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**Forest Genetic resources  
Their use and conservation**

**Erfðalindir skóga  
Nýting þeirra og vernd**

Abstracts of a conference held in Húsavík, Iceland, August 27-29, 2003  
by the Nordic Group for the Management of Genetic Resources of Trees

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## 1 Inngangur

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Á undanförunum áratugum hefur kynbótastarf á norðurlöndunum skilað mikilli þekkingu um erfðafræði og lífeðlisfræði helstu trjátegunda. Þekking þessi kemur af margvíslegri starfsemi, svo sem kvæmatilraunum og kvæmavali, rekstri og meðferð fræreita og frægarða og rannsóknum á sviði sameindaerfðafræði. Þekkingin tengist ekki aðeins mikilvægum skógartrjám heldur einnig trjám sem ræktuð eru í margskonar augnamiði s.s. landgræðsluskógræktar, útivistarskógræktar og jólatrjáæktar.

Varðandi mikilvægasta þáttinn í vernd erfðalinda skóga, þ.e. trén sjálf, er þessi þekkingargrunnur nauðsynlegur við mótun á áætlunum um varðveislu erfðalinda. Reyndar eru trjákyrbætur snar þáttur í áætlunum um vernd erfðalinda í skógrækt.

Á ráðstefnunni á Húsavík voru saman komnir sérfræðingar í skógerfðafræði og trjákyrbótum til að bera fram nýjustu rannsóknaniðurstöður og ræða þátt trjákyrbóta í norrænni stefnumótun um verndun og nýtingu erfðalinda skóga. Fyrirlestrar fjölluðu um tæknileg atriði, stjórnsýslulega afstöðu og rannsóknaverkefni sem ýmist eru í gangi eða fyrirhuguð. Í þessu hefti er að finna ágrip fyrirlestrana.

Ráðstefnan var á vegum Norræna sérfræðingahópsins um erfðalindir trjáa og var studd fjárhagslega af SNS (Norrænt samstarf um skógfræðirannsóknir). Gestgjafinn var Skógrækt ríkisins.

*Lykilorð: Erfðalindir, erfðaaauðlindir, trjákyrbætur.*

## 2 Introduction

*Eysteinnsson, T. (ed.) 2004. Forest genetic resources: their use and conservation. Abstracts of a conference held in Húsavík, Iceland, August 27-29, 2003 by the Nordic Group for the Management of Genetic Resources of Trees. IFRS Report 21/2004. 41 pp.*

Throughout the Nordic countries, tree improvement activities over many decades have yielded a wealth of scientific knowledge about the genetics and physiology of forest tree species. This knowledge has been gained through a variety of activities including provenance testing and selection, seed stand and seed orchard management and more intensive tree breeding efforts as well as molecular genetics research. These activities have involved both native and exotic species, and not only important forest trees but also trees grown for a variety of purposes such as soil conservation, amenity and as Christmas trees.

With respect to the most important aspect of forest genetic resources conservation, the trees themselves, this knowledge base is essential in formulating a genetic conservation strategy. Indeed, the tree improvement activities themselves are an integral part of any genetic resources conservation strategy in forestry.

The conference brought together experts in forest genetics and tree breeding to present relevant research findings and to discuss the role of tree improvement research within a Nordic forest genetics conservation strategy. The papers presented, for which the abstracts are included herein, included technical papers, position papers and reports of planned or ongoing projects relating to the management and conservation of forest genetic resources.

The conference was held by the Nordic Group for the Management of Genetic Resources of Trees with the support of SNS (Nordic Forest Research Co-operation Committee). The host institution was the Iceland Forest Service.

*Key words: Forest genetic resources, tree breeding, conservation.*

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### **3 NORDIC COOPERATION ON GENE RESOURCE MANAGEMENT – AN OVERVIEW**

#### ***Leena Yrjänä***

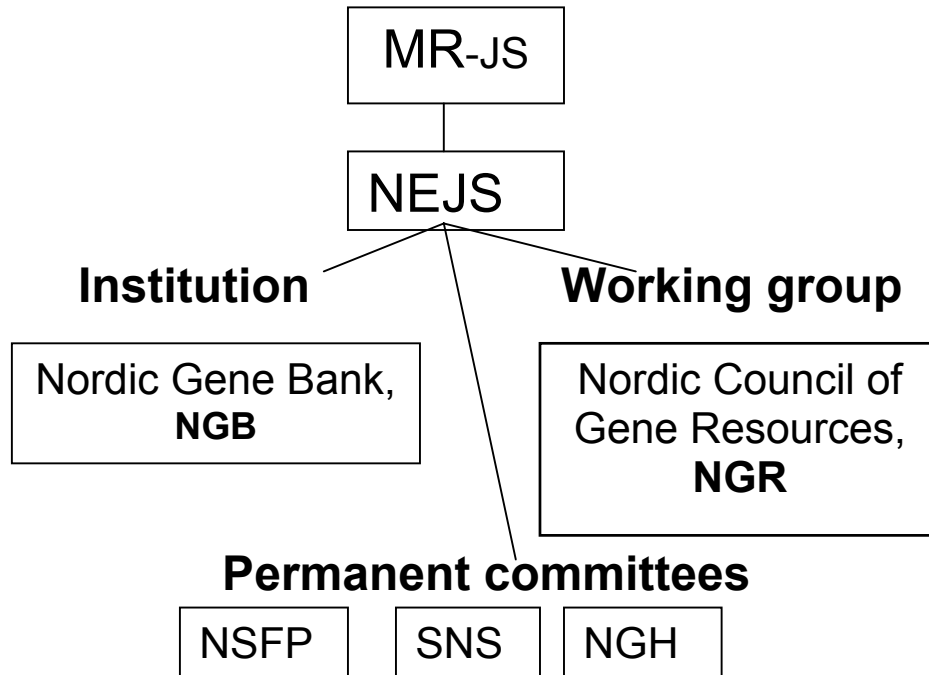
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Nordic cooperation dates back 50 years. The Nordic Council, a forum for members of the Nordic parliaments was established in 1952. Inter-governmental cooperation started in 1971 through the establishment of the Nordic Council of Ministers. Notable early successes include the passport union, the common labour market and social policy conventions. Budgets of these bodies in 2003 are DKK 30 million and DKK 800 million, respectively. Nordic cooperation also extends outside the Nordic Countries, with one fifth of the Council of Ministers budget earmarked for cooperation with the Baltic countries, Northwestern Russia and other Arctic regions.

The Nordic Council of Ministers (MR) is divided into 17 specialist ministerial councils. Cooperation within the forestry sector is guided by the Council of Ministers for Agriculture and Forestry (MR-JS). Administration and follow-up is executed through the Nordic Committee of Senior Officials for Agriculture and Forestry Affairs (NÄJS). The priorities listed for Nordic Cooperation in Agriculture and Forestry for the period 2001–2004 include genetic resources and biodiversity and 4 other main areas (sustainable agriculture, sustainable forestry, food safety and rural development).

Organigram 1. shows the gene resource related bodies under the Council of Ministers for Agriculture and Forestry (MR-JS). Work on Nordic gene resources is done in the Nordic Gene Bank (NGB) on agricultural and horticultural plants and in the Nordic Gene Bank for farm animals (NGH). NGH also coordinates the joint information service for gene resources of agricultural plants, livestock and forest trees. The Nordic Council for Forest Reproductive Materials (NSFP) deals with forest regeneration materials and is now hosting a new organization, the Network for Nordic Forest Tree Gene Resource Conservation. The Nordic Forest Research Cooperation Committee (SNS) has several research groups, one of them dedicated to the management of forest genetic resources. The Nordic Council of Gene Resources (NGR) has since 2001 acted as an advisor on gene resource questions to the Council of Ministers. NGR combines expertise in agriculture, forestry and the environmental sector.

The Nordic Strategic Program on Gene Resources 1994–1997 stated the overall framework for Nordic cooperation on genetic resources. Evaluation of this program in 1998 pointed out the need to create a forum for Nordic gene resource work on forest trees. This proposal was discussed in a meeting in 2000 and the Nordic Gene Resource Strategy 2001–2004 stated that a network organization to carry out Nordic forest tree gene resource work should be established. NSFP was then asked to plan and make a proposal on how to build that network. This proposal was discussed in a meeting in the end of 2002. The outcome was that the Nordic Council of Ministers increased the financing of NSFP for this purpose and the establishment of the Nordic Network for Forest Tree Gene Conservation was included in the NSFP work program 2003–2006. The Network is by no means a permanent body among Nordic organizations; actions and benefits will be evaluated on a yearly basis.



Organigram 1. Gene resource related bodies in the Agriculture and Forestry division of the Nordic Council of Ministers.

The Nordic Network for Forest Tree Gene Conservation should make forest tree gene resources as important an issue as the other sectors of Nordic gene resources are and raise the awareness of politicians, foresters and the public regarding gene resources of forest trees. The Network should make a full use of the joint information resources with both the Nordic gene banks. Cooperation on operational gene conservation work is another issue of importance. Members of the Network all have good knowledge of the administration and implementation of National forest tree gene resource programs. Combining and comparing methods and experiences should be useful to all parties.

National authorities have named these representatives to the Board of experts of the Network: Anna Thorman DK, Mari Rusanen FIN, Aðalsteinn Sigurgeirsson IS, Tore Skrøppa NO and Jonas Bergquist SWE. NSFP Chair Lennart Ackzell serves as the Network Chair and Leena Yrjänä acts as the Network Coordinator.

#### **4 CONSERVATION AND MANAGEMENT OF FOREST GENETIC RESOURCES – FROM STRATEGY TO IMPLIMENTATION**

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The Danish Forest and Nature Agency adopted a *Strategy for the Conservation of Genetic Resources of Trees and Shrubs in Denmark* in 1994. The strategy covers more than 70 different trees and shrubs, indigenous as well as exotic species. The strategy is based on a combination of *in situ* and *ex situ* conservation. *Ex situ* conservation is carried out as a part of breeding programmes. To implement the *in situ* conservation an extended field study has been carried out. Natural populations of indigenous broad-leaved species were identified, mapped and evaluated as to their use as *in situ* conservation stands. Special attention was put on authenticity, population size and isolation against pollution from populations of other origins. The result is a long list of potential stands.

##### **Compromises**

The road from a list of potential stands to the actual layout of protected stands is laid with compromises. The Danish forests have all been intensively cultivated for hundreds of years. This means that we can never



be 100% sure that the stands are absolutely unpolluted by foreign provenances. Even the most remote populations may have received pollen from foreign sources or supplemental plantings may even have been made. For some species the pattern of distribution shows a high degree of fragmentation, with small and scattered populations. Very often, most of the populations have been considered too small to ensure a fair level of genetic diversity and hope for future survival in terms of a more formal conservation of genetic resources.

During implementation, it has been recognized that the strategy to some extent has been too ambitious. The strategy has defined minimum requirements for each species considering the number of stands for protection. For some species, the planned number of stands may not be reached.

### **Forestry**

Pedunculate oak (*Quercus robur* L.) and common beech (*Fagus sylvatica* L.) are the most important broadleaved species in Denmark, covering 26% of the forested area. Dutch and Middle European provenances of oak and beech have been widely used in Danish forestry during the last centuries. Nevertheless it has been more difficult than expected to find pure natural stands well isolated from stands of foreign origin that will be suitable for *in situ* conservation.

### **Tolerance/politics**

Danish forestry is in these years trying to survive a major economic crisis. At the same time the Danish government has cut down funding for forestry and nature conservation. To these factors adds the yet unsolved discussions on consequences and compensation for the layout of thousands of hectares of forests for Natura 2000 habitat reserves. This surrounding reality does not leave much professional or public attention (or goodwill!) for conservation of genetic resources.

## **5 A NORDIC APPROACH TO ACCESS AND RIGHTS TO GENETIC RESOURCES**

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A project group mandated by the Nordic Genetic Resources Council has investigated various aspects related to rights and access to genetic resources in the Nordic countries, published by the Nordic Council of

Ministers in report ANP 2003:717. The project comprised all types of organisms and all existing international agreements.

The main international agreement is the Convention on Biological Diversity (CBD) from 1993. The CBD applies an ecosystem approach to conservation. Important items are *sustainable use* and *fair and equitable benefit sharing* concerning genetic resources.

Originally, exchange of plant genetic resources was based on the principle of *common heritage of mankind*, but this has been undermined as patent legislation was interpreted to cover innovations in biological material. Also the concept of *breeders' rights*, as defined by the International Union for protection of New Varieties of Plants (UPOV) is inconsistent with this undertaking. Therefore CBD gives each state the possibility to proclaim *national sovereign rights* over genetic resources.

Compared to other groups of organisms, forest trees can in many cases be considered as being between wild and domesticated. Most outstanding for forest trees is the long generation time, the right of common access to private land in some Nordic countries and the geographically widespread material. The project group analysed three legal situations applied to forest genetic resources; exclusive rights of the owner of the biological material to genetic resources, consider the genetic resources as in *public domain*, an unclear legal status, and recommends the Nordic countries to determine the legal status of their forest tree genetic resources.

Referring to access to the resources, the project group can foresee a wider use of *material transfer agreements* (MTA), but recommends that the present legal situation with simple and non-bureaucratic access to forest tree genetic resources should be maintained. If a future proliferation of exclusive private rights should occur, the Nordic countries are recommended to introduce measures to promote access. Concerning patents, the conclusion is to keep the resources available as freely as possible, and if necessary consider changes in relevant regulations to ensure future access.

The Nordic Council of Ministers (for fishery, agriculture, forestry and food) decided on the 25. of June this year, to launch a Nordic project for developing a platform for decisions concerning the legal status of forest tree genetic resources. The ministers have so far not found it necessary to initiate regulations for access.

## 6 GENE CONSERVATION IN THE SWEDISH PINE AND SPRUCE BREEDING PROGRAMS

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Most tree breeding programs aim for increased value production in the breeding populations and ultimately in the production populations. In dimensioning them, we seek a level of genetic variation, which will serve the value production goal optimally during a number of generations, given certain cost constraints. Gene conservation programs for more long-term purposes are commonly managed separately from the breeding programs. The Swedish pine and spruce breeding programs combine gene conservation and preparedness for environmental change with that of increased yield. In that way the need for renewing and complementing the breeding population with unimproved material in advanced generations is avoided.

In this presentation we discuss different measures of genetic variation and their relevance and impact on genetic status of future generations, referring to the Swedish pine and spruce breeding programs. Based on analytical models and simulation studies, under normal genetic parameter assumptions, the breeding populations will retain large additive genetic variation for sustainable improvement as well as a high level of allelic variation to meet unpredictable future changes in breeding goals and environment. Within a subpopulation of initially 48 unrelated founders the additive effect in response to selection for quantitative traits is still linear and almost unaffected after 10 generations of breeding. At that time, inbreeding coefficient and group coancestry is around 0.05, demonstrating that most of the potential additive genetic variation is preserved. Allelic diversity expressed as proportion of original heterozygosity will be 94%. The number/share of alleles remaining will vary, depending on the original allele frequencies in the population. When allele frequencies are low, i.e. many different alleles present, fewer alleles are retained. If we assume the extreme that all alleles ( $2N=96$ ) in the founder population are different (allele frequencies app. 0.01) only 17% of them will be retained at an average after 10 generations, which is reflected in the status number dropping from 48 to 8. However, if we assume 10 different alleles in equal frequencies (0.1) all of them will be retained at the probability of 60%. Moreover, referring to the metapopulation of 1200 individuals (without sublining) the probability of losing a rare allele ( $< 1\%$ ) after 10 generations

is less than 1%. Subdivision of the metapopulation into several (>20) smaller (sub)populations further improves the probability of keeping rare alleles. Consequently, the pine and spruce breeding programs will produce sustainable gain for tens of generations without exhausting genetic variability either for further breeding or evolutionary development.

A prerequisite for the predictions is balanced within-family recruitment. Fine-tuning of the breeding programs, for instance through implementing positive assortative mating, can improve the genetic gain with no effect on genetic diversity. Other methods, such as increased contribution from superior parents and elite line breeding with open nucleus within subpopulations, can also increase gain at the cost of only minor reduction of genetic diversity. Since the breeding programs are dimensioned with high safety margins there is room for such enhancements.

## **7 THE GOOD ANIMALS AND THE BAD ANIMALS (NOT A SCIENTIFIC PAPER)**

***Þröstur Eysteinnsson***

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### **Introduction**

There is an ever increasing body of both popular and scientific literature dealing with genetic material being where it “does not belong”. By this I mean 1) species taking up residence in places where they did not reside before and 2) genetic material taking up residence in species where it did not reside before. This out-of place genetic material is usually referred to in variably negative terms as 1) exotics, invasive aliens, exotic weeds and 2) GMOs, Frankenfood, clones (although clone is in fact an unrelated term and its use in this context shows ignorance). In this non-scientific paper, I will discuss this subject as it relates to trees.

### **Out-of-place genes - exotics**

Most agree that national borders are not criteria for determining whether a species is exotic or native on a specific site, although that can be the outcome legally speaking. Usually, historical distribution is used as the main criterion – if there is evidence (written records, pollen, fossils) for a tree species having been somewhere earlier, then it is considered native, otherwise not. In discussion of biodiversity, this thinking is sometimes taken to the extreme, with populations replacing species as the unit discussed – a population of a species belongs somewhere if it has resided there before.

Tree species (or populations) can grow in new places as a result of several different processes. They can arrive in a new place without the intervention of humans or start thriving where they did not thrive before due to environmental change not attributable to humans. They can arrive accidentally or start thriving in a new place through human activity that had nothing to do with the tree species in question. They can escape from cultivation, i.e. spread through human activity without that having been a specific goal. Finally, humans can distribute them purposefully to new places.

Of these, the most commonly occurring is the first. Palaeobotanical and palynological evidence indicates that the ranges of tree species change constantly over time. Natural climate change is one factor in this, but as important are factors such as pest-host co-evolution. These processes do not stop just because we all of a sudden notice that things are where they are now.

Human activity such as disturbance due to urbanization or agriculture or decreasing disturbance such as fire prevention can affect distribution of tree species. Land use practices having nothing to do with trees are the human activities that most affect the distribution of tree species. Agriculture is by far the most important of these activities and has mostly led to decreased cover of tree species but often increased range, especially for tree species that are able to take advantage of disturbance.

Much less important in terms of area affected is the purposeful distribution of trees to new areas and accidental escapes from cultivation into pristine ecosystems are even less common. These are however the ways in which tree species are moved very long distances and the way in which it is done often involves technology such as nursery production and site preparation.

#### **Out-of-place genes - GMOs**

The term Genetically Modified Organism (GMO) refers to an action and not a state of being. It matters how the gene got into the genome, but not how the gene is different or not different from the rest of the genes in the genome nor what the gene does. GMO refers specifically to human manipulation of the genetic makeup of an organism using specific (new) technology.

Genes move between species on a regular basis and on a large scale, to say nothing of movement between populations within a species. Hybridisation is a common occurrence in nature. Is the Icelandic birch *Betula pubescens* with some *Betula nana* genes that make it crooked and

low growing or is it *Betula nana* with some *Betula pubescens* genes that make it a bit taller? Has it been genetically modified? Yes, only not by humans.

Hybrids made by controlled crossing in tree breeding programmes are certainly genetically modified, and by humans. Yet they are not called GMOs because the method used was normal sexual reproduction.

There are several ways of inserting specific genes into an organism, but it most often involves using bacteria such as *Agrobacterium* as a shuttle followed by multiplication via some sort of tissue culture. This involves some technology and people in white lab coats but mostly it involves a great deal of knowledge of biology, of the nature of genetics, reproduction and growth. This, finally, leads to something that is called a GMO.

When the range of a species or its genetic make-up changes and humans had nothing to do with it (by far the most common way for genes to become "out-of-place"), it is not a cause for controversy. If the same thing happens and it is an indirect result of human activity (less common but wide-spread), it may cause some controversy but is usually accepted as at worst a necessary evil. If the same thing happens and it involves purposeful action or complicated technology (least common), it becomes unacceptable to many people. Why?

### **Practical objections**

Trees can become weeds. They can invade agricultural land, roadside areas and other places where they are a nuisance, and it costs money to control them. These are economic concerns that certainly have a basis in fact. People also cite biodiversity concerns, that invasive species could "take over" and dominate entire ecosystems. There are some grounds for this as well. Examples of economically important or biodiversity threatening invasive organisms are mostly of annual herbs (weeds) in agriculture or anthropophilic animals colonizing new lands, specifically oceanic islands, leading to the extinction of native animals and plants. Examples involving trees are few and the problem therefore mostly theoretical.

That is where the dogma comes in; that exotic weeds are worse than native ones. However, in Iceland at least, the native birch is the most invasive tree species we have and every bit as effective at pushing out open-land birds and light demanding plants as the exotic larch. Yet birch regeneration is "natural" and good whereas regeneration of exotic tree species is to some people "cause for concern". So it seems that it is the exoticness and not the invasiveness of a tree species that bothers people. Or perhaps

“exotics are bad” is a more easily promoted dogma than “invasive are bad”. This leads to a purist attitude against all exotic species, where no distinction is made between those that are invasive and likely to cause problems and those that are not. Practicality does not enter the picture.

I know of no practical objections to GMOs, only theoretical or “moral” ones.

### **Public opinion**

We are all raised on stories about the good animals and the bad animals. In the older stories, wolves and foxes are bad animals as well as lions and tigers and bears, while sheep, goats and chickens are good animals. Humans can even be heroes, such as the forester who frees Little Red Ridinghood and her grandmother from the belly of the wolf. More recently, lions and tigers and bears and even wolves are often depicted as good animals while humans are the bad animals, reflecting the fact that humans are now in a position of dominance over other animals. Children today are raised on the idea that humans are dangerous to the environment, not that wolves are dangerous to humans, a notion that is not entirely without merit. Despite increased knowledge, the good animal - bad animal dichotomy is still as strongly ingrained in us as ever.

The makeup of today’s society is such that the vast majority of people have a limited understanding of science, while a few have very great understanding within their respective fields. With advances in science, this knowledge gap widens. Most peoples’ knowledge of genetics is limited to Mendel’s peas and they don’t even understand them very well. The result of this situation is that when a new technology is introduced, be it automobiles, nuclear power plants or genetically modified organisms, the public is basically asked to trust the scientists, “they know what they’re doing”. With time however, the general public, if given the chance will, through market forces, judge whether a new technology is a good animal or a bad animal.

I listed automobiles, nuclear power plants and GMOs for a reason. Automobiles kill millions of people globally each year and are a major source of pollution, yet they are considered a “good animal”, a technology, accepted by nearly everyone. Nuclear reactors kill practically nobody and pollute very little, yet they are considered “bad animals” by the majority of people. And GMOs seem to be going the same way as nuclear reactors, in Europe anyway. The proverbial person from Mars might conclude that humans embrace technology that is harmful to themselves and the environment and reject technology that is not harmful.

This is of course not the case, since the decision by society on whether or not to embrace a new technology is not an informed one. That wolves were bad animals was not an informed decision, it was a purely economic one, since wolves sometimes ate livestock. There was no thought as to the ecological significance of the wolf, its complex social behaviour or its role in curbing deer and rodent populations. No, we humans as individuals and as a society make snap decisions on whether a thing is a good animal or a bad animal based on very limited information, usually information provided by someone who seems to have more knowledge than we do or is in a position of authority, such as people on television.

To easily become suspicious or even afraid is an adaptive trait of humans as well as other animals, whether it is has a genetic or a cultural basis. It is therefore much easier for a person with an agenda against something new to get people to reject it than it is for a person with an agenda for something new to get people to accept it. After all, who are we more likely to believe? A person who says "trust me, I know what I'm doing" or someone who says "be careful, this could be harmful", the extreme form of which is "be very very afraid, this will kill you", incidentally the message about wolves in many of the tales collected by the brothers Grimm almost 200 years ago. This is known as the rhetoric of fear.

People on television, who's agenda it is to appear to be in a position of authority, are likely to travel the easiest road possible to that position. After all, their jobs depend on getting the public to watch them. The same applies to people trying to get you to give money to environmental NGO's. An environmentalist stating that exotic Sitka spruce is spreading through Pingvellir National Park like wildfire, exterminating the native birch and causing eutrophication of the lake (a rhetoric of fear statement with no basis in fact) made the news here in Iceland last year, whereas refuting that statement (the truth) was not considered news.

There is an insidious version of the rhetoric of fear that we are all familiar with and many of us accept as axiomatic and is therefore dogma. This is the so called precautionary principle, which is about as difficult to find a definition of as biodiversity, but in general states that when planning or carrying out actions that affect the environment, one should take every reasonable precaution to minimise environmental damage. More commonly, this principle is worded something to the effect that: "where doubt exists as to the effect of an action on the environment, then don't do it". And of course there is always doubt when predicting the future. And this extreme form becomes accepted as dogma and used as a basis for setting laws and regulations because this is what politicians think it says in the Rio accord.



Example: According to regulation 583/2000 based on the Icelandic Nature conservation act, there is a ban on cultivating exotic plant species at elevations greater than 500 m. Why? There is no evidence or even indication that an exotic plant species is likely to damage the environment at higher elevations, although theoretically, some exotic plant might exist that could vegetate barren land at that elevation. No, this is simply the case of someone believing in the dogma that exotics are bad animals as well as the dogma that the precautionary principle says to prevent all actions of humans that could affect nature. Both based on the rhetoric of fear.

It is my conclusion that public opinion, regulations and even laws against out of place genes are nothing more than the result of people who want to be in positions of authority taking the easy road there, simply by classifying them as "bad animals" to a public that is raised on the good animal - bad animal dichotomy. And after having said it often enough it becomes the accepted dogma. I am not optimistic that this will ever change and it can certainly affect those of us who are involved in management and conservation of forest genetic resources. Therefore, we as scientists must realise and accept this state of affairs, deal with it by resisting dogmatic thinking and doing the best we can to provide information to the public.

## **8 THE PRESENT STATUS OF NORWAY SPRUCE BREEDING IN FINLAND IN LIGHT OF GENETIC CONSERVATION**

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Norway spruce is the dominant tree species on 24% of the forest land area in Finland, and the proportion of spruce in the total volume is 34%. The increased need of raw materials during the last ten years has led to large scale felling and clearcutting of natural mature spruce stands. The total area of Norway spruce planted in 2002 was almost 40 000 ha. Eighty per cent of the 91 million seedlings planted were of seed orchard origin. Although plus tree selection of Norway spruce was started in 1950's and a majority of the present 25 seed orchards were established in the 1960's, the breeding of spruce has advanced slowly and has been less intensive compared to the breeding of pine or birch. This has lead to the present unsatisfactory situation with the existing spruce breeding material available

for long term breeding purposes, especially for the composing and conservation of breeding populations.

The Forest Research Institute (METLA) is responsible for the conservation of Norway spruce gene resources in Finland. Since 1993, eight gene reserve forests, a total of 1540 hectares, have been established to meet *in situ* conservation demands. The number of registered seed production stands (plus forests) has decreased rapidly because of ageing of the stands and in many cases because of private ownership and partitions due to inheritance. There is no legislation to allow for the full protection of seed production stands for conservation purposes. This same situation also gives rise to concern for the preservation of the original plus trees. By 2002, only 36% of the 2642 selected plus trees have been grafted into clonal collections.

The typical, current *ex situ* conservation practises with Norway spruce are clonal archives of breeding and research material, seed orchards, provenance stands and trials and most progeny trials. The latest tree-breeding programme was published in spring 2003. According to the adopted open nucleus breeding strategy, a breeding population for Norway spruce will be compiled for each of the six Finnish breeding zones. For the conservation of the breeding material, all plus trees chosen for the main and nucleus populations will be grafted and maintained in clonal archives until the progeny selections are made for the next breeding cycle. Breeding material will be conserved partly as containerised graft collections at Haapastensyrjä Breeding Station.

If the rate of felling of the registered plus forests and plus trees continues to be high in the near future, one solution for the conservation of spruce genes from high quality natural stands might be in existing provenance trials. The Forest Research Institute established more than 40 provenance trials with original plus forest open pollinated material in the 1960's and 1970's. The plot size of the trials is large, comprising of 25 or 49 tree plots with 5 to 6 replications. These trials have acted and will act in the future as a source for new plus tree selections for breeding populations. This is especially the case in Central-Finland, where only 24 % of plus trees have been grafted into collections and seed orchards. It is very likely that in the future, when climatic warming advances, provenance trials and plus tree progeny trials will offer one possibility for the conservation of Norway spruce genes for future needs.

## 9 A CD-ROM PROJECT ON FOREST TREE GENE CONSERVATION

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There are several misconceptions among laymen about evolution. The most prominent being the beliefs that natural selection is the sole factor in evolution and that adaptedness is perfect in nature. The concepts of domestic and Darwinian fitness are frequently mixed leading to erroneous conclusions. The need for inclusion of tens of thousands of individuals in the gene resource population is still another misconception. The latter is related to the belief in the need to capture rare alleles in gene resource populations. Since rare alleles do not contribute to additive variance their presence or absence in a population is dependent on random events even if the rare alleles contribute to fitness.

Currently a CD-rom on *forest tree gene conservation* is under development at our department. High school teachers in biology are the main target group.

In order to design forest tree gene conservation, basic knowledge in quantitative and evolutionary genetics is a necessary prerequisite. Gene conservation of species included in intensive breeding is mostly taken care of in the breeding activity. Besides the file on forest tree gene conservation our CD-rom has files on molecular genetics, quantitative genetics, evolutionary genetics, and breeding. By aid of animations, the dynamic evolutionary processes leading to among- and within-population variability may be better understood than from a "frozen" picture.

In the basic files, DNA, gene transfer, additive variance, breeding value, heritability, genetic gain, genotype x environment interaction, inbreeding, selection differential, selection intensity, selective environmental neighbourhood, phenotypic plasticity, adaptation, adaptedness, adaptability, fitness, and evolutionary factors (natural selection, genetic drift, mutation, and gene flow) are treated and illustrated. Observations in experiments are discussed in light of the basic concepts. Some genetic aspects of global climatic change are treated in a separate file. Principles of tree breeding are illustrated and some results obtained in tree breeding are presented. The different functions of breeding population, gene resource population, and production population are emphasized.

The main focus in the CD-rom is the file on gene conservation of tree species grouped according to breeding activities, genetic knowledge, and utilization. Different objectives and methods in gene conservation are discussed. The multiple population breeding system, MPBS, concept is emphasized. Consequences of species hybridisation are touched upon. There are two files with simulations, the first deals with response to selection at different gene frequencies and selective advantage. The other file deals with simulations of the number of generations it takes to allele fixation when number of individuals and starting gene frequencies are varied. Finally, questions and answers to these questions will be included in each file.

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## **10 CONSERVATION OF GENETIC RESOURCES OF FOREST TREES – A WEB-BASED APPROACH**

***Tor Myking<sup>1</sup>, Arne Steffenrem<sup>2</sup> and Tore Skrøppa<sup>2</sup>***  
Norwegian Forest Research Institute, Fana<sup>1</sup> and Ås<sup>2</sup>

A strategy for conservation of genetic resources of forest trees was recently adopted in Norway by the Committee for Genetic Resources of Forest Trees (see <http://www.skogforsk.no/genress/default.cfm>). The target groups are research colleagues, forest officers, conservation bodies and bureaucracy at the national and regional level. The strategy consists of six items: monitoring, conservation, operating a national network, research, publishing and cooperation.

In the summer of 2003 a pilot project on monitoring of genetic resources of rare and scattered trees was initiated, e.g. for tracing tree migration associated with climate change. Abundance, size distribution (including regeneration) and damage by browsing ungulates are scored at the species level on 0.2 ha permanent plots in a 3x3 km grid in three counties.

Conservation has a dual approach – conservation by the use of forest reproductive material, and by dynamic gene conservation of tree species in forest reserves. A searchable database was established containing the selection of tree species in the different forest reserves.

The national network aims to increase the contact between researchers, forest officers and NGOs by disseminating research news and updates on gene conservation by e-mail and by publishing on our web pages.

The ongoing research on Norway spruce will be supplied with molecular genetic studies. In addition, genetic structure and migration patterns of broadleaved tree species will be studied by genetic markers.

Publishing is to take place at different levels, in refereed international journals and in national forestry journals and newspapers for public awareness purposes.

At the national level cooperation on management of genetic resources occurs in the Committee for Genetic Resources of Forest Trees and in the National Genetic Resources Council. This also includes cooperation among gene conservation activities in agricultural plants, domestic animals and forest trees. At the Nordic and European level NSFP (Nordic Council for Forest Reproductive Material) and EUFORGEN (European Forest Genetic Resources Programme) are the main cooperation units.

## **11 OPTIMAL NUMBER OF TESTED CLONES IN SEED ORCHARDS**

***Dag Lindgren***

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A model has been constructed based on maximizing a goodness criterion ("benefit") for a seed orchard. The criterion is a function of:

- Number of candidate clones (1) available; (2) selected for use in the seed orchard;
- Contribution to pollination from (1) the ramet itself; (2) the closest neighbours; (3) rest of the orchard; (4) outside the orchard (pollen contamination);
- Variation among genotypes for fertility;
- Efficiency of selfing;
- Production of selfed genotypes;
- Gene diversity (=status number);
- Production influence of contamination;
- Genetic variation among candidates;

- Correlation between selection criterion (e.g. height in progeny test) and value for forestry (e.g. production over the range of environments the seed orchard is targeted for);
- and the number of clones harvested.

Numeric values of the entries were discussed, and values were chosen to be relevant for the Swedish conifers and loblolly pine in south US. Benefit was maximized considering the number of clones. The optimum was 16 clones for the Swedish scenario. Intentionally unequal number of ramets, which I argue for, was not a part of this study. A sensitivity analyses was performed to study the relative importance of the entries. The most uncertain factor is the quantification of the value of gene diversity. Other considerations are if the genetic variance in value for forestry or our ability to predict that is lower than assumed and the value of flexibility in future use of the orchard.

## **12 CRYOPRESERVATION AS A TOOL FOR GENE CONSERVATION AND BREEDING OF NORDIC FOREST TREES**

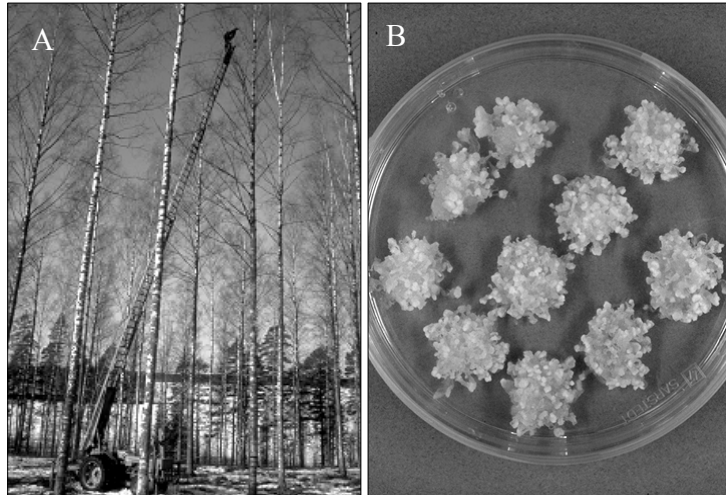
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Forest genetic resources, as a part of biodiversity and nature conservation, are highly appreciated in all the Nordic countries. In forest tree breeding, their value as a source of variation, i.e. as raw material for current and future breeding activities, has been understood for a long time, and different strategies for maintaining genetic variability are applied in connection to breeding programmes. Cryopreservation – storage of tree germplasm in liquid nitrogen (LN) at ultra-low temperature – provides a modern tool for conservation of forest genetic resources and their use in breeding.

Cryopreservation is considered an alternative or a duplicate storage for traditional *ex situ* clone collections. There are, however, also cases in which conservation of genetic material in clone collections is not possible, like genetically modified trees that can be planted outdoors only for a limited period, and after an extensive licence application process. Characteristics, technical demands and costs of both cryopreservation and

conventional clone collections of Nordic forest trees are compared in Table 1.



**Figure 1.**

- A) Collection of *in vivo* birch buds cold-hardened in nature that are ready for cryopreservation.
- B) Tissue cultured material, like embryogenic cultures of conifers, need to be pre-treated with cold acclimation (optional) and cryoprotectants prior to cryopreservation.

Different types of plant materials, such as *in vivo* buds, samples of *in vitro* tissue cultures, seeds, and pollen can be cryopreserved (Fig.1). In deciduous tree species, such as birch and aspen, in which the initiation of micropropagation from bud explants originating in mature trees is possible, cryopreservation can be applied to conserve specific genotypes. Endangered elite trees, and trees expressing rare, valuable or interesting characteristics can thus be saved with the minimum of space and maintenance, and without the risk of contamination, somaclonal variation or genotypic changes due to external factors.

**Table 1.** Cryopreservation versus *ex situ* –clone collections.

<b>Cryopreservation</b>	<b>Clone collections</b>
<b>Space required:</b> -1400 vials in a container (at 1m <sup>3</sup> ); at the maximum as many genotypes	-approximately 100 genotypes /ha
<b>Establishment:</b> -sampling <i>in vivo</i> trees or <i>in vitro</i> cultures -cold acclimation & cryoprotection (if needed) -freezing & immersion in LN	-site preparation -grafting & planting -herbivore protection
<b>Maintenance:</b> -adding LN when needed	-weeding
<b>Costs:</b> -programmable freezer & LN container, 30 000 € -LN & consumables, 200 € labour; depending on material	-land, 3300 €/ha -grafting, depending on material -planting, 600 €/ha -vole nets, 350 €/ha -weeding, 50-200 €/ha
<b>Risks:</b> -uncontrolled thawing of samples -renewal not successful	-climatic extremes -diseases, herbivores -human activities (building etc)
<b>Storage time:</b> -in theory, unlimited	-renew every 50-100 years?

In breeding programmes, cryopreservation can be applied together with vegetative propagation. If clonal testing is used instead of progeny trials to evaluate breeding material, and tissue culture methods are applied for producing clones, the genotypes that are cloned can simultaneously be stored by means of cryopreservation. Of the main Nordic forest tree species, tissue culture methods suitable for mass propagation exist currently for birch (organogenesis) and spruce (somatic embryogenesis). Cryopreservation of embryogenic cell lines maintains their juvenility and regenerability enabling prolonged field-testing of the cloned material. Even if maintaining juvenility is not needed, such as in the case of silver birch, cryopreservation of the genotypes to be tested can be utilised for scheduling tissue culture initiations in a convenient way.



Feasibility of cryopreserved material for different applications differs from that of trees conserved by conventional clone collections. When compatibility of clone collections with vegetative propagation depends on the species and the age of collection, only material regenerable by tissue culture is put into cryostorage. DNA analyses can be performed with both types of collections, although cryostored samples probably need to be regenerated prior to DNA extraction. For sexual reproduction, cryopreserved material can be used only after regeneration and maturation of plants that may require rather long time depending on the species. On the other hand, utilizable pollen and seed production in clone collections is restricted to mature but not senescent grafts, and may vary from year to year.

For all the applications of cryopreservation, genetic fidelity of the cryostored material is of utmost importance. The fact that cryoprotective chemicals, like DMSO, widely used for storing tissue cultured material, are known mutagens, also underlines the necessity of studying the genetic stability of cryopreserved material. At the Finnish Forest Research Institute, genetic fidelity of cryopreserved forest tree material has been studied using molecular markers (RAPDs) in several species: No variation was observed in embryogenic cultures of Scots pine, while in embryogenic cell lines of *Abies* sp. hybrids remarkable genetic changes were found in DMSO-treated but non-frozen samples. In this *Abies* study, however, no variation could be detected in cryostored samples or in samples treated with cryoprotectant mixture. Also, no changes were found in RAPD profiles or in transgene copy number in transgenic lines of both silver birch and hybrid aspen. Furthermore, an extensive study in silver birch is currently under way, covering potential effects of different cryopreservation protocols using both *in vivo* and *in vitro* material on the growth, morphology and genetic fidelity of regenerated plants. For evaluating genetic stability, both markers (RAPDs) and chromosome analysis are employed.

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### **13 OVERVIEW OF OAK GENETICS RESEARCH IN DENMARK AND THE APPLICATION OF GENE RESOURCE MANAGEMENT OF OAK**

***Jan Svejgaard Jensen and Ditte Christina Olrik***

FSL and KVL

*Quercus robur* and *Q. petraea* (Matth.) Liebl. have been the subjects of a number of studies in Denmark within the last 10 years. A brief review of these studies is given, including discussion on how they support breeding, utilization and conservation of oak in Denmark.

Provenance trials have exposed large provenance variation within both species for growth and stem straightness, which are important for forest practice. Human influence can be traced in all oak stands, which is partly indicated by the low floristic diversity and partly by racial differences in stem form. Large provenance variation in phenological traits has been shown in common garden tests. Large variation has been revealed for frost resistance and response to drought stress. The adaptive potential seems to be remarkable. Progeny trials have revealed large within-population variation in growth, straightness and epicormic branching.

Isoenzyme and nuclear microsatellite markers showed limited provenance variation but indicate high gene flow. Other studies of gene flow in oaks reveal extensive pollen flow, while seeds spread slowly. Hybridisation between the two species is mainly unidirectional.

Extra-nuclear markers have indicated the migration routes following the last glacial period. These markers are promising for certification purposes, and most useful for gene conservation.

The outline for a gene conservation plan is suggested. A few large areas with native oaks should be protected from replanting with non-local oaks. A number of smaller stands should be protected and a number of seed orchards should be established in order to produce adequate amounts of seed from Danish seed sources.

## **14 ALIEN GENES IN FORESTRY: HYBRID ASPEN AS A MODEL SPECIES FOR ALIEN GENE FLOW**

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Hybrid aspen (*Populus tremula* x *P. tremuloides*) has been planted in both Finland and Estonia in order to serve as a material for fine quality paper. Hybrid aspen is however an alien in European nature, and the genetic and ecological consequences of hybrid aspen plantations are not known. *Populus* species are among the most studied forest tree species where GMOs are concerned. Since plantations of GMO aspen are restricted by law, hybrid aspen can act as a model species for the environmental, ecological and genetic effects of GMO aspen. In addition to this, hybrid aspen has been planted in Finland for several decades allowing evaluation long-term effects.

We have studied aspen (Finland and Estonia) and hybrid aspen (Finland) stands in order to see whether the offspring are of clonal (root suckers) or sexual (seedlings) origin. Young trees as well as old clones (possible mother trees) have been analysed using microsatellites. Our preliminary results from three Estonian stands show that there are several clones and both suckers and seedlings in all of these stands. Some of the offspring had unique alleles, which could not be found in the adult trees in the stands, indicating that they are result of sexual reproduction, where at least one parent is from a different population. The proportion of these trees varied from 33 to 78% between the different stands. We also found suckers that shared the same genotype, but their mother tree was not among the sampled adult trees. In Finland we have established a trial area, where a hybrid aspen stand produced a large amount of viable seeds and seedlings. However, the number of seedlings decreased rapidly during the first growing season.

In addition to these studies we have made controlled crosses to reveal the possible differences in pollen germination capacity between hybrid and native aspen pollen. We have also tested seed production differences

between different clones of hybrid and native aspens to estimate the possibilities of seed oriented gene flow.

Hybrid aspen is the most rapidly growing tree species in Finland and may spread very effectively through root suckers. To test this hypothesis we have compared root differentiation characteristics between clones of hybrids and native aspens. Large variation in many physiological and anatomical properties has been found in studies of aspen and hybrid aspen clones. Our results with respect to vegetative propagation of hybrid aspen show that there is high variation (29-57%) between clones in their ability to reproduce from root cuttings. Thus we believe that native aspen clones also have very large variation in their vegetative reproduction capacity.

The results from these studies will give knowledge on the reproductive biology of aspen and hybrid aspen. This information will help to evaluate the possible hazards and probability of alien tree gene introgression (e.g. GMOs) into nature.

## **15 CLIMATIC ADAPTATION AMONG NATIVE AND INTRODUCED POPULATIONS OF DOWNY BIRCH (BETULA PUBESCENS Ehrh.) IN ICELAND – IMPLICATIONS FOR GENE CONSERVATION**

***Aðalsteinn Sigurgeirsson***

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Downy birch (*Betula pubescens* Ehrh. or *Betula pubescens* Ehrh. ssp. *tortuosa* (Lebed.) Nyman) is the only woodland-forming tree found in the Holocene record of Iceland. At settlement in the 9th century A.D. birch woodlands are believed to have covered 25-35% of the total land area of Iceland. Eleven centuries later, this figure is down to 1.2%. Nevertheless, birch woodland remnants are found in most regions of Iceland, at elevations from sea level to 620 m a.s.l. Concomitant with the degradation and reduction in birch woodland distribution, the effects of dysgenic selection since settlement are believed to have affected morphological variation among present-day birch populations in Iceland.

In 1998, a series of field experiments were planted on nine test sites in Iceland, with as many as 50 seedlots from native, autochthonous birch stands and woodlands, from throughout Iceland. The objectives were to elucidate the genetic and environmental effects on morphological and adaptive characters in Icelandic birch populations, as well as to identify

local adaptations and populations with good genetic traits. The experiments have been evaluated twice since planting, in 1999 and 2001.

The results so far indicate high genotype x environmental interaction for survival and growth, but at the same time they suggest broad adaptation among birch populations from Southeast Iceland. The latter result may however be due to warmer-than-average growing season temperatures since the initiation of these experiments.

## **16 VARIATION AND COVARIATION IN ANNUAL FLOWERING PHENOLOGY AND FROST HARDINESS IN NORTHERN POPULATIONS OF SCOTS PINE**

***Matti Haapanen, Pakkanen, A. & Pulkkinen, P.***

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Field observations suggest that there is some variation in frost hardiness of the open-pollinated seed crops collected in different years from the northern seed orchards of Scots pine established in southern Finland. It is unclear, however, whether such annual variation is also present in the seed collected from natural forests. Although the variation in hardiness within and between populations of Scots pine is fairly well documented, there is a shortage of studies addressing the possible year-to-year fluctuation in this characteristic.

In Scots pine, female flowers normally become receptive before pollen shedding commences in the same trees. Our hypothesis is that this lag, which we have found to show a slight increasing tendency toward the north, might result in a decreased adaptability of the seed due to gene flow via pollen from the south. To study this, we made daily observations on the flowering phenology and the presence of airborne pollen in four consecutive years (from 1997 through 2000) and in six Scots pine stands representing two geographically distinct areas in northern Finland. Furthermore, we carried out freezing tests to determine the rhythm of autumn hardening of seedlings that were raised from the seed collected from these same stands in several years following the phenology observations. The state of hardening of the seedlings was determined by visually observing primary needle injuries resulting from the freezing.

The main objective of the study was to find out whether the annual differences observed in the relative time of female and male flowering are

related to the frost hardiness of the respective seed crops. A possible corroboration of this hypothesis could have implications both for genetic field testing (the performance of control seed lots being dependent on the year the seed was collected) and for the use of stand seed by forest tree nurseries.

## **17 LENGTH OF GROWTH PERIOD OF SILVER BIRCH (*BETULA PENDULA*) SEEDLINGS AS A FUNCTION OF LATITUDE OF SEED ORIGIN AND SOWING TIME**

***Jouni Partanen*<sup>1</sup>, *Risto Häkkinen*<sup>2</sup>, *Anneli Viherä-Aarnio*<sup>2</sup>, *Alpo Luomajoki*<sup>2</sup> and *Veikko Koski*<sup>2</sup>,**

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Characterization of different photoperiodic ecotypes and other seedling materials is needed, for instance, in gene mapping of photoperiodism and winter hardiness in birch. For proper characterization, we need to know better, how the timing of growth cessation of birch seedlings varies according to their sowing time and the stage of development. The aim of our study was to examine the height growth cessation of first-year silver birch seedlings determined by seed origin and sowing time. This presentation demonstrates how the length of growth period, i.e. the age at growth cessation, of first-year silver birch seedlings varies as a function of latitude of seed origin and sowing time.

The material consisted of seven seed origins of silver birch from different latitudes, i.e. from different photoperiodic conditions. The southernmost was from Viljandi, Estonia (58°10'N) and the northernmost from Kittilä, northern Finland (67°44'N). A greenhouse experiment at Haapastensyrjä Breeding Station (60°37'N, 24°26'E) was based on natural light conditions and natural variation of photoperiod during the growing season. Each origin was sown at one to two week intervals eight times during the summer from May 21 to July 30. By using this experimental design, seedlings with differing stages of development were obtained to meet the lengthening night towards the end of the summer. Each combination of origin and sowing time was replicated twice with 12 seedlings each. The temperature in the greenhouse was kept at a constant +20°C during daytime (8:00 to 18:00 hours) and at +10°C during night (20:00 to 6:00 hours). During the intervening times the temperature was changed steadily at the rate of 5°C per hour. The height of the seedlings was measured twice a week until the

height remained constant over three successive measurements (cessation).

The leading pattern of behaviour of the length of the growth period was evident. The later the sowing time and the more northern the seed origin, the shorter was the growth period, i.e. the lower was the age of the seedlings at growth cessation. Accordingly, the rate of physiological development from the seed phase to the growth cessation phase was higher in later than in first sowings. The growth period shortened almost linearly as a function of the sowing time, but the rate of shortening depended on the latitude of the origin. There was an interaction between the latitude of seed origin and sowing time. In the first sowing (May 21), the observed growth period of the northernmost Kittilä origin was 89 days and that of the southernmost Viljandi origin 121 days, i.e. 32 days longer. In the last sowing (July 30), the observed growth period of Kittilä origin was 43 days and that of Viljandi origin 57 days, i.e. only 14 days longer.

Conclusion: The length of growth period, i.e. the age at growth cessation, of the first-year silver birch seedlings can be predicted with high precision as a function of latitude of the seed origin and sowing time.

## 18 THE HISTORY OF NATIVE ICELANDIC WOODLANDS

### **Ólafur Eggertsson**

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Birch woodlands and scrub (*Betula pubescens*) are the only indigenous woodland types in Iceland. Before landnám, in the 9<sup>th</sup> century, it has been estimated that birch woodland covered 15.000 km<sup>2</sup> to 25.000 km<sup>2</sup> (15-25% of the total land area of Iceland). According to recent mapping of the forest woodlands, Iceland has lost 92-95 % of its original woodland. The present day area of natural birch woods in Iceland is only 1200 km<sup>2</sup>.

The explanation for the severe decline in forest cover since landnám is normally blamed on human activities, e.g. overgrazing, clearing for pasture and hay fields and use of firewood and charcoal. Natural factors are also involved e.g. volcanic eruptions with extensive ash fall, catastrophic floods related to volcanic activity, jökulhlaup and climatic factors like cooling due to the Little Ice Age followed by more extensive glaciers overrunning vegetated land. The combination of all these factors, both natural and human, is probably the main cause for this extensive deforestation since landnám.

The methods used to estimate the change in forest cover over time are the climatic demands for different species, pollen and macrofossil analysis and historical data like sagas and place names.

Today's woodland probably survived because of factors like favourable climatic conditions, areas were access of man and animals was limited and regions rich in other natural resources like driftwood.

## POSTERS

### 19 THE MATERNAL TEMPERATURE DURING ZYGOTIC EMBRYOGENESIS INFLUENCES THE ADAPTIVE PROPERTIES OF NORWAY SPRUCE PROGENIES: A “MEMORY” INVOLVING DNA METHYLATION AND DIFFERENTIAL TRANSCRIPTION OF PHYTOCHROME GENES?

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Survival and competitive successes of boreal forest trees depend on a balance between exploiting the full growing season and minimising frost injury through proper timing of hardening in autumn and dehardening in spring. Our research has shown that the female parents of Norway spruce adjust these timing events in their progeny according to the prevailing temperature conditions during sexual reproduction. Reproduction in a cold environment advances bud-set and cold acclimation in the autumn and dehardening and flushing in spring, whereas a warm reproductive environment delays these progeny traits by an unknown non-Mendelian mechanism.

We have performed identical crosses in combination with timed temperature treatments during shorter and longer periods from female



meiosis, pollen tube growth, syngamy and embryogenesis, tested the progenies for bud-set and frost hardiness, and concluded that the effect of temperature most likely is a response to accumulated heat during embryogenesis and seed maturation. Our first attempt to look for a molecular mechanism has revealed that transcription of PHYO, PHYP and PHYN (using RealTime PCR) all show higher transcription levels in progenies born under cold conditions than their full-sibs born under warmer conditions. This result is consistent with preliminary findings that methylation of cytosine in total DNA is higher in progenies reproduced under warm conditions than their colder full-sib counterparts. If these observations are related to methylation of phytochrome genes, we may explain why progenies with a memory of a past time cold embryogenesis are more sensitive to short days than their full-sibs with a warmer embryonic history.

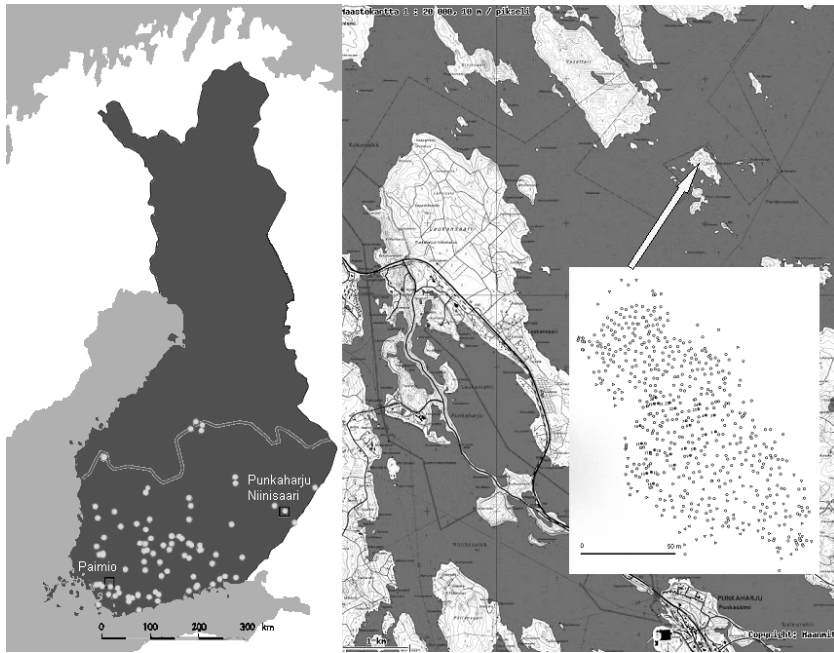
## **20 CONSERVATION AND UTILISATION OF GENETIC RESOURCES OF SMALL-LEAVED LIME (*TILIA CORDATA*) IN FINLAND**

### ***Teijo Nikkanen***

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Small-leaved lime (*Tilia cordata*) is one of the most widely distributed broadleaved trees in Europe. In Finland, this species is clearly the northernmost of the so-called noble hardwoods. Before influence by man and competition by Norway spruce, small-leaved lime was far more common than it is at present. Despite its relative rarity, lime is a rather competitive tree species on fertile forest soils due to its vigorous sprouting and good ability to tolerate shading. However, regeneration from seed is poor under boreal conditions.

Gene resources of small-leaved lime are conserved in special gene-resource collections (*ex situ*) as vegetatively propagated grafts. Branches were collected from 566 trees in 86 natural stands of lime in different parts of Southern and Central Finland. Representative examples of natural stands are also selected as gene-reserve stands (*in situ*). Four gene-reserve stands have been selected in Finland for lime; two of them are also being used for conserving maple. Gene-reserve forests are an additional method of gene conservation for lime, and can be used as seed collecting stands.



**Figure 1.** Material for a gene-resource collection was collected from the 86 natural stands of small-leaved lime marked on the map. The location of the Niinisaari gene-reserve stand and the northern limit of the range of small-leaved lime are also marked on the map.



**Figure 2.** Seed collection was focused on the Niinisaari stand with the aim of obtaining good quality seed originating from several trees. A tractor equipped with a stepladder was ferried to the island on a raft to enable seed collection from trees over 20 metres in height.

An exceptionally large stand of lime, one ha in area, is located in the centre of Niinisaari Island. The stand area is occupied by an almost pure stand of lime. The number of lime trees in the stand is more than 500. However, the number of genetic individuals of lime may be smaller because lime is mainly regenerated vegetatively.

Seed collection was focused on the Niinisaari stand with the aim of obtaining good quality seed originating from several trees. 2.5 kg seed was collected from 25 trees in 1997 and 7.8 kg from 31 trees in 2002. The collected, ripe lime seed is in deep physiological dormancy. After stratification (3 months at +20°C and 3 months at +4°C), 27% of the seeds (varying from 3 to 49% between trees) germinated. A total of 8000 seedlings from the 1997 seed collection were planted in 12 of the Finnish Forest Research Institute's research areas in Southern and Central Finland. The aim of collecting Niinisaari lime seed is to promote the use of indigenous lime both in forest cultivation and in landscape management.

A start has been made on studying the genetic structure of the Niinisaari stand using DNA markers. The aim of the study is to analyse the number of

genetic individuals and the degree of consanguinity of growing lime trees, and the genetic diversity of the seed produced in the stand. Information on the population structure and mating patterns of the lime populations is important from the genetic conservation point of view, and in the case of the Niinisaari stand especially, considering the future management of the stand. The genetic information about the stand is used when thinning and regeneration as well as seed collections of the stand are planned.

## **21 IDENTIFICATION OF ECTOMYCORRHIZA FORMING FUNGI AND IMPACT ON BIRCH AND PINE SEEDLING GROWTH IN ICELANDIC SOILS**

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Iceland has suffered from severe ecosystem degradation since it's settlement in 874 and has lost more than 50% of the original vegetative cover. The cover of birch (*Betula pubescens* Ehrh.), the only native tree species forming forest in Iceland, has reduced greatly. Today it is the most common species used in afforestation and is extensively used in land reclamation on degraded sites. Due to low survival rates of young tree seedlings at these sites, it is hypothesized that the lack of beneficial soil biota is hampering afforestation. A Nordic co-operation programme aims to study the beneficial microorganisms (mycorrhiza, insect pathogens). The aim of the mycorrhizal sub-project was to compare a) mycorrhiza species diversity and b) early root colonization dynamics in birch and pine seedlings grown in birch humus and soil from adjacent eroded areas.

Root colonization dynamics over 3 months was followed in microcosm systems. Ectomycorrhizal diversity was assessed by morphotyping and PCR (ITS) RFLP/sequencing. Differential birch mycorrhizal development in the two test soils was characterised by heavier colonization and more diversity in humus. Birch growth in eroded soils was significantly reduced compared to humus. Similar soil-specific differences were not as apparent with pine, which supported heavy colonization by suilloid-type mycorrhizas in both humus and eroded soils. These preliminary results indicate that the requisite mycorrhizal fungal biota supporting seedling development exists in old birch stands in Iceland and the lack of specific mycorrhizal species

may be an important factor contributing to high mortality of young seedlings in eroded areas.

## 22 PROGENY TRIALS OF *LARIX SUKACZEWII* AND HYBRIDS IN ICELAND – 3 YEAR RESULTS

### ***Throstur Eysteinnsson***

Iceland Forest Service

Selection for adaptation is one of the most important steps when working to improve an exotic tree species, especially when attempting to introduce production forestry as a viable land-use option in marginal (with respect to forestry) areas such as Iceland.

*Larix sukaczewii* (Dylis) is the most important exotic tree species in Icelandic forestry. It is most often used for afforestation of nutrient poor heathland and eroded land. With currently available seed sources, it can be grown for timber production in interior valleys in north and east Iceland. Spring frost damage and other types of damage, resulting in poor form, preclude use of *L. sukaczewii* and *L. sibirica* (Ledeb.) for timber production in the more maritime south and west Iceland, whereas fall frost damage results in poor form of all *L. decidua* (Mill.) provenances tried thus far. *L. leptolepis* (Sieb. & Zucc.) as well as the *L. decidua* x *leptolepis* hybrid are too poorly adapted to be of use in Icelandic forestry. Nevertheless, larches are potentially the best trees available for nutrient poor sites throughout Iceland so better adapted provenances or hybrids are needed.

In 1992, an accelerated breeding program was set up with the aims of creating a well adapted land race of larch for N- and E-Iceland and to find hybrids that grow well in S- and W-Iceland, with breeding and seed production taking place in greenhouse orchards.

The first progeny tests of this material (23 families) were planted in 1999 on sites in East and South Iceland. Assessment at age 3 showed good survival (98% in S Iceland, 100% in E Iceland), significant family differences in height growth and frost damage and significant family x site interaction.

The significant family x site (GxE) interaction was to some extent due to a difference in the behaviour of the *Larix sukaczewii* families on the one hand and the *L. sukaczewii* x (*L. decidua* x *leptolepis*) hybrid families on the other. The *L. sukaczewii* families were taller, suffered less frost damage

and had better form at the east Iceland site than at the south Iceland site whereas the opposite was true for the hybrid families. The hybrid families were generally taller than the *L. sukaczewii* families at both sites.

Frost damage was the most important single factor in determining both form and height at this early age. The frost damage in question most likely occurred in late winter or early spring and was caused by frosts coming after loss of frost hardiness during warm periods. Tolerance of such frosts, or resistance to losing frost hardiness during winter warm spells is an important adaptive trait for trees in northern maritime climates. Thus, selection for this trait is clearly important when breeding larch for use in maritime areas of Iceland.

These trials are young and it is still much too early to use them as criteria for selection. However, they provide an early indication that gains can be made in adaptation of larch to spring frosts by creating hybrids.

### **23 EFFECT OF SPACING AND PRUNING HEIGHT ON EARLY PRODUCTION IN A SCOTS PINE CLONAL SEED ORCHARD**

***Curt Almqvist***

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In 1990, an experimental clonal seed orchard of Scots pine was established in central Sweden (Lat. 59°37' N). In total 16 different combinations of spacing and pruning height were tested. The spacings ranged from 178 stems/ha (7.5×7.5 meter) up to 4000 stems/ha (1.0×2.5 m), and the pruning heights ranged from 2 to 7 meters.

The objective of the pruning regime employed was to initiate the pruning when the grafts reached a height 0.2-0.5 meter below the intended height of the plot. Through annual pruning, the crown was formed and the grafts reached the aimed height in 5 years. After that, the annual pruning continued with the aim to reduce the height development to a maximum of 0.1 meter per year. Average graft height in autumn 2002, before this year's pruning, ranged from 2.9 meter to 5.1 meter.

Pollen production started at the same age in all treatment combinations. In 1999, pollen was produced on 23 % of the grafts. In 2001, the percentage of grafts producing pollen had increased to 74 %. Based on measurements

of pollen production per graft the pollen production per hectare was calculated for data from 2002. The highest production, 19.5 kg pollen/ha, was obtained on plots with 1600 stems/ha and at a pruning height 2 meters. The lowest production, 2.7 kg pollen/ha, was obtained on plots with the widest spacing of 178 stems/ha aiming at 7-meter height (as yet unpruned grafts).

Cone production has been measured annually 1996-2002. The highest average production occurred in 2001, with the highest production, 33.9 hl cones/ha on plots with 4000 stems/ha and 2-meter pruning height. Lowest production, 6.1 hl cones/ha, was obtained on plots with 178 stems/ha aiming at 7 meter.

## **24 TIMING OF HEIGHT GROWTH CESSATION OF SILVER BIRCH (BETULA PENDULA) SEEDLINGS DETERMINED BY SEED ORIGIN AND SOWING TIME**

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Proper timing of growth cessation and subsequent hardening is crucial to the survival of birch as well as other temperate zone tree species. Photoperiodic ecotypes with different critical night lengths causing different timing of growth cessation have been demonstrated in several birch species. The photoperiodic response, i.e. the effect of night length on growth cessation of birch is known to be modified by other factors during the growth period, temperature being the most important. The aim of this study was to examine the timing of height growth cessation of first-year silver birch seedlings determined by seed origin and sowing time.

The material consisted of seven seed origins of silver birch from different latitudes, i.e. from different photoperiodic conditions. The southernmost was from Viljandi, Estonia (58°10'N) and the northernmost from Kittilä, northern Finland (67°44'N). The design of greenhouse experiment at Haapastensyrjä Breeding Station (60°37'N, 24°26'E) was based on natural light conditions and natural variation of photoperiod during the growing season. Each origin was sown at one to two week intervals eight times during the summer from May 21 to July 30. By using this experimental design, seedlings with differing stage of development were created to meet the lengthening night towards the end of the summer. Each origin x sowing

time combination was replicated twice with 12 seedlings each. The temperature in the greenhouse was kept at a constant +20°C during daytime (8:00 to 18:00 hours) and at +10°C during night (20:00 to 6:00 hours). During the intervening times the temperature was changed steadily at the rate of 5°C per hour. The height of the seedlings was measured twice a week until the height remained constant over three successive measurements (cessation).

The timing of height growth cessation varied significantly according to the latitude of the seed origin. The more northern the origin, the earlier was the timing of growth cessation in all eight sowings and thus, the shorter the night length at growth cessation. However, the timing of height growth cessation of each ecotype was not constant, but was strongly modified by the sowing time. Within each origin, the later the sowing time, the later was the growth cessation in the autumn, and thus the longer the night length at growth cessation. Differences among the seed origins in date of growth cessation were biggest within the earliest sowing times decreasing towards the later sowings. There was a significant interaction between the latitude of the seed origin and sowing time. The growth cessation of the origins in the first sowing (May 21) varied from August 18 (Kittilä origin) to September 19 (Viljandi origin), and in the last sowing (July 30) from September 11 (Kittilä) to September 25 (Viljandi).

Conclusion: In silver birch seedlings of the sowing year, the photoperiodic regulation of height growth cessation is modified by the sowing time, i.e. the stage of development of the seedlings. Only one value of critical night length parameter may be insufficient in characterization of photoperiodic ecotypes, and stage of seedling development should be integrated in ecotype characterization when using first-year seedlings.



## 25 APPENDIX 1 - PARTICIPANTS

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