infer genetic information on individual trees or pre-selected candidates. Clonal testing combines high accuracy with moderately high selection intensity and short cycle time, and is thus the most efficient method of testing in terms of test individuals per candidate tested. It is superior for testing for individual-tree flexibility traits like phenotypic plasticity.

The mating design for the breeding population has little importance with respect to long-term average genetic gain in the breeding population. However, the mating design can be used to create a within-population structure to enhance gain in a part of the breeding population, and thus increase

potential gain in the production populations, i.e., by using PAM. Strategies using backward selection will result in a list of trees with accurate breeding values that could be used for selection of the 50 final breeding parents and to develop a mating design with more precise assortment. Strategies that plan the mating on breeding values of selected trees from forward selection will have less precise assortment, due to less precise breeding values.

In Strategy 4 “Field forward with expanded BP”, that seeks to save time and avoid progeny testing, an excess of forward-selected trees are mated with less accurate breeding values. In this case, the increase in among-family selection options must compensate the reduced forward selection intensity. Here, more than one mating per candidate is a prerequisite for increasing the probability of mating the best trees in the breeding population and avoid mating a good tree with those that will later be rejected.

Strategy 5 “Field candidates polymix forward with expanded BP” does not use PAM, although the polycross will produce any mate combination including those of PAM. It has high accuracy for the family-selection component, but less family selection intensity. In polycross mating,

there will be few progeny from each father mated to a given mother (averaging 2 at the intermediate resource level), but still many trees per father (averaging 200) over all mothers, although paternity is determined only for a selected subset of the progeny. Our simulation is idealistic, since we have assumed equal representation of males in the PX families and determined paternity on a large number of candidates to achieve the diversity target (2500). Undoubtedly, this strategy warrants further study. For example, it should be interesting to study in more detail what the effective selection intensity can be for fathers by optimising selection of fathers over all polymix families. It will also be interesting to see how many trees must be paternity tested before finding an acceptable representation of fathers. More has to be learned also about the variation in mate success of fathers in a pollen mix.

The strategies also differ in the breeding population's ability to support selection to production populations, such as seed orchards and clone mixes. Progeny testing and clone tests give rise to more accurate breeding values, which may be preferred in a production population as compared to forward selected trees with a slightly higher, but less accurate individual breeding values. Seed orchards, however, are assembled from many selections resulting in expected high mean orchard value, despite a large variation in true individual breeding values (Burdon and Kumar 2004). Strategies maintaining higher genetic variance among selected trees also provide a greater additional gain in a selected subset deployed to seed orchards, given that the strategy also maintains a suitable coancestry structure within the breeding population.

### A fair comparison?

We have illustrated how a number of conditions will influence the outcome of a comparison among breeding strategies. In our case, the rank order of breeding strategies is much changed considering one economic and one genetic factor, i.e. the input of resources and the loss of genetic diversity. By combining them with the third factor, time, we have been able to express annual gain at the same annual loss of genetic diversity and equivalent use of resources. By calculating gain per unit time, the comparison reveals that breeding strategies with less gain per cycle but short cycle times are much improved. But by compensating for the lower use of gene diversity and resources from strategies with long cycle times, these strategies also improved and the variation in outcomes among strategies was decreased. We applied the lowest target possible for annual loss of gene diversity, i.e., that resulting from balanced within-family selection from the strategy with the fastest cycle turnover,

but other rates of loss might be tolerated and used for the comparison. To obtain additional gain in an optimised breeding programme, the restrictions could be relaxed even more. In the actual case of Scots pine in Sweden, the long-term diversity objective is reached if  $N_e$  is reduced from 100 to 50 for a single population (Danell 1993a, b). But we also showed how relaxed selection restrictions reduce additive variance by selection in the breeding population, counteracting the effect of PAM, and giving less additional gain from selecting a more-related elite part of the breeding population for a seed orchard. On the other hand, using strategies with long cycle times and accurate restricted selection will reduce annual loss of diversity even further than our target.

It can be difficult to estimate the use of resources in terms of monetary costs and these might change in the future. From our experience, the number of test trees used in field experiments is driving much of the costs. The cost per tree involves controlled crosses, seedling production, field test establishment, measurement and analysis of the data.

Since many of the activities take place over a short period of time at the turn of the breeding cycle, they are in operational practice about equal among strategies if considered on a per cycle basis. Therefore, calculating the annual input and outcome is very sensitive to the cycle time. Small differences will have large impact and future technology for example to stimulate flowering might change cycle time considerably. It is worth studying the influence of cycle time by sensitivity analysis.

Breeding strategies are more complex than can be studied by computer simulation, although simulation comparisons are helpful as part of a larger decision process. First, all information is not included in the simulations and second there are differences in basic as well as marginal costs not suitable to include when making annual effort equal. From our example, we have emphasised the additional value of clonal replication for testing individual genotype performance over multiple sites and the uncertainty in future cost and accuracy of DNA analysis to determine paternity. Mass propagation by seed orchards can accept much higher relatedness in breeding populations, provided seed orchard trees are selected from a number of unrelated breeding populations, in this case favouring less-restricted selection or low-cost breeding with less control of relationships.

As a practical concern, trees selected forward will often be small and produce few suitable scions, while backward selections often are represented by many older grafts, providing a better supply of scion material for orchard establishment. Such differences have to be considered when the best options for long-term breeding are chosen. In the end, it is the realised gain in forest plantations that should guide the decisions.

## Improving comparisons for real life applications

These simulations were made to illustrate how breeding strategies can be compared, taking into account a number of important factors. To be applied in real situations, the strategies should be individually optimised and subjected to sensitivity analyses. For example, whereas we illustrate our comparisons at one level of heritability, the conclusions might differ at other heritabilities. At lower heritability, strategies with more effort on test accuracy might be more favourable, while at higher heritability forward selection might be preferred.

The allocation of resources among activities within the individual strategies shown in our example was done as best guesses and often chosen to make strategies parallel. Parameters that could be further optimised include the number of seedlings used for progeny testing versus the number of candidates tested and the factor by which the breeding population is expanded in Strategies 4 and 5. More emphasis should be put on comparing the options for deploying a selected subset to a seed orchard or a clone mix. It is the seed orchard progeny gain rather than the breeding population mean that should guide the evaluation a breeding strategy.

## Summary

Forests and forestry within Europe constitute a broad variety of growth conditions, tree species and intensity of management. In this introductory chapter we emphasise that a breeding strategy can have a variety of objectives, including improving economic traits, adaptation to new environmental conditions and gene conservation. Given the objective or a combination of objectives for a given breeding programme, an array of constraints including biological, economic, institutional, and socio-political factors will need to be investigated. To find the best practice for any specific situation, one must understand and consider all these decision factors.

We use a Swedish example with Scots pine to illustrate how breeding strategies can be developed and compared. The programme objective is both to improve economic traits and conserve a high degree of gene diversity. The biology of Scots pine allows for both progeny and clonal testing, and the economic, institutional and socio-political factors are favourable for intensive breeding. In this context, six breeding strategies are developed and compared by computer simulation. These strategies vary in population size, selection and mating system and cycle length.

To make these strategies comparable, we refer to the annual genetic gain with equal annual loss of gene diversity

and equal annual input of resources in terms of number of planted trees in field experiments. This implies that the genetic gain of strategies with longer cycle times is punished by the additional years used, but it also implies that these strategies can utilise more trees per cycle and selection is allowed to exploit more genetic diversity per cycle.

In general, strategies using progeny testing for backward selection of the breeding parents are very accurate producing the highest gain per cycle, but the long cycle time reduces annual gain as compared to strategies using forward selection. The simplest forward selection programme selecting the final breeding population directly in field experiments with control-pollinated trees results in higher annual gain. The annual gain can be further improved by using clonal replicates to improve selection accuracy, despite decreased selection intensity when using the same annual resource and the extra time required for cloning.

The gain by forward selection can also be improved by “expanding” the breeding population, from 50 to 100 in our example. By this expansion, more sibs ( $F_n$ ) can be selected from one family and crossed with trees from other families. Using BLUP, selection among the  $F_n$  sibs occurs in the next generation ( $F_{n+1}$ ) based on family mean values, while new trees are simultaneously selected in the best  $F_{n+1}$  families. The trees of an expanded breeding population can also be progeny tested using a pollen mix of the selected BP members. By selecting an excess of candidate trees for paternity analysis, using DNA markers, a new breeding population can be selected forward in the polycross families, while maintaining a genetic diversity target; the higher the target, the more paternity determinations are needed.

In general, strategies using forward selection also produce relatively higher annual genetic gain at lower resources compared to those using backward selection, since there is a starting cost in terms of trees used for progeny testing or ramets used for clonal testing.

Our demonstration shows a change in rank order among strategies depending on how the comparisons are made. It emphasises the importance of adjusting parameters to make a fair comparison. Our results are based on computer simulation, which is of great help, but all decision factors cannot be incorporated in a simulation model. In a real situation, each strategy must be optimised and the sensitivity of result comparisons analysed. This might include the influence of model parameters like heritability, but also changes in technology that might improve selection accuracy, crossing options, mass propagation options and their effect on breeding-cycle time. Finally, we emphasise that the genetic gain in progeny of a selected subset deployed to a seed orchard or clone mix should be used to evaluate a breeding strategy, rather than the mean of the breeding population.

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# Norway spruce – *Picea abies* (L.) Karst

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2

## Introduction

The natural distribution of Norway spruce, *Picea abies* (L.) Karst., covers large parts of northern Europe (Country groups according to FOREST EUROPE 2011) (Fig. 2.1). The species also occurs naturally in mountainous regions of central Europe (central-west and central-east) and is scattered in mountainous regions of south-eastern Europe. In northern Europe, extensive coverage of Norway spruce is common. In Sweden, the species covers more than 25% of the total land area and more than 40% of the forest area (Spiecker 2000). A large coverage of the forest land can also be found in Finland and the Baltic countries. In central-west Europe, extensive coverage of Norway spruce occurs in Austria, where the species covers more than 25% of the total land area and more than 40% of the forest area. In Germany, the species covers 10–15% of the total land and more than 30% of the forest land. In central-eastern Europe, large coverage of forest land by Norway spruce can be found in several countries. Plantations with the species are common outside the area of natural distribution, especially in central-western and northern Europe.

## Economic Importance

Norway spruce is the most economically important conifer tree species in Europe (Skrøppa 2003). Since the 1950's the general trend is a considerable increase in the annual growth rate of Norway spruce in Europe (Spiecker 2000). The cause for this accelerated growth may be a change over time of several interacting factors influencing growth, for example changes in land use, forest management, natural disturbances, climate changes and nitrogen deposition. On average, the annual increment of Norway spruce in Europe during the last 20-year period has been about 7.3 m<sup>3</sup>/ha (von Teuffel et al. 2004). The highest volume production of Norway spruce is found in pure spruce plantations and often

outside its natural range (Schmidt-Vogt 1977; von Teuffel et al. 2004). For example, in western Norway the production in unthinned Norway spruce plantations reached 15 m<sup>3</sup>/ha/yr after 60 years on good sites (G26) (Øyen and Nygaard 2007). Today the growth of the forests in Europe exceeds the harvest. For example, the total annual harvest in Norway is less than 50% of the annual growth or about 10 million m<sup>3</sup>. A rough estimate of the annual net harvest of spruce in Sweden is 60 % of the total net harvest, about 43 million m<sup>3</sup>, which is approximately 60 % of the annual growth of spruce (Swedish Statistical Yearbook of Forestry 2011). In Finland, the annual net harvest of spruce is about 20 million m<sup>3</sup>, which is approximately 60% of the annual growth of spruce (Finnish Statistical Yearbook of Forestry 2011). The annual net consumption of coniferous round wood as sawlogs or pulpwood in the Swedish and Finnish industry was 67 million m<sup>3</sup> (2009) and 58 million m<sup>3</sup> (2010), respectively. On top of these figures, an annual 6 million m<sup>3</sup> and 8 million m<sup>3</sup> of stem wood used as fuel wood can be added. Of the round wood consumption by the Swedish and Finnish forest industry about 52 % and 34% is spruce, respectively.

## Annual harvest, Areas managed

Altogether Norway spruce covers some 30 million ha in Europe, which corresponds to 38% of the coniferous area (Jansson et al. 2013). Norway spruce is managed more-or-less intensively in large parts of its area of natural distribution. The predominant method of regeneration is planting after clear cutting. The tending of young stands varies. During the last 20-years, a trend in central Europe has been to achieve more mixed stands in contrast to existing pure spruce stands. This trend is reflected in a shift in regeneration methods as natural regeneration is considered the preferred method and clear-cutting is undesirable and less common but to some extent needed to transform pure spruce stands to mixed stands (Klimo et al. 2000).

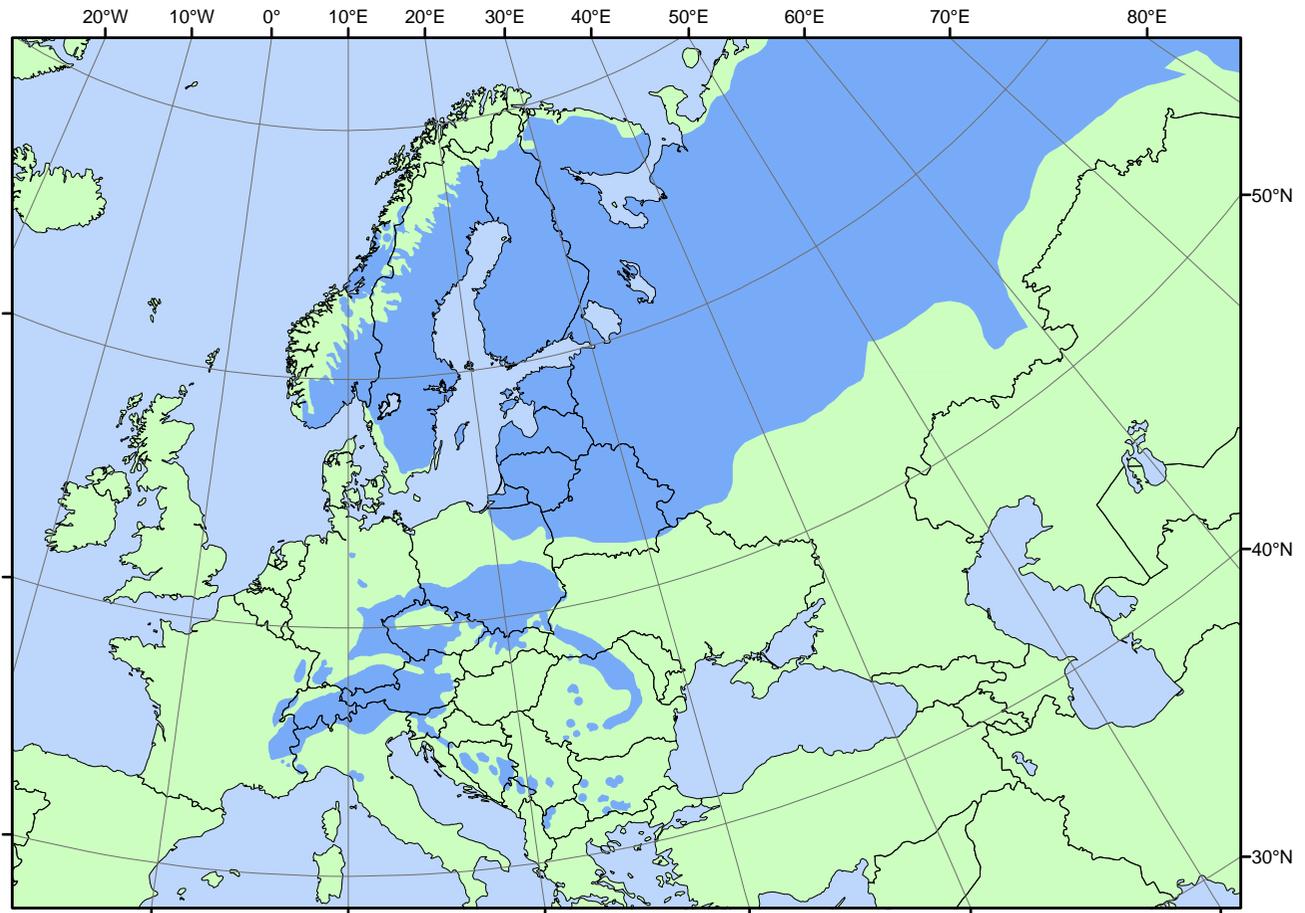


Fig. 2.1. Natural distribution area of Norway spruce compiled by members of the EUFORGEN Networks (2009), after an earlier map published by Schmidt-Vogt (1977).

### Forest management, processing and end-use

Norway spruce produces large volumes per unit area of straight timber that is suitable for structural applications, panelling and furniture. In Sweden, sawn wood production in 2009 included 9 million m<sup>3</sup> of spruce (Swedish Statistical Yearbook of Forestry 2011). Its relatively fine branching and long, lean and straight fibres makes it particularly attractive as raw material for the pulp and paper industry. It is therefore a widely used and valuable tree species for the forest industry in Europe. The industrial consumption of spruce round wood in Sweden in 2009 was 35 million m<sup>3</sup> (Swedish Statistical Yearbook of Forestry 2011) and 21 million m<sup>3</sup> in Finland 2010 (Finnish Statistical Yearbook of Forestry, 2011). In recent years, an increase in the use of wood resources for energy purposes is evident, e.g., in Sweden and Finland. An increase in competition for wood resources, including spruce, may be further triggered by the ambitions within EU to increase biofuel production to reach climate/environment goals in 2020, together with environmental demands to abandon the productive functions of forests on larger areas.

### Economic impact of tree breeding

Breeding of Norway spruce started in the 1940's in many countries in Europe with phenotypic selection of plus-trees, primarily in natural populations. A common early motive was to establish base material for production of seed. The actual breeding objectives differed among breeding programmes, but most of them include adaptation and health, volume production and wood quality. In many programmes, plus-trees were selected to establish seed orchards, but the numbers of tested plus-trees in the seed orchards are often very limited. Over the years some breeding programmes have been down-sized or curtailed, but other programmes are expanding. Today, the largest long-term breeding programmes for Norway spruce exist in Sweden, Finland and Norway, and tested plus-trees have been selected to form breeding populations. Based on test results, gains from the original plus-tree selection have been estimated at 6% genetic improvement in height growth at stand closure (Danell 1991). The present seed orchard programmes in Sweden are predicted to result in a future increase in annual income of 1.7 billion SEK (Rosvall 2011).

## Historical perspective and current situation

### Regeneration and planting systems

The regeneration of Norway spruce forests is based both on natural regeneration and planting, with the former often preferred, although in northern Europe spruce stands are predominantly regenerated by means of planting. While seed orchards are common in many countries, the bulk of Norway spruce seeds is still collected in natural or planted stands. The proportion of plants produced from seed collected in seed orchards varies among countries, with estimates between 0% and 90%. The highest proportion of seed orchard seeds is in northern Europe and in regions outside the area of natural distribution. Seed lots are normally identified by the geographic origin of the stand, and in several countries it is required that the seed stand should be selected for superior performance. The relative amounts of seeds from forest stands and from genetically improved seed harvested in seed orchards vary considerably among countries and regions within countries. In the Nordic region, there has been a considerable increase in the use of seed orchard seed during the last five-year period. In Norway, 77% of the 300 kg Norway spruce seeds sold in 2007 in the south-eastern region originated from seed orchards (Mullin et al. 2011). In Sweden, 62% of spruce seedlings produced in 2011 originated from seed orchards (Swedish Statistical Yearbook of Forestry 2011), which is a 10% increase since 2006. The nearly 12 tonnes of Norway spruce seeds that were produced in Swedish seed orchards in 2006 would be sufficient to produce 1.2 billion plants and regenerate 450 000 ha (Almqvist et al. 2008). In Finland, nurseries have used from 1000 to 1500 kg of spruce seed annually. In the peak year of 2001, 80% of the seed was obtained from qualified first-generation seed orchards. The share of the seed orchard seed has since declined due to consecutive poor seed crops and was only 24% in 2010 (Finnish Statistical Yearbook of Forestry 2011). The seed orchards generally start to flower 10–15 years after grafting, although the periodicity and amount of flowering are very much dependent on climatic conditions at the orchard site. To promote flowering, orchards have often been located on warmer sites, relative to those from where the parents originated and where the orchard seed is intended for use.

### Populations and seed origin

Field experiments have revealed substantial variation in growth performance among populations from different geographic origins. Both results from old provenance trials that cover a large portion of the native range and empirical knowledge based on actual seedtransfers suggest that

Norway spruce can adapt to a fairly wide range of climates and is relatively tolerant to transfer. The results from provenance trials have clearly demonstrated that the local provenance is not always the best in terms of growth performance (König 2005), although in extreme environments like in northernmost Scandinavia and at high altitudes in the Alps, the local provenances appear to be the best if large plant mortality is to be avoided.

The timing of budburst in spring is a crucial trait for adaptation. The use of maladapted provenances has resulted in damage and reduced yield in plantations. Central-eastern European populations and south-eastern seed sources are late flushing and may, when grown in northern locations, more successfully avoid early frosts in spring than the local origins. Late flushing origins are also characterised by higher growth performance compared to the local origins. In many regions of Europe, a gain of 10% in volume production is often realised by choosing a non-local more-southern seed source (Jansson et al. 2013). Seed sources from Belorussia and the Baltic Republics have shown excellent performance in southern Sweden. Current recommendations for seed transfer both in Sweden and Finland advocate cultivation of seed sources originating south of the reforestation site.

### Seed orchard and nursery production

There are currently 166 Norway spruce seed orchards covering an area of 1414 ha in 17 European countries (Jansson et al. 2013). Another 28 seed orchards are in preparation and will increase the total area by 375 ha. About two-thirds of the seed orchard area are located in Sweden (379 ha), Finland (321 ha) and Lithuania (256 ha). Of the total, about 15 % are defined as “tested”. In Finland and Sweden, some 100 and 200 million spruce plants are produced annually, respectively.

### Genetics research

The first provenance trials with Norway spruce were established in the late 1800s in Austria and were followed by several series of national and international experiments (König 2005). The aim of these trials was to study among-population variation in order to find most suitable provenances in terms of wood production. In many trials large among-population genetic variation in growth performance has been shown.

In the early breeding of Norway spruce in northern Europe, provenance hybridisation attracted much interest. The idea of provenance hybridisation was that it would be possible to exploit heterosis via crosses between differentiated Scandinavian and continental populations. Later it was shown that these effects were rather minor and the idea of hybridisation was no longer used in breeding (Kaya and Lindgren 1992).

Selection of plus-trees started in the best provenances based on results from provenance testing. The first genetic trials with progenies from individual spruce trees were established in the early 1900s (Eriksson et al. 1973). Genetic variability has been found within all natural populations studied, also for traits that show clinal variation at the provenance level (e.g., Dietrichson 1971; Eriksson 1982; Skråppa 1982; Ekberg et al. 1985, 1991). Traits that show such variation characterise germination, early and later height and diameter growth, the timing and duration of the shoot-growth period, autumn frost hardiness, survival in the field, branching, crown and stem form, and wood quality (e.g., Skråppa 1991, 1993; Hylén 1997). The variation within a provenance may sometimes be as large as that found among geographically distant provenances.

Genetic variation of Norway spruce has also been extensively studied using various genetic markers. As gene flow is mediated through wind transport of pollen, little differentiation can be expected over large areas with similar wind and temperature conditions, provided that there are no mountainous barriers that would hinder long distance transport of pollen. Many studies have confirmed a large amount of genetic diversity in Norway spruce within populations, while relatively little differentiation has taken place among populations (Goncharenko and Potenko 1990; Paule et al. 1990; Giannini et al 1991; Paule and Gömöry 1993; Breitenbach-Dorfer 1996). However, these studies have been carried out within each of various sub-regions of Europe and therefore do not rule out considerable differentiation among populations across a larger clinal gradient.

Over the years, research on flowering and measures to improve flowering in Norway spruce have been considered important, in order to both increase the production of seeds in seed orchards and facilitate controlled crossings between selected individuals. Mechanical and chemical treatments have been used to increase flowering. Hormonal treatments (e.g., gibberellins) have been studied frequently (Bleymüller 1976; Chalupka 1979, 1981, 1997; Dunberg 1980; Luukkanen 1980; Bonnet-Masimbert 1987) and are recommended when reproductive induction and synchrony are fundamental prerequisites for the production of seeds (Kang 2001). Recently, Almqvist (2008) reported that although practical use of GA4/7 in Norway spruce seed orchards does not always have a clear positive effect, the treatment is still a cheap and highly profitable way of increasing the cone production.

### Operational breeding programmes

Operational breeding programmes of Norway spruce were initiated in many countries in the late 1940's (1930's in Sweden). A total of about 25 000 plus-trees were selected in 17 countries in Europe as the founding material for first-generation seed orchards and breeding programmes. Most of the plus-tree selections were made in Norway, France and Sweden (5500, 6743 and 6000, respectively) (Table 2.1). About 45 % of the plus-trees have been tested in field tests (Table 2.2).

The largest long-term breeding programmes for Norway spruce are on-going in Sweden, Norway and Finland. In a number of countries, such as France, Denmark, and the Netherlands, Norway spruce breeding programmes have been suspended or closed due to limited resources. In a few countries, such as the Baltic States (Estonia, Latvia and Lithuania), there are currently new attempts to start or revive former programmes.

### Achievements

Genetic tests in Sweden have demonstrated about 10% gain in volume production per hectare over a rotation period with seedlings originating from untested first-generation seed orchards compared to unimproved seed lots (Rosvall et al. 2001; Andersson 2002). A Finnish study (Ruotsalainen and Nikkanen 1998) found similar genetic gain (10%) for height growth from progeny trials in northern Finland. Differences in quality traits are positive but smaller. The predicted gain in volume production from the second round of Norway spruce seed orchards established during the 1980s varies from 10% (untested plus-trees) to 25% (intense selection from tested plus-trees) (Rosvall 2011). Genetic thinning of seed orchards can increase the gain further. The gain in volume production from the second round of seed orchards established using a mix of untested and tested parent trees is estimated to be in the range of 12-25% (Rosvall et al 2001). In a third round of seed orchards, based on the best tested plus-trees, a gain of some 35% is anticipated (Rosvall et al. 2001).

Even higher genetic gains are associated with the use of clonal reforestation material. Many plantations were established with rooted cuttings of selected clones in the 1970s in Germany, Denmark and Sweden, although such materials are now used only on a small scale. The same is true for bulk propagation of rooted cuttings from selected full-sib families. Clonal forestry based on somatic embryogenesis (SE) has potential to become a valuable tool for intensive wood production, but is not yet implemented operationally in Europe.

**Table 2.1.** Number and size of breeding populations in some countries with spruce breeding programmes (Jansson et al. 2013)

Country	Plus-trees	Tested plus-trees	Breeding populations	Individuals in each subpopulation	Total number of individuals
Finland	2770	1700	5	160	900
Lithuania	239	80	4	50	250
Norway	5500	2800	22	50	1150
Slovakia	225		2	55 and 100	155
Sweden	6000	All	22	50	1200
	+ 18000 clones				

**Table 2.2.** Existing field trials of Norway spruce among Treebreedex partners (Jansson et al. 2013). Empty cells = data not available.

Country	Trial type					
	Provenance		Progeny		Clonal	
	No. trials	Area (ha)	No. trials	Area (ha)	No. trials	Area (ha)
Austria	22	33				
Belgium	14	19	3	10	4	5
Czech	16	35	29	42	20	12
Denmark	23	28	54	23	32	11
Finland	137	287	168	192	59	69
France	29	48	42	72	13	45
Germany	57	118	45	23	46	38
Great Britain	22	46				
Italy	55	9				
Lithuania	13	42	15	26		
Norway	52	26	130	130	2	2
Poland	11	29	16	21	3	4
Romania	15	31	18	32	3	4
Slovakia	11	14	4	4		
Sweden	65	83	199	204	349	222
<b>Totals</b>	<b>487</b>	<b>839</b>	<b>723</b>	<b>779</b>	<b>586</b>	<b>421</b>

## Genetic resources and biology

### Distribution

Norway spruce is one of the main tree species of Europe. The natural distribution covers, to a large extent, the boreal and mountainous region of the temperate zone (Fig. 2.1). During the last ice age, the distribution of Norway spruce was restricted to two refugial areas; one refuge was in western Russia and another refuge in the eastern parts of the mountain ranges in central-east and south-east Europe (Huntley and Birks 1983). A separation into a northern and a central European group has been confirmed by using both analysis of quantitative traits and random amplified polymorphic DNA variations (Collignon et al. 2002). After the ice age, the population in western Russia spread north and westward to Fennoscandia, and the east European population spread north and westward in central Europe.

Plantations of the species are common outside the area of natural distribution in central-western and northern Europe. For example in Belgium, the Netherlands and Denmark, Great Britain, Ireland and most parts of France, but also in south-western Sweden. The proportion of Norway spruce in Europe has substantially increased by reforestation since the beginning of the 19th century. The main aim of the reforestation was to establish forests for timber production. This process started when many forests in Europe had been affected by forest loss due to over-exploitation and soil degradation.

### Silvics and reproductive biology

Norway spruce is a shade-tolerant secondary coloniser with few distinct site requirements for good survival. For high germination and seedling growth, soil conditions with sufficient moist are important, especially in early summer. Fertile soils, preferable with mobile soil water during the growing season, are generally highly productive. Despite the often high production of Norway spruce the soil nutrient level generally appears to be a limiting factor for growth as addition of nitrogen has been shown to result in substantial increase in stem growth (Bergh et al. 1999). On dry soils shoot growth is closely related to occurrence of rainfall during the vegetation period whereas on wet soils other factors like air temperature is more important (Mork 1960). Soils with soil water contents of approximately 10% or more are sometimes referred to as wet soils whereas soil water contents of approximately 5% or less are dry soils (Helenius et al. 2005). Soil water content appears to be a limiting factor for growth in some areas. For example, in southern Sweden addition of water has been shown to have a positive effect on stem growth but not in northern Sweden (Bergh et al. 1999).

Norway spruce shows a wide geographical natural distribution with clinal differentiation of natural populations into provenances. It can easily be established artificially outside its natural range, provided that shoot growth phenology is reasonably synchronised with the climatic conditions at the growing site. When adapted to the local climatic conditions, Norway spruce is an extremely frost-tolerant species; however, when regenerated artificially on open land it may be exposed to several stress factors simultaneously. Of these factors, weed competition and frost damages appear to be the most important. Frost during the period of active shoot growth, especially in spring or during growth cessation and hardiness development in autumn, may affect survival and growth performance of both plants and young trees. Repeated frost damage has the strongest selective effect before reproductive maturity in northerly regions (reviews by Hannerz 1998, 1999).

Timing of phenology is of adaptive significance for Norway spruce. Genetic variation in phenology traits is a driving force in the differentiation of spruce into natural populations or provenances. The phenology traits of natural populations are significantly correlated with geographic variables representing photoperiod, temperature and continentality of the climate at the site of origin (Dormling 1973, 1979, 1993; Persson and Persson 1992).

Timing of bud flush in Norway spruce is influenced by both environmental and genetic factors. Of the environmental factors especially high daily mean temperatures from March and onwards promotes rest break, early flushing and subsequent risks of frost damages. When seed sources, provenances, with different origins are compared at the same site, onset of shoot growth and growth cessation occur earlier with increasing latitude and altitude of origin. Early-flushing provenances, usually flushing in mid- to late-May as a response to relatively few degree days, are found in northern Europe and at high altitudes in the Central European Alps. Late-flushing ones, usually flushing in early June, are common from central-eastern Europe. The latest flushing sources come from central Russia, north-eastern Poland and the interior of the Baltic Republics. In Western Europe, with the exception of the Alpine region, early flushing is common.

Seedlings end their height growth earlier and build up hardiness earlier at higher latitudes and altitude (e.g., Dietrichson 1969; Krutzsch 1974; Giertych 1976, 1979, 1984; Qamaruddin et al. 1993; Danusevičius 1999). Provenances from northern Europe end their shoot growth period early, often in early August, and in this region growth cessation exhibits a strictly clinal variation, correlated with latitude of origin. Maritime populations show later shoot growth cessation than continental origins (Leinonen 1996).

In general, provenances from central-western Europe finish shoot growth relatively late in the autumn, for example in October. The more southern sources from central-eastern Europe are intermediate in terms of growth cessation.

Norway spruce is a wind-pollinated simultaneously monoecious species. The species usually has a rather long juvenile period, during which it will seldom flower and set seeds. In open stands, sexual maturity will generally be reached after 20-30 years, while it occurs later in closed stands (Schmidt-Vogt 1978). The process of seed development requires almost two years from the initiation of female and male flower primordia at mid-summer in the year of initiation. The year after, the actual flowering takes place involving pollination of female flowers, fertilisation, seed maturation and shedding of seeds. Close to northern and alpine timberlines, cold summers can restrict seed maturation leading to poor regeneration in most years. In northerly regions it often takes 15 to 20 years for Norway spruce to reach reproductive maturity (reviews by Hannerz 1998, 1999). The amount of flowering and to some extent the flowering age may be enhanced by environmental factors like high air temperatures and stress treatments like drought during the period of flower induction in mid-summer. Hormonal treatments on spruce grafts during the shoot growth period (with gibberellic acid alone and in combination with heat) have been efficient in promoting female flowering (Johnsen et al. 1994). However, irregular flowering is often a problem causing uneven seed production.

The root pathogen *Heterobasidion annosum* is important in mature stands, as it causes root and butt rot. The fungus spreads primarily via spores that colonise the stump and is then spread to adjacent trees via root contact. The damage causes economic losses both in growth and wood quality. Root rot causes increased rates of mortality and wind throw, but also increased risks for infections of future generations.

Norway spruce has the ability, under certain conditions, to reproduce vegetatively through layering (Skoklefeld 1993). Vegetative propagation of spruce by the use of cuttings has been widely used for production of plants in great numbers (Kleinschmit et al. 1973). In older stages the rooting ability decreases and the cuttings show plagiotropic growth and irregular branching (Roulund 1975). To maintain the rooting ability, different methods like hedging have been used to extend juvenility 10-20 years. Today, somatic embryogenesis is a new technique for vegetative propagation. Somatic embryogenesis enables a mechanised production of thousands of identical plants from a single cell without ageing effects, together with relatively cheap long-term cryo-storage of clones in liquid nitrogen. When practical applications are available, clonal forestry will be facilitated by this method as crossing of superior parents followed

by vegetative propagation of the offspring and subsequent testing of the cloned progeny is possible without any ageing effects (Lindgren 2009).

### Genetic variation and genetic parameters

The patterns of variation observed in Norway spruce provenance trials show geographic variability on a large scale. However, genetic variation is also present among offspring from natural populations within the same provenance region and among progenies from trees in the same population. A large number of studies have demonstrated large within-population genetic variation in quantitative traits in Norway spruce (Tables 2.3 and 2.4). Genetic variability has been found within all natural populations studied, also for traits that show clinal variation at the provenance level (e.g., Dietrichson 1971; Eriksson 1982; Skrøppa 1982; Ekberg et al. 1985, 1991). Traits that show such variation characterise germination, early and later height and diameter growth, the timing and duration of the shoot growth period, autumn frost hardiness, survival in the field and branch, crown form, stem and wood quality (e.g., Skrøppa 1991, 1993; Høyen 1997). These variations, however, should be considered as normal patterns of variation within a widespread species and should at most be used to designate different climatic varieties (Schmidt-Vogt 1977, 1978).

### Resources

The full wealth of natural genetic resources is still available to Norway spruce breeders. The natural range of Norway spruce has not been fragmented by human activity and most spruce stands are still naturally regenerated. There are exceptions like in southern Sweden where the forest is fragmented and heavily influenced by human activity and seed transfer. Plantations of open-pollinated offspring of first-generation plus trees have become more common during the past decades, however, the genetic structure of the plantations is representative of natural stands. Abundant samples of natural variation, consisting of hundreds of genotypes, have been collected and stored in *ex-situ* grafted archives such as seed orchards or specific clonal archives established for breeding purposes. In Finland, for instance, there are 1831 plus-trees available in such archives. Field trials established for genetic testing of first-generation plus-trees (progeny trials) or testing of second-generation candidates (clone trials) or provenance seedlots make up another resource that may be exploited for breeding purposes. In Europe, the area of genetic field experiments established in Norway spruce exceeds 2000 hectares (Table 2.2). Surplus-seeds of open-pollinated and cross-pollinated seedlots (F1) of plus-trees are also archived in seed storage for possible later usage, e.g. in DNA studies. The Swedish review suggested that it would

**Table 2.3.** Estimates of individual-tree narrow-sense ( $h^2$ ) and broad-sense ( $h^2$ ) heritabilities, and additive ( $CV_A$ ) and genotypic ( $CV_G$ ) coefficients of variation (%). (Jansson et al. 2013)

Trait	Age	Method	Population	$h^2$	$CV_A$	$H^2$	$CV_G$	Reference
Height	6-15		31 progeny, 81 clonal tests	0.13	12.0	0.18	12.0	Rosvall et al. 2001
Height	9-14		201 half-sib families	0.05-0.36	6.4-27.8%			Hannerz et al. 199
Height	22		55 half-sib families, two sites	0.16	10			Steffenrem 2008
Height increment	9-14		201 half-sib families	0.07-0.47	10.7-35.6%			Hannerz et al. 1999
Height increment	5-6		192 half-sib families	0.04-0.30	8.2-39.1%			Kowalczyk et al. 2009
DBH	15		OP and Nested designs	0.09-0.27	7.2-11.8%			Costa e Silva et al. 2000a
DBH	28		Partial diallel	0.18	12			Hallingbäck et al. 2008
Density	15	Pilodyn	OP and Nested designs	0.18-0.36	5.0-8.2%			Costa e Silva et al. 2000a
Density	28	Core X-ray	47 OP families	0.47	5			Hyllen 1997
Density	21	Water displacement	OP	0.55				Danusevičius and Gabrilavičius 2002
Spiral grain	12	Surface	Clones			>0.30	2.6°	Hannrup et al. 2003
Spiral grain	15	Surface	OP and Nested designs	0.29-0.47	39-44%			Costa e Silva et al. 2000b
Spiral grain	28	Surface	Partial diallel	0.36				Hallingbäck et al. 2008
Branch diameter	22	Breast height	55 half-sib families, two sites	0.14	8			Steffenrem 2008
Stem straightness	22	Scores 1-5	55 half-sib families, two sites	0.28				Steffenrem 2008
Microfibril angle	33	Increment core	13 half-sib families	0.38	8			Steffenrem et al. 2009
Bud burst	5	Scored				0.72		Karlsson and Högberg 1998
Bud burst	3-4	Scored	201 half-sib families	0.82				Hannerz et al. 1999
Shoot lignification	2-4		201 half-sib families	0.21-0.48	18%			Hannerz et al. 1999
<i>Heterobasidion</i> infection	20	From discs	50 clones			0.18		Karlsson and Swedjemark 2006
<i>Heterobasidion</i> infection		Fungal growth	Four ramets of each of 252 progenies in one full-sib family			0.11		Arnerup et al. 2010
<i>Heterobasidion</i> infection	17		24 clones			0.18		Swedjemark and Karlsson 2004
<i>Heterobasidion</i> infection	4	Fungal growth Lesion length	98 clones			0.35		Swedjemark et al. 1997

**Table 2.4.** Genetic correlations between traits (Jansson et al. 2013).

Trait 1	Trait 2	Age	Genetic correlation	Reference
DBH	Spiral grain	28	0.37	Hallingbäck et al. 2008
DBH – age 16,18	Spiral grain surface – age 11-12		0.32-0.47	Costa e Silva et al. 2000b (2 field trials)
DBH – age 16,18	Pilodyn – age 16,18		0.19, 0.80	Costa e Silva et al. 2000a (2 field trials)
Pilodyn – age 16,18	Spiral grain surface – age 11-12		0.19, 0.33, 0.33	Costa e Silva et al. 2000b (3 field trials)
DBH	Micro-fibril angle	33	Positive (approx. 0.5)	Steffenrem et al. 2009
Height	Density	28	-0.68	Hysten 1997
Height	Branch diameter	22	0.69	Steffenrem 2008
DBH	Branch diameter	22	0.60	Steffenrem 2008
DBH12	Grain angle 12		0.11	Hannrup et al. 2003
HT9	Grain angle 12		0.06	Hannrup et al. 2003
Root rot	Growth traits	11-20	-0.01-0.09	Karlsson & Swedjemark 2006
DBH	Basic density of whole increment core	21	-0.71	Danusevičius and Gabrilavičius 2002

be beneficial to have this kind of material, for example from all founders, archived as extracted DNA-sample to facilitate association genetics and to allow for tracking their genetic contributions over generations (Rosvall 2011).

## Breeding objectives

### Economic traits

All breeding programmes generally aim at improving the value of future Norway spruce forests for a variety of end products and mitigating the risks under variable environmental conditions. The principal goal traits are increased dry biomass production of stem wood per unit area and good adaptation to the environment of the planting site. Increased dry biomass production includes both volume production and basic wood density. Studies have shown compound growth indicators such as height and diameter, to be very important selection criterion, while a wood density indicator was of moderate value (Rosvall 2011). Selection for yield is mostly based on height and diameter growth (Mullin et al. 2011); however, wood density must be maintained to give a stable, uniform and high-value yield. Wood quality is given attention to various degrees in different programmes (Steffenrem 2008; Steffenrem et al. 2009). Norway spruce produces straight structural timber that is applicable to a wide range of industrial uses. Due to the relatively good quality

of Norway spruce timber, many breeding programmes do not consider branching characteristics or wood properties as important objectives, but rather aim at keeping them at the same level as in unimproved material. Of the specific wood properties, spiral grain is a major cause of twist developed in sawn timber, and is already being addressed by some programmes (Hallingbäck et al. 2008). For pulp and paper, tracheid structure and wood chemical composition are important traits to determine quality. Spike knots and forks have a detrimental effect on timber quality and their absence is usually required in selected material.

### Adaptive traits

Adaptability is a complex trait involving traits such as overall vitality, tolerance of spring and autumn frosts, cold hardiness, resistance to pests and diseases and stable performance over a wide range of climatic conditions. The relative importance of growth and adaptive traits varies along with the harshness of the climate. Adaptability (cold hardiness) becomes an increasingly important breeding goal toward northern regions and higher altitudes. Various target traits of direct adaptive value are selected, most importantly, the timing of flushing and bud-set, frost hardiness during the dormant period and lack of injuries in field or freezing tests. Norway spruce plantations are occasionally heavily damaged by spring frosts. Late-flushing genotypes are less sensitive

to frost and favoured in selection, which may have consequences as a delayed growth cessation in autumn for some genotypes. Phenological traits are under fairly strong genetic control, enabling genetic progress in flushing time to be made quite rapidly by simple phenotypic screening (Karlsson and Högberg 1998). A corresponding simple phenotypic screening of the internal growth cessation, i.e. 1-2 months after the end of shoot elongation in late summer, is still needed.

Resistance to root rot (*Heterobasidion annosum*) is considered as an important trait in Norway spruce. The genetic variation in root rot resistance also indicates the possibility to breed for increased resistance (Swedjemark et al. 1997). The damage by the root pathogen causes great economic losses in Norway spruce forests throughout the northern hemisphere. Thus, substantial commercial gains could be achieved if resistance to pathogens could be included in the breeding programme, although techniques to select resistant genotypes are still under development (Mullin et al. 2011). The genetic component of root-rot resistance may take 20 years to express (Karlsson and Swejdemark 2006), which makes selection for resistance difficult in ordinary field testing.

### Diversity and genetic resource conservation

The objective of conservation of genetic resources is to maintain the potential for continuous future evolution. Various strategies are applied in the genetic conservation of Norway spruce. In many EU countries, the conservation takes place *in situ* in protected areas. In some countries, however, protected areas alone are not seen to fulfil the requirements for genetic conservation. These countries have set up specific gene reserve forests. A gene reserve forest consists of a large area (~100 hectares) of natural stands that are managed silviculturally to ensure the potential for successful natural regeneration. Genetic improvement programmes underway in Norway spruce are also important gene conservation units as they comprise tonnes of genetic material maintained *ex situ* in archives such as clone banks, seed orchards, progeny tests and seed storages. Another advantage of the materials maintained for breeding purposes is that they have known genetic properties that can be used to generate new populations with known adaptive and wood-production characteristics (Skrøppa 2003). For this reason, some countries with an active breeding programme, such as Sweden, have not established a separate gene reserve network for conservation of genetic resources.

### GxE, plasticity, stability

The concern over expected climate change has become more pronounced in the last decade. Measures to prepare for climate change have been introduced to Swedish, Norwegian and Finnish programmes for Norway spruce. In the Swedish programme, adaptation to changing climate has been specifically addressed by establishing a network of populations that are bred for adaptation to different combinations of photoperiod and temperature, including combinations that are not present under the currently prevailing climate (Andersson 2002). Genetic testing across a wide range of climate conditions is a prerequisite for selecting genotypes that can perform well in many environments. Both Swedish and Finnish breeding strategies advocate selection of stable genotypes for advanced breeding as a means of meeting the challenge of changing climate.

## Breeding strategies

### Activities

A detailed operational plan is required for all the breeding activities, including timing of field measurements, planting of breeding archives, top-grafting selected clones, flower stimulation and propagation archives, etc., which must be carefully planned and prepared in advance.

Double-pair mating (DPM) is the preferred mating design used for generating the recruitment population. When the DPM crossing scheme is difficult to complete, for example due to delayed flowering, the scheme can and should be relaxed. Single-pair mating (SPM) is sufficient for forwarding the breeding population and is used in lower-ranking strata of the breeding population. Positive-assortative mating (PAM) is used if possible to create a within-population structure to enhance gain in a part of the breeding population, and thus increase potential gain in the production populations.

Clonally replicated field testing with rooted cuttings has proved efficient for Norway spruce and will be the standard procedure in Sweden and Finland in the near future. Somatic embryogenesis (SE) has the potential in the future to replace or supplement cuttings from zygotic seedlings for routine clone testing. Clonal testing of a sufficient number of ramets within each full-sib family combines high accuracy with moderately high selection intensity and short cycle time. A general testing strategy uses factorial designs with, for example, the two basic climate factors temperature and photoperiod. The environmental conditions at the test

sites should be well defined. Among test series, there should exist sufficient genetic connectedness in order to improve testing efficiency. Nursery practices are needed for growing of seedlings and replicating them by rooted cuttings in a way that avoids or reduces possible C-effects (common environmental effects confounded with genetic entries). In clonal testing there is the option of keeping one (or two) ramets in a breeding archive.

The genetic evaluation of field trials is dependent on adequate measurements and continuous accumulation of tree data and pedigree information. The field trials should be evaluated at roughly 20% of the expected rotation time. Routines for optimising experimental designs, connectedness among trials, spatial analysis, correction for competition, efficient measurement of traits etc. are emphasized to ensure that data can be used to infer genetic effects. The continuous accumulation of data calls for new tools, such as TREEPLAN®, for genetic evaluation and managing very large amounts of data, derivation of economic weights and reporting of breeding values.

Within-family selection is the basic concept in breeding. Index selection (combining several traits based on their economic values and heritability) is considered to be more efficient than individual-trait selection. A suitable level of imbalance in selection is often sought to maximise gain, within the limits set by objectives for maintaining genetic diversity. More trees are generally selected from excellent parents and fewer trees from less good parents. Imbalance in founder contribution will be dealt with by introducing optimisation methods maximising gain subject to constraints on relatedness. As time goes on, generations will overlap, which will also affect the selection of trees.

Seed orchards are currently the predominant method to deliver genetic gains from spruce breeding to commercial forestry. Seed orchards may be established vegetatively with grafts, cuttings, rooted cuttings or tissue culture plantlets, or with seedlings (El-Kassaby and Askew 1998). The vast majority of seed orchards are established with grafted propagules of the selected parents. Common problems associated with the production of spruce seed in seed orchards include background pollination, the long unproductive phase before the onset of flowering, infrequent flowering, and damage to seed crops by insects and fungi. There is currently a great interest in vegetative propagation methods such as somatic embryogenesis that would avoid the main difficulties of sexual propagation and offer a more effective means of exploiting the genetic gains made in spruce breeding programmes and transferring them to production forests.

## Population structure

Breeding materials deployed in long-term breeding processes are normally organised in a system of multiple breeding populations with different adaptive and economic targets. Breeding activities are ordinarily carried out within each sub-population by means of recurrent selection and clonal testing. Many programmes have breeding populations that consist of around 50 individuals (Denmark, Norway, Sweden). Correspondingly, the number of populations varies from very small (two populations in Denmark and Slovakia) to quite large (22 populations both in Sweden and Norway) in different programmes. Some programmes with fewer larger populations (such as the Finnish programme with 5 breeding populations, each comprising 160 individuals) deploy other structures, especially sub-lining, to control the development of inbreeding and retard the loss of genetic diversity (Jansson et al. 2013).

## Strategy alternatives: evaluation and comparison

The recent review of the Swedish tree breeding programme explored seven strategies for within-family selection in Scots pine (Table 2.5). The strategies available for pine were explored and investigated with simulation studies including full costing so that genetic gain/year and cost/year and generation could be investigated (Rosvall 2011). The costs of a breeding strategy were largely determined by the number of trees in field tests.

The cycle times have a large impact on annual costs but the annual costs of the most interesting strategies are roughly the same. The options investigated in Scots pine are also applicable to Norway spruce breeding. However, due to the much easier vegetative propagation of young spruce seedlings, compared to that of pine seedlings, the outcome of the analysis of Norway spruce is different. Computer simulations have demonstrated that the current breeding strategy for Norway spruce, using clonal testing, is already the best strategy from all perspectives, genetic as well as economic (Rosvall 2011). Experiences from practical breeding also favour this strategy.

Therefore, only the two main strategies are analysed here, i.e., “Seedling candidates backward” (Strategy 1, Rosvall and Mullin 2013) and “Field forward clonal replicates” (Strategy 6). Both strategies commence with producing the new F+1 generation of full-sib candidates to be tested by seedling or clonally replicated tests. Strategy 1, referred to in Chapter 1 of this report as “Seedling candidates backward” (Rosvall and Mullin 2013) was previously practised as the main strategy in the Swedish tree breeding. First, a breeding archive is established with full-sib F+1 progeny from the current breeding population. These progeny will produce F+2

**Table 2.5.** Characteristics of the alternative breeding strategies (from Rosvall and Mullin 2012).

Strategy	Realised selection intensity	Selection accuracy	Assortment accuracy	Number of crosses	Breeding archive complexity	Field testing	Cycle time	Potential gain per year
1. Seedling candidates backward	Low	High	Precise	2 rounds many PX <sup>1)</sup>	Low	1	Long	Moderate
2. Field candidates backward	High	High	Precise	2 rounds OP <sup>2)</sup>	High	2	Long	Moderate
3. Field forward	High	Low	Moderate	Few	High (few)	1	Short	Moderate
4. Field forward with expanded BP <sup>3)</sup>	Moderate	Moderate	Moderate	Moderate	High	1	Short	High
5. Field candidates polymix forward with expanded BP <sup>3)</sup>	High for mothers Low for fathers	High and low	Moderate	Moderate	High	1	Short	High
6. Field forward clonal replicates	Moderate	High	Precise	Few	Low (many)	1	Short	High

<sup>1)</sup> PX is progeny testing with polymix

<sup>2)</sup> OP is progeny testing with open pollination

<sup>3)</sup> BP is breeding population

progeny (polymix pollination) for a progeny test, after which selection is made backward of the final F+1 breeding population. Some form of early selection may be performed to select the seedling candidates, but the main criterion is to have 10-20 vigorous seedlings. The Swedish review (Rosvall 2011) also lists a more intense variation of this strategy. It is basically identical to the Strategy 1, except that the development of F+1 seedlings is forced by intense cultivation, top-grafting and flower stimulation, to shorten the breeding cycle.

In Strategy 6, “Field forward clonal replicates”, F+1 full-sib progeny are replicated by cloning to produce a population for forward selection. Best clones within each family are selected for a new breeding population. This strategy is the current breeding strategy applied in both the Swedish and the Finnish Norway spruce programmes. This Swedish Review (Rosvall 2011) considers two variants of this strategy, *a* and *b*. In the variant *a*, scions for grafting of selected clones are transferred from the field trials, after assessment, to a grafted small breeding archive to facilitate crosses, while in variant *b* copies of each clone are kept in a parallel large breeding archive. The current Swedish strategy for Norway spruce is based on variant *a*, but slightly adapted to the biology of spruce. Most likely a pre-assessment of the clonal tests will be used to collect cuttings for rooting of

candidate trees instead of using top-grafting after the final assessment. The current Finnish strategy is more reminiscent of variant *b* (taking advantage of clonal tests as potential breeding archives).

The “Seedling candidates backward” strategy combines high selection accuracy with low to moderate cost per year. The main handicap of this alternative is associated with the long breeding cycle time arising from the difficulty of making small seedlings to produce offspring for progeny tests. Although flowering can be promoted in various ways, experience has shown that it generally takes a long time to obtain a sufficient number of progeny from each seedling candidate which prolongs the onset of the testing phase. Consequently, the genetic gain per year from this strategy tends to be only moderate. Computer simulations suggest that the currently applied “Field forward clonal replicates” is more efficient, yet somewhat more costly strategy than “Seedling candidates backward”. At fixed annual investment, the gain per year is clearly higher for “Field forward clonal”. The clonal forward selection alternative is based on the ease of vegetative propagation of Norway spruce by rooted cuttings. The opportunity for clonal testing allows accurate and immediate genetic evaluation of F+1 seedlings, entirely avoiding the long waiting time associated with the flowering

of seedlings in “Seedling candidates backward”. That genetic testing by rooted cuttings is more expensive than progeny testing by seedlings is counterbalanced by the considerably shorter cycle time of the clonal testing strategy. Hence, “Field forward clonal replicates” is likely to yield a higher genetic gain per year than “Seedling candidates backward” (Rosvall 2011; Rosvall and Mullin 2012).

### Guidelines for implementation

- Clarify long-term breeding objectives
- Divide breeding material into multiple breeding populations (in many programmes these consist of around 50 individuals, although number of populations can vary)
- Genetic improvement programmes serve as important gene conservation units - emphasise archives and DNA storage
- Create a detailed operational plan for all the breeding activities.
- Regenerate breeding materials using controlled matings (designed DPM/SPM) with PAM
- Breed often and establish tests - rather than keeping seeds in storage.
- Use vegetative propagules in genetic field testing as a means to reduce breeding cycle and obtain the results cost-efficiently

### Case study/example

We present here a case study that applies to the breeding strategy employed for Norway spruce in Finland, while noting important deviations from the Finnish strategy for that used for spruce breeding in Sweden. Both breeding strategies are based on recurrent within-family selection and clonally replicated testing in multiple populations. The material contributing to the next-generation breeding consists of about 900 trees. The meta-population is structured into five populations, each comprising 160 members. The breeding populations in Finland are larger than those in Sweden, which have about 50 members in each population. Breeding zones are also larger in Finland, where altitudinal variation is considerably smaller than in Sweden. The populations have different climatic targets delineated by temperature sum, thus corresponding to the length of the vegetative growth period. Most breeding materials are representative of the local Finnish populations. In addition, 100 plus-trees of Baltic origin have been included as a supplementary population to be tested along with the domestic populations.

Controlled crosses between progeny-tested plus-trees (grafted in clone banks, seed orchards or pots in the breeding centre) are used primarily to create recruitment

populations. In the Swedish strategy, controlled crosses are mainly performed among progeny-tested plus-trees grafted into conventional rooted archives (grafted in pots in the breeding centre). Both SPM and DPM are used, conforming to the principle of unbalanced investment of resources. Single-pair mating is the basic method of regenerating the bulk of a breeding population, resulting in a single full-sib family per plus-tree (the target family size is 120 individuals). The plus-trees in the top quarter of the breeding population, the 40 highest-ranked trees, are mated twice (additional matings are possible in the top tier). All matings are carried out in a positive assortative manner (PAM): the members of a breeding population are ranked by their genetic value after which the nearest-ranking pairs of trees are assigned as mating partners (some flexibility is practised to ensure a maximum number of matings in each year). In addition to controlled crosses, open-pollinated families in progeny trials have also been deployed as a supplementary recruitment material. This has been done due to practical constraints in breeding zones where sufficient numbers of plus-trees have not been available for crosses. In the Swedish strategy, with its smaller breeding population size, double-pair mating and PAM is the basic method of regenerating the bulk of a breeding population. When single-pair mating is used, it is mainly used for lower-ranked parents. In both programmes, deviations from the basic methods occur due to practical considerations such as female flowering and pollen availability.

In the Finnish programme, the full-sib seedlings are raised in a nursery and screened mainly for vigour and the timing of flushing. Seedlings that show a tendency for early flushing are removed. Eventually, the 30-35 most vigorous seedlings from each family are marked as candidates to be tested more extensively in clonal trials. In some cases, candidates are preselected on their phenotype (phenology, growth, defects) in older, 5-7 year-old progeny trials. When clonal testing is preceded by preselection, a smaller number of trees per family (10-15) are selected as candidates.

In Sweden, the procedure for the screening step is still not decided until good measures for phenology estimations, both for growth start and growth cessation, have been evaluated. One phenology-neutral approach so far is to raise the full-sib seedlings, approximately 120 seedlings per family, in a nursery during the first year of growth. Day/night-length conditions are controlled to avoid height variation due to differences in photoperiodic responses. From late summer, artificial light is used to prolong day lengths and, later, short-day treatments are applied to achieve simultaneous growth cessation. Screening for vigour (height) is then done after the first year of growth and the 40 most vigorous seedlings from each family are marked

as candidates to be tested more extensively in clonal trials. Another approach, until good tools for phenology estimations have been decided, is to sow fewer seedlings and reduce the effect of screening. About 60 seedlings of DPM full-sib families are then sown in a nursery, somewhat more for SPM families. After the first year of growth, abnormal seedlings are discarded and the most vigorous seedlings from each family are marked as candidates to be tested more extensively in clonal trials.

Each set of clonal material is tested preferably at six locations. Four is accepted as a practical minimum number of sites, partly due to difficulties of finding sufficiently uniform sites (formerly agricultural fields) for testing. The testing effort per candidate is six to eight ramets per test location, requiring a total of 24–36 cuttings. The testing effort per candidate is slightly lower in Sweden with four ramets per test location and a total of 16 cuttings. The replicated trials are dispersed to cover a wide range of climate, taking into account the variation both in temperature and continentality. One of the trials is planted in the vicinity of the breeding centre to serve as a breeding archive where future crossings among selected clones are performed. The final assessment is to take place around at age 15–18 even though preliminary selection may take place some years earlier. The field-selected trees of selected clones are promoted to flower in various ways. Selected clones are grafted into the breeding centre (pot grafts, top-grafts) only as a secondary measure, if attempts to promote flowering in the field trial fail. Most likely, regeneration of the breeding population will take place as a combination of both these methods.

The emphasis is on within-family selection to avoid reduction in the effective population size. Each plus-tree will eventually contribute its genes into two members of the next generation breeding population. The 2<sup>nd</sup>-generation breeding population will consist of 40 sub-lines (four genotypes per subline) as a measure to control the development of inbreeding with the breeding population.

## Issues for breeding of Norway spruce in Europe

### Climate change

The uncertainty of future climate needs to be addressed by increasing plasticity/adaptability and resistance of bred materials. To increase plasticity/adaptability, assessments of performance in environments under change are needed. Genetic testing should be carried out over a wider range of climate conditions than today. As a consequence, the exchange of breeding material between neighbouring countries or countries with similar climates, in terms of photoperiod

and temperature, needs to increase. Generalist genotypes should be favoured in the selections, even more so than today. The environmental test conditions in the field need to be better defined in order to select for adaptation rather than testing on sites characterised only by their geographic location. Temporal variation should also be implemented in testing by establishing tests over multiple years. Climate change is also expected to increase the risk of damage from pests and diseases. An increased emphasis on testing and selection to improve tree resistance and tolerance may be needed.

### Shortening of breeding cycle

The adoption of rooted cuttings for clonal testing as the standard procedure in the main Norway spruce breeding programmes has already resulted in a substantial reduction in the length of the breeding cycle compared to strategies based on progeny testing and backward selection (ref. Table 2.5). The remaining main challenge is associated with the regeneration of the breeding population. Norway spruce has a prolonged juvenile phase (20–25 years), thus only a limited number of clones selected have reached flowering competence at the time of selection, which takes place about ten years earlier. This either causes a delay in the breeding cycle, or forces the breeder to select trees with early flowering competence, which might have unexpected side-effects. To avoid the awkward choice, better knowledge is needed of measures to speed up the ontogenic development of juvenile trees. Several such measures to stimulate precocious flowering have been studied, including applications of gibberellin and other growth regulators (Almqvist 2008), girdling, and top-grafting. Top-grafting is a method adopted from fruit-breeding where the selected material is grafted into the crown of reproductively mature clones in order to transfer the flowering competence of the rootstock clone to the top-graft scion (Almqvist 2001).

## New directions

### Epigenetic effects

The influences of the maternal environment on the phenotypic variability of the progeny may have important implications, in particular in the boreal region (Bjørnstad 1981; Johnsen et al. 2009). Results from both field tests and practical plantings have demonstrated that the change in phenotype, in particular of the annual growth rhythm, may influence both survival and other fitness traits. The observed phenomenon suggests that an epigenetic mechanism exists in the developing embryo, being either zygotic or somatic, that senses important environmental signals such as temperature, which in turn influences adaptive traits.

The consequences may either be positive or negative for the plantation, depending on how climatic extremes are related to the timing of growth start, its cessation and development of autumn frost hardiness. The effect should be recognised in practical tree breeding. Consideration should be given to the choice of locations for seed orchards and the conditions under which controlled crosses are made for progeny testing and for generating breeding material for further selection.

### Technologies at the frontier

Marker-assisted selection (MAS), which uses genetic polymorphism detection based on quantitative trait loci (QTL) analysis, has long been proposed as a method to accelerate forest tree breeding. However, QTLs generally tend to show weak linkage disequilibrium among families, which limits the use of significant markers to within-family selection. Genetic polymorphisms for MAS have also been identified by means of association genetics. So far, however, this approach has yielded only a few useful polymorphisms, largely of modest effect, mainly due to the abundance of minor frequency alleles in tree populations (Harfouche et al 2012). In Sweden, research is underway using SNPs to identify candidate genes for the terminal budset. Genome-wide association (Meuwissen et al. 2001) is the most novel approach and seems to have perhaps the greatest potential to become a powerful tool in the selection of valuable traits in combination with conventional methods. Genomic selection has already been suggested to replace MAS in crop plant breeding. It seems to be particularly well suited for species with long generation time such as Norway spruce, as well as for selection on low-heritability traits expressed late in the life cycle (Grattapaglia and Resende 2011; Harfouche et al. 2012; Resende et al. 2012).

A project aimed at sequencing the Norway spruce genome has recently been completed in Sweden (Nystedt et al., 2013).. The results are expected to facilitate the development of genetic markers and dissection of complex traits, and likely lead to applications in breeding; however, neither marker-aided or genomic selection has not yet been applied to breeding of Norway spruce. Introduction of novel biotechnological methods into tree breeding programmes is a fairly slow process and they will not likely influence breeding of Norway spruce much over the next five to ten years (Andersson and Lindgren 2011). Cost-effective markers developed for population-wide application have the greatest potential of becoming rapidly applied in current breeding programmes, e.g., for pre-selection among seedlings for phenology.

One biotechnological method already now in use is the genetic identification of individuals in the breeding stock using DNA markers such as microsatellite loci. Another method close to practical application is vegetative propagation by somatic embryogenesis. Clonal forestry based on somatic embryogenesis has potential to become a valuable tool for intensive wood production, and methods for somatic embryogenesis in Norway spruce are now to a point where operational testing and deployment programmes can be launched (Devillard and Högberg 2004). Technical challenges encountered mainly in the automation of the processes, most important the steps where somatic embryos and plantlets/plants are handled one-by-one, still need to be solved before such programmes can be launched (Högberg 2009).

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3

# Scots pine – *Pinus sylvestris* L.

Seppo Ruotsalainen and Torgny Persson

3

## Introduction

Scots pine (*Pinus sylvestris* L.) is an important species in the many European countries where it occurs abundantly, especially in the countries around the Baltic Sea. In 17 countries participating in the EU Research Infrastructure Action TreeBreedEx, covering most of the Scots pine distribution area in Western Europe, the Scots pine forests cover an area of 41 million hectares (Krakau et al. 2013). This is, however, less than 20% of the total Scots pine forests, as 60% are in the area of the former Soviet Union (Boratynski 1991). In Finland, Poland and Sweden, it is a dominant species on more than 50 % of forest land (Krakau et al. 2013).

## Economic Importance

Scots pine is widely used for sawn products (construction timber), for pulpwood and paper (Mátyás et al. 2004; Mullin et al. 2011). The main countries for Scots pine timber production are Russia, Finland and Sweden (Mullin et al. 2011). In 15 Treebreedex countries, the annual harvest of Scots pine timber is ca. 95 million m<sup>3</sup> (Krakau et al. 2013).

Natural Scots pine forests grow predominantly as pure even-aged stands, but can also occur in mixtures (Mátyás et al. 2004; Mullin et al. 2011). It is regenerated in both natural woodland and cultivated in plantations. In Finland, about one third of the Scots pine regeneration is done naturally (FFRI 2011). In many countries, most of the regeneration material for plantations comes from seed orchards, e.g., 80% in Sweden, 70% in Germany, and 50% in Finland (Krakau et al. 2013).

As Scots pine is planted over such large areas, its genetic improvement can have a massive impact. For instance, it can be estimated that in Finland some 800 000 ha of Scots pine has been regenerated to date with seed orchard material (corresponding to 16% of all Scots pine cultivations and 6% of all Scots pine dominated forests). The current Scots pine

forests that have been established with genetically improved seeds can be estimated to produce some 370 000 m<sup>3</sup>/ha/yr more wood than if the same stands had been regenerated with natural stand seed. In the future, this added growth can be considerably increased by expanding the area cultivated with seed orchard material and by further improvements in the genetic gain.

From a private forest owners point of view, the use of seed orchard seed in direct sowing at an extra cost of 70 € per hectare was economically feasible with increases in bare-land values per hectare of several hundred euro when assuming genetic gain of 7% in height and diameter and a discount rate of 3% (Ahtikoski et al. 2012). At least in Finland, planting genetically improved material is even more profitable as the nurseries do not transfer the extra seed price to the price of seedlings. Thus, planting stock has the same price irrespective of its level of genetic improvement.

The value of forest product exports in 2010 was €13 440 million in Sweden, which accounts for 11% of all exports and 4% of GDP (<http://www.skogsstyrelsen.se/>). In Finland, the corresponding value for the same year was €11 000 million, which was 21% of all exports and 6% of GDP (FFRI 2011). The Swedish forestry sector employed 95 700 people in 2010 (2.1% of all workers) and the Finnish forestry sector 69 000 people (2.8%). The contribution of Scots pine to these figures is roughly 30-40% when calculated from the share of total harvest volume.

## Historical perspective and current situation

Scots pine has been planted in Central Europe since the early 19<sup>th</sup> century. It has been planted for timber, covering areas disturbed by mining, etc., and for establishing shelterbelts for protection from wind (Boratynski 1991). Both in Sweden and Finland, Scots pine planting really started in the 1950's. Scots pine has been traditionally cultivated in Finland by direct sowing since the beginning of the 20<sup>th</sup> century, and even currently more than half of artificial

regeneration is by this method. There are currently about 4.8 million hectares of planted or sown Scots pine forests in Finland, which is about 1/3 of all pine forests (Varmola 1996; FFRI 2011). In recent decades, the importance of Scots pine in forestry in many European countries has diminished and amount of planting has similarly decreased (Mikola 1991).

Plantings have been made in Central Europe extensively with foreign provenances (Mátyás et al. 2004). In northern Europe, the use of foreign provenances has been more limited, although in northern Finland large amounts of seed from central and southern Finland were used in the 1960's and earlier due to lack of local seeds (Pohtila 1979). Many of these uncontrolled long-distance seed transfers have led to unwanted results with regards to the adaptation of the trees, their quality or growth (de Vries 1990, Krabel et al. 2010). There are currently 534 Scots pine seed orchards covering an area of 4928 ha in 20 European countries (Table 3.1). The largest areas of seed orchards are in Finland (1739 ha), Sweden (835 ha) and Poland (672 ha). About 14% of the seed orchards belong to the tested category. As compared to the situation described 25 years ago by Mátyás (1991), the total area of seed orchards has clearly decreased (831 ha), mainly due to the great decrease of seed orchard area in Finland. Minor decreases have occurred also in most other countries, except in Sweden and Poland, which have considerably increased their seed orchard areas.

According to the statistics given by Krakau et al. (2013) about 600 million Scots pine seedlings were planted annually in 11 Treebreedex countries at the millennium shift. The greatest Scots pine planters are Poland (387 million seedlings), Sweden (123 million) and Finland (55 million). In later years, the trend has been toward a slight reduction in planting levels.

Genetic studies on Scots pine started as provenance research that was motivated at least partly by the poor success of many haphazard seed transfers in Europe (Giertych and Oleksyn 1992). The first known Scots pine provenance experiment was established by a French nurseryman called de Vilmorin in 1820. Later on, huge numbers of provenance experiments were established across Europe (Krakau et al. 2013), and several international test series were also established, the first being the IUFRO trial in 1907 (Giertych 1991b). In fact, Scots pine is likely the most intensively studied tree species as regards provenance questions (Mullin et al. 2011).

Olof Langlet (1936) demonstrated that the dry matter content of Scots pine needles in autumn increases clinally with latitude of origin and thus reflects the adaptation in development of frost hardiness in the trees. The early observations by Kalela (1937) sum up nicely the dependencies:

when southern or maritime provenances are compared to more northern or continental ones, they usually show better growth rate, later growth onset and cessation, poorer quality and lower survival. It depends on the local growing conditions which of these factors is most decisive for the forest production. In harsh conditions in northern Europe, short transfer from north to south increases survival and thus the volume production per hectare. In more favourable conditions in central Europe, northern provenances are inferior in growth to local ones (Eriksson 2008). At the species margins or other extreme site conditions, local populations have proved to be the ones best adapted (Mátyás et al. 2004).

In addition to provenance studies, Scots pine has also been the focus of intensive studies on flowering, seed production, genetic diversity, genetic variation within populations, etc. (Sarvas 1962; Koski and Tallqvist 1978; and reviews by Giertych and Mátyás 1991; Ericsson 2008). These studies have been much inspired and facilitated by the operational breeding of Scots pine that revealed important gaps in knowledge to be filled and provided researchers with plenty of suitable material for their studies.

Breeding activities for Scots pine sprouted from the genetic research that was initiated more than one hundred years ago (Krakau et al. 2013). The breeding started often as simple mass selection, but special breeding plans were created in many countries between the 1950s and 1980s. Since then, breeding programmes have been suspended in several countries, so that active breeding of Scots pine is currently going on only in Sweden, Finland, Lithuania and France. In the other countries, activities are concentrated on testing and use of seed orchard material (Krakau et al. 2013).

The first round of plus-tree selection and establishment of seed orchards has led to considerable improvement in volume production and quality of Scots pine. According to young field tests, the volume growth of first-generation seed orchard material is usually 10-20% higher than that from natural stands (Mullin et al. 2011). In many countries, improvements in stem quality have also been reported. The greatest efforts for improving quality have taken place in France, where stem straightness and branch angle have been considerably improved and forking has been decreased in both the indigenous and foreign breeding populations (Krakau et al. 2013).

When long-term breeding programmes were initiated, it was observed in some countries that the initial number of plus-trees was too small, which necessitated additional selections. There were also problems in the functioning of seed orchards. The synchronisation of female and male flowering was not always good and background pollination decreased the realised genetic gain. In cases where seed orchards have been transferred to more favourable climates, pollen

**Table 3.1.** Number and area (hectares) of Scots pine seed orchards in different European countries, divided into "qualified" and "tested" categories in 2012 and at the end of 1980's. Current situation is based mainly on the EU Community list<sup>a)</sup> and Krakau et al. (2013). The situation in the 1980's is based on Mátyás (1991).

Country	Current situation						1980's total area	Gain (loss) in total area
	Qualified		Tested		Total			
	Number	Area	Number	Area	Number	Area		
Austria <sup>b)</sup>	7	14			7	14		
Bulgaria	4	10			4	10		
Czech Republic	29	107			29	107		
Denmark	4	7			4	7	9	(2)
Estonia	12	164			12	164		
Finland	94	1560	10	179	104	1739	3100	(1361)
France	2	15			2	15	17	(2)
Germany	44	173	10	30	54	203	238	(35)
Great Britain			4	14	4	14	23	(9)
Hungary	6	53	5	46	11	99	140	(41)
Ireland	2	3			2	3	4	(1)
Latvia	53	472	4	132	57	604	700	(96)
Lithuania	18	186	17	120	35	306	325	(19)
Netherland <sup>b)</sup>	3	15			3	15	10	5
Norway <sup>b)</sup>	2	7			2	7	12	(5)
Poland	77	672			77	672	285	387
Romania	11	57			11	57		
Slovakia	23	57			23	57		
Spain	3	11			3	11		
Sweden <sup>b)</sup>	64	678	9	157	73	835	477	358
<b>Totals</b>	<b>458</b>	<b>4261</b>	<b>59</b>	<b>678</b>	<b>517</b>	<b>4939</b>	<b>5340</b>	<b>(821)</b>

<sup>a)</sup> [http://ec.europa.eu/food/plant/propagation/forestry/index\\_en.htm](http://ec.europa.eu/food/plant/propagation/forestry/index_en.htm)[accessed 19-01-2012]

<sup>b)</sup> Krakau et al. (2013)

contamination from local sources has led to difficulties in the utilisation of the seed crops, as they are not sufficiently hardy for the more northern target areas (Pulkkinen et al. 1995).

In Finland, seed procurement for the northern areas of the country was intended to be carried out with seed orchards established with northern plus-trees in central Finland, motivated by higher seed production and better seed ripening in the warmer climate. Based on studies in flowering of Scots pine, it was estimated, that the genetically different temperature-sum requirements for flowering would be great enough for isolating temporally the seed orchards of northern origin from the surrounding population (Sarvas 1970). In fact, most of the Finnish Scots pine seed orchards were similarly transferred, because there was an intention to produce large amounts of Scots pine seed for direct sowing in northern Finland (Hagman et al. 1963). However, the high levels of background pollen contamination proved them unsuitable for their original purpose (Pakkanen et al. 1991; Mikola 2010). Their seed has since been utilised on the intermediate area, but large areas of these orchards have been decommissioned (Table 3.1). One reliable option to decrease the maladaptation from contaminating pollen is to establish the seed orchards in the same breeding zones as their ultimate deployment.

### Genetic resources and biology

The natural distribution of Scots pine covers about 33 degrees of latitude from south (Spain) to north (Norway), about 3700 km. In the longitudinal direction, the distribution of Scots pine stretches over a distance about 10 000 km from Portugal and Scotland close to the Pacific Ocean in Russia (corrected from Boratynski 1991, Maria Varela, pers. comm.). It must also be noted that Scots pine earlier had a larger natural distribution in western Europe, but became extinct from areas such as the Netherlands and Ireland (de Vries 1990; Krakau et al. 2013). Scots pine occurs from sea level to 2 600 m (Boratynski 1991). In the boreal forests in northern Europe, it is a dominant species and in Europe as a whole it grows naturally in about 30 countries (Figure 3.1); in more than 10 of them it is a ubiquitous species with distribution covering most of the country (Mátyás et al. 2004; Mullin et al. 2011). In addition, it has been cultivated in several countries outside its natural distribution area (Boratynski 1991).

The northern limit of distribution is determined by continentality (Boratynski 1991) and low temperatures during the growing season (Veijola 1998). Scots pine is not as continental a species as Norway spruce (*Picea abies* [L.] Karst.), which gives rise to a distribution area that penetrates further west and north in the maritime regions in western

and north-western Europe (Sarvas 1964; Hustich 1966). Scots pine seems to be unable to grow in areas of permafrost (Hustich 1966; Boratynski 1991). The southern limit is determined primarily by soil edaphic factors and moisture, as well as by human influence (Boratynski 1991). Distributional limits of Scots pine in temperature sums (+5 °C threshold) vary from 500 to 2400 degree-days (Prentice and Helmisaari 1991). The distribution area is not constant, but responds over time to climatic changes by extending further north with climatic amelioration and retreating with deteriorating climate (Hustich 1966; Sirén 1998).

Scots pine has experienced several cycles of glaciation in Europe during the last millions of years and consequently has been forced to retreat and recolonise multiple times. By using palaeoecological and DNA techniques it has been possible to identify several possible refugia in southern Europe (Spain, northern Italy, etc.), where Scots pine survived during the last Ice Age, and from which it later recolonised more northern areas (Hyvärinen 1987; Krakau et al. 2013). According to recent studies, Scots pine may have survived the last Ice Age in other refugia located south of the permafrost and north of the European peninsulas (Salmela 2011) and even on the Norwegian coast (Kullman 2002; Parducci et al. 2012). At the species margins, especially in the south, the distribution is very dispersed and concentrated on the most favourable sites (in the south the mountain areas, in the north sheltered valleys) (Mátyás et al. 2004; Eronen 1979).

Scots pine is a light-demanding pioneer species that regenerates naturally after catastrophic events, such as forest fires. It also benefits from disturbances caused by human activities (Mátyás et al. 2004). Because it does not compete well with broadleaved species and herbaceous weeds, it usually does not manage to occupy fertile sites and in natural conditions is confined to poorer, sandy and rocky sites or peat lands (Mátyás et al. 2004; Krakau et al. 2013). With good care and tending, Scots pine can also be grown on more fertile sites under managed conditions.

Scots pine has relatively thick bark at the base of the trunk that is an adaptation to resist fire (Nikolov and Helmisaari 1992). It has a rather extensive and deep root system with a taproot. It also uses, like other conifers, symbiotic fungi for forming mycorrhizae to assist in nutrient extraction from the soil (Woodward 1995).

Scots pine can reach large dimensions and considerable age. The maximum values are 45 metres, 150 cm and 780 years for height, diameter and age, respectively (Krakau et al. 2013). In practical forestry, trees are shorter lived and the dimensions considerably smaller. For instance, in southern Finland, the rotation age of naturally regenerated forests at a site with medium fertility is reached at 80 to 100 years

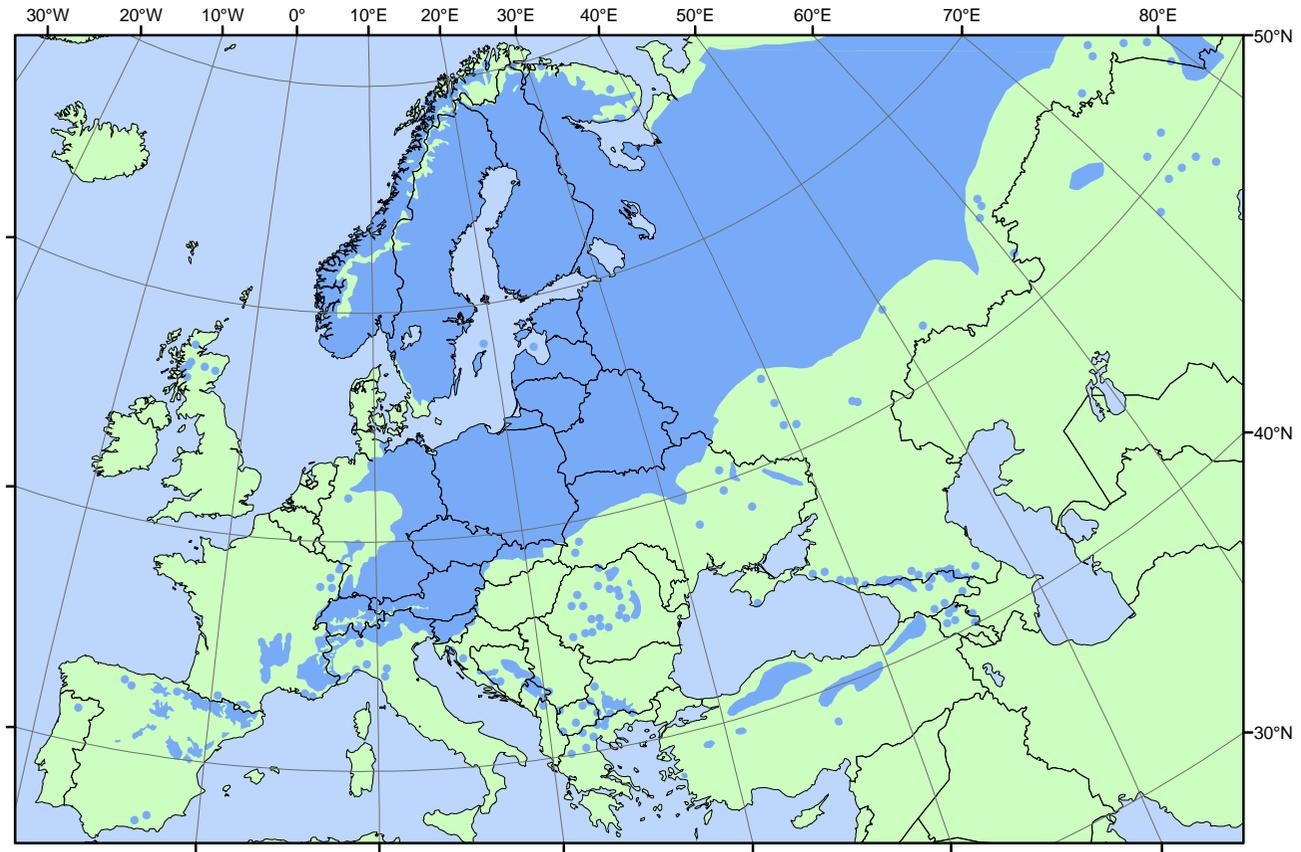


Fig. 3.1. Natural distribution area of Scots pine in Europe, compiled by members of the EUFORGEN Networks (EUFORGEN 2009).

age when the trees are about 22 metres tall and 25 to 30 cm diameter at breast height.

Scots pine is a wind-pollinated, monoecious species with prolific flowering that commences at a rather young age (10–30 years, depending on stand density). In grafts, flowering begins some years earlier than in saplings (Chalupka 1991; Mátyás et al. 2004). Female flowering starts somewhat earlier than male flowering (Mátyás et al. 2004). Seed development requires almost three years from the initiation of female flower primordia in the bud to maturation and release of seed. In the first year, the female strobilus primordia are formed within the developing buds, and flowering and pollination take place in early summer of the second year. The female flower develops into a small conelet in the flowering summer and overwinters in such a state. Fertilisation and cone maturation occur in the third summer, and the cones open and shed their seeds the following spring (Koski 1991). For silvicultural purposes, the cones can be collected in the autumn or the winter. Close to northern and alpine timberlines, cold summers restrict seed maturation

leading to poor regeneration in most years (Kujala 1927).

In natural stands in southern Finland, the seed crop in average years is about 3.5 kg/ha on fertile pine soils (Myrtillus type) (corresponding to 900 000 seeds/ha), and the average annual pollen production is 35 kg/ha. The maximum values for both female and male flowering can be 3- to 4-fold the average values (Sarvas 1962; Koski 1991). Seed and pollen production increase with dominant height of the stand (Sarvas 1962). Seed crops are also larger at more southern latitudes (Koski 1991). Heavy pollen crops lead to effective outcrossing, but monoecism, overlapping female and male flowering of an individual, and absence of any self-incompatibility systems lead inevitably to some degree of self-pollination. However, competition among multiple fertilised archegonia within the seed ovule likely eliminates selfed individuals expressing embryonic lethal genes, or postzygotic selection favours non-selfed progeny later in the seedling or sapling phases, so that the mature Scots pine forests are predominantly of outcrossed origin (Kosinski 1991; Koski 1991).

Being a pioneer species makes Scots pine well-suited for cultivation. It can be regenerated without any special problems on clear-cut areas, either by direct seeding or planting. Rather early and regular flowering also facilitate breeding activities and seed production. Scots pine is also amenable to grafting (Mátyás 1991). Especially important for practical seed production, it does not suffer from any major seed-damaging pest or fungus; only pine cone weevil (*Pissodes validirostis*) has been reported to cause occasionally remarkable seed crop losses (Eidman and Klingström 1976).

Due to its wide distribution over many climatic conditions, there is lots of genetic variation within the species. The variation is usually clinal, as mainly contiguous distribution and effective pollen flow prevent local specific adaptations. The decisive adaptation is the length of growing season, so that northern and continental origins have genetically shorter growing periods than their southern or maritime counterparts. Also, stem and crown form show adaptive differences among the populations, so that trees from northern areas or high elevations have straight stems and narrow crowns adapted to shedding snow (Mátyás et al. 2004).

A further contribution to the large amount of genetic variation within populations is the effective wind pollination that introduces genes even from distant populations (Mátyás et al. 2004). There are many studies concerning different aspects of genetic variation and heritability of Scots pine, as summarised by, e.g., Giertych and Mátyás (1991), Eriksson (2008) and Mullin et al. (2011).

## Breeding objectives

The general breeding objectives of economic importance for Scots pine are similar across European countries and include improved growth performance, quality (stem, branch and wood properties), survival and resistance against biotic and abiotic stresses. In addition, programmes often stress stable performance covering a range of sites and climatic conditions (Mullin et al. 2011).

Examples of main objectives found within European countries are: adaptation, growth and branch characters in Finland (Haapanen and Mikola 2008); improved adaptation/survival, yield and stem quality in Sweden; growth traits, stem straightness and branch quality in Latvia (Jansons and Haapanen 2009); height, survival and stem shape in the Czech Republic (Mullin et al. 2011). In France, special emphasis has also been put on resistance to pathogens and adaptability to poor sandy sites, whereas genetic conservation is of high importance in Spain and Poland (Mullin et al. 2011; Bastien 2008). In Finland and Sweden, adaptation to harsh environmental conditions is a fundamental goal.

Selection criteria meeting the general objectives are also

often similar across countries. Of prime interest are wood production (volume, height and diameter), but quality traits describing the stem form and branch characters (diameter, angle, forking) are also important. In addition, adaptability measured primarily as survival ability is important in many breeding programmes (Mullin et al. 2011).

Information about genetic parameters is available for many selection traits in Scots pine (see reviews, e.g., Eriksson 2008). Growth, quality and adaptive traits have been found heritable with significant genetic variance (e.g., Haapanen 2001; Persson and Andersson 2003; Hannrup et al. 2008). According to Hannrup (1999), phenological traits have high additive genetic variation and also high heritability; growth traits have also high genetic variation, but lower heritability, whereas wood characteristics in contrast exhibit little genetic variation, but high heritability. High or moderate heritabilities have also been reported for fungal disease and pollutant resistance as well as tree form (Stephan 1991; Oleksyn 1991; Giertych 1991a). Estimates of genetic correlation between tree heights or diameters at different test sites are commonly reported as moderate to high (Haapanen 1996; Zhelev 2003; Persson et al. 2006; Hannrup et al. 2008), demonstrating low genotype-by-environment interaction for these traits. Reported inter-site genetic correlations for survival exhibit a more heterogeneous pattern, with larger variation in both magnitude and reliability compared to growth characters (Persson et al. 2006). The heterogenic pattern among inter-site correlations for survival is probably attributable to across-site variation in the underlying factors causing field mortality.

## Diversity and genetic resource conservation

Relatively common alleles are of greatest importance, from the point of view of species adaptation to current situations and stand stability. While rare alleles do not contribute much to the current variation and diversity simply because of their rarity, rare alleles have significance in the long term evolution of the species. Theoretically it can be calculated that a meta-population with a census number between 500 to 1000, divided to smaller populations bred for different targets, is large enough to maintain enough genetic variation for sustainable long-term breeding (Namkoong 1984; Danell 1993). According to Eriksson et al. (2006) common tree species included in intensive breeding programme under multiple population breeding system does not necessarily need any other measures for gene conservation. The *ex situ* breeding material takes care of the gene conservation.

There are 15 countries in Europe from the 30 countries participating the European Forest Genetic Programme (EUFORGEN) which have established some specific gene reserves (gene conservation units) for Scots pine. Altogether

**Table 3.2.** The number and area (hectares) of Scots pine gene conservation units by countries in Europe.

Country	Units	Total area
Austria	26	962
Bosnia and Herzegovina	15	142
Croatia	1	18
Estonia	4	1137
Finland	21	3614
Germany	2	43
Latvia	10	2880
Lithuania	64	1604
Luxembourg	3	32
Poland	8	135
Romania	34	302
Serbia	1	12
Slovakia	14	1106
Spain	7	30 <sup>a)</sup>
Turkey	18	1715
<b>All</b>	<b>228</b>	<b>13 733</b>

Sources: <http://portal.eufgis.org/data.html> [Accessed 13-02-2012] and Michele Bozzano, pers. comm.

<sup>a)</sup> Estimated area, exact figures not available

these stands number 228 and total area almost 14 000 ha; in some countries there is only one stand (e.g. Croatia), but in Lithuania as many as 64 (Table 3.2). By area most gene reserves have been established by Finland, Latvia and Turkey. At least in Luxembourg also non-autochthonous stands are selected as gene reserves. The sizes of the gene reserves vary from a few hectares to several hundred hectares. The minimum requirement is, that the tree population is large enough to allow natural evolutionary processes within the stand (EUFGIS 2008). However, Mátyás et al. (2004) set as a minimum size for gene conservation units of Scots pine 100 ha, which leaves many of the existing gene reserves below the limit.

The countries that have established specific gene reserves for Scots pine are mainly those countries with no or low breeding effort for the species. This follows the deductions of Eriksson et al. (2006) that in the case of intensive breeding,

gene conservation can be connected to the management of breeding material. Finland and Lithuania can be regarded as deviating in this respect; they have both intensive breeding programmes and *in situ* gene reserves. Also Sweden is planning to establish a network of gene reserve forest (based on the existing nature protection areas) for Scots pine in the future (Black-Samuelsson 2012).

In Europe, especially in the native areas in the south west and south east, there is a good coverage of gene reserves, which is justifiable, as these are in many cases physically threatened small populations that can harbour rare specific genes and adaptations (Mátyás et al. 2004). The northern edge of the distribution area is not as well covered by gene reserves, but the risks for population extinction are not as obvious there as in the south.

## Breeding strategies

Around Europe, the amount of breeding activity is linked to the economic importance of the species. Breeding programmes were introduced in several countries between 1950 and 1980. In most countries, breeding started with founder plus-trees selected in natural or artificially regenerated stands (Krakau et al. 2013), mostly within-country, but also of foreign origin (Germany, France, Finland, Sweden). Generally, first-generation seed orchards were established using the non-tested first round of plus trees. From this starting point, there are large differences in how the different countries have continued and further developed the breeding programme. A large number of genetic field trials have been established across Europe. To date, around 250 provenance trials and 1300 progeny test have been established (Krakau et al. 2013). Sweden, Finland, Lithuania and France are today using more advanced breeding methods (Krakau et al. 2013).

Realized gains from Scots pine breeding activities are generally obtained by harvested seed crops from clonal or seedling seed orchards (Mullin et al. 2011). Vegetative propagation methods are at present not used in large scale for plant production.

Deployment of regeneration material of Scots pine is regulated differently across European countries. The European Union has prescribed common guidelines for classifying the forest regeneration material and directed the information to be given with it (EU 1999), but each country then applies its own rules for the use of the material.

Some countries are rather restrictive in the transfer of forest regeneration material, whereas in others transfer of the material is used as a basic tool for improving the success of forest regeneration and growth. A large number of European countries (Poland, Lithuania, Slovakia, Czech Republic, Germany, Italy, Spain, Romania, Austria) delineate the deployment areas for improved material according to regions of provenance (Krakau et al. 2013). On the other hand, Sweden and Finland determine the utilisation areas for forest regeneration material on the basis of climatic adaptation (Nikkanen et al. 1999; Rosvall 2003; Anon. 2006). In the climatically harshest areas in Sweden, it is recommended to transfer regeneration material several hundred kilometres southwards to improve survival and productivity (Persson 1994).

Material transfer from other countries is allowed in 13 of 19 European countries investigated (Krakau et al. 2013). Of course countries with no indigenous pines must rely on foreign material. In some cases, such countries can develop better-adapted land races with such introduced material. For instance, in the Netherlands, material from the local

non-autochthonous stands has been superior to foreign provenances in comparative trials (de Vries 1990).

## Activities and population structure

In Finland, pine breeding, together with that for other species, has undergone great changes during the last decade, when the geographically delimited breeding zones were replaced with six climatically defined target areas and the sizes of the breeding populations were greatly reduced (Anon. 1989; Haapanen and Mikola 2008). This reduction was motivated by new results concerning the optimum size for breeding population (Namkoong 1984; Danell 1993) and is accomplished by selecting backward the best plus-trees based on the results of progeny tests. Breeding populations are divided into sublines according to the breeding values of the plus trees. Sublining facilitates selection of unrelated trees for seed orchards, and when sublining is done in an assortative way, there remains options for higher genetic gains, when only the best sublines are utilised for seed production (Ruotsalainen and Lindgren 2000).

The intended breeding population sizes are 160 for the most target areas, except for the northernmost zone, where the aim is for just 60 trees. There is also no long-term breeding to be practiced in that zone, but the best 60 trees are selected to fulfil the needs for seed procurement for that area (Haapanen and Mikola 2008). One breeding population of size 60 trees with Estonian, Swedish and southernmost Finnish material will also be established, in preparation for the warming climate. Altogether these separate breeding populations sum up to a metapopulation of about 1000 trees (in the two southernmost target areas the breeding population sizes are due to historical reasons somewhat larger than intended). As the number of tested first-generation plus-trees (candidate population) is about 5800 trees, this means re-selection of the best 17% (selection intensity  $i = 1.5$ ) of trees to breeding population following progeny testing.

The trees in breeding population are crossed mainly with single-pair mating within the sublines to create the next generation recruitment population. The best 25% of the trees are, however, used in 2 or 3 crossing combinations. The targeted size for the full-sib families is 120 progeny, in the best families somewhat higher, in the lower-ranking ones lower. The recruitment populations are planted on 2-3 environmentally uniform test sites within the target area. At about 15 years of age, 5 to 20 best trees (depending on the genetic value of the family) are selected within the families to create the candidate population. The candidates are progeny tested with open-pollinated material in 4 to 6 progeny tests that are spread over the target area, and possibly also outside it, to test the climatic robustness of the material. Testing time is about 15 years and 80 to 120 offspring are used to

test each candidate. In both selection of the candidates and progeny testing, the selection criteria are adaptation, growth and stem quality (Haapanen and Mikola 2008).

Currently, crossings have been completed in the two southernmost target areas, and the second generation recruitment populations are growing in the field tests. Crossings are underway for the third target area, and the selection of the first-generation breeding population will be made within one year for the remaining three northern target areas.

The Finnish breeding plan follows Strategy 2 in Rosvall and Mullin (2013), where six breeding strategies for within-family selection in Scots pine (representing the majority of options available) are outlined.

In France, Scots pine is mainly used in afforestation on the low elevation areas in northern and central France. Selection criteria are endurance of summer drought, stem quality and wood density (Bastien 2008). Three breeding populations were selected based on the results of provenance tests. Two of them are native French populations and one is of Polish origin. The French population “Haguenu” displays good volume production but poor stem form, whereas the other native population “Bitche” has lower volume production but better quality. Also, the Polish population “Taborz” is a compromise between volume growth and quality. The number of selected  $F_0$  plus-trees is somewhat above 200 for both native populations and slightly below 200 for the Polish population. Progeny testing using open-pollinated seed was performed on three sites. In addition to this, progenies produced by selfing have also been used in testing. Selfing is used to purge the deleterious alleles from the population. After testing, about 100 plus trees from each population will be selected into the breeding population. The selected trees are double-pair mated within each population to produce the next-generation recruitment population (Catherine Bastien, pers. comm.).

In Sweden the majority of the 6000 founder plus-trees in the first generation are of indigenous origin, but plus-tree materials from Finland, Norway, Russia and Estonia have also been introduced (Danell et al. 1993). The majority of the first-generation plus-trees are now progeny tested. The first-generation progeny trials contain full-sib families (partial diallel mating scheme) or half-sib material, either from polycross crossings or open-pollinated seed collected in the original plus-tree stands. Trees have been evaluated, selected and crossed to form the second generation. The selected trees will form a meta-population of around 1500 best trees, further divided into 24 breeding populations (with 50-70 individuals in each sub-population) and linked to target areas defined by photoperiod and temperature climate (Danell et al. 1993).

Within each breeding population, the candidate trees will be crossed using a double-pair mating (DPM) design with positive assortative mating (PAM) (i.e., each tree is crossed with two parents and in rank order). Until recently, testing has generally followed one of two alternative strategies: Strategy 1 “*Seedling candidates backward*”, or Strategy 6 “*Field forward clonal replicates*”, as described by Rosvall and Mullin (2013). In Strategy 1, 10-20 full-sib  $F+1$  seedlings per family are planted in breeding archives where they are mated using controlled polymix pollination. Progeny tests are established to select backward (one selection per DPM family) a breeding population of size 50. In Strategy 6,  $F+1$  progenies are clonally replicated and planted on four test sites to select forward a  $F+1$  breeding population of size 50. Quite recently, the Swedish programme has introduced Strategy 4 “*Field forward with expanded breeding population*” as described by Rosvall and Mullin (2013) and described in greater detail in the case study section below.

In Lithuania, a meta-population of about 500 founder plus-trees was selected from six provenances covering the whole country (Krakau et al. 2013). Clones of these trees were planted in clonal archives and seed orchards. The first breeding cycle also involved progeny testing of the selected founder trees. Progeny tests were established in different parts of the country and three breeding populations were formed based on progeny test results. These test results have also been used to establish 139 hectares of 1.5- and 2<sup>nd</sup>-generation seed orchards. A second breeding cycle is planned, but no decision of suitable breeding strategy has yet been taken.

There has also been previously tree breeding for Scots pine in many other European countries, but currently the breeding activities in these are at a low level with no plans for advanced-generation breeding (Mullin et al. 2011; Krakau et al. 2013).

## Case study/example

We refer here to Strategy 4 “Field forward with expanded breeding population” (Rosvall and Mullin 2013) for our case study of Scots pine breeding, as currently conducted in Sweden. In the Swedish breeding programme, the strategy will be practiced, mostly in those breeding populations where  $F+1$  field experiments already exist. These field trials originated from crossing 50 tested founders per population in breeding archives using DPM under PAM. The benefit from using PAM is that the genetic gain in selected seed orchard populations can increase without reducing genetic diversity in the breeding population. One hundred seedlings per  $F+1$  full-sib family were planted in five field experiments (in total 25000 seedlings). At 3-5 m average tree height

(around 20% of the rotation time) the field tests are measured.

On average, two candidates per F+1 full-sib family (in total 100 candidate trees) are selected forward in the field, based in estimated BLUP breeding values. Scions from selected trees are collected and top grafted into a breeding archive (5 scions per clone, two clones per established interstock). Flowering is promoted by flower stimulation treatments, such as gibberellins. Pollen collection and controlled crosses are performed on the top grafts in the breeding archive using DPM and PAM, forming a new recruitment population with 100 F+2 full-sib families.

The number of F+1 selections per family in the crossing scheme forming the new F+2 generation of size 100 is allowed to vary (preferably more selections from the top ranking families). The imbalance among selected parent trees can be optimised using mathematical programming that simultaneously maximises gain, subject to a constraint on group coancestry. The inputs to the algorithm is predicted breeding values and pedigree of the candidates. The increased population size of the “expanded” breeding population, combined with relaxing the restriction of completely balanced within family selection, will promote selection among the top-ranking families, which will form an “elite” that can increase genetic gain in selected production populations.

Approximately 250 progeny per F+2 family are planted across four field trials (in total 25 000 seedlings). When the F+2 experiments are ready to be measured, BLUP breeding values estimates are available for all progeny, as well as for their relatives. With the additional families in the “expanded” population, some selection can be applied among-families, while still achieving a target constraint on relatedness within the subpopulation arising from 50 founders. Forward selection and crossing work follow the same design as for the foregoing F+1 generation (the breeding cycle is repeated).

## Issues for breeding of Scots pine in Europe

The differing environmental conditions and role of Scots pine in forestry in different countries pose difficulties for wide ranging international cooperation in Europe. However, countries sharing similar interests and conditions have already been cooperating e.g. by exchange of breeding material. This will probably become increasingly more important, when tackling the challenges of climatic change (Krakau et al. 2013).

The large array of provenance experiments provides possibilities for developing common basis for delineating deployment areas for forest regeneration material. However,

the practical applications must usually be developed country-by-country, as the administrative regulations differ. These experiments can also be used to study the effect of climate change on the growth and condition of Scots pine.

European countries can learn from each other. Shortening of breeding cycle by developing vegetative propagation methods for testing and by enhancing flowering is of prime importance for effective breeding work (Rosvall 2011). The Swedish web-based decision-support tool “Plantval” for assigning most suitable forest regeneration material to the planting site (Knowledge Direct 2011) is one example of developments, which are worth considering also in other countries.

## New directions

Changing environmental and market conditions set new challenges for the breeding objectives, as trees in forest face new threats and limiting factors and the marketplace demands new products. Climate change can expose the trees to new damaging climatic conditions, associated with new pests and pathogens. Wood is required today in increasing amounts for non-traditional uses such as energy, chemicals and composite materials as well as solid timber for construction. Although the possibilities to modify the composition and chemical content of the wood by breeding are limited compared to industrial processing technology, there can appear opportunities for specific sub-populations aimed to some specific products (Andersson et al. 2011).

Preparation for climate change is already considered in some breeding programmes by multiple breeding populations and testing of the material in different climatic conditions to select for robustness and stability (Haapanen and Mikola 2008; Andersson et al. 2011). An example of being prepared for new threats is the newly initiated co-operation between Finland and Japan to test Finnish Scots pine breeding material against the pine wood nematode (*Bursaphelenchus xylophilus*).

There are also efforts to breed for new wood-based products. High resin acid content of Scots pine heartwood is connected to its decay resistance (Harju et al. 2002) and resin acids show high heritabilities (Ericsson et al. 2001). The resin acids are also the main components in crude pine oil, which can further be processed to biodiesel. Resin acid content has high genetic correlation with phenolic compounds, which is another group of chemicals in the heartwood increasing decay resistance (Ericsson et al. 2001). Phenolic compounds (e.g., stilbenes) are also responsible for the resistance of Scots pine to attacks by biotic and abiotic stress factors (Chong et al. 2009). So, breeding for better decay resistance of construction wood is also beneficial when

aiming for higher productivity for biofuels and creating more pest- and pathogen-tolerant forest regeneration material.

As breeding for generations in closed populations will inevitably mean accumulation of some degree of inbreeding, it is important to consider tolerance to inbreeding, so that inbreeding depression will decrease the genetic gain obtained by selection as little as possible. For that purpose methods like selfing tests in progeny testing applied in France (Krakau et al. 2013) and inbreeding among close relatives currently being studied in Sweden (T.J. Mullin, pers. comm.) are interesting innovations.

New biotechnological methods are not expected to greatly influence the breeding of Scots pine over the next five to ten years (Andersson and Lindgren 2011). Even in the best case, these methods add to the present tool kit of tree breeding, but do not revolutionise it. The extent of use of many new technologies depends not only on their technical applicability but on their scale and price.

One biotechnological method already now in use is the genetic identification of individuals in the breeding material using DNA markers. Another method close to practical application is vegetative propagation by somatic embryogenesis. This would give huge benefits in shortening the breeding cycle by enabling clonal testing. Somatic embryogenesis would also facilitate the delivery of highly improved forest regeneration material to forestry (Andersson and Lindgren 2011).

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# Maritime pine – *Pinus pinaster* Ait.

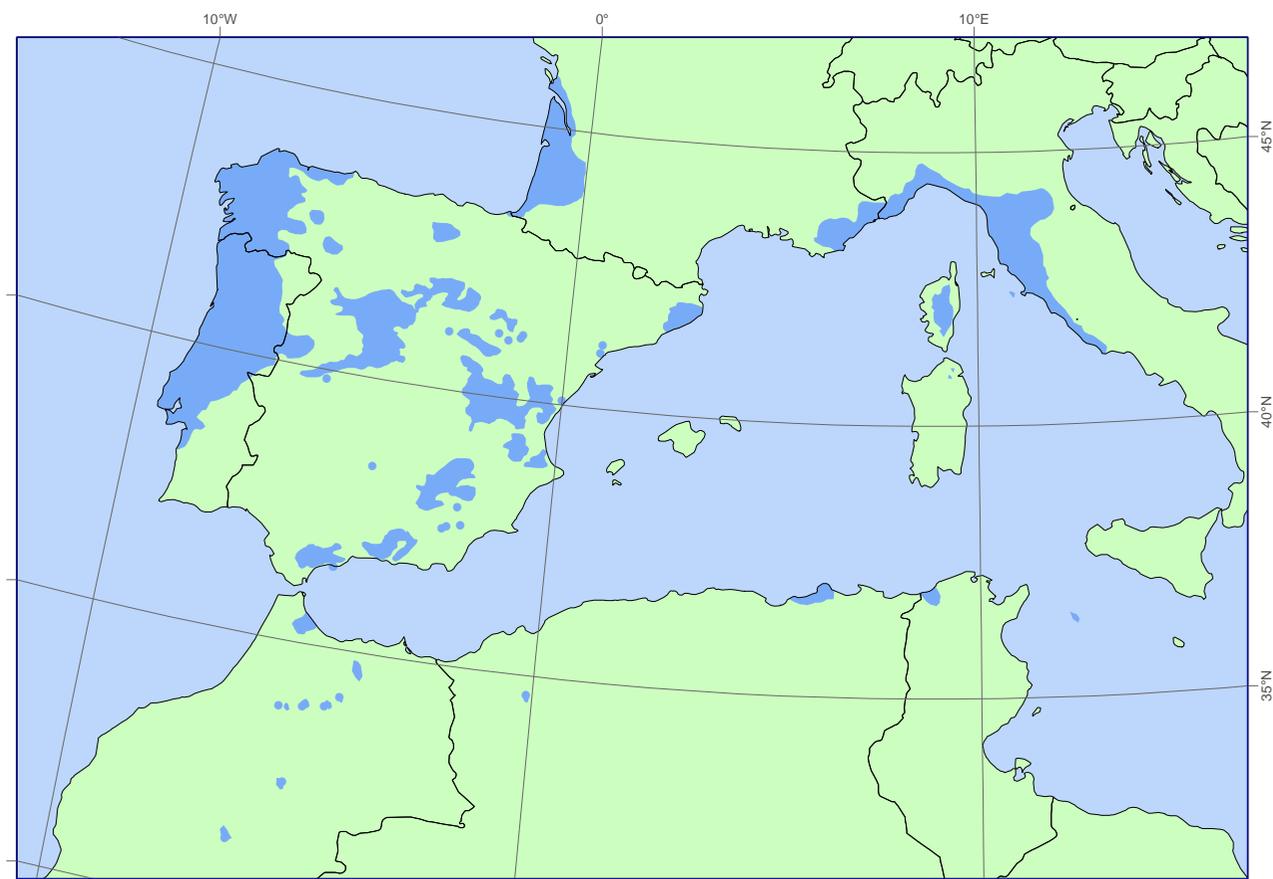
4

Laurent Bouffier, Annie Raffin and Ricardo Alía

## Introduction

Maritime pine (*Pinus pinaster* Ait) is distributed in the western Mediterranean Basin, in Southern Europe (France, Italy, Spain), North Africa (Morocco, Tunisia), and the Atlantic coast in France, Portugal and Spain (Figure 4.1). It is also found in Corsica and to a very limited extent, northern Sardinia and a marginal stand in Pantelleria Island.

The species is broadly distributed by forestation in different countries, both within and outside the natural range. The area within the natural range is around 4.2 million ha, with about 200 000 ha outside the natural range (Australia, South Africa, New Zealand, Chili, Argentina and Uruguay).



**Fig. 4.1.** Natural distribution area of maritime pine in Europe, compiled by members of the EUFORGEN Networks (EUFORGEN 2009).

Maritime pine covers areas with contrasting productivity. For instance, in Spain the mean productivity varies from 0.5 to 10 m<sup>3</sup>/ha/year, depending on the region. This affects the management of maritime pine, rotation age (from 40 to 120 years), the objectives for breeding or conservation, and also the importance of natural versus artificial regeneration. So when we speak about breeding strategies in maritime pine, we need to focus on activities in the three more productive areas: Aquitaine; Galicia; and Portugal. In these areas, the species represents an average of 33% of the total forested area, producing 60% of the solid wood harvested. The main uses here are pulp and paper production, solid wood for construction, chipboards, floor boards and pallets. Traditional uses, such as resin tapping, are no longer practiced.

However, we cannot forget that in other regions, maritime pine plays an important role for conservation of genetic resources (e.g., EUFORGEN programme), and therefore activities are now carried out across a wider area. Also, in many of these regions, which are somewhat less productive, some low-input breeding activities support afforestation purposes, especially through selection of seed sources, selection of provenances and quality control for artificial regeneration.

### Historical perspective and current situation

The most advanced maritime pine breeding programme is carried out in south-western France, in the Aquitaine area. In this area, maritime pine stands expanded from a natural forest of 250,000 ha at the end of the 18th century, to the current cultivated area of one million ha. Such a progression was the result of the determination of the local land owners and public authorities to stabilize coastal dunes, drain 700 000 ha of marshes, and plant a new forest. Once the forest was established, the challenges of nature and of a changing economic background had to be addressed repeatedly (Riou-Nivert 2002). Between 1939 and 1950, fire destroyed 400 000 ha. In the 1950s, the resin market collapsed, due to international competition and the emergence of oil by-products. Production objectives were reoriented towards timber, supported by the progress in silviculture and the breeding of improved varieties, marketed in the early 1980s. During the winter of 1985, an intense cold wave in south-western France destroyed 30 000 ha of maritime pine plantations from Spanish and Portuguese provenances, which had been established during the major reforestation effort following the fires of the 1940s. The genetic origin of seed source stands is now systematically verified by a biochemical test based on terpene composition, and seed harvest from non-local stands is forbidden. A hurricane in December 1999 felled more than 100 000 ha in Aquitaine: 28 million cubic meters were levelled

(<http://agreste.agriculture.gouv.fr/>). Once more, the maritime pine forest resource had to be reconstituted. Reforested areas increased, reaching 23 000 ha/year, while 100% of plantations have been established with seedlings from second-generation seed orchards (GPMF 2002).

Maritime pine is by far the most planted tree species in France with 10% of the forest area and 24% of wood harvest (French Ministry of Agriculture, <http://agreste.agriculture.gouv.fr/>). Average productivity is about 10 m<sup>3</sup>/ha/y in the Aquitaine area, but can reach 20-25 m<sup>3</sup>/ha/y on the best sites. Around 8.5 million m<sup>3</sup> are harvested annually, most of which is processed locally, 60% as saw-timber, and 40% as industrial round wood.

### Genetic resources and biology

Maritime pine is a wind pollinated conifer species, with a high proportion of outcrossed progeny (>80%) from a large number of pollen contributors, resulting in half-sib families when collecting seeds in stands (Gaspar et al. 2009). Also, it is interesting to notice the high gene flow (via pollen) with a clear J-shape kernel distribution (e.g., González-Martinez et al. 2006) that could affect the isolation of populations, and the location and distribution of seed orchards. Maritime pine is an early cone and pollen producer that can be used for reducing the breeding cycle. Also, it is interesting to notice the high seed production in seed orchards (up to 15 kg/ha), and the high number of viable seeds per kg (around 17 000). Many of the breeding strategies include deployment strategies based on seedling production, although it is possible to propagate the species by micro-cuttings obtained from very young material in order to include mass propagation in a breeding programme (Majada et al. 2011).

The species can be found in quite different environments: from sea level to 2100 m elevation in the High Atlas (Morocco); from areas with more than 1400 mm of annual rainfall and no dry season, to others with 350 mm and more than four dry months. The soil conditions are variable; mainly in acid soils, but also in basic soils and even in sandy and poor soils, where few commercial species can grow, but in which maritime pine has been used in highly productive plantations.

Fragmentation across the natural distribution is a result of those contrasting conditions in which the species is found, and also the discontinuity and altitude of the mountain ranges. It is also necessary to stress the importance of the human impact on the distribution of the species. As a result, maritime pine is a conifer with clearly defined geographical meta-populations (e.g., Eveno et al. 2008), displaying several adaptations to fire (e.g., early flowering, presence

of serotinous cones and a thick bark (Tapias et al.2006)), drought tolerance (e.g., differences in water use efficiency (WUE), allocation pattern among roots and stem), and growth (e.g., growth rhythm, polycyclism). This geographical structure of the genetic diversity has been studied both by neutral and adaptive traits, indicating a relatively high differentiation among populations (e.g.,  $F_{st}=0.10$  for nuclear SSR) and variable differentiation for quantitative traits (e.g., high differentiation for height growth and survival, intermediate for WUE and no differentiation for cavitation resistance) (González-Martinez et al. 2002; Lamy et al. 2012). Those differences have shown the importance of selecting the best material for use in afforestation and reforestation programmes. In this context, maritime pine was one of the first species where this type of study was carried out (e.g., Duff, 1928).

Conservation of genetic resources is of prime importance in many areas, where forest fires, land use and plant cover changes, introduction of exotic species, overexploitation, climatic change, and pests and diseases are imposing various threats to genetic diversity of the species within its natural range (Alía and Martin 2003). Within the EUFORGEN programme, different activities, including seed source selection, *in-situ* conservation (e.g., EUFGIS portal) and *ex-situ* conservation activities (mainly by germplasm and plant collections) are available for some of the populations or areas of distribution.

Many studies have focused on characterization of genetic parameters of the species (Table 4.1). Considering the efforts devoted to maritime pine breeding in Europe, a large number of genetic trials are now assembled in TreeBreedex/Tree4Future database, established in different environmental

conditions at both the population (provenance) and progeny (half-sib or full-sib) level. The main limitation of many of those genetic trials is the low connectivity of material among different organizations involved in breeding activities, especially at the European level.

The importance of efforts devoted to development of improved methods of phenotyping and genotyping should be stressed, especially in the past few years due to opportunities offered by genome-wide selection. Platforms for phenotyping of different traits are being developed (e.g., Noveltree Deliverables 1.5 and 1.6, platforms available as transnational access facilities in Tree4Future). At present, there are efficient methods for assessing wood density and different chemical components in maritime pine (e.g., lignin content, different extractives) using near infra-red (NIR) technologies. It is possible to estimate density and radial growth by micro-densitometry analysis. For other adaptive traits, research and development is still needed. Water use efficiency can be phenotyped by measuring  $\delta^{13}C$  (carbon isotope discrimination), but the cost to perform routine analysis is high, and indirect approaches using NIR technologies are being tested. All these phenotyping platforms could improve significantly the genetic evaluation and the efficiency of selection methods in a very near future.

Maritime pine is also one of the model forest tree species used worldwide for the discovery of genes related to wood quality and water stress resistance by quantitative trait loci (QTL) detection, association studies, and in the near future, genome-wide selection. Those new methods are expected to change the future breeding of forest species (Neale and Kremer 2011).

**Table 4.1.** Heritability estimates for maritime pine, extracted from Deliverable D1.2 and Noveltree annual reports

Trait	$h^2$ (min-max)
Height	0.13-0.65
Diameter	0.10-0.35
Stem straightness	0.22-0.34
Wood density	0.30-0.61
Spiralgrain	0.60-0.63
Lignin content	0.25-1.5
$\delta^{13}C$	0.26-0.30

Development of genotyping tools based on DNA information is progressing rapidly in maritime pine. Development of a large set of single-nucleotide polymorphisms (SNPs) has provided high quality fingerprinting tools and allowed characterization of the geographical origins of the species (see Noveltree Deliverable D2.2 and annual Newsletter 3). Such development of polymorphic markers in a large list of candidate genes associated to wood quality and responses to water constraints provide the bases for the first association studies in the species. Those resources are already being applied in different breeding populations of maritime pine (Lepoitevin 2009; Jaramillo-Correa et al. 2012).

## Breeding objectives

In the early days of maritime pine breeding in Europe, the main objectives were the improvement of growth potential and stem straightness. These are still currently the two main breeding objectives but new selection criteria are under study, more particularly on pests and diseases resistance (Jactel et al. 1996; Kleinhentz et al. 1998; Burban et al. 1999; Lung-Escarmant et al. 2004), wood quality (Pot et al. 2002; Bouffier et al. 2008, 2009), and drought tolerance (Dubos et al. 2003; Dubos and Plomion 2003; Nguyen-Queyrens and Boucher-Lannat 2003; Eveno et al. 2008). Some new criteria have already been included in the selection process: twisting-rust resistance (*Melampsora pinitorca*) evaluated in a cut-shoot assessment (Desprez-Loustau 1990) would be considered in the selection of future seed orchard parents. Wood density is evaluated at the family level in progeny trials with an IML-Resi tool (Bouffier et al. 2008), and branch quality is scored visually in progeny tests (GPMF 2002).

Breeding forest tree varieties in the context of changing climate is another challenge. Models predicting the evolution of climate in south-western France in coming decades all show an elevation of average air temperature and a seasonal shift in precipitation distribution from spring and summer to winter, which will likely result in decreased forest productivity (Loustau et al. 2005). Although precision of these scenarios is rather low, interest has increased in the improvement of drought tolerance. Current varieties of maritime pine in Aquitaine were selected, tested and used in only one local breeding zone. Selection is aimed at producing multipurpose varieties adapted to the different soil types of Aquitaine, including dry, semi-humid and humid podzol soils. In the near future, seed orchards could be rogued to favour clones that are better adapted to the drier sites as and when their progeny tests are assessed in a changing climate. As for future varieties, different strategies of performance

evaluation are considered: locating progeny trials in more southern and drier sites in anticipation of future climate change, infusing new diversity into the breeding population either by selecting better adapted trees in the local provenance, using the national network for maritime pine natural genetic resources conservation, or by selecting adapted interprovenance combinations (Landes x Portugal and Landes x Morocco progenies are already being tested), as well as by introducing new selection criteria for drought resistance, e.g., WUE (Brendel et al. 2002), and resistance to cavitation (Lopez et al. 2005), and molecular markers associated with genetic variation for these traits.

## Breeding programmes

### Breeding programme in France

The French maritime pine breeding programme has been described in detail elsewhere by Annie Raffin (Mullin et al. 2011). We report here the main characteristics of this breeding programme.

#### 1. Plus tree selection

The breeding programme started in the 1960s, when early provenance trials had already shown the superiority of the local Landes provenance for growth and cold resistance (Illy 1966). Aquitaine is the most northern region of the species' natural distribution, which is otherwise localized on the Atlantic coast of Spain and Portugal and around the Mediterranean basin (Spain, South-Eastern France, Italy, Tunisia, Algeria, and Morocco). Cold resistance was identified as an important issue, especially when lowest night temperatures in Aquitaine can reach -10°C or -15°C every few winters (-20°C in February 1985). The local provenances were thus chosen to build up the breeding population, despite their form defects: stem flexuosity and poor branching. A total of 380 plus-trees were selected phenotypically on the coastal sand dunes of Aquitaine, based on height and diameter, and visual scoring of stem form. This first phenotypic selection proved to be efficient for improving growth and stem straightness, as shown by a progeny test comparing plus-tree progeny with their non-selected neighbour-tree progenies on two locations, 10 years after planting (Danjon 1995). In addition, genetic variation among provenances and performance of crosses between provenances were explored (Harfouche and Kremer 2000; Harfouche et al. 2000). Among all tested combinations, Landes x Corsica families proved to be the best material for growth and form in Aquitaine conditions. A few hundred clones from the Corsica provenance were selected in provenance trials located in Aquitaine, based on growth, stem straightness,

branch quality, pyralis resistance (*Dioryctria sylvestrella*), and cold resistance. The objective of this second population is to produce improved Landes × Corsica varieties for better stem straightness and branch quality.

## 2. Long-term breeding strategies

Development of the breeding programme followed a classical intra-population recurrent selection approach, with a main population composed of the Landes plus-trees. Following this strategy, the main population has cycled through three generations, with more than 4500 individuals selected, and 5000 families tested over 500 ha of trials.

For the next generation, the focus is on a reduction of population census size and better management of pedigrees, to optimize selection efficiency while producing regularly renewed varieties with increasing genetic gains. Eight unrelated sublines have been assembled within the breeding population based on pedigrees and breeding values, allowing the deployment of unrelated selections in clonal seed orchards. Status number is used as an indicator of genetic diversity. Double-pair mating designs are used to produce material for progeny tests and the base of the next generation, while polycross testing is performed for parental ranking. Trials are replicated on several contrasting sites, usually with single-tree plots and a large number of replications per site (GPMF 2002).

## 3. Breeding achievements

Up to now, three generations of seed orchards have been produced. For economic and technical reasons, the deployment strategy for maritime pine varieties in Aquitaine is based mainly on open-pollinated orchards. The first-generation orchards were seedling seed orchards based on a very large number of full-sib families, corresponding to the progeny tests of plus-trees, and were rogued after genetic assessment. These orchards demonstrated genetic gains of 10-15 % in volume and stem straightness at about age 15 years.

Second-generation orchards were characterized by a reduced genetic basis and greater expected genetic gains, compared to those in the first generation. They were based on a few tens (usually around 30) of backward-selected genotypes, either as a classical grafted clonal orchard or as a randomized plantation of polycross families obtained by controlled pollination between selected clones (Baradat et al. 1992). The polycross-family seed orchards were planted over 180 ha and are open-pollinated. The expected genetic gain was estimated from progeny trials at age 13 years to be 30% for both volume and stem straightness over unimproved

material. Since hurricane “Martin” in 1999, as the area reforested with maritime pine in Aquitaine increased annually from 15,000 to 23,000 ha, 70% have originated from second-generation orchards.

Third-generation seed orchards have been established over 180 ha, and will enter in production in the coming years. They are either clonal or polycross-family seed orchards. This third generation also includes a Landes × Corsica variety, to be produced by controlled pollination.

In the future, seed orchards will have to be renewed more rapidly, to better respond to likely climate change and developments in the marketplace. Recent adaptations in the maritime pine breeding programme, such as the optimization of population management through sublining and of selection efficiency with BLUP evaluations, are expected to be augmented with marker-assisted selection for complex traits such as wood quality and drought resistance.

## Breeding programmes in other countries within the natural range (Morocco, Portugal, and Spain).

### 1. Breeding zones

Provenance studies started in Spain in the 1960's (Molina 1965), in order to select provenances for use in northern Spain (mainly Galicia) where the productivity is high. Some selection and breeding activities were also implemented for resin production, a highly heritable trait (Tadesse et al. 2001). The strategies followed were based on recurrent selection, with selection of plus-trees in the most important regions of provenance, for establishing clonal seed orchards and progeny tests from open-pollinated progenies (Pardos and Gil 1984), and resulting in different breeding zones corresponding to major geographical regions (Galicia, Central Iberian Planes, Iberian Range, Central System, and Cuenca Forest). In Galicia, the breeding programme was based on the autochthonous provenance, with two breeding zones: coastal and interior (Toval and Vega 1982). Those two zones are distinguished by altitude (being 400 m the limit between the two zones). A similar approach was undertaken in Portugal (Aguiar et al. 2003), where the local provenance displayed superiority over other material (Correia et al. 2010).

This division in different breeding zones has been demonstrated to be ineffective (Zas et al. 2004) as it would be possible to redefine the breeding zones to a larger extent, and providing material suitable for the different conditions by managing only one breeding population. This approach is being implemented, by establishing a single breeding programme over the entire area (Majada et al. 2012).

## 2. Breeding objectives

Initially, the main objective was to increase volume production and improve stem form (especially in some of the populations); but at present the wood quality characteristics are also considered essential for inclusion, along with biotic and abiotic risks (drought and cold tolerance, diseases, pests). This is becoming necessary as threats to maritime pine population are emerging, such as *Fusarium circinatum* and the pine nematode, which are affecting the area. According to Zas and Merlo (2008), the main objectives pursued in breeding programmes with maritime pine in northern Spain are: (1) genetic gain in growth and stem straightness; (2) preserving diversity in the breeding population; (3) supply of high quality reproductive material for use in reforestation; and (4) improvement of traits of interest such as resistance to pathogens and pests, nutritional efficiency or quality of the wood (among others). Breeding for resin production is no longer pursued.

## 3. Current activities

**3.1 Testing.** A complete set of progeny tests and provenance-progeny tests have been established for testing the material from the different selections. Usually plus trees were tested, in combination with material from other more advanced programmes or regions. At present, there is a network of genetic tests both with selected populations (e.g., Portugal, Galicia, Central System). Recently, different genetic trials have been established with more than 30 populations covering the distribution range of the species and about 450 families across those populations. These genetic trials are essential for defining the future breeding population of Maritime pine in the Iberian Peninsula.

**3.2 Evaluation.** Spatial analysis has been implemented as a routine analysis of experiments to increase the efficiency of estimates (e.g., Correia et al. 2010; Zas

and Merlo 2008; Saldaña et al. 2012), with improvement of 9-23% (because of a spatial autocorrelation higher than 0.97 for height and diameter). Different traits have been evaluated in these populations, such as tree height, carbon-isotope discrimination, stem diameter, stem form, nutrient efficiency, and fecundity (Correia et al. 2008; Zas and Merlo 2008; Martins et al. 2009; Santos et al. 2011). In most cases, a large level of genetic diversity has been demonstrated to exist in the populations analysed, creating a basis for long-term breeding. Also, the genetic control of the different traits is important.

In some cases, a significant G×E interaction has been detected at the different levels (both populations and families; see Figure 4.2). As has been shown in other species, G×E interaction can be reduced to an acceptable level for breeding by selecting the less interactive families in a given experimental area (Correia et al. 2010; Zas et al. 2004; Saldaña 2012).

**3.3 Selection.** Despite the different activities being carried out with maritime pine, selection has been made using simple selection indexes, in which tree height and diameter have been considered. This is a limiting factor in the different breeding activities in these regions. Genetic gains have been estimated for different traits (Table 4.2).

**3.4 Mating.** The only activity being carried out was started in the Galicia population, using an incomplete factorial mating design.

**3.5 Deployment.** Most of activities are based on deployment of planting material from the local population, and some recommendations have been established (Alfa et al. 2009). Some approaches using vegetative material obtained from mass propagation of young seedlings have been tested, in order to produce material from parents of families (Majada et al. 2011).

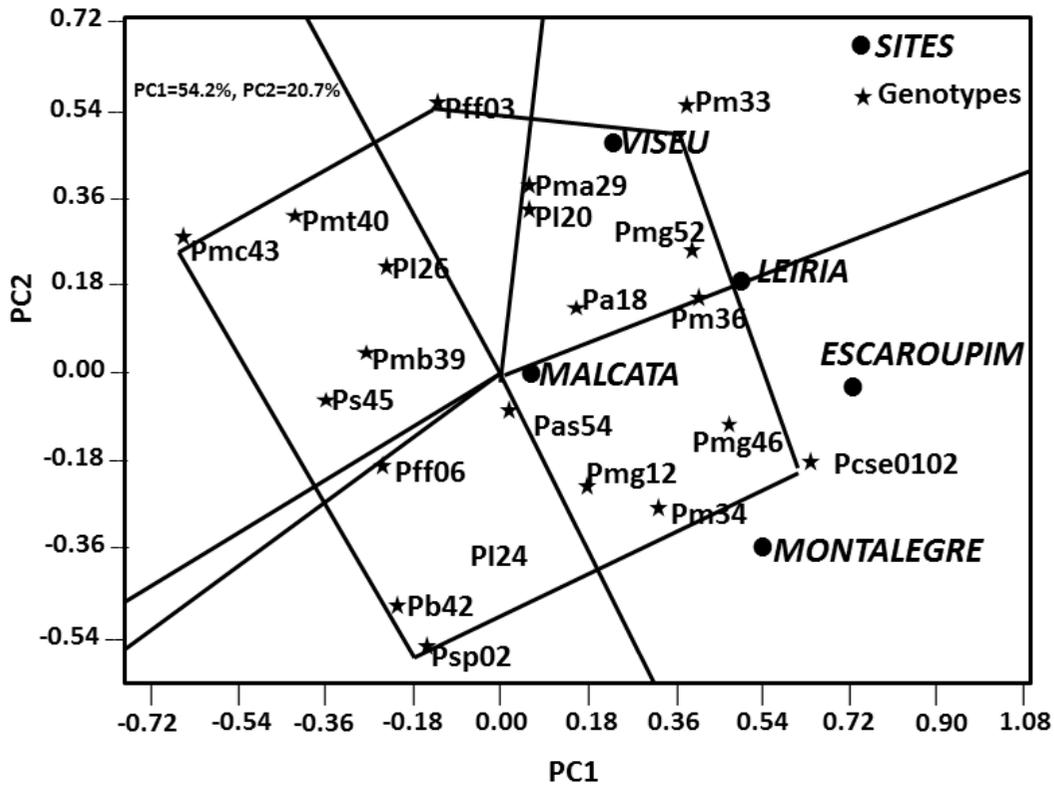


Fig. 4.2. Genotypexenvironment patterns for tree height in a subset of 22 seedlots from Portugal. Identification of populations with the highest growth regarding the mega-environments (which-won-where view) (taken from Correia et al. 2010).

Table 4.2. Expected genetic gain from different selection methods in northern Spain (from Saldaña 2012)

Selection method	Trait	Genetic gain %	
		Top 5 %	Top 10 %
Individual	Diameter	6.4	5.5
	Height	5.0	4.3
Family	Diameter	6.6	5.6
	Height	4.9	4.2
Combined	Diameter	8.1	6.9
	Height	6.2	5.3
Family and within family	Diameter	11.3	9.6
	Height	8.7	7.4

## Evaluation of polymix breeding strategy with forward selection as an alternative breeding strategy for maritime pine

The French maritime breeding programme is currently managed in two steps:

1. double-pair mating designs to produce the base of the next generation; and
2. polycross progeny testing performed for parental ranking.

Polymix breeding associated with paternity analysis (Lambeth et al. 2001) has been proposed as an alternative to the current strategy. It consists of establishing polycross families with pollen mix(es) on a set of female parents to obtain good estimates of parental breeding values and a large number of parent combinations. Molecular markers are then used for paternity analysis (and maternity analysis if the female identification was not conserved), allowing better estimates of breeding values and control of relatedness. The main advantages of this strategy are simplification of the mating design, a shortening of selection cycle (forward selection), and reducing possibilities of pedigree mistakes.

To evaluate this strategy in the context of the French maritime pine breeding programme, simulation with the POPSIM™ software (Mullin et al. 2010) was carried out with support from the Noveltree project, by Tim Mullin (Skogforsk) and Laurent Bouffier (INRA). We will focus here on the elite population (~60 G1 and G2 genotypes), as these crosses will soon be made to establish new progeny trials.

The following parameters have been considered:

- Population of 60 unrelated trees
- Single trait (or index) with only additive effects:  $h^2=0.3$ ;  $CV_a=7\%$ ;  $CV_p=13\%$
- Mating designs to generate a recruitment population of 1800 trees: double-pair mating, polycross breeding
- Selection based on BLUP breeding values
- Restriction on relatives: none or maximums of 3 or 15 progeny per parent
- Seed orchards established with 5 trees (maximum 2 progeny per parent)
- 5 generations simulated (50 iterations)

The objective of this study was to compare the current strategy (double-pair mating) with the strategy proposed by Lambeth et al. (2001). These two strategies were simulated and results are presented with the following notations:

- DPM = double-pair mating with BLUP: each genotype crossed with two genotypes [60 crosses]
- PMX = polymix breeding with BLUP, with or without paternity analysis:
  - PMX0% : no paternity test
  - PMX50% : paternity test for 50% of the recruitment population
    - PMX50%\_BT : the 50% best trees are tested
    - PMX50%\_BTWF : the 50% best trees within each half-sib family
  - PMX100% : paternity test for the entire recruitment population

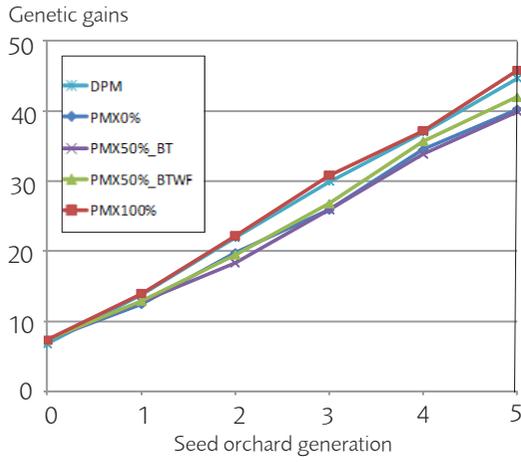
For each strategy, genetic gains in seed orchards and genetic diversity (measured by group coancestry) in the breeding population were simulated and reported in Figure 4.3. Results from these simulations suggested that:

- DPM and PMX100% give similar gains, but PMX100% maintains lower relatedness in the breeding population
- PMX50% performs better than PMX0% only if applied on the best 50% trees within each half-sib family
- PMX0% brings less genetic gain, but is low cost and conserves high genetic variability
- if a more restrictive constraint is chosen for the genotype selection in the recruitment population, advantage of PMX100% vs. PMX50% decreases

We can conclude that polymix breeding appears to be a cost-effective strategy as it achieves gains similar to double-pair mating without progeny testing. The main limitation is that relatedness is better controlled with the current strategy.

As the polymix breeding strategy allows substantial simplification of field work (polymix crosses are simpler than double-pair mating) and based on these promising simulations, it will be implemented for the elite population. Studies are in progress to recover paternity in a maritime pine polycross trial with SNP markers. It will allow to best implement polymix breeding in the breeding programme.

Genetic Gains in Seed Orchards



Group Coancestry in the Breeding Population

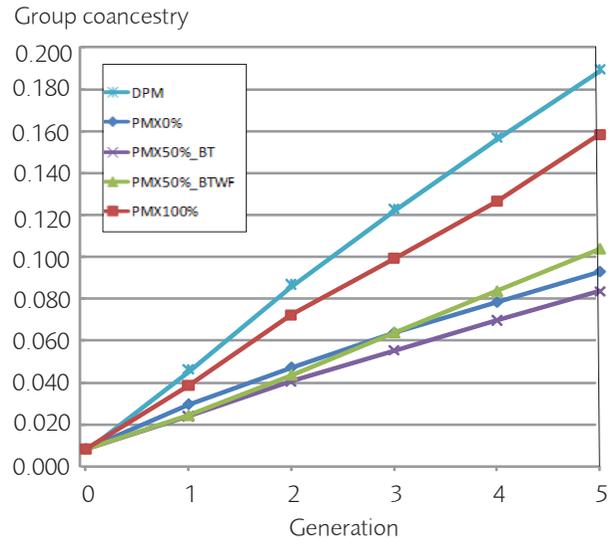


Figure 4.3. Genetic gains and group coancestry for polymix breeding strategy versus double-pair mating breeding strategy.

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# Extension of best practice to other species in Europe

Bengt Andersson Gull and Tim Mullin

## Introduction

In this chapter, we consider the practice of tree breeding extended to the many other tree species in Europe. As emphasised in the first Chapter, there is no such thing as a “one-size-fits-all” breeding plan that can be applied. Not only is every species different, but so is the context of breeding for that species in a given environmental, economic and social situation. “Best practice” is not so much about technical specification of breeding plans as it is about informed decision-making processes that recognise the constraints imposed and the context of application.

We begin this chapter by illustrating the diversity of planted forests in Europe, and consider the constraints these situations pose when analysing the various decision factors for breeding strategy introduced in Chapter 1. We go on to discuss in a general sense the approaches that might apply to various levels of intensity, depending on the objectives of the programme and resources available. We then discuss the potential and opportunities for geo-climatic breeding efforts across national boundaries and close with some thoughts on how technologies advanced through Noveltree might find best application in European forestry now and after further development in the future.

## Species important to European forestry

Forests have been an important part of European culture and tradition for centuries. Providing essential materials for shelter, transportation and fuel, as well as habitat for game and protection of water quality, forests in Europe have a long and complicated history of management and utilisation, with deforestation and over-grazing in the Mediterranean region, even while timber frontier advanced through Scandinavia in the 19<sup>th</sup> century. Today, forests cover over one-third of the landmass across Europe, while the forest cover of individual countries varies from 1% in Malta to 73% in Finland (Table 5.1). More than one quarter of

forests in the European Union (EU) are classified as “planted forests” by the FAO, although again this varies tremendously from less than 5% in Greece and Slovenia, to more than 95% in Poland, the Czech Republic and the Netherlands (FAO 2010).

Tree breeding activities in the EU display similar variation in species and intensity (Table 5.2). In general, conifer genera are well-suited to intensively managed plantation management and the species covered by this report in detail, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.), constitute the bulk of planted forests in Europe. While having a smaller overall area, several other conifers are important across international boundaries, including: maritime pine (*Pinus pinaster* Ait.), larches (*Larix* spp.), including hybrids between native and exotic species, as well as Sitka spruce (*Picea sitchensis* [Bong.] Carr.) and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), both also introduced species (Mullin et al. 2011). Other conifers, although limited in use to only one or two countries, are also important plantation species, including lodgepole pine (*Pinus contorta* L.) in Sweden (Elfving et al. 2001) and radiata pine (*Pinus radiata* L.) in Spain (Crecente-Campo et al. 2009), various true firs (*Abies* spp.) in the eastern Mediterranean region (Alizoti et al. 2011), and Italian cypress (*Cupressus sempervirens* L.) (Papageorgiou et al. 2005).

While conifers dominate the planted forest landscape across Europe, the importance of some broadleaf species has been established, albeit on a smaller scale. Of general importance in planted forests across several countries are species and inter-specific hybrids of poplar (*Populus* spp.). Other broadleaf species including ash (*Fraxinus excelsior* L.), sycamore maple (*Acer pseudoplatanus* L.), wild cherry (*Prunus avium* L.), eucalypts (*Eucalyptus* spp.), silver birch (*Betula pendula*), and both native and exotic species of willows (*Salix* spp.) have significant commercial importance in planted forests. While many other hardwoods are important

**Table 5.1.** Extent of forest and forest plantation area in the European Union, sorted by overall land area in country (adapted from FAO 2010).

Country	Country land area (1000 ha)	Forest area		Planted forest area	
		1000 ha	% of land area	1000 ha	% of forest area
France	55 150	15 954	29	1 633	10
Spain	50 537	18 173	36	2 680	15
Sweden	45 029	28 203	69	3 613	13
Germany	35 705	11 076	32	5 283	48
Finland	33 842	22 157	73	5 904	27
Poland	31 269	9 337	30	8 889	95
Italy	30 134	9 149	31	621	7
United Kingdom	24 417	2 881	12	2 219	77
Romania	23 839	6 573	29	1 446	22
Greece	13 196	3 903	30	140	4
Bulgaria	11 100	3 927	36	815	21
Hungary	9 303	2 029	23	1 612	79
Portugal	9 212	3 456	38	849	25
Austria	8 387	3 887	47	-	-
Czech Republic	7 887	2 657	34	2 635	99
Ireland	7 026	739	11	657	89
Lithuania	6 530	2 160	34	521	24
Latvia	6 459	3 354	54	628	19
Slovakia	4 903	1 933	40	959	50
Estonia	4 523	2 217	52	168	8
Denmark	4 310	544	13	407	75
Netherlands	4 153	365	11	365	100
Belgium	3 053	678	22	396	58
Slovenia	2 027	1 253	62	32	3
Cyprus	925	173	19	31	18
Luxembourg	259	87	33	28	32
Malta	32	n.s.	1	n.s.	n.s.
<b>Total EU</b>	<b>432 916</b>	<b>156 778</b>	<b>36</b>	<b>42 503</b>	<b>27</b>

n.s. = not significant, indicating a very small value

- = data not available

**Table 5.2.** Relative importance of commercial tree species in European planted forests.

<b>Extent of deployment</b>	<b>Conifers</b>	<b>Broadleaves</b>
Extensive	<i>Picea abies</i> <i>Pinus sylvestris</i>	<i>Populus tremula</i> <i>Populus hybrids*</i>
Moderate	<i>Larix</i> spp. and hybrids* <i>Picea sitchensis</i> * <i>Pinus contorta</i> * <i>Pinus pinaster</i> <i>Pseudotsuga menziesii</i> *	<i>Acer pseudoplatanus</i> <i>Betula pendula</i> <i>Eucalyptus</i> spp.* <i>Fagus sylvatica</i> <i>Fraxinus excelsior</i> <i>Prunus avium</i>
Minor	<i>Abies</i> spp. <i>Cupressus lusitanica</i> * <i>Cupressus sempervirens</i> <i>Pinus brutia</i> <i>Pinus canariensis</i> <i>Pinus halepensis</i> <i>Pinus nigra</i> <i>Pinus pinea</i> <i>Pinus radiata</i> * <i>Pinus strobus</i> *	<i>Aesculus hippocastanum</i> * <i>Alnus cordata</i> <i>Alnus incana</i> <i>Alnus glutinosa</i> <i>Alnus rubra</i> * <i>Acer campestre</i> <i>Acer lobelia</i> <i>Acer platanoides</i> <i>Betula pubescens</i> <i>Carpinus betulus</i> <i>Castanea sativa</i> <i>Corylus avellana</i> <i>Fraxinus angustifolia</i> <i>Juglans regia</i> <i>Malus sylvestris</i> <i>Prunus padus</i> <i>Pyrus amygdaliformis</i> <i>Pyrus pyraeaster</i> <i>Quercus petraea</i> <i>Quercus rubra</i> * <i>Quercus robur</i> <i>Quercus suber</i> <i>Salix</i> spp. and hybrids <i>Sorbus aria</i> <i>Sorbus aucuparia</i> <i>Sorbus domestica</i> <i>Sorbus intermedia</i> <i>Sorbus torminalis</i> <i>Tilia cordata</i> <i>Tilia platyphyllos</i> <i>Tilia x vulgaris</i> <i>Ulmus canescens</i> <i>Ulmus glabra</i> <i>Ulmus laevis</i> <i>Ulmus minor</i> <i>Ulmus procera</i>

\* Exotic species or hybrids

in European forestry, and in particular beech (*Fagus sylvatica* L.), various oaks (*Quercus* spp.), and the so-called “Noble Hardwoods”, most are long-lived or otherwise difficult to manage under typical plantation conditions, and thus are less commonly targets of breeding efforts. While these species are not currently prominent in European planted forests, they are generally recognised as having potential under changing climate conditions, and for increasing the genetic diversity and biodiversity of the forest landscape, enhancing environmental quality, recreational opportunities and other important social values.

## Implementing best practice in breeding programmes

Intensity of tree breeding varies considerably across species and regions in Europe, from simple seed collections from better phenotypes in stands of unknown origin, to advanced breeding programmes with controlled crosses over several generations and extensive field testing. Objectives are equally varied. Productivity and quality are the primary goals when trees are established in industrial plantations, relieving pressure on other forest areas and ensuring raw material for higher-value products. While adaptability is not generally an issue for plantations in milder areas, the deployment of material to harsher sites, such as those in northern Europe and drier areas of the Mediterranean, motivates breeding objectives for adaptability, as does the general concern that changing climate conditions will pose adaptation issues for many other areas in the future. Gene conservation, as a hedge against changing climates and markets, as well as the potential for breeding for new traits in the future, is an element of virtually all breeding programmes in Europe, but of particular concern where native populations have been largely displaced by agriculture and urban development. As suggested in Chapter 1, best practice in such varied landscape of breeding objectives and species starts with the realistic assessment and understanding of key decision factors on a case-by-case basis.

### 1. Biological factors

Across the range of species used in European planted forests, we see striking contrast in biology that must be respected to succeed in breeding. While almost all conifer species in Europe are wind-pollinated and monoecious (male and female reproductive structures on the same individual), the reproductive biology of broadleaf species is highly diverse. Poplars, ash and willows are normally dioecious (male and female structures on different individuals), while of those genera that are normally monoecious, some such as eucalypts and cherry have perfect flowers (male and female

structures in same florescence) while others including birch, beech and oaks have imperfect flowers (separate male and female flowers). Still other genera, such as the maples, can be both monoecious or dioecious, with the former having both perfect and imperfect flowers. Species pollination in several broadleaf species is at least aided by bees and other insects, including the eucalypts, sycamore maple, cherry and willow. Once seeds ripen, cleaning and long-term storage of most conifers is usually straightforward, while several of the broadleaf genera pose challenges for long-term seed storage and handling, including beech, ash, and oaks. The variation in reproductive biology, particularly in broadleaf species, suggests that considerable knowledge and skill is required to successfully carry out breeding whenever a programme is initiated for a new species.

Vegetative propagation is an extremely useful tool for both testing and deployment, where it is possible. Clonally replicated individuals are effective for efficient breeding (Rosvall and Mullin 2013) and are also the best (only) way to predict spatial plasticity for individual genotypes. Some conifers, such as the Cupressaceae, are so easy to propagate by rooted cuttings that this is the normal method for production of operational planting stock. Others, such as Norway spruce, can be propagated easily when the donor plants are quite young, so that clonal replication of genetic field tests is a standard and effective practice. The pines, in general, are difficult to root, although some success has been achieved with Scots pine and lodgepole pine, so that there is at least potential for clonal replication of tests, even if clonal deployment of operational planting stock is impractical (Rosvall 2011b). For the broadleaf species, the ease of vegetative propagation also varies, with the willows and most poplars being rather easy, while the oaks and beech are virtually impossible. Most other genera offer some possibility for vegetative propagation, given judicious selection of the plant part and appropriate nursery treatment (Dirr and Heuser 1987). Somatic embryogenesis (SE) can be extremely useful tool for those species where it has been developed. Not only can SE be utilised for clonal deployment, it can also facilitate the long-term storage of genotypes that can be regenerated in a juvenile state for use in breeding or as donor plants for rooted cutting production.

Perhaps of greatest importance in planning an effective breeding programme is detailed knowledge of the genetic structure of the species and parameters related to the traits of greatest concern. While the distribution of some tree species in Europe is extremely wide (e.g., Norway spruce, Scots pine, aspen, beech), others are more narrowly distributed and usually mixed with other species (e.g., oaks, maple and cherry). Because of the long history of human civilisation in Europe, much of the original genetic resource for black

poplar (*Populus nigra* L.) was lost to the impact of human activity in its natural habitat (Steenackers 1996). Genetic parameters are rather well known for the most widely planted species, thanks largely to provenance research that started in Europe in the mid-1700's by Duhamel du Monceau, Inspector-General of the French navy, and continued throughout the 19<sup>th</sup> and 20<sup>th</sup> centuries (Langlet 1971), but for most species not already in cultivation, genetic parameters remain virtually unknown.

Trees are usually regarded as long-lived, but the rotation times of planted forests can vary considerably, from just a few years for willows cultivated for biomass energy production, to over a century for pine and spruce in boreal areas of Scandinavia. Rotation time is an important consideration for the integration of breeding activity with deployment, and for determining appropriate assessment times for important traits.

#### 4. Economic factors

Tree breeding is just one of many options available in the silvicultural toolbox to enhance progress towards the objectives of forest management. The results of such analyses often confirm that breeding can increase land expectation value, shorten rotation time and enhance the immediate allowable cut with great positive impact on economic return (Binkley 1980, 1984; Mullin 1994; Rosvall 2011a). There is no single reason to use breeding over other methods of achieving the same goals, so some sort of comparison with other options is necessary before declaring breeding as “best practice”. Economic analysis provides a disciplined approach to evaluating the benefits that might be sought from a breeding programme and the cost of pursuing them. It also helps to determine the appropriate weight on identified target traits, which can often have adverse genetic correlations. The larger, established programmes in Europe have visited the economic question of costs and benefits in the light of product markets and well-documented historical costs. This information is often not readily available for species of lesser commercial importance, but evaluation of even non-timber values is of critical importance to establish new programmes with adequate economic rationale to ensure sustainability. While programmes have, in the past, been established “on faith”, sooner-or-later they are called upon to justify the investment.

The price-tag placed on breeding strategies can vary tremendously, depending on the technologies employed, the intensity of selection applied, and the speed with which gains are pursued. Any species that has found a place in planted forests is a candidate for some level of investment in breeding. Obviously, large programmes, such as those established for Scots pine and Norway spruce, warrant

considerable investment to maximise their efficiency and returns, as the opportunities to deploy gains are very large. Nevertheless, even very small planting programmes warrant some effort in controlling seed source and protection of genetic resources. The cost of conserving genetic resources *in-situ* and regenerating valuable seed sources is rather low, if integrated into the planning of planted forests. There really is no such thing as a plantation species in Europe that does not warrant some level of investment in genetic management. The keyword here describing best practice is “planning”.

Even those programmes that are well-regarded as economically viable often lack good economic information to optimise the weight placed on various selection traits, often with adverse genetic correlations (Berlin et al. 2012; Ivković et al. 2010). Appropriate economic modelling will guide the emphasis breeders place on a suite of traits and optimise the overall economic returns from a programme.

#### 5. Institutional factors

Even when biology is well known and the economic returns well established, the implementation of a tree breeding programme is always in the context of an organisation with its unique operational and cultural character. Economic viability cannot be translated into a complete skillset within the human resources available without a considerable search for or development of highly specialised staff. Existing programmes in Europe usually reflect the organisational history associated with their implementation: sometimes highly stable and competent, but many times strongly affected by reorganisation, shifting priorities and the inability to develop and retain suitable staff, such that time is lost and strategies reinvented. New programmes must wrestle with this same reality and find innovative solutions if they are to be effective and stable. Collaboration among other organisations and institutions is one approach that has proven effective, and will be discussed in greater detail in a later section.

#### 6. Socio-political factors

Finally, breeding programmes must respect the expectations of society. Many times this will affect the goals set for the programme and the technologies that are acceptable in achieving them. The use of genetic modification is perhaps the most obvious example of a technology with potential for large impacts on genetic gains, but has polarised public and political opinion to the point that its use is greatly restricted. Less obvious challenges are the acceptance of efficient plantation systems that are sometimes viewed as “unnatural”. Any new breeding programme must have a clear understanding of the socio-political factors at play, and to seek opportunities rather than threats.

## The principal components of breeding strategy best practice

With a clear understanding on the aforementioned decision factors, best practice then considers approaches to the five building blocks of a breeding plan: population management, mating, phenotypic assessment, genotypic assessment and selection. Together, these components define the breeding strategy and its implementation.

### Population management

Genetic gains are made possible by the genetic variation that is maintained within populations by the genetic processes of natural selection, migration, genetic drift, mutation and the mating system. Most breeding programmes begin with natural populations, which can vary considerably in overall size and distribution, or with local populations established with imported material. In its simplest form, genetic improvement applies selection pressure in addition to that which occurs in nature, to increase the performance of deployed offspring. This can be as simple as selecting the best phenotypes in seed collection stands and avoiding the poorer trees.

In more intensive programmes, populations may be isolated to a greater or lesser degree, and some form of pedigree management practiced. Pedigrees may be incomplete, consisting only of seed-parent identification for selections made in open-pollinated (OP) families, or may record also the male parent used in controlled-pollination (CP) crossing. Both seed and pollen parent pedigree may also be reconstructed at a later date through genetic markers.

A decision on population size usually considers the requirement to conserve effective population size to a number necessary to conserve common alleles that represent potential for future improvement – from a few hundred to a thousand. Populations conserving many of the rare alleles will necessarily be much larger, perhaps several thousand. Most programmes will adopt some kind of two-tiered population management, such that a single population is not utilised for genetic resource conservation as well as for recurrent selection, at the same time. Breeding populations managed for selection with OP families are somewhat “open” and there can be inflow of new alleles into the population, as pollen is not controlled. This pollen “contamination” can assist in maintaining high levels of diversity, but will also reduce the gain that is possible under recurrent selection within “closed” groups.

If programmes are sensitive to the need to move forward quickly, there will inevitably be some structuring of the population. Rather than trying to manage the population through discrete generation cycles, the population can either

be subdivided as overlapping generation groups, or managed under a “rolling-front” concept (Borralho and Dutkowsky 1998).

While genetic gain is always maximised when mating designs connect all parents in the population, there are several effects that can be achieved through subdivision of the population. It’s well known that population subdivision can help conserve diversity, as genetic drift can drive allele frequencies in different directions in discrete sub-populations (Baker and Curnow 1969; Madalena and Hill 1972). In tree breeding, subdivision can be hierarchical, to help focus efforts on elite portions separate from those portions managed for conservation of genetic diversity (Cotterill et al. 1989; Williams and Hamrick 1996; Williams et al. 1995) or as a strategy to maximise gain through a positive assortative mating effect (Ruotsalainen and Lindgren 2000). Subdivision can also be made as replicate sublimes, such that unrelated individuals from each subline can be used in production populations, and thus avoid the detrimental effects of inbreeding (McKeand and Beineke 1980; Zobel and Talbert 1984). Finally, multiple population breeding strategies (MPBS) apply different selection criteria to different subpopulations and thus couple gene conservation with breeding, while maintaining options to change direction (Burdon and Namkoong 1983; Eriksson et al. 1995). This approach is used in Sweden to apply MPBS across photoperiod and temperature gradients, thus conserving diversity through population subdivision and maintaining options for deployment under climate change (Danell 1993a).

### Mating

Fundamental to the principles of breeding is that populations must be regenerated by mating in order to expose new genotypes to selection. The breeding itself cannot change gene frequencies in the recruitment population compared to those of the selected parents, but selection can only act on genotypes, so recombination of alleles is essential to capture additional gain.

Breeders have an array of mating designs from which to choose; the complexity often arises from a desire to accomplish more than one goal when mating selected parents. The most common goal in the past has been to generate additional information about parental breeding values, in order to select more intensively on the parents, i.e., progeny testing. Good estimates of parental breeding values can be generated with any mating design that produces large half-sib groups. The simplest design for this purpose is simply to collect OP seed; if the pollen parents are comparable for each OP lot, the average performance of the progeny will give an excellent indicator of breeding value for the seed parent. Comparability of pollen parents can be assured by

using controlled pollination with a standard pollen mix, i.e., a polycross. There are many such progeny tests established in Europe, using one or the other of these options. While they are known to produce excellent information on the parents, and thus improve selection accuracy among parental candidates, they are much less efficient in generating recruitment populations of new genotypes for forward selection, as nothing can be known about the breeding value of the pollen parent.

If the objective is simply to recombine the alleles of the best parents in a recruitment population while conserving maximum genetic diversity in the next breeding population, there is no mating design better than single-pair mating (SPM). For a given level of effort and no selection among families, SPM gives the highest within-family selection intensity. Unfortunately, producing a single cross per parent produces a rather poor estimate of parental breeding value. If a mating design is to serve the dual purpose of progeny testing AND recruitment of the next breeding population, the mating design must necessarily trade off one against the other.

The approaches most commonly used in the past to address this dilemma have been either to conduct a progeny testing step separately from generation of the recruitment population, or to produce many more crosses per parent, in order to generate accurate parental breeding values. Both approaches have short comings, but the most important of these is that the time required to complete a breeding cycle is usually longer. A separate progeny test will effectively improve the accuracy of selecting breeding parents, but breeding itself must be delayed. Complex mating designs require considerable effort to complete and inevitably delay the completion of breeding. As demonstrated in Chapter 1, the length of the breeding cycle has a profound effect on programme efficiency and rate of improvement.

This emphasises the need for simple mating designs, with only one mating event per cycle and where all progeny are candidates for selection. The simplest design by far is to collect OP seed from parents with the highest breeding values. Selections made within OP families will have estimated breeding values that reflect those of the seed parent and relatives of that seed parents, and the individual's deviation from the OP family mean; it will lack information about the pollen parent. If instead of OP seed we produce a polycross with a standard pollen mix, the sources of phenotypic information available will be the same as those for OP, but the estimates will be improved to some degree by the standardisation of the pollen pool.

Mating designs relying on CP can exercise control over male pedigree; breeding value estimates reflect information from both parents and their relatives. Smaller numbers of

crosses per parent will maximise selection intensity within family. Positive assortment of the parents will not change the mean value of the recruitment population, but will expand the variance and produce better genotypes to be selected into production populations, where gain is realised (Rosvall and Mullin 2003). “Expansion” of the breeding population by a factor of two, as described in Chapter 1, will permit a level of selection among families, while conserving the same level of diversity, so that this additional crossing effort can achieve a return. Expansion beyond this level produces much less return for the added effort.

Finally, a programme might wish to consider crosses using a pollen mix of selected parents, followed by paternity testing of the best selection candidates (Lambeth et al. 2001). While this is certainly possible, it still requires as many controlled crosses as would double-pair mating and has the added cost of marker generation and analysis for paternity. The simulations conducted to date do not indicate large gains from using markers for paternity testing alone (Bouffier et al. 2011; Rosvall and Mullin 2013). On the other hand, the average relationship matrix usually used in BLUP procedures and derived from pedigree data could be replaced by the realised genomic relationship matrix derived from marker data and thereby increase the accuracy of BLUP breeding values, so-called marker-based BLUP or GBLUP (El-Kassaby et al. 2012; VanRaden 2008). Indeed, some tree breeding programmes outside Europe have already adopted rather simple OP mating for most breeding, on the assumption that marker-based relationships will be available for future BLUP estimates of breeding value (Dungey et al. 2009).

Time-to-maturation is a bottleneck for many species, delaying generation turnover and slowing the rate of progress in breeding programmes. Research on the biology and regulation of flowering is progressing (Day and Greenwood 2011; Flachowsky et al. 2009), but stimulation techniques are still largely cultural, such as improved growth conditions, stress through root pruning, partial girdling, hormonal treatments, etc. (e.g., Almqvist 2003; Chalupka 2007). The costs for such treatments, including movable breeding archives where selected individuals can be transferred between outdoor and greenhouse conditions, should be evaluated in relation to the achieved impact and the benefits from shortening of the cycle time.

The lesson to take from this to other programmes is that mating designs need not be complex to be effective, and that breeders must consider carefully the consequences of their choices on the rate of generation turnover. The mantra “*breed early, breed often*” will generally hold and describe best practice.

## Phenotype assessment

Application of selection in any breeding programme attempts to infer genotype from phenotypic information. Quite often in the breeding of forest trees, the objective trait of greatest concern is economic value at time of harvest. Economic value is, itself, a complex quantitative trait and rarely is it feasible to wait a full rotation before selection is applied, so breeders will normally rely on surrogate “selection traits”, applying selection on an index of genetic worth where traits are weighted by an economic weight correlated with value at time of harvest.

Generally speaking, breeders rely on observed phenotype combined with information on pedigree relationships in order to infer genotype, described quantitatively as breeding value. Assessment of phenotype for selection traits often consumes the majority of resources of a breeding programme, as these traits must be expressed and observed in some combination of field, nursery or laboratory tests. Best practice requires careful planning of these tests in order to maximise the quality and usefulness of phenotypic assessments used to estimate genetic worth.

Most forest trees exhibit adaptive variation to different parts of their planting area. At the same time as field tests must characterise performance of established genotypes, they must also characterise the role of environmental factors in performance and the nature of any genotype-environment interactions. This is “a big ask!”

The options for testing are well beyond the scope of this document. Best practice requires careful planning of the testing scheme to avoid some of the shortcomings of phenotypic data currently available. Important principles that must be addressed in the planning include:

1. **There must be a strong genetic correlation between assessment and objective traits.** Generally speaking, age-age correlations are stronger when assessment ages are similar. Early assessment must evaluate the trade-off between weaker genetic correlations and shortening the breeding cycle. While a first assessment of survival might be made to establish a baseline for seedling and planting quality, site harshness, etc., most programmes will not want to assess productivity traits until a field test has reached about 20% of the expected rotation age, but this must be assessed on a trait-by-trait, case-by-case basis. Likewise, weaker genetic correlations between selection traits assessed on small plots and those normally expressed in a fully-stocked stand condition, such as productivity per unit area, must be compensated by the statistical efficiency and lower cost of small plots.
2. **Environments must be adequately sampled.** Phenotypic assessment of forest trees will normally require

observation on multiple sites, in order to evaluate plasticity of expression and the role of genotype-environment interactions. One would expect that the environments sampled in field testing will maximise the quality of data, yet also sample at least the current range of environments, if not additional sites representing future conditions.

3. **Assessments must be well-connected, by pedigree and by environments.** Today’s mixed-model analysis techniques are capable of combining information from a wide range of genetic groups, environments and experimental designs, but only if there are data connecting the different groups. To fully utilise the additional information, genetic tests and test series should be well connected by pedigree and common control lots. The degree of overlap can be optimised, but to-date, the connectedness among trials has been weak or lacking.

Vegetative propagation as rooted cuttings and somatic embryogenesis are under development also for species that lack natural vegetative reproduction. Clonally replicated individuals are effective for efficient selection (Rosvall and Mullin 2013) and are also the best (only) way to characterise spatial plasticity of individual genotypes. A breeding strategy should take into account possibilities to utilise vegetative propagation in future activities, even if it is not an option today.

Phenotypic data collected by a breeding programme will quickly overwhelm most desktop computer storage systems. A purpose-built database system, such as that provided with TREEPLAN® (McRae et al. 2004), will ensure that the data can be properly checked on entry, integrated with complete pedigree information, secure, and readily available for mixed-model analysis of BLUP breeding values.

## Genotype assessment

Most of the models and techniques developed through standard quantitative genetics attempt to draw inferences about genotype or genetic value, given observations of phenotype and relationships among individuals. Many of these same models can be used to infer phenotype, given observations of genotype. With technology for deriving genomic information becoming more cost effective and available to tree breeders, the possibility may soon exist to replace costly and time-consuming collection of phenotypic information with lab-based DNA analysis and derivation of genomic data.

At this point, reliance on such “reverse quantitative genetics” is premature and the technologies not yet applicable to most breeding programmes. Nevertheless, lab technologies are rapidly decreasing in cost and best practice would dictate that programmes plan ahead to be in a position to utilise

genomic assessment, when it is more generally available. A key aspect of genomic assessment is that it relies on having large amounts of high-quality phenotypic data in order to train prediction models against genomic data (Jannink et al. 2010; Meuwissen et al. 2001). Best practice would suggest that tree breeders should at least keep samples properly stored, either as tissue or extracted DNA, in order to have well-characterised phenotypes available as training populations for genomic selection.

### Selection

Selection in tree breeding focuses on assembling groups of selected genotypes, for breeding or deployment or both, usually under a variety of technical or pragmatic operational constraints. Selection would normally be applied to a calculation of “genetic worth”, defined as an index of observed selection traits, weighted by heritability and economic value. For a breeding programme to be successful, appropriate economic weights are as important as appropriate genetic parameters, but are often overlooked or crudely handled (Berlin et al. 2009; Berlin et al. 2012; Ivković et al. 2010).

Calculations of genetic worth can be extremely simple, such as the sum of the products of simple economic weights and phenotypic values for each trait (Cotterill 1985; Cotterill and Jackson 1985). Nowadays, breeders are more likely to utilise mixed-models of genetic and environmental effects, where variances and covariances between relatives and between traits are incorporated in an BLUP prediction of genetic value. Tools for calculating BLUP, such as the mixed-model analysis package ASReml (Gilmour et al. 2009), are now found on the desktop computers of virtually all tree breeders. High-capacity BLUP tools are also built into purpose-built tree breeding data management and analysis software, such as TREEPLAN® (McRae et al. 2004).

Regardless of how genetic or breeding values are calculated, the breeder must decide how these values will be used in a selection procedure, depending on how the selected group is to be used. Selection can be “backward”, using data from progeny to improve efficiency of selection on parents, or may be “forward”, including the progeny as selection candidates. Progeny testing makes backward selection highly accurate, but also time consuming. Forward selection can be made more accurate by cloning the test material, so that there are multiple phenotypic observations for each genotype.

With better estimates of breeding value comes the risk of selecting groups where the individuals are highly related. For most selection applications, whether it is for seed orchard deployment or to form a breeding population, some kind of restriction will be necessary on relatedness among the selections. The optimal balance between genetic value and

relatedness was formulated by Lindgren and Mullin (1997) who expressed “group merit” of a selected set of candidates as a function of their average genetic value, penalised by the group coancestry (relatedness) among members in the set. The problem becomes one of maximising group-merit, with a declared constraint on group coancestry. Solving the optimisation problem has progressed to mathematical programming approaches. Meuwissen (1997) proposed an “Optimum Contribution” (OC) algorithm, based on Lagrangian multipliers, which was recommended for application to forest trees by Kerr et al. (1998). The application of OC has been demonstrated in the management of breeding populations (Hallander and Waldmann 2009a), and in the optimal selection of Scots pine parents for a seed orchard with unequal numbers of ramets (Hallander and Waldmann 2009b). While the method showed promise in both selection of breeding parents and seed orchards, there were difficulties to impose other realistic constraints, such as total number of selections, minimum or maximum numbers of contributions, etc.

The selection optimisation problem has been revisited recently, which has led to new optimisation models using mathematical programming. A software package is about to be released by Skogforsk that will implement mixed-integer linear programming to optimise selection of a fixed-size breeding population and semi-definite programming to optimise unequal contributions of clones to a seed orchard, both incorporating constraints on relatedness (or effective population size) as well as pragmatic constraints reflecting availability of material (Mullin et al. 2013).

### Opportunities for best practice through “geo-climatic” cooperation

Cooperation in human endeavours is usually beneficial for all parties. Some level of cooperation among public and private entities, both within and among countries, in breeding and deployment of improved reforestation stock has long been practiced across Europe, even if not always formalised.

There are several examples of non-local species and provenances showing superior performance in survival and growth, which stimulates agreements between regions. Other reasons for interest in foreign seed sources may be the lack of local seed, a comparatively higher level of genetic improvement, enrichment of the gene pool, preparation for climatic change, etc. For species of minor economic importance or of limited/scattered distribution, a joint breeding and mass production programme can lower costs and increase effectiveness over what might be possible for a single country or partner, each managing a small population in a MPBS-style

programme. Collaborative approaches may target native species as well as introduced species. In the case of introduced species, knowledge about ecological suitability and genetic architecture in the novel environment is often limited, making cooperation even more attractive.

Different types of cooperation can be identified, based on the nature of sharing and exchange:

### **Germplasm**

Seed and seedling exchange among countries in Europe, as well as with continents such as North America, has been practised for centuries. Both successes and failures have been experienced, where the failures from the earliest introductions often were due to lower awareness of the importance of genetic origin or adaptation profile. Consequently, there exist within Europe forests of exotic but unknown genetic origin, and younger generations of forests that may be a mix of unknown and local origin.

Establishment of designed and documented field experiments with known genetic origins started mainly in the 20th century. As a result of such experiments, e.g. the IUFRO series, much knowledge about performance and adaptation of various genetic origins in various environments has been gained (Morgenstern 1996). Based on experiments and practical experience, there has been an exchange of forest regeneration material as well as breeding material among countries. As an example, founder populations for Scots pine, Norway spruce and birch in Sweden include genotypes from other Nordic/Baltic countries and Russia. Some of the genetic testing is also performed in other countries.

The potential benefits of sharing germplasm are increased economic productivity and effectiveness, given that genetic background and its suitability for the environment are known. A potential drawback is a potential reduction in genetic variation, since a given resource is used over a larger area. If we formulate the exchange as part of an MPBS cooperative programme, it can serve to conserve genetic resources, without the need for a separate, and expensive, conservation programme.

### **Information**

Formalised collaborative projects such as GeneCar, Treebreedex, Adapcar, and Noveltree are examples of more recent networks in Europe facilitating the exchange of information and knowledge, including operational tree breeding. In Treebreedex, one of the Activities (no. 3) had the aim to define European-wide breeding zones for a number of tree species.

Establishing species networks for exchange of information on breeding strategies, available germplasm, etc., will increase effectiveness as well as serving as a base for merged

deployment programmes. Such exchange would be a natural outcome of collaborating on joint breeding programme, structured under an MPBS.

### **Expertise**

Sharing expertise can be favourable where the critical mass of resources is insufficient to carry out tasks with the most advanced and appropriate methods. This can be especially true for analytical tasks that are conveniently carried out remotely. Despite formal networks for exchange of information on both scientific and technical issues, exchange of expertise where staff work directly on operational breeding tasks with other organisations is probably less common.

Support agreements with outside organisations can improve breeding efficiency and be seen as a form of expertise sharing for operational breeding. An actual example of expertise-sharing is the formal agreement between Skogforsk and the Australian PlantPlan Genetics group, developing innovative software tools such as TREEPLAN® to assist breeders in operational tree improvement activities (McRae et al. 2004). As breeding becomes more advanced with complicated pedigrees, genomic relationship and multiple generations of phenotypic data, sharing of expertise and cooperative development of methods and tools may prove quite advantageous.

### **Implementation and operational cost**

Large breeding programmes are usually established for species of significant importance for a major land area or a country. Even in these cases, there are economies and increased efficiency to be gained by linking these established programmes under an MPBS framework with other neighbouring countries. Not only can the workload be shared, there is greater ability to maintain suitable backup to respond to changes in climate and markets.

Even when breeding activities for major species are conducted separately, merged deployment programmes (e.g., Scots pine in Sweden and Finland), driven by market factors, may be strategic and beneficial for both partners. Commercial trade is supported by EU and is likely to develop, provided that transfer distances are within adaptation limits.

Pan-European cooperation for minor (and major) species can include testing in neighbouring countries. Total cost for testing could then decrease and at the same time cover larger climatic and environmental gradients. Advantages with such testing cooperation are both improved testing/breeding (e.g., stability), exchange of breeding material, and possibilities to define response norms and facilitate preparation for climatic change.

As mentioned earlier, some aspects of expertise and skills are highly specialised. Rather than attempting to duplicate these skill sets in all countries, collaborative training and development could establish common expertise to be shared on specialised tasks.

### Optimum deployment across political boundaries

Co-operation and exchange of breeding and deployment material should preferably be based on scientific results from field experiments where a variety of genetic entries are tested in ecological zones or gradients of interest. Both climatic factors and physical site conditions should be considered. Adaptive traits, such as survival, fertility, resistance to damaging agents, growth, etc., are of major importance, as are temporal and spatial plasticity, for these will have an impact on the performance in a new environment. Consequently, in addition to provenance experiments, clonal and seedling field test of bred material will contribute valuable information. Norms of reaction and transfer functions (e.g., Thomson et al. 2009) may then be constructed and guide cooperative breeding and deployment. Such an approach also gives possibilities to predict performance in a changing climate.

Even when genetic performance data are lacking, ecological zones or gradients may be identified, and these can help guide deployment in new areas. In general, the most important are:

- photoperiod (latitude)
- temperature conditions (where altitude is a strong agent)
- soil humidity or precipitation (water-use efficiency)
- soil conditions
- biotic resistance/ susceptibility

In addition to geo-climatic factors, biotic factors also need to be considered. Pests and parasites as well as damage from herbivores can have large impact when species are grown in novel environments. The biotic factors may either disqualify or favour novel reforestation material, depending on susceptibility or resistance, respectively. Most obvious are those at the species level where there are lots of examples on resistance/susceptibility when grown in new environments (such as radiata pine, lodgepole pine, poplar, etc.), but different genetic origins within species also show variation in resistance/susceptibility and are important to consider.

If norms of reaction or transfer functions can be established for a species and its ecological requirements known, the performance of a genetic origin can be predicted for a novel environment. The development under Noveltree (WP4) of joint deployment functions for Scots pine in

Sweden and Finland is an example. Scots pine is a major species in both Sweden and Finland, where each operates large, intensive breeding programmes (Ruotsalainen and Persson 2013). There is exchange of breeding material, but to a rather limited extent. Both countries have a strategic interest in managing a national breeding programme, but there is a market-driven interest in using material from Sweden in Finland and vice versa. In Sweden, transfer functions for Scots pine have been developed (Andersson et al. 2007; Persson 1994), which together with estimates of genetic gain in improved stock (Andersson et al. 2007; Rosvall et al. 2001) constitute the basis for deployment guidelines. The guidelines are demonstrated in the form of a web-based program with free access ([www.kunskapsdirekt.se/plantval](http://www.kunskapsdirekt.se/plantval)). By supplying parameters for a given reforestation site (latitude and altitude), the user receives information about the performance of different reforestation stock in terms of growth and survival, and can make an informed choice to source their planting stock.

Under Noveltree WP4, new production and transfer functions have been developed (Berlin et al. 2011). The functions predict Scots pine performance, i.e. growth and survival, in both Sweden and Finland, and for both current and future climate conditions. Geo-climatic indices for the site of reforestation as well as for the genetic origin of the reforestation material are used. The climatic indices are taken from global (GCM) and regional (RCM) climate models used in climate scenario research, and then bias-corrected based on observed station data. Since the indices originate from those used in scenario analyses, it will be possible to predict Scots pine performance in a future climate by adjusting the indices according to the chosen scenario. The GCM and RCM data cover the entire Europe and are thus available for similar modelling of other species and areas. The goal in WP4 is to produce a web-based decision support tool where the performance of all possible seed sources of Scots pine, with origins from Sweden and Finland, is predicted for any reforestation site in Sweden and Finland. The level of genetic improvement for each seed source is added to the functions, thereby allowing for accurate comparison of all available seed sources under operational conditions.

## New tools and techniques from Noveltree projects

The main objectives for Noveltree include:

- Identify new selection criteria to increase the competitiveness of the European forest-based sector and guarantee sustainability of European forests and ecosystems, including tolerance/resistance to pests and diseases, biomass production, and wood properties for present and future use, and plastic response to climate change.
- Develop and integrate genomic information in breeding, including both neutral markers for identification of individuals/populations and for monitoring genetic variation and relationships, and markers for associations with traits of importance.
- Develop novel/improved breeding strategies and demonstrate their efficiency in different case studies with simulation, with particular attention given to multiple-goal breeding constraints, trade-offs among selection objectives, balance between short- and long-term genetic gains, and multigeneration schemes.
- Provide training in emerging technological approaches in connection with on-going European projects, disseminate the results to different publics and transfer technology to the forest-based sector.

Significant progress has been made and reported in Noveltree presentations, deliverables, and scientific articles. Some of the findings in Noveltree that are more directly connected to improved breeding strategies are summarised here.

### Tools for Strategy Evaluation

Simulation programs such as POPSIM™ (Mullin et al. 2010) and METAGENE (Sanchez 2010) constitute new decision support tools that have been developed and made available to European breeders. POPSIM, a parameter-driven simulation program, assists in evaluation of alternative breeding strategies over multiple cycles. Genetic gain per year and/or cycle is maximised, controlled for costs and/or loss of genetic diversity. Multiple combinations of mating, testing and selection are possible to evaluate with detailed inputs on species and population prerequisites (Rosvall and Mullin 2013). A second simulation tool, METAGENE, is an allele-based model to predict the development of correlated traits under selection. Depending on linkage and/or pleiotropy, the outcome in trait expression and their correlations will differ over generations (Wu and Sanchez 2011; Yanchuk and Sanchez 2011).

### Understanding plasticity

Phenotypic plasticity and its genetic background are important components when developing a breeding strategy, especially when considering changing climate conditions. Norms of reaction for wood density in maritime pine have been evaluated to relate phenotypic response to environmental gradients, i.e., drought index (Bouffier 2010). The method gives a measure on phenotypic plasticity of water use efficiency. Candidate genes controlling variation in annual growth rhythm in Norway spruce and Scots pine have been identified (Lagercrantz and Avia 2012). The analysis of the available polymorphism at these strong candidate genes will offer new perspectives for the design of gene-assisted selection for bud phenology plasticity in conifer species.

Phenotypic plasticity/stability to mitigate climate change is a focus of Noveltree and has also been stressed in other cooperative programmes and networks, e.g., EUFORGEN (Koskela et al. 2007). A long-term breeding strategy should consider plasticity/stability of pronounced traits (adaptive traits and economic yield traits) as a priority. Both spatial and temporal plasticity/stability are of importance, where spatial can be evaluated from multiple test sites and temporal from multiple registrations in long term experiments. A large variation among test environments facilitates the construction of relevant reaction norms.

### Improving assessment of genetic worth

Genetic parameters for height increment, the most important selection criteria have been compiled for Norway spruce and Scots pine in Sweden (Kroon et al. 2011). The results can be used for fine tuning breeding strategies with POPSIM for individual breeding populations over climatic gradients. Genetic correlations between traits of interest for European key tree species have started to accumulate (Climent 2010). Together with genetic parameters, the correlations are crucial to develop a selection index and optimise breeding with given constraints.

As mentioned earlier, the average relationship matrix usually used in BLUP could be replaced by the realised genomic relationship matrix derived from marker data and thereby increase the accuracy of BLUP breeding values. Pedigree reconstruction with markers over multiple generations has been developed in Noveltree and constitutes a powerful tool to control relatedness, improve accuracy of breeding values, and guide mating designs (Fernández 2010, 2011).

A comprehensive review of MA-BLUP showed a gap between the theoretical developments of marker-aided selection and their operational applications (Muranty et al. 2010). Finding the associations between markers and

expressed traits, and validating those for actual environments, is still to be done before large-scale operational applications are possible. Often, the main stumbling block is lack of accurate phenotype data to associate with the large amount of available genomic data.

## And what about the future?

With support from Noveltree and other collaborative efforts, genomic tools are under rapid development and a new generation of sequencing technologies for SNP discovery has increased the number of background or neutral markers to monitor genetic diversity and control population structure and relatedness. This track is rather straight forward regarding methodology to be applied in operational breeding programmes. There remains the large practical task to extract the information from the vast number of individuals making up a breeding programme – and the cost associated with such an effort. Still, the potential of controlling population structure and relatedness suggests the strategic importance of securing DNA samples of selected germplasm, particularly from founder populations, to facilitate the analyses needed.

Marker technologies already in-hand can be applied for verification of identities in seed orchards, clone archives, etc. They also make possible the paternity determinations required by advanced polymix breeding, as outlined in Rosvall and Mullin (2012), and to estimate rates of pollen contamination in seed crops from seed orchards. The application of this technology is more a question of whether or not the information is worth the cost and effort. Markers also offer possibilities to dissect evolutionary processes and the influence of human society on the genetic structure of the forest.

Another application of genomic markers is their associations to functional polymorphisms. Here, genome-wide scans seem to have the largest potential to add valuable information in operational breeding (Harfouche et al. 2012; Meuwissen 2009; Meuwissen et al. 2001; Woolliams 2010). Traits that are expressed in later life stages and/or are expensive to collect (e.g., disease resistance, wood and fibre properties, etc.) are of greatest interest. Before any large-scale implementation is to be expected, the cost for whole (or informative) genome scans need to be lowered. Associations between phenotypic performance and genomic patterns need to be established with appropriate “training populations” and validated in varying genetic and environmental backgrounds. Some studies with real data on trees (Grattapaglia and Resende 2011; Resende et al. 2012a; Resende et al. 2012b) have now followed earlier simulation studies (Iwata et al. 2011). The development of genome-wide scans requires large amounts of data, both marker and

phenotype, ultimately related to the founder population. Learning from the experiences in livestock breeding suggests that forward-looking tree breeding programs in Europe should be archiving pedigreed DNA samples with associated phenotypic records, in anticipation of affordable scans of very dense SNP maps in important tree genomes.

The future of best practice for tree breeding must also consider the application of genetic modification (GM) technologies. In principle, new gene constructs can be inserted into an individual with recombinant DNA methods affecting genotype and gene expression, giving rise to a GM organism with more or less novel traits (Flachowsky et al. 2009; Harfouche et al. 2011; Osakabe et al. 2011). These techniques are used commercially on a wide scale for herbicide resistance, insect control and several other characters in agricultural crop species. For trees, there remains a hurdle for GM technology to find suitable gene constructs, rather than the modification technology itself. GM also requires a system for propagation of transformed tissue, and somatic embryogenesis is most commonly suggested for this purpose. Even if GM comes to large-scale practical application in forestry, it is most likely to be used to introduce specific traits rather than for general improvement, so that strategies for best practice of recurrent selection and breeding remain valid.

## Summary

In summary, we have emphasised that there is no such thing as a “one-size-fits-all” breeding strategy, even for a given species within Europe – and there are many! The design of a breeding programme must consider a number of important factors and we have provided a framework that can assist in a disciplined analysis of each case according to the relevant biology, economics, institutional and socio-political factors at play.

In establishing a new breeding programme, or in reviewing one already underway, best practice is to consider the five building blocks of a breeding plan: population management, mating, phenotypic assessment, genotypic assessment and selection. Together, these components define the breeding strategy and its implementation. Best practice may well be to implement more than one strategy within a species, where, for example, part of the breeding population might be regenerated in clonally replicated tests, while others are tested as seedlings. Having flexibility to combine strategies can capture opportunities to minimise time lags and shorten the rotation time for a breeding cycle. At any point, you will have the best information about the trees in the breeding population, produced in shortest possible time. Time is the biggest cost.

We have also explored the potential for improved efficiency through “geo-climatic cooperation”, both in operational breeding and in deployment of bred material. A multiple population breeding strategy, or MPBS, will often facilitate collaborative approaches to breeding across regional borders, and collaborative development of production and transfer functions can help ensure that the best material is available to forest owners through the market place, regardless of its source.

Finally, we have highlighted some of the contributions made to tree breeding in Europe through the various projects carried out in recent years through the Noveltree collaboration. New tools and techniques are now available that can assist European partners to squeeze the most out of their investment in tree breeding, and the collaborative research culture encouraged under Noveltree will continue to ensure that research contributes to best practice of tree breeding across Europe.

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