Conifers Network

Report of the fourth meeting (18–20 October 2003, Pitlochry, United Kingdom)

J. Koskela, C.J.A. Samuel, Cs. Mátyás, and B. Fady, *compilers*

European Forest Genetic Resources Programme (EUFORGEN)

IPGRI and INIBAP
operate under the name
Bioversity International

Supported by the CGIAR

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Bioversity International is an independent international scientific organization that seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. It is one of 15 centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. Bioversity has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

The international status of Bioversity is conferred under an Establishment Agreement which, by January 2007, had been signed by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mali, Mauritania, Morocco, Norway, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

Financial support for Bioversity's research is provided by more than 150 donors, including governments, private foundations and international organizations. For details of donors and research activities please see Bioversity's Annual Reports, which are available in printed form on request from bioversity-cgiar.org or from Bioversity's website (www.bioversityinternational.org).

European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring effective conservation and sustainable utilization of forest genetic resources in Europe. It was established in 1994 to implement Resolution 2 of the Strasbourg Ministerial Conference for the Protection of Forests in Europe. EUFORGEN is financed by participating countries and is coordinated by Bioversity International, in collaboration with the Forestry Department of FAO. It facilitates the dissemination of information and various collaborative initiatives. The Programme operates through Networks in which scientists, managers and policy-makers work together to analyse needs, exchange experiences and develop conservation methods for selected tree species. The Networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong and promote integration of gene conservation into sustainable forest management. Network members and other experts from the participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme. EUFORGEN is overseen by a Steering Committee composed of National Coordinators nominated by the member countries. Further information on EUFORGEN can be found from its website (www.euforgen.org).

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Fourth EUFORGEN Conifers Network meeting, Pitlochry, Scotland, 18-20 October 2003

Opening of the meeting

C.J.A. Samuel welcomed the participants on behalf of local organizers and provided a short presentation on forestry in Scotland and the UK. He described how the forest sector is structured in the UK and stressed the importance of conifers, especially the role of exotic conifers in Scotland. He also introduced his colleagues from the Forest Research, i.e. C. Baldwin, E. Ker and R. Sykes.

C. Mátyás, Chair of the Network, welcomed the participants from 26 countries and thanked the local organizers for the arrangements. All participants then briefly introduced themselves.

The Chair reminded the Network members of the importance of producing visible and useful outputs. He highlighted the task of EUFORGEN which should support conservation activities in the participating countries. At the same time, EUFORGEN should raise awareness of forest genetic resources among forestry professionals, decision makers and the public in general.

J. Koskela introduced himself as the new EUFORGEN Coordinator since 1 January 2003. He then presented the tentative agenda of the meeting which was adopted with some amendments. J. Fennessy, B. Fady and T. Skrøppa were nominated as rapporteurs for the meeting.

EUFORGEN update

J. Koskela provided a short update on the two recent external reviews which were carried out on IPGRI[1]: one assessed the performance of Bioversity International as a whole and the other focused solely on the Bioversity Regional Office for Europe. The two reviews were carried out as the regular five-year monitoring cycle of CGIAR and Bioversity activities, respectively. Results of both reviews were positive and several recommendations made during the Europe review were specifically addressed to the EUFORGEN Steering Committee. These recommendations will be presented to the Steering Committee for discussion during its next meeting in May 2004. He thanked those participants who responded to the Bioversity surveys which were sent to Bioversity's partners in Europe as part of the reviews.

J. Koskela informed the meeting that the new Director General of Bioversity, Dr. Emile Frison, commenced his duties on 1 August 2003. He then presented the reports on recent meetings and outputs of EUFORGEN and listed various other meetings where the Secretariat promoted the EUFORGEN Programme. He briefly highlighted the outputs of the 4th Ministerial Conference on the Protection of Forests in Europe (MCPFE), held in Vienna, Austria at the end of April 2003. The MCPFE Liaison Unit will move from Vienna to Warsaw by the end of 2003 as Poland takes the Chair in the MCPFE process. Relating to EUFORGEN activities, he informed the meeting that a collaborative capacity-building programme, 'Conservation and use of biological diversity for development', is being developed between Bioversity and the Government of Austria. It is planned that this initiative will include an international training programme on forest genetic resources. Its focus is on providing short-term training courses and scholarships, especially for developing countries and European countries in transition.

Introductory country report by Iceland

T. Eysteinsson gave a general presentation on forestry in Iceland. The native tree species are birch, rowan and aspen, while common juniper is the only native conifer. Before human settlement, the native birch forests covered about 30% of the land area but currently the figure is only 1%. Modern forestry in Iceland started in 1899 with the first pine enclosures. In the 1940s, large-scale seed import took place for afforestation purposes and the period from 1960 to 1990 was known as a learning period with development of forest research. The present forest management goals are mainly multiple uses, with emphasis on timber production. Other important elements are soil conservation, land reclamation, amenity and recreation. The most important exotic conifers are Siberian larch, Sitka spruce and lodgepole pine.

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[1] From 1 December 2006, IPGRI and INIBAP operate under the name Bioversity International.

Progress made in various countries

Central and Eastern Europe (Czech Republic, Hungary, Poland, Russian Federation, Slovakia and Slovenia)

Hungary, Slovakia and the Czech Republic reported that general insecurity in forest management has increased due to the ongoing transition process towards market economies. In the Czech Republic, for example, there are too many owners of small forests, and some of them do not have any particular interest in forest management. Some good results from the past have been lost after many personnel changes in various organisations. This situation has also increased lack of responsibility among those organisations which offer services in forestry and their staff have inadequate capacity to monitor activities at an appropriate level. Substantial changes in ownership structure have also taken place in Slovenia. In Hungary, large private owners have greater interest and they are doing more for FGR (forest genetic resources) conservation than state forest services. However, the privatisation process is sometimes less transparent and probably based on particular interests of individuals and groups.

The forests still face serious health problems, although the situation is improving in the Czech Republic with decreasing industrial pollution and emissions. Too high a number of large game species is a problem in Hungary and the Czech Republic. In recent years, extreme weather conditions, e.g. flood and drought, have increased stress on the forest ecosystems. Consequently, the frequency of bark beetle outbreak is increasing which creates a specific and long-lasting problem.

There are problems in communication between environmental NGOs, state agencies responsible for forests and those responsible for the environment. There are conflicting views regarding NATURA 2000 between private forest owners and representatives of environmental agencies. Russia reported that illegal logging and trade is continuing, particularly in the Far East, Siberia and the Caucasus region. Decreasing profitability of forestry and lack of funds for forestry activities is creating problems to conservation of FGR in Central and Eastern Europe.

Legislation changes in most countries are connected to the preparatory phase of accession to the EC requiring harmonisation of national legislation with the acquis communautaire (the entire body of European laws). In addition, national strategies and/or programmes on conservation and utilisation of FGR are in preparation in several countries and many newly adopted national forest programmes include chapters on FGR. Some of the ongoing projects on FGR and tree breeding include:

- Development of molecular and biochemical databases on forest trees for monitoring movement of forest reproductive material;
- Tree breeding and preservation of valuable and threatened populations;
- Silvicultural techniques and genetic composition of tree species;
- Reproduction methods such as vegetative propagation and *in vitro* culture;
- Approval and record keeping of forest reproductive material and information services for forest owners.

Increased public awareness is considered important and publications and videos are under preparation in Central and Eastern European countries.

Mediterranean region (Bulgaria, Croatia, Cyprus, France, Italy, Macedonia, Malta, Serbia and Montenegro, Spain and Turkey)

The Mediterranean eco-geographical group was represented by 10 countries. The species of interest for this group are: *Abies* spp., *Picea abies, Pinus pinaster*, *Taxus baccata*, *Pinus nigra*, *Pinus pinea*, *Pinus halepensis*, *Pinus brutia*, *Pinus peuce*, *Pinus heldreichii*, *Cupressus* spp., *Cedrus* spp., *Juniperus* spp. and *Tetraclinis articulata*.

Research projects on characterization of genetic resources of some Mediterranean conifer species (e.g. *Abies alba* in Croatia, *Pinus nigra* in Macedonia, Turkey, Serbia and Montenegro) are in progress using a combined approach with neutral and adaptive molecular markers. In France, Spain and Italy demography and parentage analyses are being carried out for *Abies alba*, *Pinus halepensis* and *Pinus pinaster*. The main objectives of these studies are to gather information on the distribution of neutral and adaptive variation in the natural range of the species, to identify 'hot spots' of diversity and to monitor gene flow. Maps were prepared using neutral markers regarding the distribution of genetic diversity of *Pinus pinaster*, *Pinus halepensis* and *Picea abies*. The results of these projects can be summarized as follows:

- New information on colonisation dynamics, gene flow and hybridisation processes as well as on migration of populations;

- First evidence on the distribution of adaptive variation at the molecular level for *Pinus pinaster, Pinus halepensis, Pinus nigra* and *Picea abies*;
- Development of methods for provenances and seed lot identification for *Pinus pinaster*;
- Establishment of new provenances trials and progeny tests for *Abies alba, Pinus nigra, Pinus brutia* and *Pinus halepensis* and analysis of quantitative traits.

Various *in situ* conservation measures have also been undertaken, including establishment of four additional conservation units for *Abies alba* in south-eastern France, revision of *Abies alba* seed stands in Croatia and identification of three additional gene conservation units for *Pinus brutia* in Turkey. For *ex situ* conservation, the number of *Pinus pinaster* and *Pinus halepensis* populations stored in seed banks has increased in France and Italy. Additional samples for DNA banks of *Pinus halepensis*, *Pinus pinaster*, *Picea abies*, *Abies alba*, *Picea omorika* and *Pinus brutia* have been stored at -80°C in Italy and France. In Bulgaria, the number and size of gene reserves for *Picea abies* and *Abies alba* have also increased.

Regarding changes in relevant policies, Spain, France, Turkey and Bulgaria have harmonized the Council Directive 1999/105/EC on the marketing of forest reproductive material in their legislation. In Spain, a National Committee on Breeding and Conservation of Forest Genetic Resources has been established to coordinate the activities of the different autonomous communities. Spain has recently also prepared a new forest law including aspects related to breeding and conservation of FGR. New laws on forest reproductive material have also been adopted in Serbia and Montenegro, and Macedonia. Public awareness and training activities include:

- Organization of the 2nd edition of an international course on conservation of FGR in Spain;
- Participation at the French Agricultural Exhibition "Our forests: a genetic treasure";
- Participation in TV programmes;
- Contribution to the preparation of books devoted to conservation genetics;
- An international course in Colombia was organised by Spain in collaboration with some South American countries.

Northern Europe (Finland, Iceland, Lithuania, Norway and Sweden)

At the Nordic level, conservation of genetic resources is one of the areas that have a high priority in the agricultural sector. The Nordic Genetic Resources Board was established with one representative from the Ministry of Agriculture and one from the Ministry of the Environment in each country.

On request from the Nordic Council of Ministers, a Nordic Network for Forest Tree Gene Conservation was established in 2003 with one representative from each Nordic country. This is similar to the Nordic Gene Bank for agricultural plants and the Nordic Gene Bank for farm animals. The objective is to promote and co-ordinate the conservation of genetic resources of forest trees in the region. Raising public awareness at different levels has a high priority in Nordic co-operation. The joint annual publication "Nordic GENresources" with contributions from all three sectors is one of the public awareness initiatives.

A report of the legal issues of genetic resources of agricultural plants, agricultural animals and forest trees was presented to the Nordic Council of Ministers in June 2003 (A Nordic Approach to Access and Rights to Genetic Resources, ANP 2003:717, 99pp, available free of charge from nmr.dk). It recommended that legally, forest trees should be considered to be in the public domain. The Council decided, however, that the legal status of the genetic resources of forest trees is still unclear and that the topic should be further evaluated.

In Norway, efforts have been made to evaluate the role of nature conservation reserves in gene conservation. It seems that their role will be different both for species and countries. Investigations along the same lines are being initiated in Sweden and Finland. In Iceland, gene conservation has the highest priority in state-owned forests.

A pilot project monitoring the genetic resources of rare and scattered tree species was initiated in Norway. Abundance, size, distribution and damage by grazing animals was assessed in 1000 m^2 plots in a 3 x 3 km grid in three counties, as an additional registration in the national forest survey.

Breeding of Norway spruce and Scots pine is continuing both in Sweden and Finland with the establishment of new generation seed orchards. A long-term breeding strategy for Finland was presented this year. It has been evaluated and will be revised by the end of 2003. The breeding strategies in these two countries combine long-term breeding and gene conservation.

In Lithuania, a law on genetic resources of plants, including forest trees, came into force in 2003. A new gene bank as a state-funded institution for the management of plant genetic resources is under establishment. The area of the Norway spruce gene reserves in Lithuania has been reduced due to bark beetle outbreaks.

In both Iceland and Norway, it has been observed that rather fast development of landraces may take place in forest trees. This has been shown as adaptation to climatic conditions and susceptibility to the green spruce aphid in Sitka spruce.

Characterization using molecular markers in Norway spruce from the assumed refugia and along the migration routes is underway in Norway. Combined with results from dating pollen profiles, it will most likely provide new information about the immigration of the species after the last glacial period. In a joint Norwegian–Finnish project isozyme studies have been made for a large number of *Taxus baccata* populations from the western and northernmost range of the species.

Western Europe (Austria, Belgium, Germany, Ireland, Switzerland and United Kingdom)

In general, few major developments were reported among this group of countries. Difficulties in obtaining data were highlighted. These were due to insufficient resources and low interest in genetic conservation in conifers.

Switzerland reported the successful publication of a Gene Conservation Concept, a document covering all forest tree species (published in French and German only). Forests of special genetic interest are presently being identified.

A symposium on Genetic Conservation and Breeding of *Taxus baccata* was held in Austria. Forthcoming work is in hand to investigate molecular methods to be used as diversity measures and for seed lot identification across a range of species.

In Germany, the National Concept on the conservation of forest genetic resources was last reviewed and published in 2000. All activities are coordinated by a national working group and the reporting period runs from 2001 to 2004. At this stage, only reports from some federal states have been received as levels of activity among states are variable. The following list summarises these activities:

- For several species (*Abies alba, Picea abies, Taxus baccata, Pseudotsuga menziesii*), stands, populations in experiments or individuals have been characterized by isozyme markers;
- The establishment of clonal archives, seed orchards and conservation plantations has continued;
- A IUFRO (International Union of Forest Research Organizations) meeting on 'Ecology and silviculture of Silver fir' was held in September 2002 with some 30 contributions;
- For *Pseudotsuga menziesii* a number of states have checked several hundred stands to assess their adaptation and suitability for further propagation (seed collection) and natural regeneration. Isozyme analyses have been applied using the allele frequencies of the 6-PGDH-A locus to distinguish between sources of the coastal and interior type. However, morphological characters such as needle cast, poor stem form, resin flow, early and heavy coning and mortality have been considered as undesirable characters;
- In an EU-funded project, possible changes in the genetic structure of populations transferred from North America to Europe have been investigated.

Documentation, information and public awareness

EUFORGEN website and bibliography

J. Koskela presented the new structure of the EUFORGEN website, which is accessible directly through a new address (www.euforgen.org) while the old address under the Bioversity website also remains operational. The website is database-driven and includes a number of new features such as a 'what's new' section, species summary pages and an improved search engine. The EUFORGEN grey literature database is maintained as part of the new website and it currently includes nearly 2000 references. There is a need to include information on where to find a given reference and it was decided to indicate network members as contact persons in each country.

Conifers and EUFORGEN posters

An updated version of the Conifers Network poster and a general EUFORGEN poster were displayed and copies in A4 size were distributed to the participants. The Network poster was originally developed for the DYGEN (Dynamics and Conservation of Genetic Diversity in Forest Ecosystems) Conference and revised for the World Forestry Congress. These posters as well as other Network posters are available from the EUFORGEN website.

Image database

B. Fady updated Network members on the progress made on the image collection database. The participants were asked to provide additional slides on threats (pollution, pests and diseases), silviculture, utilization, genetics and breeding before 30 November 2003. Slides should be provided with the following information: name of photographer, country, species, item number and any additional comments. A reminder will be sent to all participants after the meeting. It was agreed that photos can be used freely for public awareness purposes provided that the name of the photographer and EUFORGEN (e.g. B. Fady/EUFORGEN Conifers Network) are acknowledged.

T. Skrøppa gave a presentation on the Norwegian website on conservation of forest tree genetic resources (http://www.skogoglandskap.no/temaer/genetic_resources). On this website, detailed information can be found on strategies for forest tree gene conservation in Norway, and related issues.

Meetings, projects and other initiatives

Outcomes of the DYGEN Conference

C. Mátyás reported outcomes of the Dynamics and Conservation of Genetic Diversity in Forest Ecosystems (DYGEN) Conference, which was held in Strasbourg in December 2002. Most topics discussed during the conference have relevance for developing strategies for genetic conservation, such as mating and population size, effects of post-glacial migration and of human activity on genetic structure, or the impact of past and future climatic changes.

There is no doubt that maintaining the dynamics of evolution and adaptation must remain the main goal of gene conservation. In view of expected long-term environmental changes, adaptability and phenotypic plasticity are traits of primary importance. However, the gap between molecular marker data and adaptive traits is still not overcome. In this respect the legacy of post-glacial migrations (organellic lineages) provides limited support, and historic differences have been erased by selection and gene flow.

Important strategic issues, such as the role of science in shaping forest policy, were also discussed during the DYGEN Conference. There was general agreement that genetic issues must be made more visible for decision-makers and the general public. The summaries of the conference sessions are published in the latest issue of Forest Genetics (2002, 9(4):333-340) and selected papers will be published in Forest Ecology and Management.

EVOLTREE proposal

G.G. Vendramin provided a short overview of the proposal submitted to the European Commission for the creation of a network of excellence on genomics of forest tree species. The title of the proposal is Evolution and Management of Diversity in European Forest Trees (EVOLTREE). EVOLTREE aims to implement genomic approaches for monitoring, predicting and managing genetic diversity in European forest trees for sustainable resource management and environmental protection. The aim of EVOLTREE is to transfer basic knowledge of gene function and genetic diversity into the forest sector, both by improving management practice and by tracing wood or seed products. It integrates interdisciplinary research (genomics, population and quantitative genetics, ecology, eco-physiology, palaeoecology, reproductive biology, modelling, bioinformatics, conservation biology, silviculture) to decipher the structure, expression and polymorphism of genes of adaptive significance and attempts to gain new insight into ecosystem function.

EVOLTREE is a consortium of 32 partners from 14 different countries. Besides the very positive scientific evaluation, the proposal was rejected because forest genomics was not considered as a high priority topic. It is the intention of the coordinator (A. Kremer) to resubmit the proposal. Possible changes in the content and eventually in the partnership will depend on the final version of the next call (Topic III.1.3) that will be published in January 2004.

Results of analyzing SNPs in conifers

G.G. Vendramin gave a talk about the detection and usefulness of a new category of markers, namely single nucleotide polymorphisms (SNPs), for population genetic studies in conifer species. Direct analysis of genetic variation at the sequence level using SNPs offers several advantages over other types of DNA marker systems. Information about the frequency and distribution of SNPs in forest tree species is still lacking.

The work consisted of a preliminary analysis of SNPs distribution in Aleppo pine (*Pinus halepensis*) and the evaluation of their usefulness in population genetic analyses. Based on isozymes, nuclear and chloroplast microsatellite data, it appears to carry high levels of variability, most of which (>85%) resides within populations. In this first phase attention was paid to a CAD gene, which is involved in lignin biosynthesis. Five populations sampled in different parts of the natural range (Israel, Greece, Italy, Spain and Morocco) of the species were analyzed.

Based on preliminary data from about 1200 bp (base pairs), the frequency of nucleotide changes appears to be high, with an average of one SNP every 143 bases overall. This frequency, which is much higher than that observed in humans, appears to be lower (but of the same magnitude) than frequencies observed in maize, *Picea abies* and *Pinus pinaster*, which are commonly considered species with extremely high levels of variability.

The estimated value of nucleotide variation appeared low. On the other hand, the genetic differentiation among populations was higher (about 42%) than that estimated using neutral markers (ranging from 2–12%), suggesting that this gene is under selection. The analysis of SNPs in candidate genes was a first attempt to detect putative adaptive variation and may provide extremely useful information for designing conservation units.

New EC Regulations on Genetic Resources in Agriculture

J. Koskela reported on the development of new EC Regulations on Genetic Resources in Agriculture. The EC is currently working on a revision of Regulation 1467 from 1994. The draft proposal was presented and discussed during two meetings held in Brussels in early 2003. The focus of this new regulation will be on animal gene conservation and the budget will be €7–10 million for a period of three years. The scope of the Regulation also includes crop, microbial and forest genetic resources with the focus on conservation, characterization, collection, utilization, documentation and evaluation. On-farm conservation and inventories are also likely to be eligible but research activities are specifically excluded. EUFORGEN is mentioned as the only forest-related framework in the draft document. The first call is expected to open by the end of 2003 or early 2004, and a second call is scheduled for 2005 after ten new countries have joined the EU. The Regulation is expected to provide funding for one or two forest-based projects. It was suggested that a proposal could be developed based on the common action plan concept.

In addition to the EC Regulation on genetic resources, the participants discussed the EC Directive on forest reproductive material. Concern was expressed that this might lead to inappropriate use of forest reproductive material.

Progress made in the Network activities

Technical Guidelines

J. Koskela provided an update on the state of the Technical Guidelines (TGs) production process. The six-page Technical Guidelines are specifically targeted for forest managers while Technical Bulletins are more comprehensive presentations of relevant information targeted for both scientists and managers. The first set of TGs was published in April 2003, including *Picea abies*, *Pinus pinaster* and *Pinus brutia/halepensis*. Two new TGs for conifers (*Abies alba, Pinus sylvestris*) will be published as part of the second set in November 2003. A third set is scheduled for publication in February 2004. The state of the various conifer TGs is as follows:

- *Abies alba* (Heino Wolf): text has been circulated and the draft map will be distributed shortly.
- *Pinus sylvestris* (Csaba Mátyás, Sam Samuel, Lennart Ackzell): draft will be sent to the Secretariat soon after the meeting.
- *Taxus baccata* (Rudolf Bruchanik, Ladislav Paul) draft text was provided just before the meeting.

- *Pinus nigra* (Vasilije Isajev, Bruno Fady, Hacer Semerci, Vlatko Andonovski): deadline for the draft text is 15 Jan 2004.
- *Pinus cembr*a (Marcus Ulber, Gregor Bozic, Felix Gugerli): final text is ready for circulation.
- *Larix decidua* (Jan Matras): draft text was provided during the meeting.
- *Juniperus communis* (Sam Samuel, Vahid Hadjiyev, Maia Akhalkatsi, Mikhail Pridnya): This was rescheduled for future publication and it was agreed that *Pinus peuce* would be prepared by Alexander Alexandrov and Vlatko Andonovski (deadline for text is 30 December 2003).
- *Pinus pinea* (Giuseppe Vendramin, Bruno Fady, Sonia Martin): draft text will be provided by 30 November 2003.
- *Pinus leucodermis*/ *P. heldreichii* (Giuseppe Vendramin, Bruno Fady): draft text will be provided by 30 November 2003.

Best practice for genetically sustainable forest management

C. Matyas informed the meeting that the paper published in Hungarian is now available in English. However, it was agreed that this issue was more relevant to the whole of EUFORGEN and that it should be directed to the next Steering Committee meeting in 2004.

Common action plan

The goal is to establish a pan-European network of *in situ* gene conservation units using *Picea abies* as a model species. Only a few contributions were received; in part due to lack of a clear definition of *in situ* gene conservation units. The table will be modified to include different categories of *in situ* conservation areas (e.g. gene reserves, seed stands, national parks, strictly protected areas). The Secretariat will send out a revised table and the definitions to the participants shortly and they should provide data currently available to the Secretariat by 15 December 2003.

List of priority species

The list of priority conifer species was discussed and it was agreed that the scoring would be changed from ten classes to four. The four classes are: 1) high priority, 2) medium priority, 3) low priority and 4) no priority. In the case of a species not used in forestry in a country, the table should indicate a blank. The Secretariat will provide an updated table with existing priority species for the participants to be scored. If needed, new species can be added into this table. The deadline for providing the priority species to the Secretariat is 30 November 2003.

After receiving feedback, the Secretary will then finalise the table and divide the last column (number of countries in which a given species received scores) into four sub-columns indicating the number of countries per each priority class.

Outputs of the MCPFE process and development of EUFORGEN Phase III

J. Koskela presented recent outputs from the MCPFE process, i.e. the fourth Ministerial Conference on the Protection of Forests in Europe (28–30 April 2003) and the Expert Level Meeting (16–17 Oct 2003), both held in Vienna, Austria. After the EUFORGEN Steering Committee meeting in Sweden in June 2002, a task force produced a EUFORGEN strategy paper for the preparatory process of the Vienna Ministerial Conference. Based on this paper and other efforts, conservation of FGR was highlighted in the Vienna outputs.

At the Vienna Ministerial Conference, the European ministers responsible for forestry and the EC committed themselves to “take further steps to maintain, conserve, restore and enhance biological diversity of forests, including their genetic resources, in Europe and also on a global scale” (paragraph 22 of the Vienna Declaration). In Resolution 4 on Forest Biological Diversity, the ministers also committed themselves to “promote the conservation of forest genetic resources as an integral part of sustainable forest management and continue the pan-European collaboration in this area” (paragraph 16).

Following the Vienna Ministerial Conference, the Liaison Unit organized the Expert Level Meeting to finalise a draft Work Programme, which was prepared by the Liaison Unit and the countries coordinating the MPCFE process. The draft Work Programme did not include FGR as a focus area. However, the EUFORGEN Secretariat provided feedback to the Liaison Unit and proposed several actions to be added into the Work Programme during the Expert Level Meeting. The adopted Work Programme now includes FGR as a focus area under the Vienna Resolution 4 (Forest Biological Diversity) with two actions: 1) Promote conservation of forest genetic resources as an integral part of sustainable forest management and continue pan-European collaboration in this area through EUFORGEN and 2) International training programme on FGR as part of the collaborative capacity building programme 'Conservation and use of biological diversity for development' that is being developed between Bioversity and the Government of Austria. Furthermore, under Resolution 5 (Climate Change and Sustainable Forest Management) and focus area 'Adaptability of forests', the Work Programme includes a workshop on the role of genetic diversity in improving adaptability of forests to climate change and in maintaining the productivity of forests under changing environmental conditions. IUFRO and EUFORGEN/Bioversity were identified as leading agencies to organize this workshop in 2005.

These MCPFE outputs imply that EUFORGEN Phase III should continue implementation of the Strasbourg Resolution S2 on Conservation of Forest Genetic Resources and as a new element, also contribute to implementation of the Vienna Resolution V4 on Forest Biological Diversity by better linking gene conservation and forest management.

The participants then discussed the future of EUFORGEN and concluded that the Programme should continue for its third phase. The participants identified several issues that are relevant to future work. The discussion emphasized that EUFORGEN should play an important role in advising national programmes on FGR and that genetic considerations should be included in forest management. Conifers should be addressed regardless of how EUFORGEN will be organized in Phase III. Exotic conifers of importance for European forestry should be included in work plans for Phase III. The participants also highlighted need for better linkages between EUFORGEN and processes on forest biological diversity at global and pan-European levels. EUFORGEN should also promote cross-sectoral co-operation in natural resource management with all relevant stakeholders.

Seminar on conserving and using exotic conifers

The International Conifer Conservation Programme (M. Gardner)

M. Gardner gave a presentation on conservation activities of threatened conifer species. Globally there are about 800 conifer species of which 355 are listed by IUCN-The World Conservation Union as being of conservation concern. They are threatened by logging, fire, insect attacks and invasive species. Seventy may become extinct in the near future. He also provided several examples of threatened conifer species at global level. The major focus of the International Conifer Conservation Programme is the integration of *ex situ* with *in situ* conservation through scientific research, education and training, and cultivation. He also described the Global Strategy for Plant Conservation adopted in The Hague in April 2002 at the sixth meeting of the Conference of the Parties (COP) to the Convention on Biological Diversity (CBD). The Strategy attempts to deliver several outputs by 2010 including an assessment of the conservation status of all threatened plant species, ensuring that 60% of all threatened plant species are conserved both *in situ* and *ex situ*.

The introduction, variation and use of non-native conifer species in Britain (C.J.A Samuel)

C.J.A. Samuel commenced his presentation by giving some historical information on the many plant collectors, mainly Scottish, who introduced different exotic conifer species into Britain. He then presented 20th century trends in the use of conifers in the UK. Around 400 provenance experiments were established over the period 1926–1985. Results have revealed good increases in production from up to ten degrees transfer northwards in latitude on appropriate sites for North American conifers.

Before the late 1960s, the problems in establishing provenance trials related to limited seed availability (mainly commercial seed lots and lack of information on collection methods), population sizes and sampling. In the 1960s, IUFRO developed guidelines on sampling natural populations, co-ordinated seed collections and improved trial design. In conclusion, he raised a number of different questions on the conservation and use of exotic conifer species in the UK.

Breeding programmes of exotic conifers in Britain (S. Lee)

S. Lee presented a paper on breeding programmes of exotic conifers in the UK (Sitka spruce, Douglas-fir, lodgepole pine, Corsican pine and hybrid larch) and one native species, Scots pine. He reported progress made since the activities were initiated and provisions made for genetic conservation. He also provided some details on tree selection and progeny testing in these species. The objective was to breed for timber suitable for the construction industry. Tested clonal seed orchards established at a number of locations were now in production and improved seed for vegetative propagation was obtained through artificial pollination. Similar programmes were developed for a number of species. In conclusion, he highlighted pressures on tree breeders due to a shift to multi-purpose forestry and the increasing use of native species.

Developing a policy for long-term archiving of breeding material (R. Sykes)

R. Sykes presented a paper on clonal archiving in the UK breeding programme. This programme started in the 1950s and all selections were routinely archived until the late 1970s. The early material grew to inaccessible heights for pollination work and resulted in the introduction of top pruning in the 1980s. This policy was reviewed in the 1990s. He described the structure and layout of the early clonal archiving system. As a result of the review, a new archiving system was introduced. In future, as well as clones from the breeding population, a proportion of those originally selected will be retained to supply any future breeding material which may be needed in response to changing selection objectives.

Introduced forest tree species: some genetic and ecological consequences (B. Fady)

B. Fady presented a paper on handling exotic species from the genetic conservation point of view using *Cedrus atlantica* as a case study. The presentation aimed at evaluating some of the genetic and ecological consequences of species introduction for the management of both local ecosystems and the introduced species. He presented evidence that introduced forest species may have a very significant genetic and ecological impact on local ecosystems. Using examples drawn from research carried out at INRA-Avignon (French National Institute for Agricultural Research) on *Cedrus* species, he also demonstrated that introduced tree species experience rapid and quite considerable ecological and genetic change. They seem to evolve quickly into new landraces as a result of selection, genetic drift, population admixture and changes in spatial structure of their mating system. Several aspects relevant for *in situ* and *ex situ* conservation of introduced resources, both in their new and original environments, were presented.

Identifying origins of Douglas-fir (*Pseudotsuga menziesii*) plantations in Germany (A. König)

A. König made a presentation demonstrating how several Federal States of Germany monitor their Douglas-fir stands to conclude on their adaptation and suitability for future silvicultural use. In general, provenance trials have demonstrated that interior provenances are maladapted and exhibit inferior characteristics when planted in Germany. Forest administrations want to exclude unsuited stands from further propagation either as seed stands or for natural regeneration. Rhineland–Palatinate, Northrhine–Westfalia and Thuringia used different frequencies of alleles at the 6-PGDH-A isozyme locus in order to discriminate between provenances/stands of coastal and interior origins. The State of Hesse, however, assessed maladaptation on the basis of several phenotypic characteristics, such as needle discolouration, needle cast, growth distortions, extraordinary coarse branches, resin flow and mortality after the establishment phase.

Douglas-fir provenance tests in Serbia (V. Isajev and V. Lavadinovic)

It is a very common practice to introduce exotic conifers as garden specimens in several parts of Serbia but little attention is paid to provenances. In a Douglas-fir provenance trial 31 different provenances were introduced from Washington, Oregon and New Mexico. They were established some 25 years ago at two sites with different environmental conditions and different elevations. Two Oregon provenances had the best performance at both sites.

Conclusions based on the seminar and the discussions

In many European countries, public opinion favours native tree species over exotics. Despite obvious mistakes made in cultivation of exotic conifers, their role in production forestry and amenity is undisputed, especially in countries/regions with a low percentage of remaining native forests or impoverished dendroflora (Ireland, UK and Iceland).

The meaning and importance of autochthony should be understood in broader terms. This is due to human impact on site conditions, extensive use of genetically improved forest reproductive material and predicted climate change. Therefore, the use of non-native material under clearly defined conditions should be considered as an acceptable option.

The cultivation of exotic species may have certain ecological and genetic consequences which have to be monitored carefully (e.g. diversity loss of ecosystems and introgression). In some species, disease and pest problems develop only after a considerable time lag and may be difficult to eradicate due to lack of natural control.

Analyses of second-generation performance of some introduced tree species show that adaptation to new environmental conditions can be relatively fast and consequently the gene pool may change significantly within one generation. This fast adaptational process may lead to the emergence of landraces. Such locally adapted genetic material should be conserved by appropriate methods.

Breeding strategy and policy changes can lead to changes in the current valuation of species. The proper conservation of selected/improved genotypes and archiving of pertinent information are important.

Traditional plantings of exotic species in arboreta are useful for public awareness. Recent initiatives of arboreta to develop conservation programmes of threatened forest tree species are also valuable from a genetic point of view.

Exotic tree species should be incorporated in both the national programmes of FGR conservation and in the multilateral programmes of EUFORGEN and other international organisations.

Any other business

- Iceland indicated its interest in join EUFORGEN.
- A. Alexandrov informed the meeting that an IUFRO 5-needle pine meeting will be held in Bulgaria 3–7 September 2004.
- Missing contributions to the meeting report should be sent to the Secretariat by 15 December 2003.

Date and place of next meeting

Cyprus indicated its interest to organise the next meeting in spring 2005. The offer was accepted, provided that the Conifers Network will continue in its present form during Phase III of EUFORGEN.

Adoption of the summary of the meeting

The meeting summary was adopted and the Chair (C. Mátyás) closed the meeting.

Introductory country reports

Conifer genetic resources in Iceland

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Forests and native species

Iceland has the lowest forest cover in Europe in terms of percentage of total land area—about 0.3%. This translates to between 30 000 and 40 000 ha of forested land. About two-thirds of this is native downy birch (*Betula pubescens* Ehrh.) forest and about one-third is planted forest of various species, mostly exotic conifers. In addition to this, about 100 000 ha or 1% of the total land area is classified as 'other wooded land', which is mostly downy birch scrub less than 5 m in height (Anonymous 2000).

Other native tree species in Iceland are rowan (*Sorbus aucuparia* L.), which is wide-spread but uncommon, aspen (*Populus tremula* L.), which is very rare, and tea-leaved willow (*Salix phylicifolia* L.), which is common but only rarely attains tree height (5 m). Other native woody species never reach 5 m in height.

Common juniper (*Juniperus communis* L.) is the only native gymnosperm in Iceland. It is fairly common throughout most of the Icelandic lowlands and is found up to an elevation of over 500 m. It is prostrate in form when growing in open land but becomes upright in the shade of a woodland canopy; however, it rarely reaches 1 m in height. The tallest Icelandic juniper known is a multiple stemmed individual of about 2 m in height growing in a birch forest in north Iceland.

Forestry

Traditionally, the birch woods of Iceland were economically important as a source of building material, fuel, fodder for livestock and charcoal, needed to smelt bog iron and make iron tools. Woodlands continued to be important as a source of fuel and winter fodder for sheep until the 1930s, after which wood fuel was replaced by geothermal heat and imported oil. Furthermore, birch was replaced as a source of fodder by cultivated hayfields. Thus, traditional forest utilisation practices, which were unsustainable, have all but disappeared.

Modern forestry is considered to have started with the planting of the 'Pine Stand' at Thingvellir in 1899, Iceland's first forest plantation. The seedlings came from Denmark and the pines that still survive are mountain pine (*Pinus mugo* Turra and *P. uncinata* Mill. ex Mirb.) and Siberian stone pine (*P. sibirica* Du Tour). In the following years, small plantations were established at four other sites and three tree nurseries were set up, one of which is still in operation and celebrated its 100th anniversary in 2003. Several exotic conifers were tried in Iceland during the first decade of the 20th century; in addition to the pines already mentioned, these include Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.), Engelmann spruce (*Picea engelmannii* (Parry) Engelm.), white spruce (*Picea glauca* (Moench) Voss), Siberian larch (*Larix sibirica* Ledeb.) and European larch (*Larix decidua* Mill.) (Blöndal and Gunnarsson 1999).

After these limited early trials, emphasis in forestry shifted from planting to protecting native woodland remnants. During the first half of the 20th century the state acquired and protected from grazing several important forests and woodlands, which today comprise our national forests.

By the 1930s it was clear that some of the conifers planted 30 years earlier were growing quite well, notably Scots pine, Norway and Engelmann spruce and Siberian larch. After an understandable delay caused by World War II, trial and error outplanting of conifers started in the late 1940s and increased greatly during the 1950s, reaching over 1.5 million seedlings per year during 1960–62 (Petursson 1997). This was of course dependent on imported seed.

Beginning in 1944, several expeditions were made to Alaska to collect seed and make contacts that would continue to collect seed and sell them to Iceland. In the early 1950s, similar connections were made with the Soviet Union, especially to obtain larch seed and contacts were renewed with northern Norway for Scots pine and Norway spruce (Blöndal and Gunnarsson 1999).

Literally hundreds of kilogrammes of Sitka spruce (*Picea sitchensis* (Bong.) Carr.), mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) and western hemlock (*T. heterophylla* (Raf.) Sarg.) seeds were shipped to Iceland from Alaska during the 1940s and 1950s; potentially, enough to plant thousands of hectares. However, most of those hectares were never planted since much of the Sitka spruce and almost all of the hemlock never made it out of the rather primitive nurseries, due mostly to frost heaving in the nursery beds as well as sensitivity of these species to autumn frosts. Lodgepole pine (*Pinus contorta* Dougl.) from Alaska fared better in the nurseries as did both Scots pine and Norway spruce from Norway and larch from Siberia. Scots pine did not die until after planting (caused by infestations by the pine woolly aphid (*Pineus* *pini* Gmelin, L.), Siberian larch survived poorly in the wet, maritime climate of south and west Iceland and Norway spruce performed poorly, although survival is generally good (Blöndal and Gunnarsson 1999).

After these early setbacks, the 30 years between 1960 and 1990 was a period of learning for Icelandic foresters. Practical experience was gained through trial and error but scientific knowledge was also gained in silviculture and choice of seed origins for conifers in Iceland. This provided a basis for embarking on afforestation on a larger scale, with multiple use (including commercial timber production) as a feasible goal.

The Iceland Forest Service

The Iceland Forest Service (IFS) is the state forestry authority. Established in 1907, it has as its mandate to protect and preserve natural forests and forest remnants, to grow new forests where appropriate and to advise on forests and forestry-related matters. To these ends the IFS manages the national forests, 58 native forests and afforestation areas, with combined conservation, recreation, research and development goals. Research is an increasingly important role of the IFS as afforestation increases. Emphasis in research is on forest genetics and adaptation, pests and diseases, afforestation conditions and establishment techniques, forest ecology, carbon sequestration and forest inventory. Forestry extension and planning has been an important part of IFS activities but this is increasingly being provided by the Regional Afforestation Projects.

For most of the second half of the 20th century, the main activities of the IFS were seedling production (with as many as six nurseries in operation) and planting trees (mostly in IFS owned lands). Seedling production has now been privatized and planting is only a minor part of IFS activities today.

The head office of the IFS is in Egilsstadir in east Iceland and the research station— Iceland Forest Research—is located at Mógilsá, just north of Reykjavík. In addition to this, there are six district forestry offices, mostly located within the national forests. The IFS has a full time staff of about 50 professionals and support staff and employs another 30–40 staff seasonally.

Regional Afforestation Projects

Beginning in 1990 in east Iceland, a total of five Regional Afforestation Projects (RAPs) have now been set up covering the whole country. The RAPs manage the government grants scheme for afforestation on farms, each in its own region of the country. They are independent of the IFS in that they each have their own board of directors, consisting mostly of local people, and an independent budget. However, the IFS have one member on each board and professional ties with the IFS are close.

Setting up the RAPs as independent projects turned out to be very positive politically, as they have been able to get increased funding for afforestation far above what the IFS would have been able to do.

Five of the six RAPs have their offices located alongside IFS offices, resulting in close professional and social contacts. The RAPs employ a total of 22 professional staff and provide grants and other assistance to over 500 landowners participating in the projects. About 70% of all tree-planting in Iceland is carried out under the auspices of the RAPs.

The Icelandic Forestry Association

The Icelandic Forestry Association (IFA) is the third player in Icelandic forestry. It is an umbrella organisation of over 50 local forestry societies and with a total membership of over 7000, by far the largest environmental NGO in Iceland. The IFA publishes 'Icelandic Forestry', Iceland's main forestry publication, and provides education and extension services. They also manage the Land Reclamation Forests programme according to a contract with the Ministry of Agriculture. This government funded programme provides seedlings for planting on degraded and eroded land; roughly one million seedlings have been planted each year since 1990, mostly by the local forestry societies in co-operation with municipalities.

Besides the Land Reclamation Forests programme, local forestry societies are mostly concerned with managing older forests and woodlands for outdoor recreation; some grow Christmas trees, some have small tree nurseries and one owns a large commercial nursery.

Forestry goals

In general, Icelandic afforestation is planned and cultivated forests are managed with multiple-use objectives (Skulason *et al.* 2003). These objectives can best be described based on the four principle functions of forests: 1) ecological (ecosystem processes, habitats, wildlife), 2) economic (wood production, non-wood products), 3) protective (soil and water conservation, shelter, carbon sequestration) and 4) social (recreation, cultural and spiritual).

In forest planning and management, greater emphasis is often placed on one or two of these functions, with less emphasis on others but without ignoring them entirely. Within the RAPs, the majority of afforestation plans to date emphasize timber production as a primary goal within areas where timber production is possible, the main timber species being Siberian larch of north-western Russian origin ('Russian larch', *Larix sibirica*, syn. *Larix sukaczewii* Dylis), Sitka spruce and lodgepole pine. In peripheral areas, emphasis is on protective functions and in some cases ecological restoration, where the main species is native birch, or on establishing shelter-belts. A few plans have been drawn up emphasizing wildlife value, improved grazing for livestock and outdoor recreation.

A combination of protective and social functions is the aim of Land Reclamation Forests. Since they are mostly close to population centres, these forests will become the most used outdoor recreation areas in Iceland. In fact, two forest areas originally cultivated on degraded or eroded land in the 1950s and 1960s, one near Reykjavik and the other near Akureyri, annually receive over 400 000 visits, well over the entire population of Iceland (Reykjavik Forestry Society, Eyjafjördur Forestry Society unpublished data).

The management goal for the majority of national forests (IFS lands) is simply protection of native forest and woodland ecosystems. Outdoor recreation, timber production, ecosystem restoration and research are also main goals in some IFS lands, whereas erosion control and reclamation are the main aims on land managed by the Soil Conservation Service.

There are no forests managed only for carbon sequestration nor is there any plan for afforestation specifically for that purpose. However, carbon sequestration can be one of the management objectives of multiple-use cultivated forests.

Use of conifer species in Icelandic forestry

Three conifer species comprise 50–60% of annual planting for afforestation in Iceland and another seven regularly planted conifer species comprise about 5%. Table 1 summarizes the conifer species used in Icelandic forestry and their relative importance.

Table 1. Conifers in Icelandic forestry

Species	Imp.†	Preferred provenances	Seed sources	Future‡
Larix sukaczewii Dylis	1	Raivola, NW Russia and Urals	Finnish and Swedish seed orchards and indoor orchards	1
Picea sitchensis (Bong.) Carr.	1	Coastal Alaska	Stands of known origin in Iceland, Alaska and Norway	1
Pinus contorta Dougl.	1	Skagway, SW Yukon and NW British Columbia	Stands of known origin in Iceland, Alaska and Yukon	1
Picea engelmanni (Parry) Engelm.	2	Southern Rocky Mountains	Imported seed used	1
Abies lasiocarpa (Hook.) Nutt.	3	Southern Rocky Mountains	Imported seed used	2
Picea glauca (Moench.) Voss.	3	Alaska	Imported seed used	2
Picea abies (L.) Karst.	3	Norway	Imported seed used	2
Pinus cembra L.	3	Unknown	Seed stands in Iceland and imports	1
Pinus sylvestris L.	3	Unknown	Imported seed used	2
Pinus uncinata Mill. ex Mirb.	4	Unknown	Imported seed used	3
Larix laricina (Du Roi) K. Koch	4	Alaska	Imported seed used	3
Larix decidua Mill.	4	Alps, high elevation	Imported seed used	2
Larix lyallii Parl.	4	Unknown	Small indoor seed orchard in Iceland	2
Pseudotsuga menziesii (Mirb.) Franco	4	Unknown, high elevation	Not currently planted in forestry	2
Juniperus communis L.	4	Native	Not currently planted in forestry	3

† Importance with respect to conservation of genetic resources:
1 Very important for Icelandic forestry
2 Of intermediate importance
3 Of low importance
4 Not important for Icelandic forestry

‡ Future:
1 Likely to remain important or increase in importance
2 Future importance uncertain
3 Likely to remain unimportant or decrease in importance

Conifer genetics strategy

It could be said that we have elements of a conifer genetic resources and breeding strategy in Iceland. This includes an accelerated breeding program for larch based on indoor seed production and with the goal of developing a well-adapted landrace of Russian larch for Iceland as well as larch hybrids for use in more maritime areas. Seed orchards and seed stands of Sitka spruce have been set up and results from recent provenance trials and frost hardiness testing will yield material for selection to set up new seed orchards. Recently, we have started to collect seed of lodgepole pine in Iceland, with very promising results. We are also conducting provenance trials on several species that provide information on selecting seed sources and which can also be a source of breeding material in the future.

Recently, discussion about forest genetic resources has increased among foresters in Iceland and it is likely that we will have a coherent strategy within 2–3 years, not only for conifers but for all forest trees. This strategy is likely to include provisions for conservation of important genetic material, seed production and procurement and research in forest genetics and tree improvement. Target species are likely to be the same ones that are most important today, namely Russian larch (and hybrids), Sitka spruce and lodgepole pine.

Research needs/capacity building

A great deal of research is needed when starting forestry with multiple-use goals using exotic species. This not only includes work in the field of genetics, such as testing and selection, but also silvicultural research including both the establishment phase and later phases. Pests and diseases require research and the ecology of a new species is always of both academic and practical interest. Finally, socio-economic aspects must be considered. This research is at various stages, depending on the species being investigated, but in general we have emphasized genetics and establishment phase silviculture. Pests and diseases have been fairly well-researched in recent years but ecological work with exotic species is just starting. Research in the field of conservation of genetic resources is practically non-existent.

The total number of people working in forest research in Iceland is less than 20 and most of them do research alongside other duties. Even though the forestry industry in Iceland is small, the need for research is proportionately great, mainly because there is limited experience and practically no tradition to build on in what we are attempting to do. Therefore, we have sought knowledge and experience abroad, especially from our Nordic neighbours.

Nordic co-operation has been a mainstay in Icelandic forest research for some time and we have gained a great deal from it both in terms of knowledge and experience. Most Icelandic forest scientists are also trained at universities in the Nordic countries. Co-operation on the European scale is more recent but we have participated in the MCPFE (the ministerial conferences themselves, not the process that goes on between them) as well as some European Co-operation in the Field of Scientific and Technical Research (COST) groups. Even though such co-operation is without doubt beneficial to Icelandic forestry, it is also costly for a small organisation like the IFS. In many cases, we simply do not have the human resources to participate. This has been the case with the European Forest Genetic Resources Programme (EUFORGEN) until now and is still the case with respect to participation in the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (IPC forests). Indeed, the emphasis within EUFORGEN and IPC forests until recently has been such that Iceland has not had much to gain from it or much to give.

We are slowly but surely building up our capacity in forest research and we hope that in the near future we can give as well as gain from both Nordic and European co-operation.

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Single nucleotide polymorphisms (SNPs) in forest trees

Review on single nucleotide polymorphisms (SNPs) and population genetic studies in conifer species

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Introduction

Forest biodiversity is one of the main components of terrestrial ecosystems. All together, tropical, temperate and boreal forests offer diverse habitats for plants, animals and micro-organisms, holding a vast amount of the World's terrestrial biodiversity. In view of growing concern about human impact and expected climatic changes, the maintenance of mechanisms generating diversity in forests has become a central issue as it determines the stability of terrestrial ecosystems and the sustainability of their resource. It is generally accepted that maintaining or restoring biodiversity is a basic precondition to give a long-term, evolutionary answer to these challenges.

Diversity can be studied at different levels, but DNA sequence data represent the highest level of genetic resolution (Järvinen *et al*. 2003). Genetic markers have been extensively used during the past two to three decades to unravel patterns of distribution of genetic diversity and infer possible mechanisms of plant evolution. The use of neutral molecular markers (e.g. simple sequence repeats, SSRs) has allowed historical patterns and the role of evolutionary forces—such as genetic drift—to be depicted because these phenomena affect all markers in similar ways. However, they are not well suited to providing information about variation in functional regions, where selection operates. Many clines for adaptive variation have been described in forest trees, e.g. bud set in *Pinus sylvestris* L. (García-Gil *et al*. 2003) and they are likely to be the products of variation in a limited set of genes and biosynthetic pathways that might not be detected by correlation with random neutral genetic markers. Thus, studies of genetic diversity could benefit from targeting genes that exhibit relevant variation on adaptive traits, rather than targeting random markers (Brumfield *et al*. 2003; Morin *et a*l. 2004; van Tienderen *et al*. 2002; see review for forest trees in Krutovsky and Neale 2001).

Dissection of complex adaptive traits in plants, including forest trees, was traditionally undertaken through genetic linkage analysis (quantitative trait loci [QTL] mapping) based on DNA polymorphisms in highly structured populations with known pedigrees. The study of the pattern of variation of adaptive traits benefited from classical tree breeding experiments, which provided information about families particularly adapted to specific environmental conditions. The opportunity to bring together population genetic and functional genomic studies by identifying candidate genes controlling target traits or underlying QTLs has been made possible by: (1) the development of molecular markers in functional regions (genes, promoters, etc.), such as SNPs (single nucleotide polymorphisms), (2) the discovery of candidate genes via transcript profiling, and (3) an extraordinary effort in EST (expressed sequence tags) sequencing (e.g. more than 250 000 ESTs are already available in pine). Subsequently, polymorphisms that are in strong linkage disequilibrium (LD) with phenotypic traits (i.e. phenotype–genotype genetic associations) have been revealed by using natural populations; this has been termed 'association mapping', e.g. see Plomion *et al*. (2003) and Neale and Savolainen (2004). Association mapping was first developed in humans, where classical approaches are not feasible, and was recently extended to plants.

Natural populations can be used to map traits by means of association analysis. Association mapping takes advantage of LD created after many generations of recombination and random mating. In these conditions, only tightly linked loci will show statistical association, allowing finer mapping than standard QTL approaches. To avoid false association, it is extremely important to have detailed information on basic population parameters, such as the extent of LD, the level of genetic variation and the degree of population structure; and also how these parameters vary across the genome of the target species. In principle, association studies can identify variation down to the single-nucleotide substitutions that are responsible for variation in phenotypes (QTNs, quantitative trait nucleotides) (Ingvarsson 2005).

Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are Mendelian, co-dominant markers that can be analysed by any statistical method that is based on genotype frequencies. They are the result of single point mutations that produce base-pair alternative sequences (alleles) in genomic DNA. SNPs are abundant and widespread in the genomes of the species studied so far; for example, they represent 90% of the genetic variation detected in the human genome. Moreover, they can potentially be associated with adaptive traits. They are more frequent in non-coding regions than in coding ones but the mean frequency varies greatly among species (Table 1). Usually, SNPs have only two alleles, but SNPs with three allelic variants appear at low frequency (~1–2% in *Pinus taeda* L.).

The use of SNPs as molecular markers became possible only recently thanks to sequencing projects of model species (e.g. *Arabidopsis* and *Populus*) that produced redundant databases highlighting the prevalence of nucleotide polymorphism in the genomes. Moreover, rapid progress in sequencing technology has made it easier to collect many sequences by automating the processes and reducing the costs.

There are two main steps that need to be followed to use SNP markers: SNP discovery and SNP genotyping.

Table 1. Frequency of single nucleotide polymorphisms (SNPs) in different plant species, including several forest trees

Plant species	SNPs/kb†	Reference(s)
Beta vulgaris L.	8	Schneider et al. 2001
Picea abies (L.) H. Karst.	13	De Paoli and Morgante 2004
Pseudotsuga menziesii (Mirb.) Franco	26	Krutovsky and Neale 2005
Pinus taeda L.	16	Brown et al. 2004
Pinus pinaster Ait.	6–10	Le Dantec et al. 2004; Pot et al. 2005
Pinus radiata D. Don	3	Pot et al. 2005
Pinus halepensis Mill.	10	Sebastiani et al. unpublished data
Populus tremula L.	60	Ingvarsson 2004
Populus nigra L.	10	Zaina and Morgante 2004
Glycine max Merr.	3	Zhu et al. 2003
Zea mays L.	16	Ching et al. 2002

† single nucleotide polymorphisms per kilo base pair

SNP discovery

SNP discovery is the process of finding the polymorphic sites in the genome of the species and populations of interest. There is not a single way to discover SNPs, and different approaches may be adopted depending on the availability of DNA sequence information. These include the re-sequencing of polymerase chain reaction (PCR) amplicons (fragments) with or without pre-screening, electronic SNP (eSNP) discovery in shotgun genomic libraries and eSNP discovery in EST libraries (Rafalski 2002a). The strategies adopted to develop SNP markers differ between model and non-model species (a model species is one that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made will provide insight into the workings of other organisms, i.e. the non-model species).

For model species, redundant overlapping databases exist, derived from sequencing, ESTs or large-scale SNP identification projects, making it possible to directly retrieve SNPs for the genes of interest. The increasing availability of software and databases is helping to facilitate SNP discovery enormously through the implementation of automatic platforms (Le Dantec *et al*. 2004). Some of these are able to provide transcript profiling information; for instance, the MAGIC Gene Discovery tool (Cordonnier-Pratt *et al*. 2004), available at http://fungen.org/genediscovery/.

For non-model species, the most direct way to identify SNPs is to sequence a genome fragment from multiple individuals. Candidate fragments for different genes can be obtained from model species or expressional studies. In order to avoid ascertainment bias in allele frequencies, it is advisable to obtain high-quality sequence from a relatively large sample of individuals representing all the populations in the study. To avoid sequencing errors and low-quality sequence due to the presence of repetitive regions, sequencing from both ends is also advisable. An inexpensive pre-screening, e.g. single strand conformation polymorphism (SSCP) of several samples can facilitate the choice of the most informative loci and provide a preliminary estimate of the level of polymorphism. Unfortunately, these pre-screening methods for SNP detection are often labour-intensive and not very sensitive. Pre-screening may be necessary for species like soybean, where the rate of SNP is low, but more rarely in forest trees which generally show high levels of standing nucleotide variation.

The high level of conservation of gene sequences across species facilitates the design of primers to amplify orthologous gene regions in related species, starting from information available in model species. PCR primers (a nucleic acid strand, or a related molecule that serves as a starting point for DNA replication) are carefully designed to amplify the loci of interest, excluding any other member of the same gene family. The PCR products are then sequenced in both directions and the resulting sequences are aligned. Taking care to distinguish true polymorphisms from sequencing errors, polymorphisms are identified (Figure 1).

For those non-model species where it is not possible to amplify orthologous genes, a random sequence approach can be followed. This approach involves sequencing anonymous nuclear loci

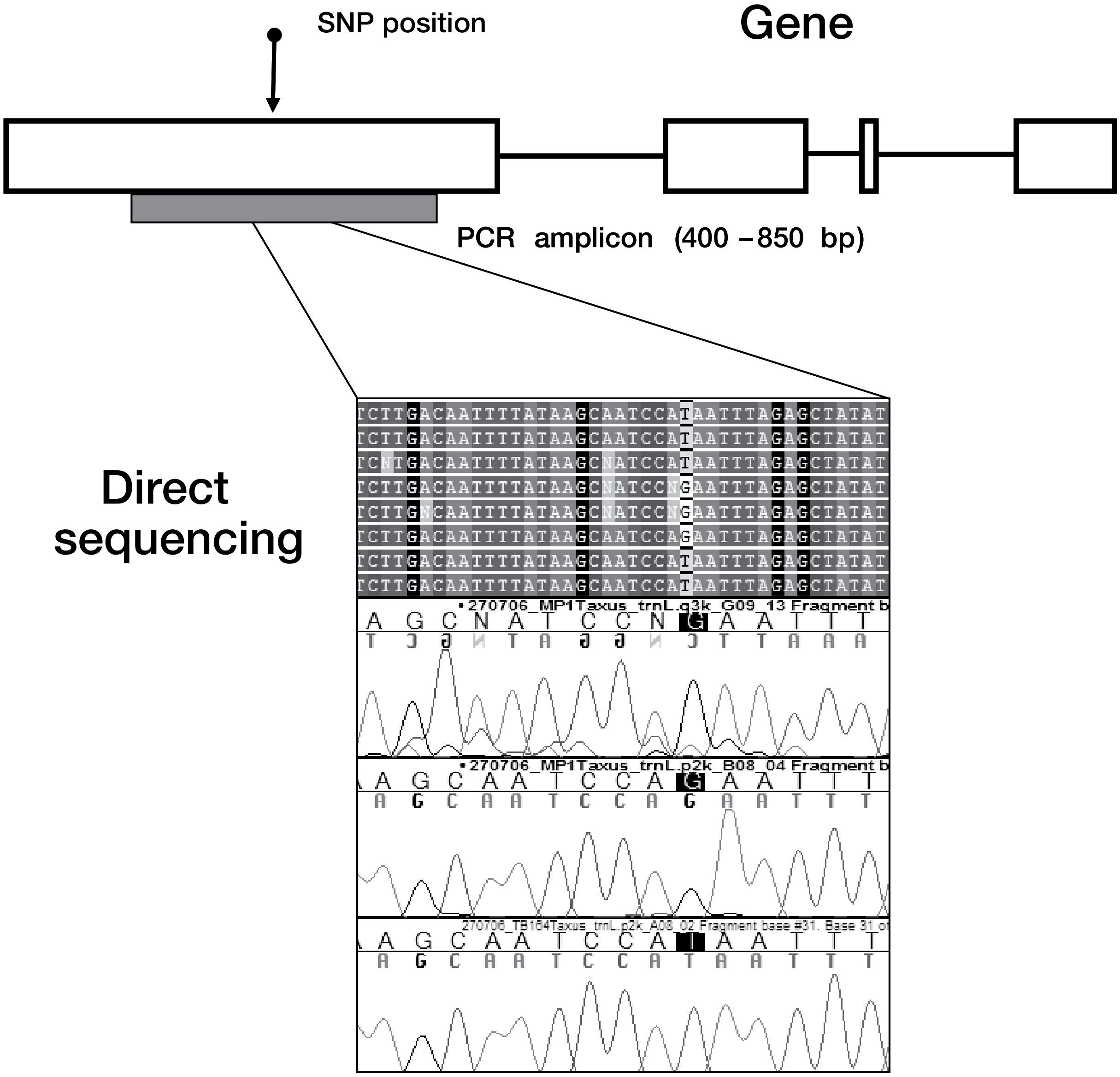

Figure 1. DNA sequence analysis for SNP discovery. After amplification from different individuals, PCR fragments (i.e. amplicons) are purified and sequenced from both ends. Sequences are then aligned and SNPs (and insertions/deletions) are identified. In this example, the SNP (G/T) is indicated in black.

derived from a genomic or cDNA (cytoplasmic DNA) library or produced through amplified fragment length polymorphisms (AFLPs) and in designing PCR primers that can be used to screen multiple individuals and find polymorphic SNP loci.

SNP genotyping

Multiple technologies are available for SNP genotyping. The choice of the method depends on the study to be performed and other criteria, such as cost, throughput level and equipment available.

PCR-RFLP (restriction fragment length polymorphism) and PCR-SSCP (single strand conformation polymorphism) are the traditional techniques used to gather information at the sequence level. The main advantage of these methods is that they only require standard laboratory equipment, and can therefore be used in every laboratory; the main drawback is that they are not well suited to high throughput genotyping.

Another category of methods relies on the direct acquisition of sequence information. One way is the traditional sequencing approach based on the standard method with dideoxy fluorescent label nucleotides. This method is useful for SNP discovery but can be expensive for genotyping, with the exception of those cases where SNP frequency is high, and therefore sequencing allows the genotyping of multiple loci at the same time. An alternative, recent method to genotype multiple loci at the same time is eco-tilling, a low-cost technique for rapid identification of haplotypes (Comai *et al*. 2004).

Fluorescent-based sequencing has an increasing role in SNP analysis because of the development and availability of programs that automate the base calling, assembly and finishing of sequences; such as: Phred, Phrap and Consed (see www.phrap.org/phredphrapconsed.html). Polyphred is another program that operates together with Phred, Phrap and Consed to identify SNPs as high-quality base mismatches in assembled sequences. Importantly, Polyphred can also detect SNPs as heterozygotes (two bases at a single position in the sequence) in diploid sequences amplified by PCR (Brumfield *et al*. 2003).

Newer methods for allelic discrimination are based on primer extension. There are numerous variations in the primer extension approach that are based on the ability of DNA polymerase to incorporate specific deoxyribonucleosides complementary to the sequence of the template DNA. However, all these methods can be grouped into two categories. The first one is a mini-sequencing technology, a single base extension (SBE) where the identity of the polymorphic base in the target DNA is determined. In this case, only dideoxy nucleotides are used, causing the addition of only the complementary nucleotide. The second one, called allele specific primer extension (ASPE), is an allele-specific PCR approach where the DNA polymerase is used with deoxy nucleotides to amplify the target DNA; the PCR product will be obtained only if the primers are perfectly complementary to the target DNA sequence.

Several ingenious methods have been devised which differ in the way they monitor the reaction. Most of these approaches combine novel nucleic acid analogues and new methods of monitoring differences in physical properties between starting reagents and primer extension products. Alleles can be sorted and detected using various methods; including gel electrophoresis, macro and microarrays and fluorescence polarization. These genotyping methods are suited to automatic machines, such as automatic sequencers, and can ensure medium to high throughput results. Finally, several commercial high-throughput genotyping platforms that can handle as many as 100 000 assays simultaneously have been developed (Hirschhorn and Daly 2005). A more comprehensive survey of SNP genotyping methods can be found in Kwok (2001).

Functional vs neutral genetic markers

The main difference between a random marker and a functional marker is the distance of the mutation causing the phenotypic effect in the trait of interest (van Tienderen *et al*. 2002). During the last 20 years, the majority of studies aiming to monitor the level and distribution of genetic diversity in natural populations were based on the use of neutral markers. Indeed, molecular markers, such as allozymes or microsatellites, provided useful information on historical demography and population evolution. However, neutral markers do not generally reflect selective processes or are related to fitness which is an indicator of the level of adaptive variation within populations and therefore of the adaptive potential of populations to changing environments (Morin *et al*. 2004).

SNPs are particularly useful markers for finding genes under selection and studying the dynamics of these genes in natural populations. SNPs are robust markers, easy to score and widespread in the genome. The availability of high-density markers, such as SNPs, opens the possibility of studying, by association genetics, the molecular basis of complex quantitative traits in natural populations of plants, taking advantage of the fact that genetic markers in close proximity to mutant genes may be in LD to them. Association studies can be carried out using a genome-wide approach (without assuming one region of the genome to be more likely to harbour the associated genetic factor than another) or with a candidate gene approach (using biological knowledge to prioritize some fragments of the genome for the study). The magnitude and distribution of LD determine the choice of association mapping methodology. When LD declines slowly with increasing distance from the mutation or gene responsible of the phenotype even a low density of markers is sufficient to identify associated markers. When LD declines rapidly around the causative gene, a much greater density of markers is required to identify an associated marker (Rafalski 2002b). Extension and distribution of LD depend on many factors including population history (e.g. the presence of population bottlenecks or admixture) and the frequency of recombination. First studies on forest tree species revealed a rapid decay in LD with distance. LD declines to negligible levels in <500 bp (base pairs) in *Populus tremula* L., although in some cases LD extends in local populations up to 1 kb (Ingvarsson 2005). Similarly, LD declines very rapidly within 200 bp in *Picea abies* (L.) H. Karst. (De Paoli and Morgante 2004) as well as in *Pseudotsuga menziesii* (Mirb.) Franco (Krutovsky and Neale 2005), *Pinus halepensis* Mill. (Sebastiani *et al.* unpublished data) and *Pinus taeda* (Brown *et al.* 2004). A rapid decay of LD in forest trees is consistent with what is expected from outcrossing species with large effective population size, and is in strong contrast with what is observed in self-fertilizing plant species. For example, in *Arabidopsis thaliana* (L.) Heynh. selfing dramatically reduces the effective recombination and LD extends up to 250 kb (Nordborg and Tavaré 2002). Some variation among genes in LD has been observed in barley (Lin *et al.* 2002), pines (Brown *et al.* 2004) and maize (Remington *et al.* 2001), suggesting that, in addition to mating systems, other factors such as demographic history or variable recombination rates across the genome, can play an important role.

The association mapping approach is very promising for long-lived, relatively undomesticated forest trees where the general high level of genetic variability of natural stands can be successfully used to identify markers linked to economically and ecologically relevant traits. Moreover, as forest trees are predominantly outcrossing organisms characterised by large effective population size, they generally show LD extending only a few hundred base pairs. For these reasons and considering that in some species (e.g. some conifers) the genomes are extremely large (> $1x10^{10}$ bp), the whole-genome scan approach is not feasible because of the too high number of SNPs required for adequate genome coverage. On the other hand, very fine-scale mapping is possible if candidate gene approaches are used and it might even be more advisable given the high variation found in tree genomes (Ingvarsson 2005; Neale and Savolainen 2004).

A limiting step in forest trees is the choice of candidate genes. The choice of appropriate candidate genes can be facilitated by the availability of information about the biochemical and/or physiological pathways related to the trait of interest, i.e. by selecting genes involved in these pathways. Unfortunately, this information is rarely available for forest trees; therefore, sequences of genes identified in model species, such as *Arabidopsis thaliana,* are used to design consensus or degenerated primers for the amplification of orthologous loci. The recent sequence of the complete poplar (*Populus trichocarpa* Torr. & Gray) genome (see http://genome.jgi-psf.org/Poptr1/Poptr1.home.html), which is four times larger than the *Arabidopsis* genome, opens exciting new possibilities to identify novel genes in forest trees. Other methods to identify candidate genes exist, e.g. via transcript profiling through cDNA and oligonucleotide microarrays (for more details see Pflieger *et al.* 2001). However, it should be stressed that substantial work is still needed to elucidate the functional role of genes and for the successful transfer to non-model species.

Both association mapping approaches (genome-scan and candidate genes) require abundant SNPs in the studied species and populations. For this reason, preliminary analyses to assess nucleotide diversity in different species and association populations are required. First estimates indicate that nucleotide diversity varies considerably between plant species, from the highest (maize) to the lowest (the highly-domesticated soybean). Interestingly, some conifers, such as *Cryptomeria japonica* (L.f.) Don (Kado *et al.* 2003) and pines, e.g. *Pinus sylvestris* (Dvornik *et al.* 2002) and *Pinus taeda* (Brown *et al.* 2004) are not amongst the most variable species, contradicting expectations from the results obtained using neutral markers and their life history characteristics. First evidences showed that broadleaved genera, e.g. *Populus* (Ingvarsson 2005) and *Quercus* (Pot *et al.* 2005) might display higher nucleotide diversity than pines or *Cryptomeria*. In fact, *Populus* displays about 2- to 10-fold higher nucleotide polymorphism than *Pinus* or *Cryptomeria*. However, other conifers, such as *Pseudotsuga menziesii*, showed levels of variation comparable to broadleaved species (Krutovsky and Neale 2005). The high level of variation detected in *Populus* and *Quercus* is in agreement with earlier studies based on allozyme analysis (Jelinski and Cheliak 1992; Petit *et al*. 1995).

Markers in specific functional regions of the genome need to be statistically analyzed in order to test for the possibility that these regions might have experienced different selective pressures. In unstructured populations, standard neutrality tests might be applied. When variation is structured in populations, an interesting and relatively easy approach is the comparison of genetic differentiation estimates, such as Wright's *F*-statistics among markers tagging a putative gene under selection and neutral markers, or expected distributions computed using coalescence theory (see reviews in van Tienderen *et al*. (2002) and Luikart *et al*. (2003)). If population divergence (F_{st}) is higher for the gene-targeted marker with respect to divergence estimates obtained from random markers, this might indicate divergent selection and local adaptation for the tagged gene (van Tienderen *et al*. 2002). Pot *et al*. (2005) found a higher differentiation among populations at the *Pp1* (glycine-rich protein homologue) gene in *Pinus pinaster* Aiton than in neutral markers. This result is consistent with diversifying selection acting at this locus in this species, which would have lead to the presence of different haplotypes; possibly adapted to local environmental conditions. On the other hand, the absence of differentiation observed for the gene *CesA3* (cellulose synthase) compared with the significant level observed at neutral markers may indicate balancing selection acting on this gene. Note that the presence of significant differentiation among populations may produce spurious associations; therefore, care has to be taken when sampling for association studies.

Currently, SNPs are used primarily in association studies; but their ubiquity, tractable levels of variation and readiness in screening suggests that they will increasingly dominate as markers for elucidating the evolutionary history of populations. Unlike microsatellites, SNPs have relatively low mutation rates. Multiple mutations at a single site are rare, thus facilitating high-throughput genotyping and minimizing recurrent substitutions at a single site (i.e. homoplasy) that would confound the population history (Brumfield *et al*. 2003). Moreover, in conservation genetics, the availability of markers able to detect functional variation could help to define functionally significant units (FSUs), based on differences in allelic frequencies for genes with important ecological functions (van Tienderen *et al*. 2002). FSUs might help managers in conservation biology to identify those conservation units that contain adaptive genetic variation that is worthwhile protecting.

Conclusions

In conclusion, SNPs are becoming the marker of choice in population genetics, ecology and evolution studies because of ease of modelling, genotyping efficiency and genome-wide coverage. Forest tree species, which comprise undomesticated and unstructured large populations where linkage disequilibrium is expected to be limited represent ideal organisms to efficiently apply a candidate-gene based approach to detect association between markers and ecologically and economically important traits.

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Genetic resources of exotic conifers

The introduction, evaluation and use of non-native conifer species in Britain

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Introduction

As part of an island grouping separated from mainland Europe, the United Kingdom (UK) has only three conifer species: *Pinus sylvestris* L., *Taxus baccata* L. and *Juniperus communis* L. These species arrived between the end of the last period of glaciation and the disappearance of the land-bridge with mainland Europe. Only *Pinus sylvestris* is of economic importance and it remains naturally distributed in small populations in the Scottish Highlands. The development of techniques of artificial regeneration of managed forests therefore brought an interest in non-native conifer species to exploit the wide range of site conditions present and exotic species now account for the major part of commercial coniferous forestry.

The introduction of exotic conifers to Britain

A summary of the introduction of exotic conifers is given in Table 1. In the table the origin, date of introduction, person who introduced the species, where known, and date when the species was first used as a commercial plantation species is given. More details are given by Macdonald et al. (1957).

Among the European species, *Picea abies* (L.) Karst., which was present before the last glaciation, was probably introduced as early as the 16th century and was grown commercially from that time. *Larix decidua* P. Mill. was introduced in the late 17th century and a number of individual specimens dating from the early 18th century are still standing. It was used as a plantation species from around 1750. *Pinus nigra* Arnold was introduced in 1759 but interest in its cultivation was slower to develop. The other European species were investigated but they have never been the subject of serious commercial exploitation, remaining important only for ornamental use.

Serious exploration of the Pacific north-west of North America began in the late 18th and early 19th centuries and established a major tradition of plant collection from around 1830. The better known collectors include Archibald Menzies, David Douglas, William Lobb, John Matthew, John Jeffrey and William Murray. In Table 1 it can be seen that Douglas can be credited with the introduction of seven of the species listed, two of which, *Picea sitchensis* (Bong.) Carr. and *Pseudotsuga menziesii* (Mirb.) Franco, play a major part on commercial soft-wood production in the UK. Other collectors, notably John Veitch, Charles Maries, Ernest Wilson and George Forrest, brought further species from China and Japan in the late 19th and early 20th centuries.

Past and current importance of exotic conifers

There have been major changes and trends in the use of conifer species during the last 100 years in which commercial forestry became established and expanded in the UK. At the beginning of this period, *Pinus sylvestris* and *Picea abies* were the major plantation species with lower concentration on *Pseudotsuga menziesii* and *Larix* spp. *P. sylvestris* saw a gradual decline but retains an important position on poorer, drier sites. In contrast, *Picea sitchensis* displaced *Picea abies* and rose to be the predominant plantation species during the 40 years following World War II. Other species, such as *Pinus contorta* Dougl. ex Loud., were found to have potential on more demanding sites beyond those suitable for *Picea sitchensis* and became the subject of considerable interest in the period 1960–1980. However, problems with poor growth form and instability in wind and snow, together with policy changes with respect to land use, caused this interest to fall rapidly in later years. Since the early 1990s, planting of conifers in general has given way to a major interest in the use of native broadleaved species with less commercial objectives.

Table 1. The introduction of non-native conifers into Britain[†]

Species	Origin[‡]	Date of introduction	Introduced by	Plantation date
		Major commercial species		
Abies grandis (Dougl. ex D.Don) Lindl.	NA	1831	Douglas	1900
Abies procera Rehd.	NA	1831	Douglas	1880s
Larix decidua P. Mill	E	17th century	–	1750s
Larix kaempferi (Lam.) Carr.	A	1861	Veitch	1895
Larix x *eurolepis* Henry	HY	1900	Arose in Scotland	1904
Picea abies (L.) Karst.	E	16th century	–	16th C
Picea sitchensis (Bong.) Carr.	NA	1831	Douglas	1852
Pinus contorta Dougl. ex Loud.	NA	1853	Jeffrey	1910
Pinus nigra Arnold	E	1759	–	1840–7
Pseudotsuga menziesii (Mirb.) Franco	NA	1826–7	Douglas	1860s
Thuja plicata D.Don	NA	1853	Lobb	1876
Tsuga heterophylla (Raf.) Sarg.	NA	1851	Jeffrey	1860s
		Other species of lower commercial importance		
Abies alba P. Mill.	E	1603	–	1790s
Abies amabilis Douglas	NA	1830	Douglas	1882
Chamaecyparis lawsoniana (A. Murr.) Parl.	NA	1854	Murray	–
Cryptomeria japonica (L. f.) D.Don	A	1842	Home	–
Picea engelmannii Parry ex Engelm.	NA	1864	–	–
Picea glauca (Moench) Voss	NA	1700	–	19th century
Picea omorika (Pani) Purkyne	E	1884	Frobel	1908
Pinus peuce Griseb.	E	1863	–	1950s
Pinus pinaster Soland. non Ait.	E	1596	–	17th–18th century
Pinus ponderosa P.&C. Lawson	NA	1827	Douglas	1940–50
Pinus radiata D.Don	NA	1833	Douglas	1850
Sequoia sempervirens (D.Don) Endl.	NA	1846	Hartweg	1856

[†] – = information not available

[‡] NA = North America; E = Europe; A = Asia; HY = Hybrid

Seed origin and provenance research

Research on within species adaptive variation began in the 1920s and Forest Research (the research agency of the Forestry Commission, the government department responsible for forestry in Great Britain) has established over 400 trials since that time. General recommendations for the UK are given by Lines (1987). These have included all the conifer species listed in Table 1, together with others of very minor interest. Early work depended on availability of seed and on fruitful contact with other researchers or seed suppliers. Samples of seed were used in which there was often no knowledge of the exact location of the source, the population size or the sampling or collection methods. This resulted in trials often comprising a more opportunistic collection of origins rather than one organized on a range-wide basis. This situation improved from the late 1960s when the International Union of Forest Research Organizations (IUFRO) began to sponsor organized representative sampling of the natural range of a number of important commercial conifer species. IUFRO laid down standards in sampling the natural distribution, making seed collections and the design of evaluation trials. In particular, Forest Research played a major part in making collections of a number of North American species and in their evaluation across a major range of site types. Table 2 summarises provenance testing of the most important conifers.

Table 2. Summary of provenance testing in commercial conifers in Britain

Species	Series[†]	Sites[‡]	IUFRO[§¶]
Abies grandis (Dougl. ex D.Don) Lindl.	3	16	11
Abies procera Rehd.	2	11	10
Larix decidua P. Mill.	14	35	–
Larix kaempferi (Lam.) Carr.	7	14	–
Larix x *eurolepis* Henry	6	7	–
Picea abies (L.) Karst.	10	18	3
Picea sitchensis (Bong.) Carr.	13	53	20
Pinus contorta Dougl. ex Loud.	23	79	5
Pinus nigra Arnold	6	14	–
Pseudotsuga menziesii (Mirb.) Franco	8	21	5
Thuja plicata D.Don	1	4	–
Tsuga heterophylla (Raf.) Sarg.	4	17	–

[†] number of years in which a series of trials was planted
[‡] total number of trials planted
[§] number of trials using IUFRO collections
[¶] – = IUFRO collections not made

Pacific north-west American species

Most of the species from this region which have been evaluated in Britain have extensive natural distributions, covering up to 20 degrees of latitude. In general it has been found that increased rates of growth in the UK are associated with the transfer of material from sources up to ten degrees south of those in which the UK lies. However, this advantage can only be exploited if attention is paid to climatic and site matching. This is illustrated in Figure 1 in which the mean performance of *Picea sitchensis* across two series of sites is compared (Samuel 1995). The British Isles have been drawn next to the Pacific north-west at their equivalent latitude and the performance of each origin is indicated as the percentage of the mean of all origins across the sites included. In Figure 1a, the sites plotted in Great Britain are those which are ecologically well-matched to those in which the species grows in its natural range. In Figure 1b, the site types lie outside those in which the species would occur.

In Figure 1a the sites are located on the western side of the country in which there is an oceanic climate with higher rainfall. Across these sites, the advantage of using more southerly origins from as far south as Oregon is clear. By contrast, in Figure 1b in which colder and drier site types are represented, the clinal relationship with latitude no longer exists; therefore, there are no clear advantages in the use of more southern sources, the best performance being found among origins from the Queen Charlotte Islands, off the coast of British Columbia.

Whilst this pattern has been shown in a number of species, factors other than growth rate need to be taken into account. For example, it has been found in *Pseudotsuga menziesii* that origins from as far south as Oregon, whilst having high growth rates, may have poorer survival with a heavy branching habit which would not produce good quality timber. Therefore, for this species and for *Abies grandis* (Dougl. ex D.Don) Lindl. (grand fir) the use of sources from further south than Washington would not be recommended. In *Pinus contorta*, clinal variation in growth rate with latitude is strongly inversely correlated with basal stem curvature, general stem form and heavy branching, causing serious vulnerability to the high wind profile encountered in Britain and to heavy snow falls. Within a further longitudinal cline across British Columbia in this species, sources with straight stem and fine branching characteristics which occur inland from the coastal distribution would be recommended, although some loss of fast growth potential would have to be accepted. In contrast to the more widely distributed species, there is much less evidence of clinal variation in *Abies procera* Rehd. (noble fir), which has a more limited distribution at higher altitudes in the Cascade Mountains in Washington and Oregon.

European species

A similar general pattern does not emerge from trials of the important European conifers. Sources of *Pinus sylvestris* native to Britain show superiority over continental sources (Worrell 1992) and commercial

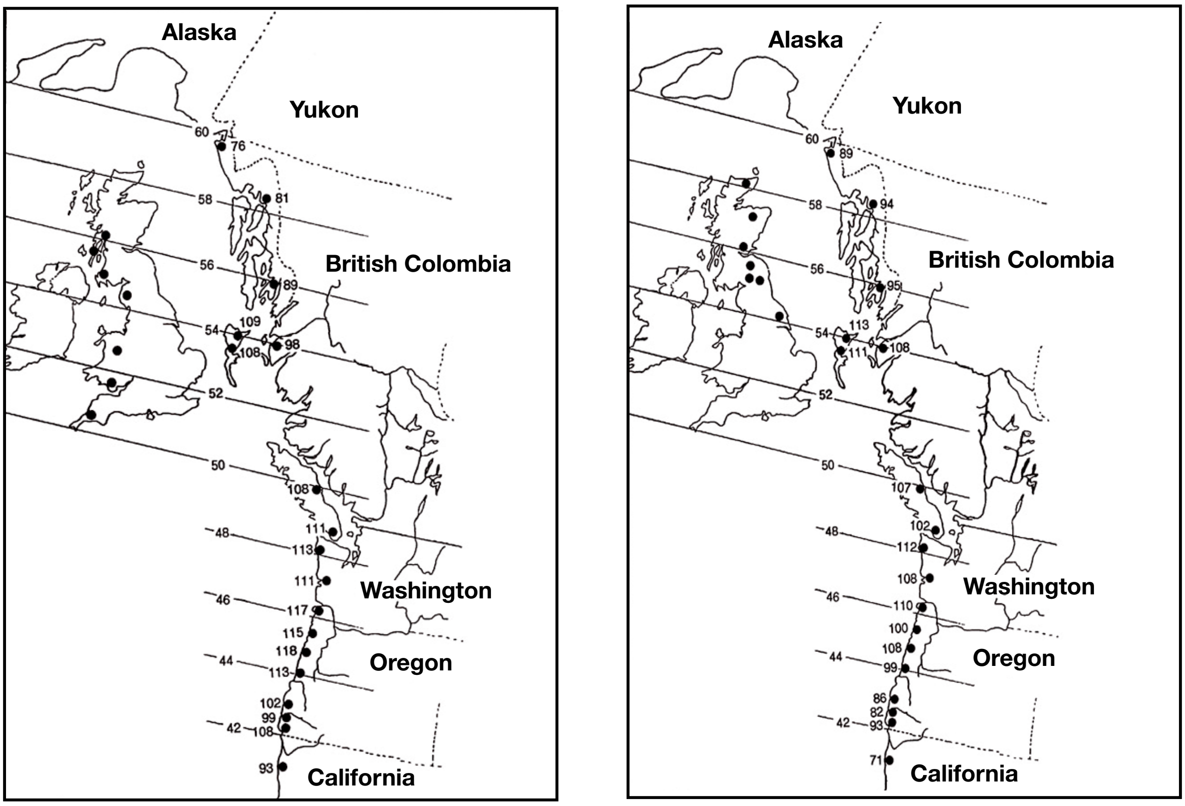

Figure 1. Comparison of mean height (expressed as a percentage of the overall mean of all origins at all sites) at 10 years of Sitka spruce, (*Picea sitchensis*) sources planted at 'on site' (Figure 1a, left) and 'off site' (Figure 1b, right) locations in Great Britain. The British Isles are shown at their equivalent latitude adjacent to the Pacific north-west coast of North America. Points in Great Britain indicate the locations of test sites. Points in N. America indicate the locations of the seed origins and relative height at 10 years is printed next to each origin location point. Figure 1a (left) shows origin performance in 'on site' locations favourable to the growth of Sitka spruce. Figure 1b (right) shows performance in 'off site' locations in which conditions would be less favourable.

plantations derive from selected stands in Britain or from seed orchards. *Pinus nigra* subsp. *laricio* (Poir.) Maire from Corsica has been found to be the best adapted source of this species for dry sites with light soils in Great Britain, whilst some eastern European sources of *Picea abies* (Carpathian mountains in Romania) and *Larix decidua* (Sudetan region of Poland) have proved to have the most superior growth rates.

Further adaptation in first generation transferred from the Pacific north-west to Europe

Seed origin trials of a number of North American species have included some material collected in phenotypically superior first generation stands growing in the Great Britain. For most of these, British sources have grown faster than most of those imported directly from the natural range, indicating that thinning management has favoured the best adapted individuals. For example, results of the IUFRO *Abies grandis* trials, in which diameter at breast height (DBH) was measured 15 years after planting revealed that a UK source of Vancouver Island origin grew 15% faster and a Danish source of Washington origin grew 6% faster than their equivalent IUFRO origins.

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Breeding programmes of exotic conifers in Britain

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Introduction

Britain has had a large afforestation programme for almost 100 years but contains only one native conifer species, Scots pine (*Pinus sylvestris* L.). Consequently, there has been a high degree of interest in exotic conifers from the Pacific Northwest, Europe, and the Far East.

First generation breeding programmes have now been completed for Sitka spruce (*Picea sitchensis* (Bong) Carr.), hybrid larch (*Larix* x *eurolepsis* Henry) and Corsican pine (*Pinus nigra* subsp. *laricio* (Poir.) Maire). A breeding programme for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was started in the 1960s, then abandoned and then started again with injection of money from the European Union (EU) in the early 1990s. A large lodgepole pine (*Pinus contorta* Franco (Mirb)) programme involving the creation of inter-provenance hybrids between selected individuals from various provenances was abandoned in the mid-1980s due to perceived lack of demand for improved lodgepole pine planting stock. Today, it is anticipated that breeding effort into a second generation and perhaps beyond will be centred on Sitka spruce which remains commercially the most important conifer in Britain.

Methods and discussion

The objective in all cases is to breed timber suitable for use in the construction industry, thereby replacing, yet meeting the same strength standards, as slower grown imported material.

Rare phenotypes, known as plus trees, which are well above average performance for growth rate and stem form have been selected by breeders since the early 1960s. Initially, this selection was at a very high intensity of approximately 1 tree in 5000 (Fletcher and Faulkner 1972) but was relaxed following early progeny test results in the mid-1970s (Samuel and Johnstone 1979) which suggested such a high level was unnecessary. Over 3000 Sitka spruce plus trees were selected, while closer to 1000 plus trees were selected for most of the other species. However, the method of selecting parent trees to be pollinated with specific pollen from a contrasting provenance led to the selection of nearly 4000 lodgepole pine plus trees.

Sitka spruce

All selected phenotypes had to be thoroughly progeny tested to determine their breeding value. Reselection of parents was carried out based on the field performance of progeny for growth rate, stem form and branching, and wood density.

Tested clonal seed orchards involving the best parents were established at various locations but maturation was slow and flowering was infrequent. In a bid to increase the rate at which improved material reached the forest, a system of artificial pollination of female flowers from up to 20 different tested clones with a mixture of pollen from 20 other tested clones was developed. The resulting seed were harvested and mixed together to form 'family mixtures'. Seed from family mixtures are raised as stock plants from which up to 1000 cuttings per plant can be harvested before age-related phase-change leads to poor rooting success.

The vegetative propagation programme proved successful and popular with the industry. Around 10 million rooted cuttings are now produced annually (6 million in the state sector and 4 million in the private sector). Predicted gains are presented in Table 1.

A Sitka spruce breeding population now exists consisting of 240 reselected, tested parent trees. A series of full-sibling crosses were created in a random manner in the mid-1980s and these have now been assessed for the usual suite of traits. Some reselection of full-sib families has now taken place such that seed from full-sib families are being released to the market place (Lee 2001).

Hybrid larch

The objective with larch has been to create a hybrid between the European (*Larix decidua* Miller) and Japanese larch (*L. kaempferi* (Lambert) Carr.). The system employed was to select good quality

Table 1. Predicted gains from propagation programmes for Sitka spruce and hybrid larch in Britain[†]

Seed source	Species	Diameter	Stem form	Wood density
Seed orchards	Sitka spruce	15–20%	2–10%	-9%
	Hybrid larch	8%	–	–
Family mixtures	Sitka spruce	15–22%	5–15%	0%
	Hybrid larch	12–20%	10–20%	–

– = no data available

[†] For updated statistics, see:
http://www.forestresearch.gov.uk/website/forestresearch.nsf/ByUnique/INFD-6JUL3Z
http://www.forestresearch.gov.uk/website/forestresearch.nsf/ByUnique/INFD-6JVBZ5

phenotypes of each species and control crosses in a clone-bank with a polymix of the contrasting species. Parents were reselected based on their ability to produce good hybrid trees when crossed with the other species in general. Progress has been slow.

Production of hybrid seed in clonal seed orchards has proved problematic due to little overlap of flowering time between the two species. A more promising line of approach seems to be controlled pollination and vegetative propagation but this has proved more expensive than the Sitka spruce programme due to poorer rooting success and fewer cuttings per donor plant (Lee 2003). Predicted gains are presented in Table 1.

Lodgepole pine

This proved an expensive species to work with due to the strategy of creating inter-provenance hybrids. No perfect provenance combinations were found. In the meantime the numbers of lodgepole pine planted dropped dramatically in favour of pure Sitka spruce plantations or mixtures of Sitka spruce and Alaskan provenance of lodgepole pine to act as a self-thinning nurse species. Further work ceased following a review in the late 1980s.

Corsican pine and Douglas-fir

Both these species are considered secondary, although each can be locally very important in part of eastern England and north-east Scotland and the area of England close to the border with Wales and milder parts of Scotland. A breeding population of Corsican pine has now been composed and a few tested clonal seed orchards exist (Lee 2004). Progeny testing of Douglas-fir continues. No Douglas-fir seed orchards are productive in UK. The short-term plan is to source suitable seed from other Douglas-fir improvement programmes around the world, including the USA and France.

Future plans

Money is being invested in the development of clonal forestry—in the first instance for Sitka spruce, although there is the possibility that other species may follow. Clonal forestry would offer greater gains due to improved uniformity, especially if clones could be selected combining good wood properties with fine stem form and good growth rate.

Somatic embryogenesis is being developed as a tool to mass-produce selected clones. The vision is that field testing of clones would take place and tissue from the clones under test would be stored in liquid nitrogen.

A programme of marker-aided selection is also being developed for Sitka spruce. The objective here is to develop markers for the economic traits under selection. Removal of undesirable genotypes in the laboratory will improve overall selection intensity prior to clonal testing in the field.

Conclusions

Great Britain has had a progressive programme of breeding exotic conifers for over 40 years. The objective has been to improve the suitability of species for use in the construction industry. The main commercial species remains Sitka spruce. The breeding programme for this species is the most advanced and includes plans for marker-aided selection and clonal forestry employing somatic embryogenesis.

The work of breeding all conifer species in Britain is described more fully in Samuel *et al*. (2000).

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Developing a policy for long-term archiving of conifer breeding material

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Introduction

In order to allow future access to selected conifer breeding material—which may be required for future breeding programmes, pollination programmes and supply of scion material—it is vital that genotypes are archived in an organised and sustainable manner. Developing a policy for long-term archiving of this breeding material is therefore an essential part of this process. This paper gives a brief summary of how a policy has been developed within Forest Research in Great Britain.

Early policy

Conifer tree breeding in Britain started in 1948, and in the early 1950s attention was focussed on grafting techniques and the clonal archiving of conifer species. This work resulted in all selections being routinely archived as grafted ramets within clone banks up until the late 1970s. More than 30 clone banks have been established, varying considerably in size and duration, covering around 15 conifer species. Not all clones of a single species are necessarily archived at any one clone bank site; some are mixed between two to four locations. Some species had only one site established while others had clones repeated over several locations.

The early clonal archiving system was established with the following practical aims:

- All clones to be planted in sequential number order on site
- Six grafted ramets to be established per clone
- Plant spacing at eight metres between rows and two metres within rows
- An alignment of rows on an east–west axis
- Location of clone banks in three geographic areas conducive to flowering (south England, central Scotland and north-east Scotland).

During the 1980s many clone bank specimens grew to inaccessible heights, making pollination work difficult or impossible. A system of top pruning was developed in order to maintain the height of clones at around two to three metres, allowing pollination work to continue from the ground or low ladders. This led to clone banks requiring a heavy maintenance programme of top pruning, grass cutting and labelling.

Review of policy

Due to the heavy maintenance programme that clone bank sites required, a review of our archiving policy was held in the late 1990s (Cahalan and Tobutt 1998). The recommendations of this review were:

- Only the six major conifer species in British forestry should be considered for archiving:
    - Sitka spruce (*Picea sitchensis* (Bong.) Carr),
    - Scots pine (*Pinus sylvestris* L.),
    - Corsican pine (*Pinus nigra* subsp. *laricio* (Poir.) Maire),
    - European, Japanese and Dunkeld hybrid larch (*Larix decidua* Mill., L. *kaempferi* (Lamb.) Carr. and L. x *eurolepis* Henry),
    - Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco),
    - Lodgepole pine (*Pinus contorta* Douglas).
- Archiving material should be planted at two geographically separate sites for each species.
- For each species, both breeding population clones (i.e. clones used within the current breeding programme) and conservation/archive clones (clones that were felt to be of some possible future use for either species conservation or as yet undefined breeding programmes) would be established.

- Clone banks would be established with fewer ramets per clone—two to four ramets per site for breeding clones and two ramets per site for conservation/archive clones.
- A low management regime would be applied to clone banks; the objective would be to manage a reserve for scion material, not for seed production. Therefore, top pruning would no longer be applied as a standard management regime.
- Re-grafting of clones and re-establishment of clone banks would be carried out on a 25–30 year basis.

Table 1 shows the numbers of clones currently held in clone banks compared with the proposed numbers under the new system following the archive policy review for the six main conifer species used in British forestry.

Table 1. Current number of clone bank sites and clones for the six main conifer species in British forestry compared with future site and clone numbers following archive policy review†

	No. sites		No. clones	
Species	Current	Future	Current	Future
Sitka spruce, *Picea sitchensis* (Bong.) Carr.	5	2	1455	1000
Scots pine, *Pinus sylvestris* L.	4	2	967	500
Lodgepole pine, *Pinus contorta* Douglas	1	2	499	200
Corsican pine, *Pinus nigra* subsp. *laricio* (Poir.) Maire	4	2	243	250
Larch, *Larix Mill*. spp.	11	2	709	400
Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco	3	2	248	150

† Current total area of clone banks = 23.2 ha; future total area of clone banks = 11.8 ha

Future seed production

Pollination work in the future will not be based within our clone bank sites; instead it will be based on potted grafts, up to eight years old, within high facility polyhouses (providing frost protection and automated irrigation etc.) using an intensive flower induction regime. Using this system new clones that are required as part of any pollination programme must be planned for, as inevitably there will be a delay period as the new clone has to be established as a potted graft before any pollination work can be carried out.

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Further reading

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Introduced forest tree species: some genetic and ecological consequences

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Introduction

Introduction of exotic resources is (and even more significantly, was) a wide-spread practice in almost every European country with a forestry tradition. It is especially so for the host country of this meeting, Great Britain, where introduced conifers play a predominant role in forestry (e.g. Samuel *et al.* this volume). The examples I will use in this paper, however, will be mostly drawn from French ecosystems and forests. They should be considered as models or case studies of a general European situation. I will very often use data from studies performed on *Cedrus atlantica* Carr. at INRA Avignon, France.

An introduced resource can be defined as a resource voluntarily or involuntarily brought by humans into an area where it was not present before. The introduction of forest trees is often the result of a voluntary germplasm (seed, cutting, graft) transfer. A forest resource can be considered introduced at several taxonomic levels—the most common levels considered are species and subspecies. The species *Pseudotsuga menziesii* (Mirb.) Franco was introduced to Europe from North America, the subspecies *Pinus nigra* subsp. *laricio* var. *corsicana* Hyl. was introduced to continental Europe from Corsica. The notion can be extended to further taxonomic levels. Plant material that comes from very different regions of provenance, new improved varieties or any plant material that presents identifiable genetic differences with the native populations of the same species, would qualify as introduced resources.

The concept of introduction should also be approached with space and time perspectives. Within a single country or region, resources can be both introduced and native. In France for example, *Larix decidua* Mill. is native in many mountain ecosystems, although its lowland forests are made of progeny introduced from the Sudeten and Poland. *Pinus pinaster* Aiton is introduced in the inland part of the Landes region (19th century plantations), and native to parts of its coastal dunes. As for time, the further we are from the actual introduction, the more we tend to consider a resource as native. At the scale of the last 15 000 years, almost no forest tree is native to its current distribution area in Europe. At human scale, it seems that a few human generations are sufficient to accept an exotic species as part of the natural landscape, and consider it as native. The acceptance threshold might be when we have no more direct contact with, or no immediate memory of, the people who were responsible for the introduction. There is evidence that *Cupressus sempervirens* L. and *Pinus pinea* L. were introduced by the Romans in south-western Mediterranean Europe (Thirgood 1981), where they are definitely considered as native today. *Cedrus atlantica* was introduced into southern France during the second half of the 19th century. It is now often considered as a natural part of the landscape by city people who enjoy walks under its now closed-canopy forests, although at the same time it is considered as invasive by local natural land managers.

Thus, most conifer resources should be considered as introduced exotics in Europe. Seeds of forests species, such as *Larix decidua, Picea abies* Karst., *Pinus nigra* Arn., *P. sylvestris* L. and *P. uncinata* Ramond ex DC are known to have been moved over thousands of kilometres in huge quantities across the whole of Europe at the end of the 19th century and during the 20th century (see Bartoli and Demesure-Musch (2003) for France). In the following discussion, I will mostly consider introduced forest trees at species and subspecies levels. I will focus on the most recent and massive introductions that occurred during the 19th and 20th centuries.

Importance of introduced conifers in Europe and France

In Europe, only 27% of forests are considered undisturbed by humans and more than 30% of forests are regenerated by planting or seeding. Conifer forests represent 42% of all forests, and mixed forests 40% (MCPFE 2003). The potential for introducing conifers is thus quite high.

In France, introduced conifer species cover more than a third of all conifer forest surface (estimated to be 5 million ha, including pure stands, mixed conifer dominated stands and conifer dominated woodlands) and account for half the annual wood production (Table 1). Introduced conifers are thus economically valuable as they are significantly more productive than native conifers.

Table 1. Introduced conifer species in France: surface area covered and wood production

Taxon	Species status	Surface area covered (ha)	Total production (m^3 per year)
Abies grandis Lindl.	Exotic	26 000	522 800
Abies nordmanniana (Steven) Spach	Exotic	10 000	53 600
Cedrus atlantica Carr.	Exotic	20 000	34 800
Larix japonica Carr.	Exotic	13 000	176 000
Picea sitkensis (Bong.) Carr.	Exotic	50 000	726 000
Pinus nigra subsp. *nigricans* Host.†	Exotic	192 000	1 200 000
Pinus nigra subsp. *laricio* Maire † (continental France)	Exotic	151 000	1 200 000
Pinus pinaster Aiton ‡	Native	~ 1 000 000	12 500 000
Pseudotsuga menziesii (Mirb.) Franco	Exotic	340 000	4 250 000
Totals (introduced conifers)		1 802 000	20 663 200
Totals (all conifers)		5 000 000	42 000 000

Source: Inventaire Forestier National (http://www.ifn.fr)

† P. nigra subsp. salzmannii (Dunal) Franco is the only native black pine in continental France

‡ Total (introduced and local provenance) Pinus pinaster forest surface area is 1 360 000 ha in France

Consequences of introductions for native forest ecosystems

Exotic forest resources have often been introduced for ecosystem rehabilitation when authorities in charge of forest management considered an area degraded, i.e. depleted of forests (for example after fire or over-grazing). This was particularly the case in the 19th century in many countries where introductions were part of massive reforestation programs for watershed management and erosion control after severe floods caused serious damage. The Mountain Rehabilitation Programme (French acronym RTM) which started during the second half of the 19th century in France is a good example (Bartoli and Demesure-Musch 2003). Exotic tree species were used because they were often proven to be better colonizers then autochthonous species in localized arboretum-like experiments. In France, *Pinus nigra* subsp. *nigricans* var. *austriaca* Loud. demonstrated better survival and growth than several other tree species in eroded Alpine regions and after several waves of plantations, now covers extensive areas (Table 1). These reforestation programmes continued well into the first half of the 20th century (Bartoli and Demesure-Musch 2003). Later reforestation programmes were based on more sophisticated scientific background, such as national and international networks of common garden species and provenance comparisons. More recently, exotic forest tree species have been used to reclaim forest areas destroyed by intense industrial air pollution in Central Europe (Küssner and Mosandl 2002).

Other cases of introduction might be linked to religious, agronomical value or a combination of several interests. For example, *Cupressus sempervirens* can be found near churches in the Mediterranean basin or as wind-breaks in southern France. *Pinus pinea* was introduced in the Mediterranean basin for its edible seeds, its value for landscaping, timber and resin production (Prada *et al.* 1997) and used as a landmark for Protestants in France during periods of religious persecution.

Two main types of impacts can be expected to occur because of the introduction of forest tree species: ecological and genetic.

Ecological consequences of exotic conifer introductions

Effects on soil and ecosystem functioning

Because introduced conifers are generally strong competitors for all environmental resources, their plantation can lead to colonization and invasion (*sensu* Richardson *et al.* 2000) of native ecosystems. Exotic conifers that become invasive can seriously affect water flow in rivers, as demonstrated by exotic pines in South Africa (Richardson and Higgins 1998).

Conifers are also notorious for decreasing litter and soil pH; for example, see Scholes and Nowicki (1998). This can alter carbon, nutrient and water cycles and soil biodiversity. Effects on biogeochemical cycles can be especially strong for those species that are planted or colonize acidic or neutral soils, e.g. *Pinus nigra* (Guende 1978). On calcareous soils, however, introduced conifers have demonstrated a positive influence for ecosystem functioning. In mountains of the Provence region of France, for example, recolonization by autochthonous species is facilitated by introduced pines (Guende 1978). These artificial ecosystems are now recolonized by native species and very strong natural recruitment dynamics can be observed (Figure 1).

Figure 1. Introduced species can facilitate recolonization by native species. Recolonization by *Fagus sylvatica* under a 19th century ecological restoration *Pinus uncinata* forest, Mont Ventoux, France. Photo: INRA Avignon.

Effects on landscape and biodiversity

Exotic conifer colonization and invasion can lead to the fragmentation, decrease in size and destruction of native ecosystems. In South Africa, invasive exotic pines have displaced native species and invaded conservation areas (Richardson and Higgins 1998). Although examples of high impact conifer invasions in Europe are as yet lacking, local cases may be found. For example, *Cedrus atlantica* is now rapidly invading native *Quercus pubescens* Willd. coppices in southern France where it was planted in relatively low numbers in the late 19th century. In Mediterranean countries, one of the main outcomes of forest colonization (and invasion) is the shift from open lands to closed canopy communities (e.g. *Pinus halepensis* Mill. or *Abies alba* Mill. (Figure 2) ‘invading’ abandoned cultivated or pasture lands in the northern Mediterranean). In addition to homogenizing natural landscapes towards continuous forest cover (which has a definite negative psychological dimension), this process reduces the very rich biodiversity open communities contain (Blondel and Aronson 1999). Conversely, introducing forest tree species in well-confined agricultural-like plantations can help reduce the economic pressure on native forest ecosystems and conserve biodiversity. In New Zealand for example, 99% of harvested wood comes from exotic plantation forests (of which 91% is *Pinus radiata* D. Don) that represent 21% of all forest cover (data from New Zealand’s official statistics agency).

Figure 2. *Abies alba* colonizing pasture lands using juniper bushes as facilitators in the Lure mountains, southern France.

Introduction of exotic tree species can lead to pest invasions, as introduced pests can sometimes shift their habits. Seed insects are notorious for spreading from their introduced host to native populations of a phylogenetically related potential host species. For example, *Megastigmus rafni* Hoffmeyer—a seed insect found on several fir species in North America—was identified in France in 1990 on the native *Abies alba* (Roques and Skrzypczynska 2003).

Finally, introductions can become health problems to humans. Millions of the Mediterranean cypress, *Cupressus sempervirens* were planted as wind breaks during the 19th century and for landscaping during the 20th century in Provence, France (Fady 2000). Cypress is a very efficient producer of very small highly allergenic pollen grains. After more than one century of contact, approximately 10% of human populations in that region suffer from severe pollen allergy (Charpin 2000).

Genetic consequences of exotic conifer introductions

Two main mechanisms can impact the genetic structure of native populations confronted with introduced resources: 1) fragmentation of native ecosystems and 2) gene flow and hybridization.

Fragmentation of native ecosystems

Forest resources introduced in high numbers and over large areas can lead to fragmentation in native ecosystems. Fragmentation increases geographical subdivision and imposes barriers to gene flow among populations. If native populations are still abundant and cover extensive areas, fragmentation will lead to independent local adaptations and geographical structuring with possible beneficial consequences in the long run. However, fragmentation may promote local extinctions either because of strong genetic drift followed by consanguineous mating or because of an increase in ecological pressure linked to the edge effect. These problems may arise when native resources are already significantly depleted. An example of this fragmentation process is well-illustrated in France by the spatial structure of native populations of *Pinus nigra* subsp. *salzmannii* (Dunal) Franco. Unlike in Spain, this black pine occurs in the Cévennes and Pyrenees in small populations localized on poor dry soils (Quézel and Médail 2003), possibly the remnants of much larger forests cut down for timber and to make way for agriculture and grazing lands. These forests are surrounded and fragmented by very large *Pinus nigra* subsp. *nigricans* and *P. nigra* subsp. *laricio* plantation forests.

Gene flow and hybridization

Introduced resources may also exchange genes with native ecosystems. Within a species, all subspecies intermix and varieties created from a breeding programme will be capable of fecundating wild-type populations. Further, reproductive isolation is often not achieved within wind-pollinated conifer genera. What we call species are often species complexes, within which extensive gene flow can occur under experimental conditions or when geographic barriers are removed. For example, Euro-Mediterranean *Abies* Mill. species intermix (Kormutak 1985) and so do Mediterranean *Cedrus* Duham. species (Fady *et al*. 2003). Genes might thus be easily passed from introduced resources to native populations of the same species and genus. If introduced resources are very numerous and/or possess genes that can quickly invade a gene pool, impacts are potentially strong and deleterious for local native resources (Lefèvre 2004). Genes with strong fitness will be passed along generations and create a new resource in replacement of the native one. The new resource could be better adapted than the replaced one if the genes passed along concern general adaptation such as better plasticity, increased tolerance to pests, frosts, drought, etc. However, if only genes linked to a very specialized and/or local adaptation are passed along and if the genetic background of the introduced resource is not polymorphic (e.g. clones), the advantage could be short-lived and the replacement of the old resource deleterious for the ecosystem—a consideration we should keep in mind in the era of genetically modified trees. Again, *Pinus nigra* subsp. *salzmannii* populations in France are a good example of this phenomenon. Not only do the very large *P. nigra* subsp. *nigra* and *P. nigra* subsp. *laricio* plantation forests reduce gene flow between its populations, but they can also be significant gene 'polluters' for this native resource, as all black pine subspecies have been shown to readily hybridize experimentally (Vidakovic 1974). *P. nigra* subsp. *salzmannii* is thus doubly threatened: by fragmentation and by exotic gene flow. This taxon is one of the few conifers recognized as constituting a high priority habitat under the EC Habitats Directive (Council Directive 92/43/EEC).

In conclusion, it is evident that introduction of forest resources can have a very significant impact on native resources and must be carefully monitored. The greater the number of the introduced resource there is, the stronger the potential effect on native ecosystems will be.

Changes expected in introduced resources

After successfully passing through the different barriers of adaptation to their new environment, new introduced genetic resources can develop into stands of various sizes and shapes (e.g. *Cupressus sempervirens* wind-breaks, *Cedrus atlantica* patchy naturalized populations and *Pseudotsuga menziesii* large planted populations). They can also occupy many kinds of ecosystems (low to high elevations, open to closed landscapes and water-deficient to humid bioclimates). They often derive from limited sample sizes (e.g. *Cedrus atlantica* in France) but can also originate from large and/or multiple introductions (e.g. *Pseudotsuga menziesii* in Europe). All these different situations will have different consequences for the genetic and ecological structure of the introduced resource.

Changes expected in introduced resources from a genetic point of view

Introduced resources are characterized by two main genetic features: 1) they are isolated from their original population and can no longer exchange genes with it and 2) they are in contact with new populations with which they can possibly exchange genes. This leads to one major genetic event for the introduced resource: rapid evolution.

Ecological conditions are often different in the new environment from that of the original environment. Population structure of the introduced population may also be quite different from that of the forest(s) where it originates. The introduced population can either come from a very limited number of original seed trees or from seeds collected on trees belonging to several different populations. Mechanisms such as genetic drift, genotypic recombination, selection and variation in the mating system, can be expected to play a strong role in the genetic make-up of the new introduced resource. The impact of mutations requires longer time scales and much larger sample sizes to be really effective; therefore, they are not generally considered as a major factor affecting the genetic make-up of these populations. However, it should be noted that mutations may have a significant effect in extreme environments such as those suffering from industrial pollution.

At this point, I will further describe the Mediterranean *Cedrus* species complex, which I have already used as model to illustrate several points on the impact of introduction on native resources. This species complex regroups three taxonomical units which colonize mountains at elevations between 800 and

2000 m: *Cedrus libani* A. Rich. in the Middle East, *C. brevifolia* Henry in Cyprus and *C. atlantica* in North Africa (Figure 3a). Although geographically distant, all Mediterranean *Cedrus* species are phylogenetically related (Figure 3b) and can exchange genes in plantation forests (Fady *et al*. 2003). *C. atlantica* from Algeria was used for reforestation in southern France ca. 1860 where it is now covering over 20 000 ha (Table 1).

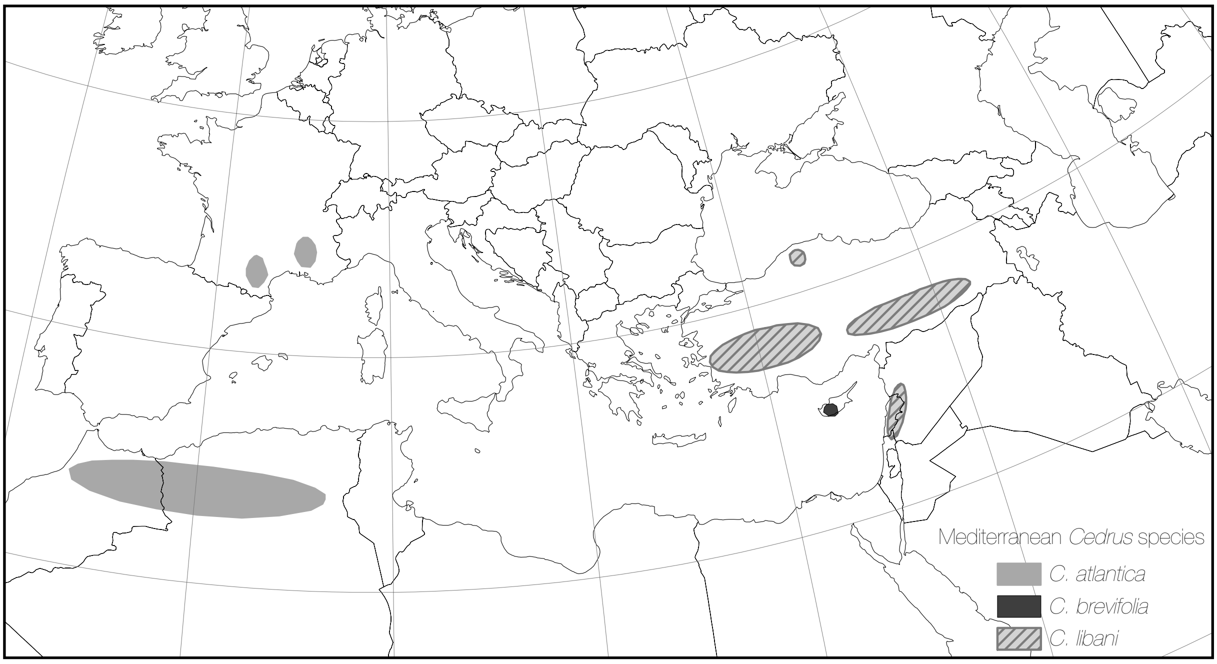

Figure 3a. The phylogenetically related and geographically separated Mediterranean *Cedrus* species: a model to study genetic and ecological consequences on introduced species. *Cedrus atlantica* is shown in solid grey (and is not native to France), *C. brevifolia* is shown in black and *C. libani* in shaded grey.

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● *Cedrus atlantica*
■ *Cedrus libani* from Turkey
□ *Cedrus libani* from Lebanon
✸ *Cedrus brevifolia*

Figure 3b: Isozyme analysis of phylogenetic relationships among Mediterranean *Cedrus* taxa (Fady, unpublished data). Branch length in the dendrogram increases with genetic distance.

Genetic drift

When introduced resources are small isolated populations, or populations with very few reproducing adults, genetic drift can be expected, leading to a modification of the genetic make-up of the population. One outcome to expect in case of demographic bottlenecks is the random loss of alleles and reproduction among few trees, leading to the rise of a consanguineous, excessively homozygous first generation population. In wind-pollinated tree species, studies indicate that such extreme bottlenecks only occur when populations are founded by one or very few seed trees, e.g. Sagnard (2001) for *Abies alba* and Ledig and Conkle (1983) for *Pinus torreyana* Parry ex Carr. (but see Ledig *et al.* (1999) for a counter example with *P. maximartinezii* Rzed.). Genetic drift is thus to be expected when arboreta or *ex situ* collections are the starting point of a new forest stand, either through natural regeneration or plantation. It might also be expected when forest management removes significant numbers of first generation adult trees in an introduced stand. Irregular flowering may increase the effect of drift by promoting reproduction among a limited subset of adult trees (Krouchi *et al.* 2003). Drift can be one of the reasons why introduced resources diverge genetically very quickly from their parent populations. This was postulated for some of the French cedar forests which have a reduced diversity compared to their parent populations in Algeria (Figure 4).

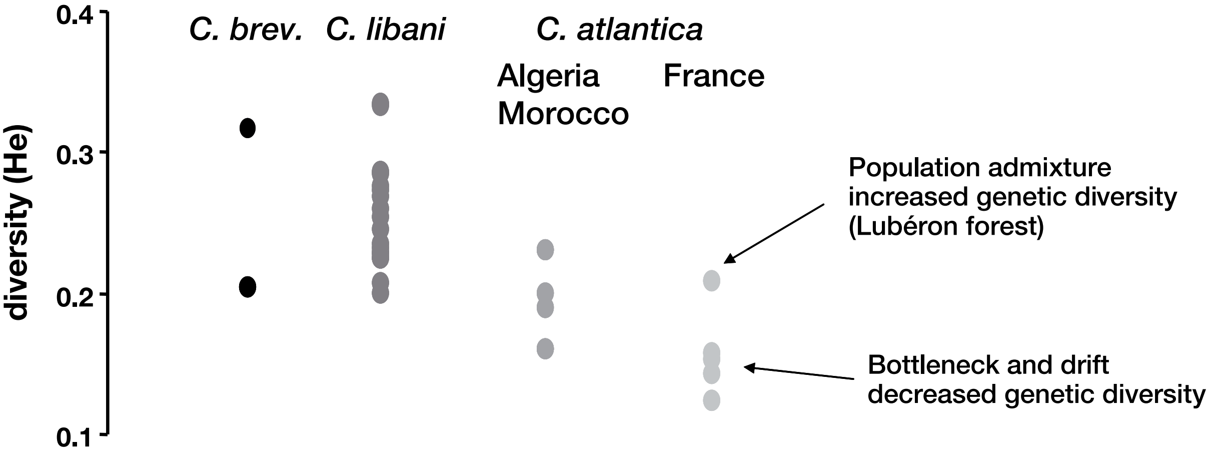

Figure 4. Effect of population admixture versus drift on the genetic diversity of *Cedrus atlantica* populations introduced in France. Data source: Fallour (1998).

Gene pool recombination (admixture)

When introductions are made from larger gene pools, and using large sample sizes, genetic drift is less likely to happen. In these populations, disappearance of the neighbourhood structures of the original populations might be expected. Unrelated trees will mate in the introduced stand, which will reduce the genetic load linked to consanguinity. Introductions can also be made from several seed sources and/or over several introduction waves; in these circumstances some level of heterosis or admixture effect can be expected. The Lubéron *Cedrus atlantica* forest in France provides an example of this phenomenon (Figure 4). Its increased diversity compared to its Algerian parent populations was explained as a result of admixture (Lefèvre *et al.* 2004).

The spatial structure of reproducing trees is of importance as it will affect their mating system (how a progeny is created) and, consequently, spatial genetic structure of the new generation. This effect will be stronger when few reproducing adult trees are contributing to the next generation.

Figure 5 illustrates this effect using a simulated tree population (Sagnard 2001). When the seed trees are randomly or regularly arranged, the seedlings they produce will show a significant spatial family structure, i.e. related seedlings will tend to be closer to each other than expected by chance alone. Such a structure will not be observed if seed trees are clumped together, as the stand will behave as one gene pool and disperse its seeds randomly in all available favourable microsites.

Such spatial genetic structures can be created when the introduction is made from a small gene pool, when strong selection or forest management has left very few trees, or when only a subset of the adult trees participate in the mating. This latter reason may explain why neighbourhood structures are found in native stands. Consequences are increased consanguineous mating and the development of a new generation of trees that might be less fit than their parents.

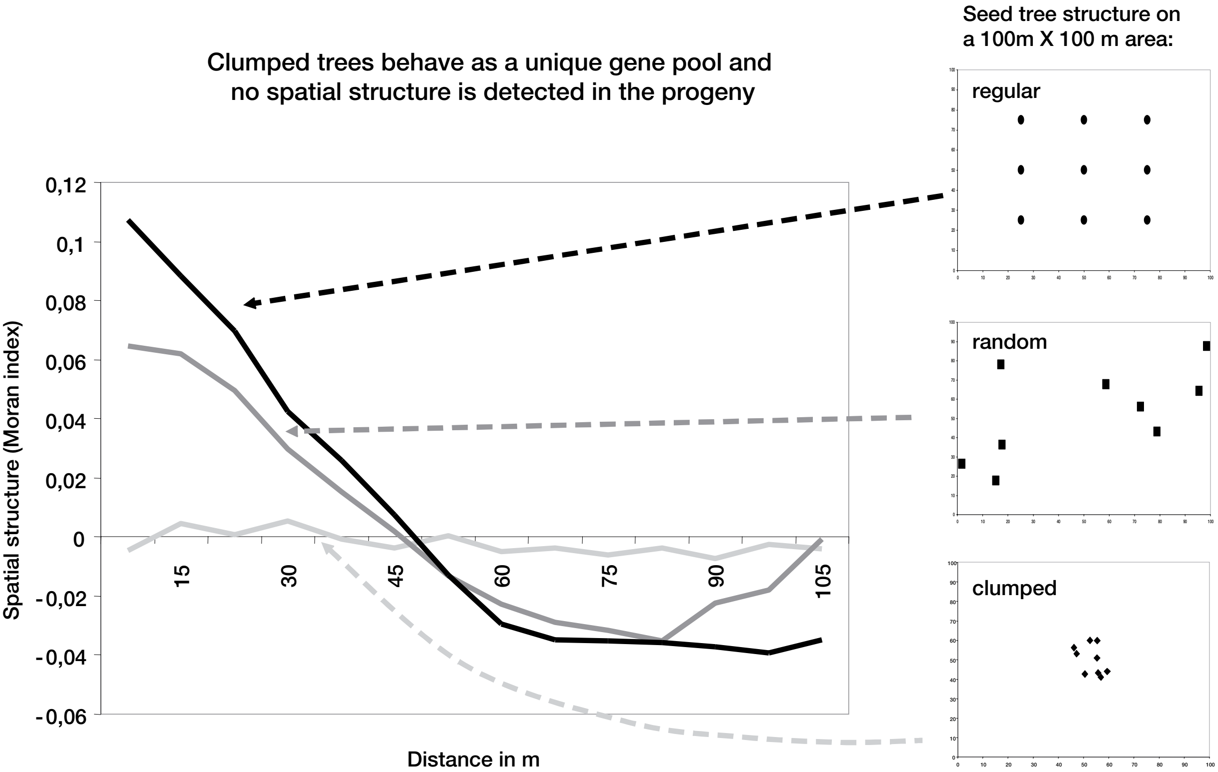

Figure 5. Effect of seed tree structure on the seedlings they produce: a simulation using a single bi-allelic locus. A positive Moran index indicates that genetically identical seedlings tend to be spatially grouped at certain distances from seed trees.

Conversely, disruption of consanguineous mating can explain why the progeny of introduced resources perform better than that of their original parent populations. Following plantation in the 1860s, very few introduced *Cedrus atlantica* trees survived. A reduction of consanguinity (mating among unrelated first generation survivors) was found to be one of the likely hypotheses to explain why the progeny of introduced stands perform significantly better than that of native North African populations in common garden experiments in France (Figure 6).

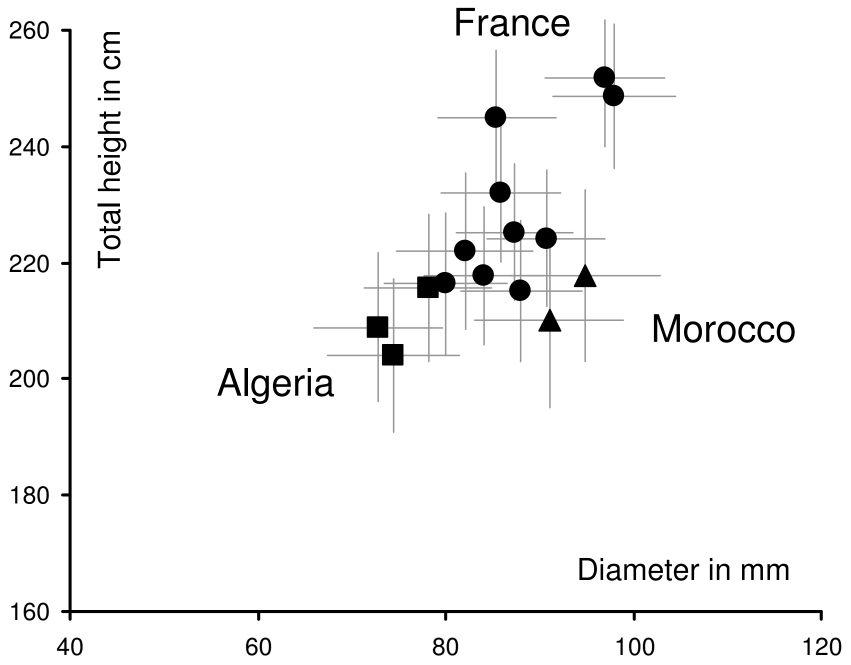

Figure 6. Possible effect of disruption of consanguineous mating on the genetic diversity of introduced populations: French *Cedrus atlantica* populations grow better in their new environment than their natural counterparts (Lefèvre, unpublished data).

Selection

When new environmental conditions differ from the original ones, the introduced resource will undergo a selection process to adapt to these new conditions. If the new environment is too different from the original one, the introduced trees will not survive. In France for example, 30 000 ha of north-western Iberian *P. pinaster* provenances introduced decades earlier in the Landes region were destroyed by the 1984–1985 winter frosts (Riou-Nivert 2002). Arboreta and common garden experiments are designed precisely to avoid introducing ill-adapted genotypes, as they test such adaptive traits as frost, water and pest resistance over decades and several environmental conditions before a resource is introduced. Although expensive to install and maintain, they provide precious safeguards over inappropriate introductions and constitute networks of high biodiversity value that should be under high priority conservation.

When adaptation is possible, divergence from the original population will be achieved faster with increasing selection pressure. A rapid adaptation and differentiation process, sometimes over a single generation, has often been observed for introduced resources. For example, first generation progeny of *Picea abies* introduced into Norway from Germany had a bud set date identical to the native local Norwegian resources, but significantly different from their original German parent populations (Skrøppa and Kohmann 1997). Selection is also a likely hypothesis for the better growth performance of the progeny of introduced *Cedrus atlantica* over that of the original populations from Algeria in southern France (Figure 6).

Hybridization

Just as introduced resources can hybridize with autochthonous and locally adapted species, native species can hybridize and modify introduced resources. Reproductive barriers are often weak between taxonomic units within genera because of insufficient elapsed time since geographical or ecological separation and because the mating system seems not to be the primary target of genetic changes that occur through evolution (Avise 1994). When introduced resources constitute small populations, the impact of hybridization can be strong. For example, hybridization has been shown to be very common when *C. libani* and *C. atlantica* are grown together—in some cases, up to 80% of the progeny was made of hybrid seeds (Fady *et al*. 2003). Acting in a similar, although more radical way, as admixture, hybridization can significantly contribute to the rapid emergence of a new resource. For example, the hybrid progeny of *C. atlantica* demonstrated increased resistance to aphids in experimental conditions, a trait transmitted from *C. libani* (Fabre and Chalon 2005), and is of significant interest in southern France.

Changes expected in introduced resources from an ecological point of view

Once planted and successfully adapted to its new environment, the introduced resource can create, or become part of, a new ecological community. Its size and spatial structure will determine whether or not it will be able to successfully reproduce and whether or not it will be colonized by members of the surrounding communities. This in turn will determine its biodiversity value in the long run.

Because they are usually introduced without their native co-adapted community of insect predators and parasites, exotic forest stands are particularly susceptible to insects, both the local ones that can modify their feeding habits and their original ones when accidentally introduced. Strong insect damage might occur several decades after introduction because resistance to insect predation will not be a primary determinant to adaptation at the time of introduction. It may take time for the local insect community to adapt to this newly available food supply, or for the original insect community to be introduced and adapt to its new environment. For example, in France, the introduced seed insect *Megastigmus spermotrophus* Wachtl has only recently started to significantly decrease seed set in Douglas-fir although it may have been introduced as early as a century ago (Rappaport and Roques 1991). A counter example is provided by *M. schimitscheki* Novitzky which was only discovered in 1994 and is already significantly reducing seed set in cedar forests in southern France (Fabre *et al*. 2004). As an example of insect adaptation to new resources, the pine processionary caterpillar, *Thaumetopoea pityocampa* Schiff., has adapted to numerous introduced conifers in France, and principally to the abundantly available *Pinus nigra* (Démolin 1969).

Native ecosystems, where phylogenetically related insect species exist along with their insect predator and parasite community, can buffer insect outbreaks in introduced forests (Roques 1983). For example, the native *Megastigmus suspectus* Borr. community found in *Abies alba* forests in France could provide such beneficial ecological buffer for the introduced *Abies* and *Cedrus* stands growing in their vicinity. When native ecosystems cannot be used for controlling insect outbreaks, collecting natural parasites and predators from the natural distribution area can be efficient. For example, the hymenoptera *Pauesia cedrobii* Starý and Leclant, collected in North Africa, significantly reduced the impact of the aphid *Cedrobium laportei* Remaudière on the French *Cedrus atlantica* forests (Fabre and Rabasse 1987). This aphid had been introduced accidentally from North Africa.

Introduced conifers remain ecologically vulnerable for a significant length of time after their first introduction, as the construction of a fully functional forest ecosystem takes at least several tree generations.

Conservation and sustainable management of introduced conifers

Introduced resources evolve very quickly. Over just one generation, they can constitute a gene pool that is quite different from their original seed stand(s) and after a few generations, they can constitute an ecologically sustainable resource. Sustainability could be considered to be effective once the second generation after foundation is in place and reproducing. Depending on the species, we can estimate the time frame for a sustainable conifer landrace to occur to be approximately 100–150 years after initial introduction.

Once ecologically and genetically sustainable, the introduced resource should be recognized (nationally and internationally) as a new gene pool—a landrace. An introduced resource will have different conservation values for the species, depending on whether it is threatened or not.

When the species is recognized as threatened in its native distribution area, the introduced resource can be used as potential seed source for ecosystem restoration. This will only be possible if interspecific gene flow does not occur, if the effective number of reproducing trees is high enough to avoid genetic drift and if ecological conditions (both biotic and abiotic) are not too different from those of the natural area. Thus, arboretum-type, garden-like or admixture-origin plantations should be avoided for seed collections, although large single-provenance *ex situ* conservation forests might be best suited.

If the species is not threatened in its original area, the ecosystems containing the introduced resource (which has now become a landrace) can be considered as an addendum to the distribution range of the species. An *in situ* approach to resource management will be best suited, in close collaboration with countries throughout the species' range. Forests chosen as part of the *in situ* conservation network should be naturally regenerated, or artificially regenerated using only local seed trees. Their reproducing population size should be large enough—optimally several thousands (Lande 1995). Populations originating from large seed collections and occupying large areas, possibly over several environmental conditions, could qualify for this *in situ* management. But forests originating from very limited gene pools or admixtures, or where interspecific gene flow occurs, could also qualify. In this case, population origin matters less than whether or not they constitute an original and sustainable genetic and ecological resource.

Conclusions

In conclusion, introducing exotic forest trees should be undertaken carefully. They should be tested over several environments and decades for adaptability in specifically designed common garden experiments. Gene flow to and from them should also be carefully monitored. Once introduced, forest management that aims to limit genetic family structures (i.e. consanguinity) and to accelerate the constitution of dynamic and functional ecosystems, should be adopted. Once introduction is successful, I suggest that introduced resources should definitively be included in gene conservation networks. Further, their genetic and ecological monitoring should provide helpful insights to understand the processes that govern evolution and speciation.

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Attempts in identifying the origin of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands in Germany

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Introduction

Douglas-fir is the most important introduced species in Germany. According to the forest inventory from 1986 to 1990, which included only the old Federal States, the area amounts to 119 520 ha (=1.6 %). However, the area varies considerably between the Federal States. Rhineland-Palatinate is the state richest in Douglas-fir; there, the species covers about 38 139 ha (=5.1%) of the forest area (Anonymous A.N., *loc. cit.* p. 48).

Douglas-fir has been planted in Germany for more than 120 years. Very successful plantations were established between 1881 and 1890. The exact origin has not been documented but it can be assumed that the collection area was the coastal region of Washington and/or northern Oregon. The nurseryman John Booth was notably very active in seed procurement. It is reported that the Prussian forest administration received 700 kg of Douglas-fir seed (Schwappach 1901). However, plantations established after 1891 frequently showed problems, and it was assumed that, at least partly, a different seed stock has been imported than in the decades before. In order to throw light into these problems the first provenance experiments were established in 1910 and 1912. Further provenance experiments followed in the ensuing decades (for a general view of German experiments see Schober, 1973). However, results from provenance experiments shall be mentioned here only as far as necessary to understand the reasons why several Federal States in Germany are at present interested in identifying the origin of their Douglas-fir stands. The provenance experiments showed that in most cases provenances from the coast range and western slopes of the Cascade Mountains have proved to be the best with regard to adaptation and quality, whereas those from the interior part of the distribution range frequently show high mortality and damage, indicating mal-adaptation.

For stands established between the two World Wars, seed stock of provenances both from the coastal and the interior race has been imported. These stands are now about 70 or 80 years old and could serve as seed collection stands. The imports continued after World War II, whereby a large amount came from interior sources, such as the Shushwap Lake region (British Columbia). According to a statistic of the Bavarian forest administration, about one third of Douglas-fir seed imported between 1965 and 1971 originated from interior provenances.

Damage symptoms became visible in the 1960s which frequently could not be associated with the impacts of damaging agents. In particular, the severe loss of needles was conspicuous. However, detailed observations revealed for example attack of the needles by the ascomycetous fungus *Rhabdocline pseudotsugae* H. Sydow (see Figure 1). Stephan (1973) described considerable differences between provenances in the susceptibility to this needle cast fungus in field experiments with ten to twelve year old trees. Whereas in 1972 not only the coastal, but also the northern interior provenances showed no infection, the situation changed in 1973–1974. In these years the degree of infection generally increased in the interior provenances; however, some of the northern ones still showed no or low infection.

Attacks by *Phaeocryptopus gaeumannii* (Rohde) Petrak, the second dangerous needle cast fungus of Douglas-fir, had not yet been detected in provenance field experiments between 1960 and 1980. Damages became more apparent from the 1980s onwards when stands had reached the second age class, i.e. they were between 20 and 40 years old. Symptoms in attacked stands are needle discolouration and yellowing, thin crowns as a result of needle cast, distorted growth characters, bark necrosis and resin flow.

Figure 1. Douglas-fir needles infected by *Rhabdocline pseudotsugae*. The light grey spots are naturally yellow to orange and in the final stage show a rust-like colour (Photo: B.R. Stephan)

As one possible reason to explain the damages the 'manganese toxicity theory' had been formulated (Schöne 1997), which concludes that a surplus of manganese in the soil contributes to an excess of manganese content in the needles, resulting in a deficiency in phosphorus and magnesium uptake. Indeed, trees showing high damage symptoms had higher manganese levels in the needles. However, this theory could not be verified in all cases. Fertilization experiments did not result in more damaged trees and sound stands or trees were also found on soils with a high manganese content. Thus, ensuing studies focused more on a genetic background, namely on the question of seed origin.

Stephan (1998) assessed the number of fungal fruit bodies of *Phaeocryptopus gaeumannii* on needles of 22-year-old trees in four consecutive years (1987 to 1990) on 31 provenances of the International Union of Forest Research Organizations (IUFRO) collection. The heaviest attack showed provenances from the interior part of British Columbia. Southern interior provenances were generally less attacked, and coastal provenances showed an intermediate degree of infection. Interestingly, the annual loss of needles did not correspond with the annual number of fruit bodies on the retained needles.

Silvicultural background

Several reasons have led to Germany's present interest in checking its stands and determining their probable region of origin:

- For about the past three decades, natural regeneration techniques have increasingly been applied in silviculture. As a consequence, less seed is needed, imports have decreased and the use of European Douglas-fir stands as seed source has gained importance.
- The majority of the original stands (old natural growth) in the Pacific Northwest, especially below 500 m a.s.l. has been cut. Therefore, the possibilities to collect in suitable stands are considerably reduced.
- The American tree seed certification scheme did and does not sufficiently fulfil European requirements and standards (Anonymous 1966, Fletcher *et al*. 1991). A national register comprising selected seed stands does not exist. For about 25 years, trade was carried out according to the OECD Scheme (OECD 1974) and only seed of the category 'source identified' was imported. However, Germany has experienced that sometimes interior provenances might have been declared falsely as coastal ones.

- Several older Douglas-fir stands in Germany might have been integrated into the national register of approved seed stands before damage became evident. However, in the meantime sanitary status may have changed making a check necessary.
- Finally, several older stands of proper origin have proved to be outstanding in provenance experiments (landrace development).

Forest administrations do not want further unsuitable material to be propagated. Additionally, many stands planted after World War II have now achieved an age of 40 or more years and can therefore be approved and registered as seed stands. Consequently, several Federal States of Germany, e.g. Rhineland-Palatinate, Northrhine-Westphalia, Thuringia and Hesse are investigating numerous Douglas-fir stands both with regard to their silvicultural suitability as well as to their affiliation either to the variety *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* (coastal race) or the variety *P. menziesii* var. *glauca* (Beiss.) Franco (interior race). This is done by using biochemical–genetic methods and by assessing phenotypic characters.

Isozyme studies

First results on range-wide patterns of allozyme variation in Douglas-fir have been published by Li and Adams (1989). They found different variation patterns for the coastal and the interior variety, and as well as for the northern and the southern part of the interior variety. In Austria and Germany, results from investigations on the variation of isozyme gene markers were published by Klumpp (1995) and Hoffmann and Geburek (1995). Due to the increasingly visible damage, comparative studies between damaged and healthy stands were also carried out in Germany (Leinemann 1996, 1998). The investigations are still going on in the Federal States of Rhineland-Palatinate (Leinemann and Maurer 1999), Northrine-Westphalia and Thuringia (Maurer *et al.* 2003; Schmitt *et al.* 2003).

In order to verify the differences between the coastal and the interior Douglas-fir races, eight provenances of each from the IUFRO collection were used as reference populations and analysed using isozyme techniques. The result was that at the gene locus 6-PGDH-A, the allele A3 occurs with high frequency in the coastal variety and low frequency in the interior variety (90% vs. 30%), whereas the reverse (4% vs. 60%) is valid for allele A6 (Figure 2) (Leinemann 1997). Variation ranges are not overlapping; therefore, the method is well suited to discriminate between the two varieties. Other isozyme loci, like ACO, also showed different allele frequencies but will not be further considered here.

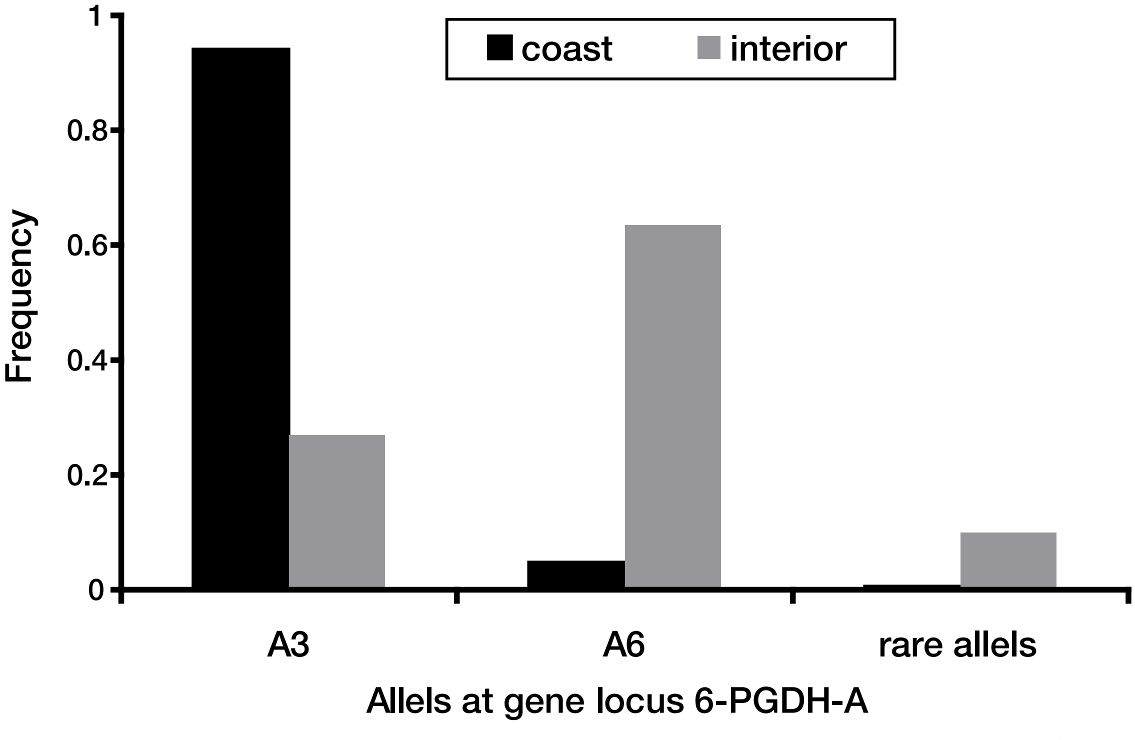

Figure 2. Mean frequencies of the alleles A3, A6 and rare alleles for the enzyme gene locus 6-PGDH-A for the coastal and interior race derived from 16 IUFRO provenances which served as reference populations (after Leinemann and Maurer 1999).

Two examples may illustrate the situation. The genetic profile of a stand with heavy damage symptoms (Dreis in Rhineland-Palatinate) shows high conformity to that of the reference profile of the interior variety (Figure 3). Consequently it can easily be associated with the interior race. However, in the past seed might have been mixed or some cultures with failed patches might have been completed by replanting with different provenances. Thus, a mixture of races might have resulted, as is assumed for the stand Mayen in Rhineland-Palatinate because it shows an intermediate profile. In this case the proportions belonging to the coastal and interior race, respectively, may be calculated by a formula developed by Bernstein (for details see Leinemann and Maurer, 1999). In the case of a mixed stand of good quality, Northrhine-Westphalia has decided to approve it as seed stand if at least 80 % of the trees belong to the coastal variety.

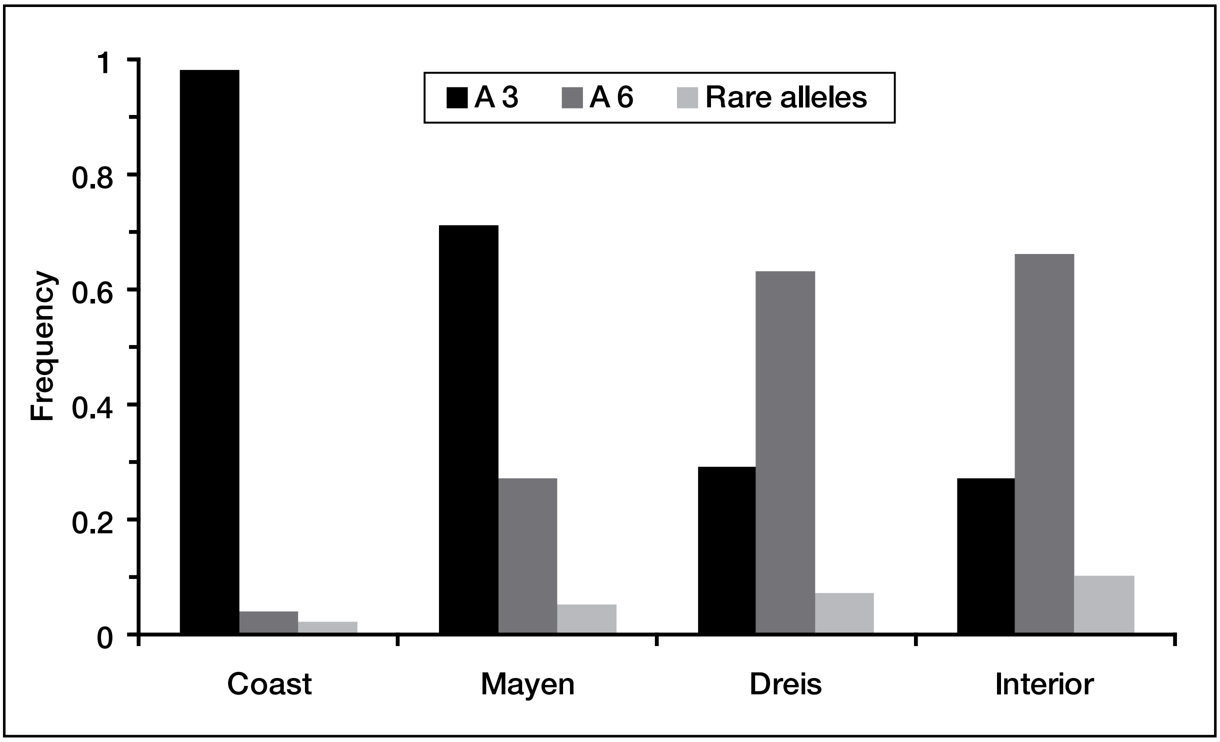

Figure 3. Genetic profiles of the coastal (left-hand columns) and interior race (right-hand columns) and two German Douglas-fir stands with regard to the 6-PGDH-A gene locus. The genetic structure of the ill stand Dreis conforms to that of the interior reference population. The genetic structure of the stand Mayen indicates a mixture of both races (after Leinemann and Maurer 1999).

Morphological characters for judging silvicultural suitability

The Federal State of Hesse based the valuation of silvicultural suitability of stands (for further propagation) on morphological traits. Rau (2002) described eight criteria which indicate mal-adaptation of a provenance:

- Needle discolouration
- Heavy needle cast (only one to two age groups on the branches)
- Heavy branchiness (extraordinary, many and notably thick branches in relation to stem diameter and growing space)
- Bad stem form (extraordinarily high proportion of trees with remarkable bends)
- Bark remarkably fissured and coarse, often grey and connected, with swellings and coarse branches
- Resin flow on the stem up into the crown which is not caused by artificial pruning
- Early and heavy fructification
- High losses after the establishment phase, even on sites well suited for Douglas-fir.

Figures 4–8 show examples of desired and undesired characters of Douglas-fir trees. Note that these examples cannot be considered in all cases to be representative for the provenance mentioned.

Figure 4. Douglas-fir stem with fine bark (IUFRO no. 1001, Stoner, BC) (Photo: E. Burchard).

Figure 5. Stem with undesired characters: bends, bumps and coarse, fissured bark (IUFRO no. 1031, Gold River, BC) (Photo: E. Burchard).

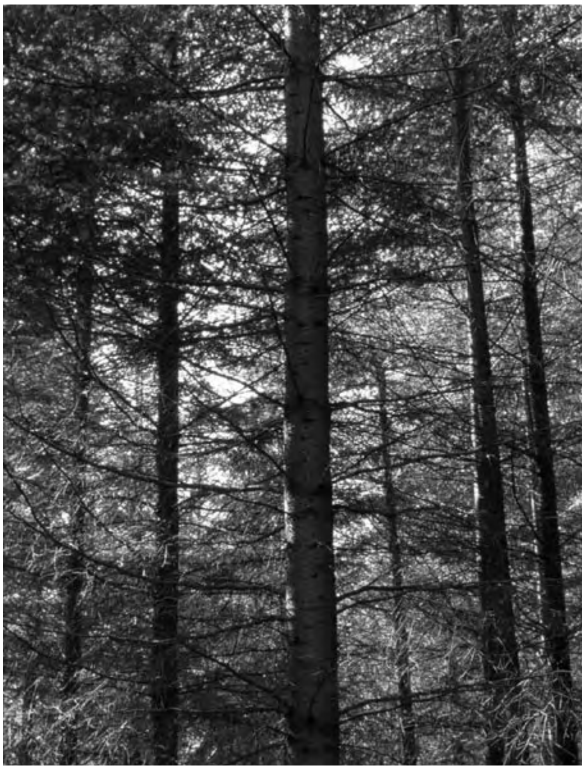

Figure 6. Douglas-fir originating from the Olympic Peninsula with fine branching in the forest district of Gahrenberg (Photo: Hessen-Forst, Forsteinrichtung, Information, Versuchswesen).

Figure 7. Heavy branching (IUFRO no. 1047, Concrete, WA) (Photo: E. Burchard).

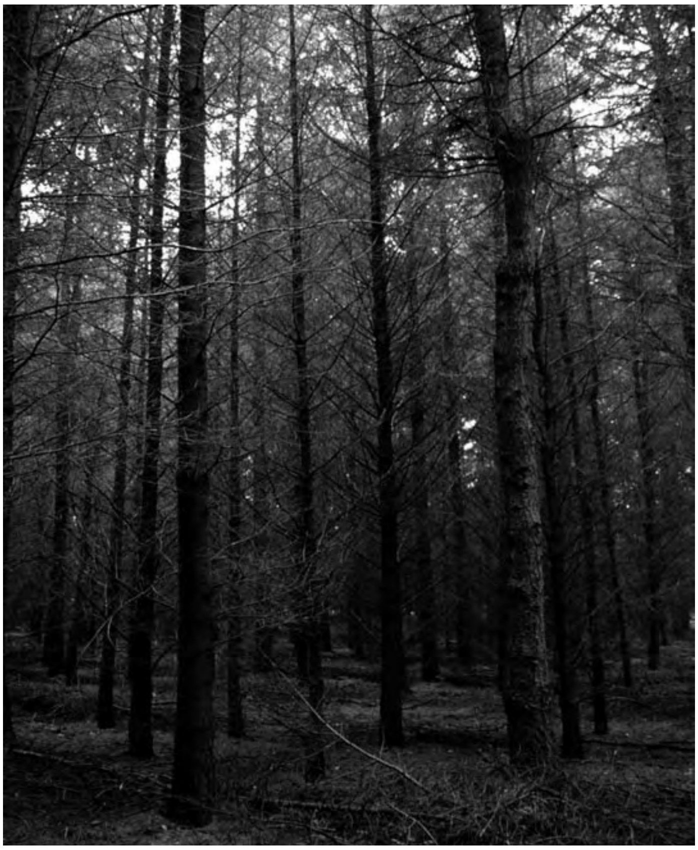

Figure 8. Douglas-fir trees with good (left, IUFRO no. 1005, Williams Lake, BC) and bad (right, IUFRO no. 1018, Salmon Arm, BC) stem forms (Photo: E. Burchard).

Results

In Germany, the minimum legal requirements for a Douglas-fir stand to be approved as seed stand are, among others, an area of at least 0.25 ha, comprising at least 40 trees and an age of 40 years. Despite these requirements, most of the stands checked were larger than the minimum area. If smaller stands were included (as in Thuringia), and if they were identified as being of coastal origin, the intention was to select trees for seed orchards in these reduced populations. Hesse carried out its field inspections in stands larger than one hectare and included plantations younger than 40 years. Results of the study are summarized in Table 1.

Table 1. Studied Douglas-fir stands: summary data

Federal State	Criteria (area, age)	No. seed stands[†]	No. other stands[‡]	Area (ha)[§]	Interior origin[¶]
Rhineland-Palatinate	>0.25 ha, >40 years	38	17	~ 500	9%
Northrhine-Westphalia	>0.25 ha, >40 years, >50 trees/ha	91	39	~ 220	27%
Thuringia	–	23	12	20	16%
Hesse	>1 ha, >20 years	–	Total no. 555	~1200	115 stands

[†] Number of seed stands checked
[‡] Number of other populations included
[§] Total area of checked stands
[¶] Percentage/number identified as populations of interior origin
– = no data available

Conclusions

Due to their excellent performance, numerous old European Douglas-fir stands can be considered as a very valuable genetic resource and should therefore be used for seed collections or for natural regeneration, as long as their population size is in accordance with legal regulations. However, many stands have also been established using unsuitable provenances, mainly from the interior distribution range of the species. Damages which have not been registered at young ages may become serious at more advanced ages. Forest administrations attempt to avoid natural regeneration of these unsuited stands as well as their use as seed collection stands. In addition to the visual assessment of stands with regard to their performance and health, isozyme analyses have proved to be a valuable tool to discriminate between coastal and interior races. For pure stands, affiliation to one of the races is no problem. In the case of racial mixtures the proportions of trees belonging either to the coastal or interior race, can be estimated. An affiliation of individual trees to a race is not possible. Nevertheless, it must also be mentioned that in years favourable for the development of needle cast fungi, infections have also been observed in stands belonging to the coastal race. However, as a rule these stands recover in the ensuing years.

In recent years, several hundred stands have been checked with regard to their silvicultural suitability and their race affiliation. In this way, Germany is increasingly using genetic resources of Douglas-fir of approved value.

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Douglas-fir provenance tests in Serbia

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Introduction

The two main objectivities of a breeding program are to: 1) improve economic traits, and 2) ensure that the resulting breeding populations are well adapted and have sufficient genetic variation for gain to continue in subsequent generations (SAF 1992).

One of the models for testing genetic diversity, variability and adaptability of introduced species is the provenance test (Rehfeld 1978, 1983). This paper presents the variability of some properties which have been analyzed in Douglas-fir provenance tests in Serbia.

Material and method

In central and east Serbia at the sites Juhor and Tanda, a provenance test was established in 1986 with 20 different Douglas-fir provenances which originated from New Mexico to Washington states in the US (Table 1).

The experimental plot in Juhor is located in a beech stand (association *Fagetum submontanum* Jov.) at an altitude of 670 m on the mountain Juhor. The second experimental plot in Tanda is in a natural stand of Hungarian oak and Turkey oak (association *Quercetum farneto-ceris* Rud.) at an altitude of 370 m (Figure 1). Based on field and laboratory analyses of soil properties, the Tanda plot has eutric brown soil (eutric cambisol), and the one in Juhor has acid brown soil (district cambisol).

Table 1. Geographical co-ordinates of the tested Douglas-fir provenances in Serbia.

Number of tested provenances	Origin code of provenance		Latitude (°)	Longitude (°)	Altitude (m)
1	Oregon	205-15	43.7	123.0	750
2	Oregon	205-14	43.8	122.5	1200
3	Oregon	202-27	45.0	122.4	450
4	Oregon	205-38	45.0	121.0	600
5	Washington	204-07	49.0	119.0	1200
6	Oregon	205-13	43.8	122.5	1050
7	Oregon	205-18	44.2	122.2	600
8	Oregon	202-22	42.5	122.5	1200
9	Washington	202-17	47.6	121.7	600
10	Oregon	201-10	44.5	119.0	1350
11	Washington	204-06	49.0	120.0	750
12	Oregon	202-19	45.3	123.8	300
13	Oregon	205-11	45.3	123.0	150
14	New Mexico	202-40	45.0	105.7	2682
15	New Mexico	202-10	36.0	106.0	2667
16	Oregon	202-31	44.3	118.8	1500
17	Oregon	205-29	42.6	122.8	900
18	Oregon	205-08	42.7	122.5	1050
19	Oregon	204-04	45.0	121.5	900
20	Washington	205-02	47.7	123.0	300

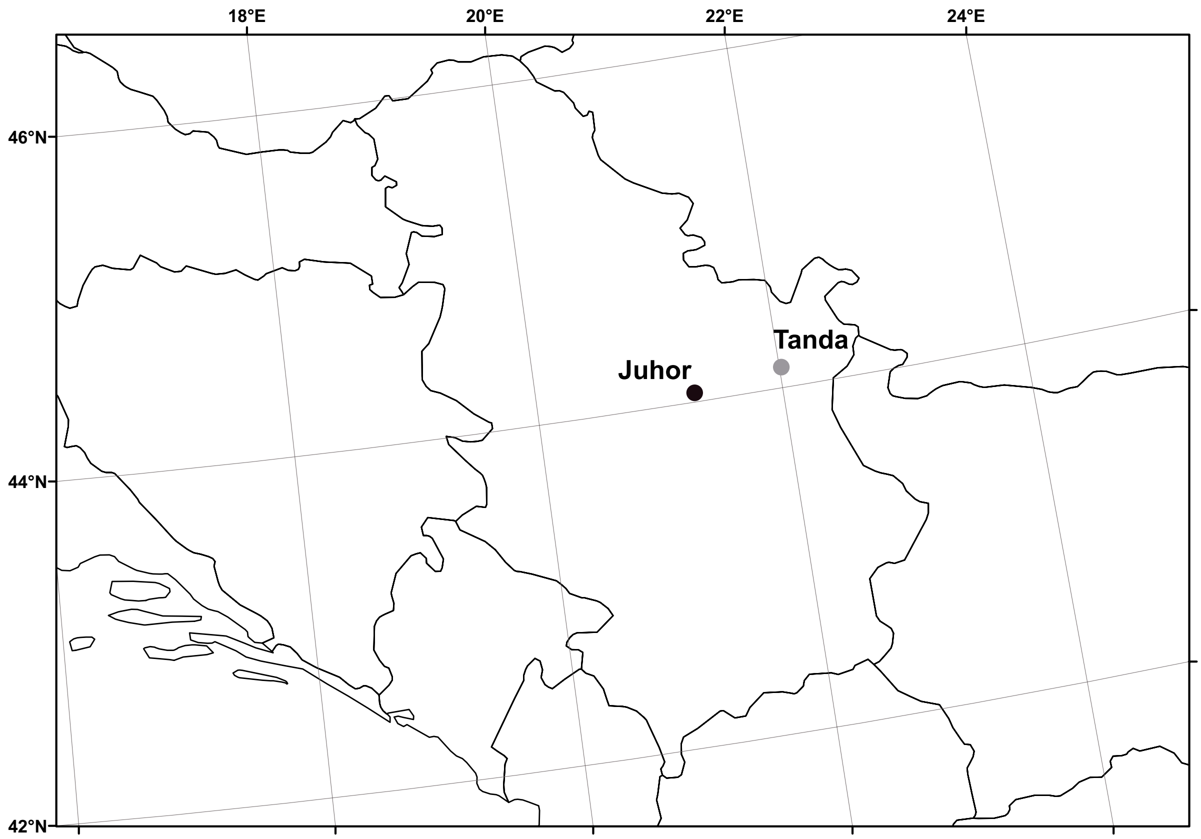

Figure 1. Location of the two Douglas-fir provenance tests in Serbia.

Table 2. Height and diameter of the Douglas-fir provenances tested in Serbia (at the age of 12 years) (Lavadinovic V and Koprivica M. 1999).

Number of tested provenaces	Origin code of provenance		Height (cm)		Diameter (cm)	
			Juhor	Tanda	Juhor	Tanda
1	Oregon	205-15	516	390	11.5	8.9
2	Oregon	205-14	517	401	11.3	8.8
3	Oregon	202-27	558	481	12.0	10.4
4	Oregon	205-38	499	477	10.8	10.8
5	Washington	204-07	282	253	6.5	6.0
6	Oregon	205-13	506	420	10.9	9.2
7	Oregon	205-18	524	463	11.8	10.7
8	Oregon	202-22	472	391	11.0	9.6
9	Washington	202-17	516	431	11.3	9.5
10	Oregon	201-10	353	298	8.2	7.0
11	Washington	204-06	348	282	8.2	7.4
12	Oregon	202-19	537	484	11.6	10.2
13	Oregon	205-11	510	475	11.6	10.7
14	New Mexico	202-40	394	394	9.0	9.2
15	New Mexico	202-10	370	346	8.1	7.9
16	Oregon	202-31	286	239	6.7	6.1
17	Oregon	205-29	486	398	11.2	8.8
18	Oregon	205-08	463	346	10.4	8.4
19	Oregon	204-04	469	367	10.3	8.7
20	Washington	205-02	529	547	10.6	11.3

During the research period (1997-1999) the experimental plots were measured every year (Table 2).

The climate of the locality Juhor (central Serbia) is predominantly moderate–continental with cold winters and rather warm summers. The climate of the locality Tanda in north-east Serbia can be characterized as sub-humid to humid.

By analyzing the values of mean height and diameter, significant differences among the provenances and between the sites were found.

The dependence of height on altitude and latitude and of height increment on longitude and altitude in the provenance trials were also analysed (Figures 2 and 3).

Results

The results from the Juhor test show that latitude has a low effect on height increment of trees of different provenances and that the effects of longitude and altitude are very important.

Dependence of height increment of the provenances (Y) on longitude (X_2) was found to be parabolic (Figure 2). With the increase of longitude, height increment also increases up to a certain level.

Dependence of height increment of the provenances (Y) on altitude (X_3) is shown in Figure 3. With an increase of altitude, height increment of trees reduces.

Parameters of regression are as follows:
$Y = -36967.0 + 604.15\ X_2 - 2.463\ X_2^2$
$Se = 5.6$ cm, $r^2 = 0.8309$
($F > F0.01$; 64.88>5.61)

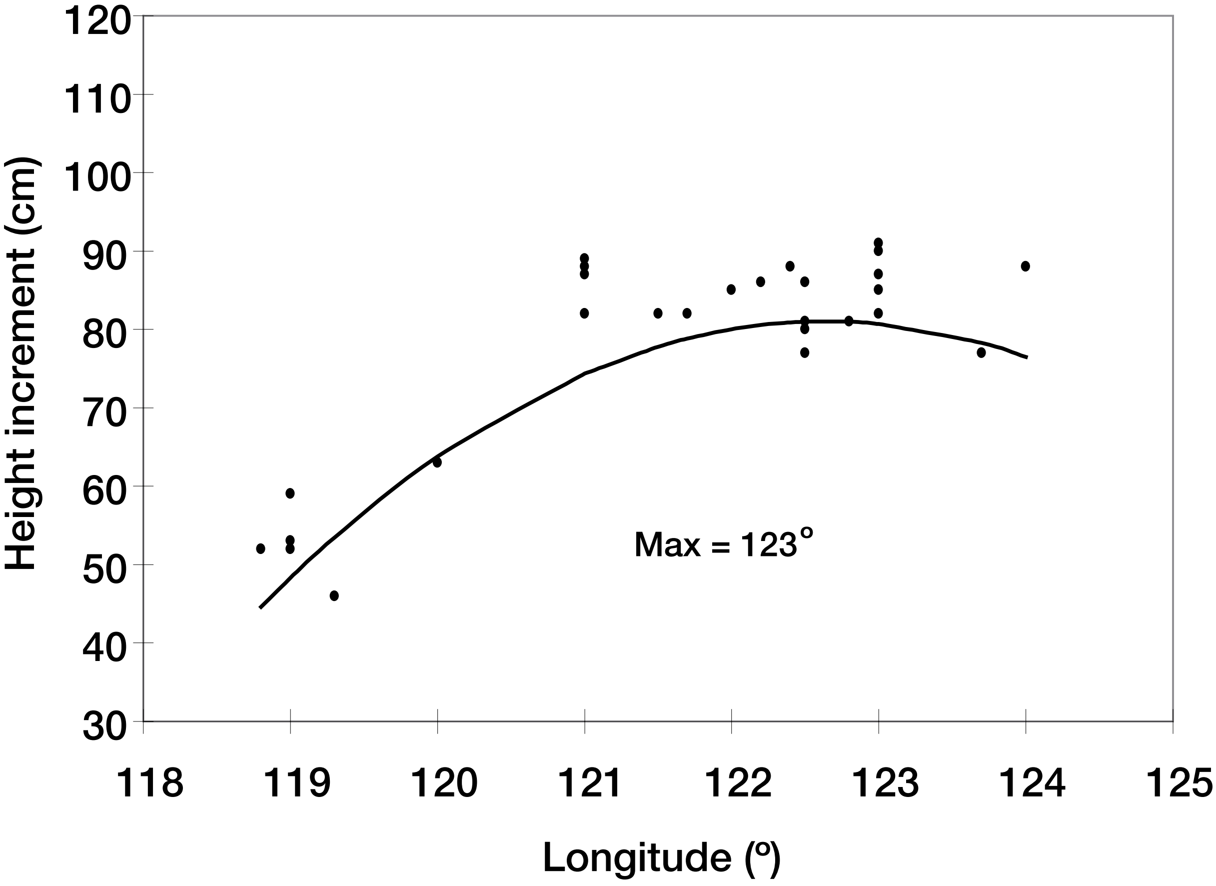

Figure 2. Dependence of annual height increment of different Douglas-fir provenances on longitude.

Parameters of parabolic correlation are:
$Y = 85.02 + 0.0102\ X_3 - 0.00002\ X_3^2$
$Se = 10.4$ cm, $r^2 = 0.4520$
($F > F0.01$; $10.04 > 5.61$)

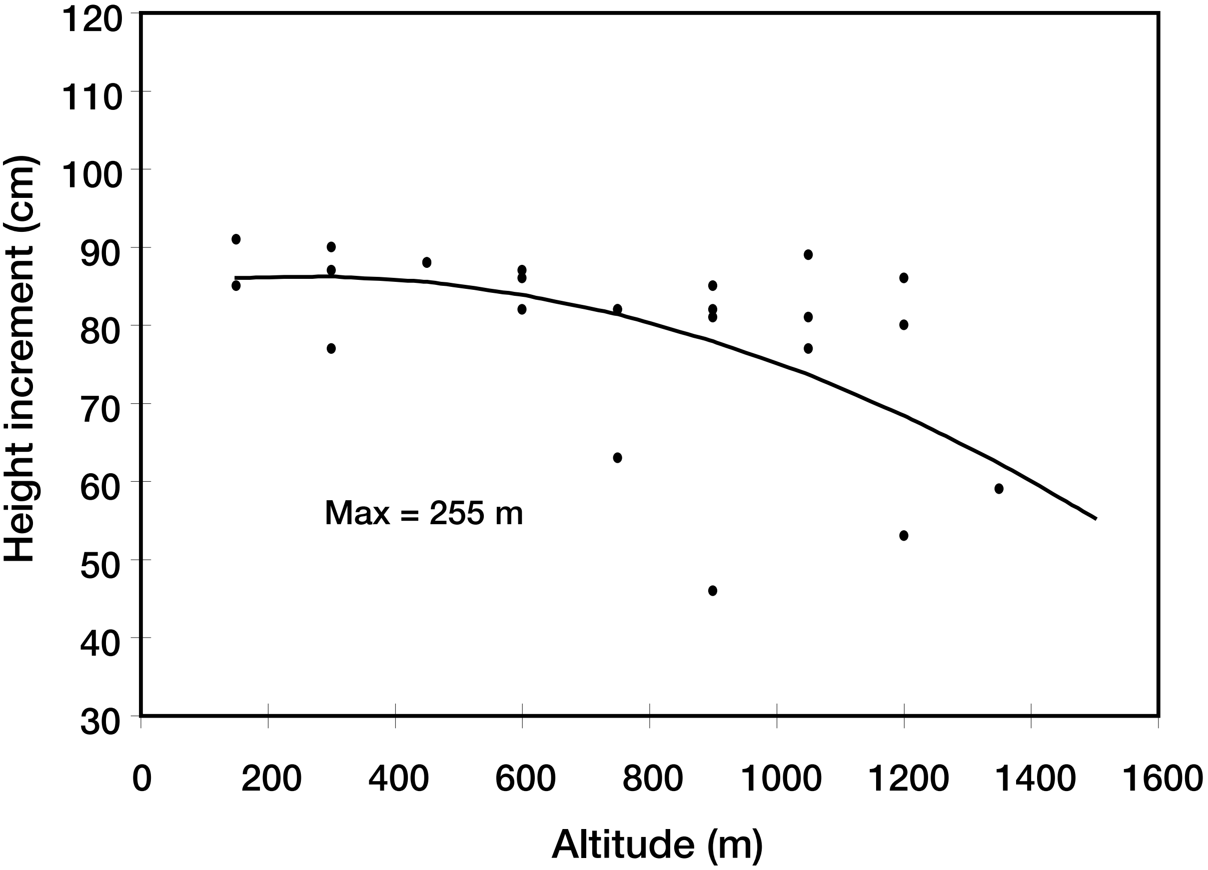

Figure 3. Dependence of annual height increment of different Douglas-fir provenances on altitude.

Conclusions

This study confirmed that there were significant differences in growth among Douglas-fir provenances at these two locations in Serbia (annual height increment of the provenances varied from 46 cm (Washington 19) to 90 cm (Washington 31)). The effect of longitude on height increment of provenances is statistically highly significant, i.e. with the increase of longitude to 123°, height increment also increases, and then decreases. The effect of altitude on height increment of provenances is statistically highly significant, i.e. above the altitude of 255 m, height increment gradually decreases.

The observed differences in tree growth are ascribed to the genetic factors. (Lavadinovic and Koprivica 1996, 1999).

The plants at the sample plot Juhor, at the same age, had a considerably higher value of diamemeter at the breast height, height, and annual height increments than the plants of the same provenances at the sample plot Tanda. This means that Douglas-fir has better adaptability to the beech sites (Juhor) than the oak site (Tanda).

Based on the analyzed characteristics, the most successful provenances are:

- At the locality JUHOR:
  - Oregon: 205-14
  - Oregon: 202-19
  - Washington: 205-02

- At the locality TANDA:
    - Oregon: 205-14
    - Oregon: 202-19
    - Washington: 205-31

There were highly significant differences between Douglas-fir provenances; thus, careful attention should be paid to the choice of provenances to be used for forestry.

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Fourth EUFORGEN Conifers Network meeting

Pitlochry, Scotland, UK, 18–20 October 2003

Theme: conservation and use of genetic resources of exotic conifer species in Europe

Friday 17 October

	Arrival of participants

Saturday 18 October

08:30	Opening of the meeting
	Welcome address (S. Samuel)
	Welcome by Chair (C. Mátyás)
	Adoption of the agenda and nomination of rapporteurs
08:45	EUFORGEN update (J. Koskela)
09:00	Country introductory report: Iceland
09:30	Country updates and discussion on the progress made in sub-regional groups
	Central and Eastern Europe (Czech Republic, Hungary, Poland, Russian Federation, Slovakia)
	Mediterranean region (Bulgaria, Croatia, Cyprus, France, Italy, Macedonia, Malta, Serbia and Montenegro, Spain, Turkey)
	Northern Europe (Finland, Lithuania, Norway, Sweden)
	Western Europe (Austria, Belgium, Germany, Ireland, Switzerland, United Kingdom)
10:30	Break
11:00	Presentations by the sub-regional groups
12:00	Documentation, information and public awareness:
	EUFORGEN website (J. Koskela)
	Bibliography (J. Koskela)
	Poster presented at the DYGEN Conference (C. Mátyás)
	Image database (B. Fady)
	Other public awareness initiatives
12:30	Lunch
13:45	Depart for Blair Castle (residence of the Dukes of Atholl for over 700 years) and Diana's Grove (major mature collection of exotic conifers)
16:45	Break
17:00	Meetings, projects and other initiatives:
	Outcomes of the DYGEN Conference (C. Mátyás)
	EC Regulation on Genetic Resources (J. Koskela)
	EVOLTREE proposal (G. Vendramin)
	Some results from analysing SNPs in conifers (G. Vendramin)
	Other initiatives
19:30	Dinner

Sunday 19 October

08:30	Progress made in the Network activities and discussion:
	Technical Guidelines (J. Koskela)
	Best practice for genetically sustainable forest management (C. Mátyás)
	Common Action Plan (C. Mátyás)
	List of priority species (C. Mátyás)
	MCPFE outcomes and EUFORGEN Phase III (J. Koskela)
10:30	Break
11:00	Seminar on conserving and using exotic conifers: experiences from the UK and implications for Europe
	The introduction, variation and use of non-native conifer species in Britain (S. Samuel)
	Breeding programmes in exotic conifers in Britain (S. Lee)
12:30	Lunch
14:00	Seminar (continued):
	Developing a policy for long-term archiving of breeding material (R. Sykes)
	Handling exotic species from the genetic conservation point of view: *Cedrus atlantica* in France (B. Fady)
15:30	Break
16:00- 18:00	Botanical gardens and gene conservation (M. Gardner)
	Seminar wrap-up and recommendations
20:00	Dinner

Monday 20 October

08:30-15:30	Field trip
16:00- 18:00	Wrap-up session:
	Any other business
	Date and place of next meeting
	Adoption of the summary of the meeting
20:00	Dinner

Tuesday 21 October

	Departure of participants

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