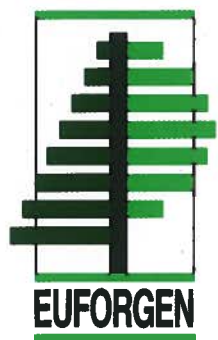




Third EUFORGEN Meeting on Social Broadleaves

22–24 June 2000—Borovets, Bulgaria

**S. Borelli, A. Kremer, T. Geburek, L. Paule and
E. Lipman, compilers**



European Forest Genetic Resources Programme (EUFORGEN)



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The International Plant Genetic Resources Institute (IPGRI) is an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advance the conservation and use of genetic diversity for the well-being of present and future generations. IPGRI's headquarters is based in Maccarese, near Rome, Italy, with offices in another 19 countries worldwide. The Institute operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme and (3) the International Network for the Improvement of Banana and Plantain (INIBAP).

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The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable utilization of forest genetic resources in Europe. It was established 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 IPGRI, 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 forest geneticists and other forestry specialists work together to analyse needs, exchange experiences and develop conservation objectives and methods for selected species. The networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong. Network members and other scientists and forest managers from 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 participating countries.

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Summary of the meeting

Opening of the meeting

Professor Alexander Alexandrov of the Forest Research Institute of Bulgaria, in his capacity as EUFORGEN National Coordinator, officially welcomed the participants to Borovets and conveyed his wishes for a fruitful meeting.

Professor Ivan Raev, Director of the Forest Research Institute, welcomed the participants in the name of the Bulgarian Academy of Science. He underlined the importance of studying the genetic resources of social broadleaves, particularly in view of the current threats to valuable native broadleaved species. Pollution, climate change and other anthropogenic factors are strongly affecting the existing forests and it is paramount to conserve and protect forest resources.

He also remarked that the third EUFORGEN Social Broadleaves Network meeting is an important step in this direction and that it fits well within the framework of the Convention on Biological Diversity, the Convention on Climate Change and the global effort towards sustainable management of forests. He wished the participants a successful meeting.

Antoine Kremer, Chair of the Network, welcomed the participants and thanked the Bulgarian Forestry Research Institute for its efforts in organizing the meeting. He welcomed the newly attending countries, Turkey and Uzbekistan.

Simone Borelli, on behalf of the EUFORGEN Secretariat, provided some highlights of the EUFORGEN activities, meetings and outputs in the year 2000 and of those planned for 2001.

Antoine Kremer presented the agenda and it was adopted. Thomas Geburek and Ladislav Paule were appointed as rapporteurs.

Joint session with the project on genetic resources of broadleaved species in southeastern Europe

Ioan Blada presented the results of the project, funded by Luxembourg for a 3-year period. He went over the objectives of the project and presented the results of the first 2 years of activities. He concentrated on the aspects related to inventories and gene conservation units. He gave information on the total surface of seed stands and protected areas in the participating countries and presented the distribution maps of some of the main species covered by the project (*Fagus sylvatica*, *Quercus petraea*, *Q. robur* etc.). He underlined the presence of virgin forest in some of the mountain ranges. He also gave some figures on the number and surface of stands that have been protected.

Myriam Heuertz presented the results of the molecular studies carried out on *Fraxinus excelsior*. She presented the characteristics of the species and highlighted the limited knowledge of the mating system. Southeastern Europe was selected because of the close-to-nature state of its forests. The objectives of the work were presented. This was followed by an overview of the results.

Simone Borelli briefly presented the objectives of the proposed Phase 2 of the project.

During the discussion, Ioan Blada indicated that Romania has the GIS facilities and is available in principle to prepare maps for other countries. The idea of joint maps at the European level was revived and the need to involve 'missing countries' in the Network's activities discussed. Ioan Blada underlined the need for additional funds, possibly from the EU.

Discussion focused on: the causes for changes in forest cover in Romania; problems related to the reproduction of oaks; the definition and distribution of virgin forests of beech and spruce; the characteristics and accuracy of the maps and the databases; the need for protection of seed stands; and several aspects related to the population genetic studies carried out on *Fraxinus*.

An important point raised was the contradiction between the status and regulations of national parks and the impossibility of collecting reproductive material in these areas. This problem appears to be common to many countries. In Austria, for example, discussion has been initiated on the possible use of genetic resources in protected areas.

Overview of research projects supported by the European Union

FAIR OAK

Antoine Kremer gave an overview of the results of the project (see Report of the last Network meeting). The project looked mainly at the distribution of cpDNA haplotypes of eight oak species throughout Europe. The coverage of the project had originally been limited to EU member countries but other countries progressively joined through bilateral agreements. Final results of the project will be soon published in the journal *Forest Ecology and Management*. Contact: Antoine Kremer, INRA Bordeaux, France.

FAIR Beech

The project started in 1983 and will end in 2001. It includes 20 countries participating in an international provenance experiment. Seed was collected for as many provenances as possible (currently 300) and trials were established in different countries to evaluate adaptation. Data have been compiled in a database and most countries already provided the information. A coarse cpDNA map (based on 3–4 markers) is being developed. A new project focused on more in-depth analysis of the existing provenance trials may be initiated and possibly submitted for funding to the EU next year. Contact: Richard Stephan, BFH Grosshansdorf, Germany.

Dynabeech

This EU-funded project (2000–2004) will look at genetic and ecophysiological differences between virgin forests and managed forests. Research teams from Austria, France, Germany and Italy participate in the project. In the genetic part of the project, predominantly microsatellites will be developed to measure geneflow within populations of *Fagus sylvatica*. Mating systems will also be studied. Contact: Thomas Geburek, FBVA Vienna, Austria.

Oakflow

The project was recently accepted for funding by the EU. Ten countries will participate (14 institutions). The main objective is to assess hybridization and geneflow in oaks as a mechanism promoting diversity. There will be three main components: (1) assessing geneflow by parentage analysis with microsatellites, mainly in mixed forests (*Q. petraea* and *Q. robur*, and sometimes *Q. pubescens*); (2) evaluating genetic and ecological consequences of geneflow; (3) identifying impacts of geneflow on the management of seed and conservation stands. In many countries, forestry agencies will be involved in examining silvicultural consequences of hybridization and the evolution of diversity through simulation models (METAPOP). Contact: Antoine Kremer, INRA Bordeaux, France.

Progress made in countries

Introductory country reports

Gursel Karagöz presented the introductory country report from Turkey and Abduhalil Kayimov, Chair of the Central Asia Working Group on forest genetic resources, presented the report for Uzbekistan and other Central Asian countries.

Update on progress made by countries

Countries were asked to present the highlights of their reports. Full reports will be provided to the Secretariat in electronic format by **15 July 2000** and will be posted on the EUFORGEN Web page. It was proposed that the meeting report should contain only a summary table of main country activities.

Legislation

Sven de Vries presented an overview of legislation related to genetic resources of social broadleaves (see pp. 27–30). To date the overview covers 26 countries. The tables were circulated among participants and they were asked to clarify some of the information. Newly attending countries were asked to compile the full questionnaire in order to complete the survey.

Lennart Ackzell presented the gene conservation aspects included in EU Directive 1999/105/EC (forest reproductive material). The preamble includes reference to genetic diversity. Article 4 touches upon plant genetic resources, *in situ* conservation, sustainable use and genetic erosion. Article 6.5 allows countries to make material for various purposes, including conservation of genetic resources, available on the market. Annex II refers to criteria for seed sources, which will be established by Member States. The new regulation is aligned with the OECD Scheme.

Results of the survey on joint conservation strategies

Thomas Geburek presented the results of the questionnaire that was distributed to members of the Network in 1999. The survey consisted of five sections:

- species distribution
- conservation
- research
- silviculture
- needs and activities of the Network.

Most urgent needs were expressed in the area of gene conservation strategies, conservation methods, and technical guidelines.

A number of countries provided maps of distribution areas of oak and beech. However, there is information missing from several countries. The Secretariat will contact countries not participating in the Network. Antoine Kremer and Thomas Geburek will lead the compilation of maps at the European scale of oaks and beech, respectively. The maps will be circulated **before the next Network meeting**.

The analysis also showed that no data were available in most countries on potential vegetation types. Most countries reported to have maps with 'regions of provenance'; however the information is too heterogeneous to be used for the development of conservation strategies.

Information on the health status of forest genetic resources was received. R. Stephan agreed to obtain the existing maps for oaks and beech at the European scale.

Tables of the status of natural regeneration in all countries were presented. While natural regeneration is of no major concern for beech, its absence is a major constraint for *in situ* conservation of *Q. petraea* and *Q. robur* throughout Europe.

In situ conservation measures are implemented in most countries for both *Fagus* and *Quercus*. *Ex situ* measures, namely conservation stands, seed orchards and clonal archives are found in some participating countries.

No additional measures seem to be necessary for *Fagus*. On the other hand, additional measures were deemed necessary by a large number of countries for *Q. robur*. The same situation can be found for *Q. petraea*. There is great interest for *in situ* measures for *Q. pubescens*, while *Q. cerris* seems to be the lowest priority.

The possibility of developing an *ex situ* network (multiple population breeding system—MPBS) was examined. A number of countries indicated the capacity to collect and establish *ex situ* stands for the different species, and *Q. robur* appears to be the priority species.

Thomas Geburek, Richard Stephan and Patrick Bonfils will prepare the results of the questionnaire for later publication. The Russian Federation and the Ukraine will provide responses to the questionnaire to Thomas Geburek by **31 July 2000**. The Secretariat will contact countries not participating in the Network and will ask them to provide a response by the same date.

Current state of knowledge on biology and genetics of social broadleaves

In order to provide scientific background for the discussion on conservation guidelines, Antoine Kremer introduced the subject of genetic processes modifying genetic diversity in oak and beech forests. His presentation covered the influence of population size, hybridization, intraspecific gene flow and selection on genetic diversity.

To illustrate the influence of population size, he presented the results of a study of mating systems in a mixed oak stand in France. Effective pollen and seed flow were monitored with microsatellites and inferences were drawn on population size. It was determined that the neighbourhood size varies between 12 and 19 ha and, therefore, if the population is kept to this size there is no risk of genetic drift.

Oaks are predominantly outcrossing species and there is unidirectional hybridization from *Q. petraea* to *Q. robur*, which tends to increase the diversity of the former species. The case studies also clearly indicated the occurrence of long-distance pollen flow and the asymmetry between pollen and seed flow. These opposing forces tend to erase any spatial genetic structure of the stand.

Finally, concerning selection, there is currently a lack of experimental results showing the amount and direction of selective pressures.

Discussion on technical guidelines on genetic conservation of European white oaks

The participants formed three groups for discussion. A set of questions related to what type of products or activities on *in situ* and *ex situ* conservation could be produced by the Network was distributed to the groups to facilitate the process. The questions referred to the target audience, style, format and dissemination of the technical guidelines; to the issue of access to protected areas for collecting reproductive material; and to the need of joint activities on *ex situ* conservation.

The rapporteurs for each group presented the results of the discussion.

The three groups agreed that the technical guidelines should be addressed to forest managers, forest owners and decision-makers and should be made available in the national language. It was proposed that the style should be less scientific, but that practical recommendations should be accompanied by the relevant references. Illustrations and graphs are also needed to make the material more immediately understandable.

It was proposed that a general document on the principles of genetic conservation should be developed that could be used for all Networks. In addition, smaller documents would be prepared for each species or group of species. However, other participants felt that this was not appropriate and that the Network should limit itself to producing a set of guidelines specific to oaks. These should also include general principles of gene conservation.

It was agreed that a decision on this issue would be aligned with the development of technical guidelines in other EUFORGEN Networks, and would follow discussion at the Inter-Network Meeting in October.

It was agreed that the material already prepared would be included in the Report of the meeting as technical presentations (see pp. 31–60). All participants will provide comments to the respective authors **by 31 July 2000**. The documents will be provided to the Secretariat **by 31 October 2000**.

It was proposed that the same authors would be responsible for preparing the final, concise version of the guidelines. The text should be no longer than 15 pages and structured along the lines agreed at the last Network meeting:

- Introduction (A. Kremer and Th. Geburek)—3 pages
- Biology and genetics of oaks (A. Kremer and P. Menozzi)—3 pages
- *In situ* conservation (P. Bonfils, A. Alexandrov and J. Gracan)—4 pages
- *Ex situ* conservation (S. Bordács)—2 pages
- Conclusions (A. Kremer, T. Geburek, L. Paule and J. Turok)—2 pages

The final draft document will be circulated to all Network members by the Secretariat **2 months before the next meeting**. The meeting will then discuss and adopt the text and will decide upon the format, layout, printing and distribution of the *Technical Guidelines on Genetic Conservation of European White Oaks*.

On the issue of access to genetic resources in protected areas, it appears that problems are different from country to country. It was proposed that additional discussion on possible EUFORGEN actions would be carried out at the level of the Steering Committee (see Report of the first Steering Committee meeting, 1995). The Secretariat will also approach relevant international organizations (i.e. CBD and IUCN) and seek to develop a common strategy.

For *ex situ* conservation activities there was a general feeling that establishing an MPBS network was premature, due to lack of knowledge on geographic variation and also to lack of breeding activities. A proposal was made for international provenance trials on *Q. robur* but, as resources are limited, these could be carried out on a bilateral basis. Although it is not the objective of the Network to become directly involved in provenance experiments, it can stimulate such activities as appropriate.

Information management

Simone Borelli presented a series of activities related to information management.

He informed the participants on IPGRI's intention to use the Web page of the Regional Office for Europe as a gateway to general information on the conservation of plant genetic resources in Europe. Users will be able to access information on national programmes and find relevant links and contacts. The feedback of Network members will be essential to complete and update this information.

He then went on to present the EUFORGEN Web page and the section relevant to the Social Broadleaves Network. The participants were encouraged to visit the page and provide comments on contents, and on additional information that could be made available.

The idea to contribute to a new EUFORGEN database on grey literature on forest genetic resources was accepted by the Network. The Secretariat will circulate the format in both Microsoft® Access and Excel by **15 July 2000**.

Public awareness

New brochure

Simone Borelli briefly presented the new brochure on *Conservation of Genetic Resources of Social Broadleaves in Europe*. Ioan Blada and Ned Cundall prepared the text for the brochure. Illustrations were taken from the Network slide collection (see below). Copies were distributed and more are available upon request.

Image collection

Dominique Jacques summarized the objectives of the collection and presented the results obtained to date. Filemaker Pro was used as a management tool and an Internet link will be made available on the Web page of Centre de Recherche de la Nature, de Forêts et du Bois (CRNFB). So far, 11 countries have provided images.

The collection is organized following a series of criteria and keywords based on general themes. The database also contains information on the source of the images. It was reiterated that the images are free for use but that the author and the EUFORGEN Social Broadleaves Network should be cited as source. All countries were invited to send additional material in order to complete the collection.

Attention was drawn on the possibilities of adding images on additional themes (i.e. cultural) and a list of existing themes was circulated for comments.

It was decided that for the time being the collection will remain in Belgium. A link to the relevant Web site will be added to the EUFORGEN site. Dominique Jacques will provide details on how to submit material and on which themes are still incomplete by **31 July 2000**. The final version of the CD will be made available to all Network members by **31 October 2000**.

It was decided that a Social Broadleaves poster would be produced. Sven de Vries, Tor Myking and Patrick Bonfils agreed to prepare a draft text and layout and to send it to the Secretariat for circulation by **31 December 2000**.

France mentioned other public awareness activities carried out through the preparation of a booklet and public events. Spain referred to a special issue of the INIA journal to raise awareness on the importance of forest genetic resources.

Date and venue of next meeting

Luxembourg and Norway offered to host the fourth Network meeting in June 2001. After short discussion, the Network members expressed preference for holding the meeting in Norway. The Secretariat will confirm the exact dates in due course.

Conclusion

The report of the meeting was adopted. The EUFORGEN Secretariat and the Chair of the Network expressed once again their appreciation of the local arrangements and thanked all participants for their contribution.

Broadleaved forest tree species in southeastern Europe

Ioan Blada

Forest Research Institute, Bucharest, Romania

Southeastern Europe, in particular the Balkan Peninsula, is a region that is rich in autochthonous broadleaved forest resources valued for their quality and natural diversity. This diversity is due to the adaptation of populations to the very different ecological conditions in several phytogeographic zones, as well as to the mountainous nature of the region with its great elevations and various soils.

A number of forest tree species are very common, like the economically important European broadleaves such as European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.) and pedunculate oak (*Q. robur* L.), while other species of these genera such as Dalechamp's oak (*Q. dalechampii* Ten.) and Italian oak (*Q. frainetto* Ten.) are endemic to the region and are quite rare.

Other broadleaved species, e.g. maple and ash (*Acer campestre* L., *A. platanoides* L., *A. pseudoplatanus* L., *Fraxinus excelsior* L., *F. ornus* L. and *F. angustifolia* Vahl), have scattered distribution patterns but are nevertheless particularly valuable for their ecological role in the species-mixed ecosystem and for the high-quality timber they provide.

Because of their ecological and economic importance, and because of the growing threats to their genetic resources such as industrial air pollution, repeated extreme summer drought and subsequent attack by pests and diseases, a number of the broadleaved tree species have received particular attention in conservation, tree breeding and afforestation programmes in Bulgaria, Moldova and Romania.

In 1997, IPGRI initiated a 3-year collaborative project with Bulgaria, Moldova and Romania on genetic resources of broadleaved forest trees, funded by the Government of Luxembourg. The project aimed at consolidating and further developing national programmes on the conservation and sustainable use of forest genetic resources in the three countries, which share similar natural conditions and forest management traditions.

An integrated approach encompassing *in situ* conservation of genetic diversity in native forests (the best way to maintain the genetic diversity in long-lived, largely outbreeding and undomesticated forest tree species), as well as further development and use of advanced technologies for *ex situ* conservation was chosen. Emphasis was given to capacity building and the project contributed to strengthening linkages between the institutions and scientists and their counterparts in other European countries.

The activities undertaken included the development of maps of ecogeographic distribution areas for 13 tree species, the compilation of databases of seed stands and gene conservation units, as well as the development and application of micropropagation techniques for priority species (in collaboration with the Centre de Recherche Public Gabriel Lippmann, Luxembourg). Databases of forest gene conservation units, including ecogeographic data, were also compiled. Seed stands and forest reserves currently represent around 2–3% of the total forest area covered by the respective species. See Table 1 for summary.

In situ conservation is, however, a dynamic process that needs to be integrated into the regular forest management, which is applied in the remaining forest area. Common principles for silvicultural measures that contribute to the sustainable, close-to-nature and multifunctional management of forests for dynamic gene conservation need to be implemented and adapted to local needs. Also, the existing knowledge of the patterns of ecogeographic variation in the region needs to be combined with genetic information from field trials or genetic marker studies.

Table 1. Overview of seed stands of broadleaved species in Bulgaria, Moldova and Romania

Species	Bulgaria		Moldova		Romania		Total	
	no.	ha	no.	ha	no.	ha	no.	ha
<i>Quercus petraea</i>	522	7101	123	5315	1010	14739	1655	27155
<i>Q. robur</i>	41	440	81	1828	400	4758	522	7026
<i>Q. cerris</i>	145	2143	–	–	60	732	205	2875
<i>Q. frainetto</i>	348	4433	–	–	90	1007	438	5440
<i>Q. pubescens</i>	4	41	–	–	5	12	9	53
<i>Fagus sylvatica</i>	898	11824	21	229	344	7628	1263	19681
<i>F. orientalis</i>	171	1863	–	–	–	–	171	1863
<i>Acer campestre</i>	161	2101	75	1528	–	–	236	3629
<i>A. platanoides</i>	49	836	51	1171	–	–	100	2007
<i>A. pseudoplatanus</i>	154	1959	6	77	5	13	165	2049
Total	2493	32741	357	10148	1914	28889	4764	71778

no. = number of stands; ha = area in hectares.

In fact, a pilot study using microsatellite markers was started on common ash (*Fraxinus excelsior*) in Bulgaria. The study assessed microsatellite variation at six specific loci. Sampling was carried out in three natural regions with different environmental conditions in western, central and northeastern Bulgaria. All the areas revealed a very high diversity within the populations, with no relationship between within-population diversity and age or management type of a common ash stand. In fact, 93.1% of the total genetic diversity was located within populations; 36% of the rest was found among regions and 64% among populations. Further sampling was carried out in Romania and will also be done in Moldova in order to obtain a complete picture of the genetic diversity in the region.

The knowledge obtained through the various project activities provides a solid basis for developing and implementing integrated genetic conservation strategies by the national programmes in the countries involved. It helped to identify priorities for the conservation of diversity both within and between species, particularly for the rare and threatened broadleaved species, which have often been neglected in the forest management and tree breeding surveys. As a result, several principles of the genetically sustainable management of forests were discussed with the national forest services:

- Natural regeneration, which supports the biological and economic stability and continuity of forest stands, should be promoted where possible.
- For artificial regeneration, planting stock should originate from mating within the same population.
- Silvicultural management should give due attention to preserving the adaptability of the principal as well as important associated species.
- Due attention should also be given to evaluating and identifying new potential gene conservation units.
- Reproductive material should be collected from seed stands and used according to the national rules and legislation.

The data related to risks and value of resources will need to be integrated into a decision-making framework that can be used for genetic conservation management in terms of priority species, populations and interventions in the future.

The consolidation of the results obtained by the project will be the main task of Phase 2 of the project, expected to start in early 2001. The main objective of this follow-up phase will be to create the conditions for sustainable management of forest genetic resources in the participating countries. Intraspecific diversity of the different species will be characterized by using molecular tools and this will provide a measure of the level of genetic diversity and a

baseline for monitoring the level of genetic change over time or as a consequence of different management practices.

These activities will be complemented with concerted actions at a policy level and activities on public awareness.

Introductory country reports

Turkey

Gürsel Karagöz

Research, Planning and Coordination Board, Ministry of Forestry, Ankara, Turkey

Introduction

Turkey has 20.8 million hectares of forest land (including only land covered with forest vegetation), which corresponds, to 26.6% of the country surface area. Much of the forested area is regarded as unproductive and requires rehabilitation. Forest ecosystems include high mountain and alluvial forests. Deciduous forests are prevalent and relatively uninterrupted at moderate elevations along northern Turkey. Coniferous forests, depending on the species and locations, are found at varying altitudes, from sea level to the timberline. Forests formations of the country include species belonging to different floristic regions, namely the Irano-Turanian, Mediterranean and Euro-Siberian regions. Approximately 800 trees and shrubs occur in the country's forests.

Productive high forest and coppice forest cover 10 million ha (48% of the total forest area) (Table 1). The remaining 10.8 million ha land is degraded high and coppice forest. Total growing stock is about 1200 million m³ (86% of which in productive high forest) with an annual increment of 34.2 million m³ (77.8% of which in productive high forest). Predominant and commercially important species are *Pinus brutia*, *P. nigra*, *P. sylvestris*, *Fagus orientalis* and *Quercus* spp.

Table 1. Status of forests in Turkey

	Productive high forest	Degraded high forest	Productive coppice forest	Degraded coppice forest	Total
Area (million ha)	8.2	6.2	1.8	4.6	20.8
Growing stock (million m³)	1032.0	63.7	80.4	24.9	1201.0
Gross annual increment (million m³)	26.6	1.6	4.7	1.3	34.2
Increment (m³/ha)	3.9		2.7		

Note: areas above include only areas covered with forest vegetation. Open areas within the forest land are not included.

Occurrence and origin of social broadleaves in Turkey

Beech

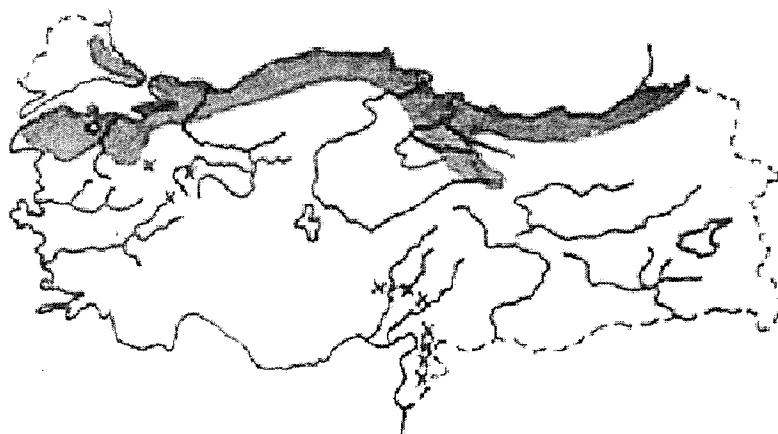
Oriental beech (*Fagus orientalis* Lipsky)

Oriental beech forests occupy about 1 323 500 ha as high forest (1 060 000 ha productive and 263 500 ha degraded) and 11 000 ha as coppice forest. The total 1 334 000 ha account for about 13% of the forest area of Turkey. These forests have 198 683 561 m³ growing stock in high forests and 284 107 m³ in coppice forests. Their increment is 4 208 162 m³ in high forests and 6548 m³ in coppice forests. Beech forests have an allowable cut of 3 333 872 m³ in high forests and 334 441 m³ in coppice forests.

Figure 1 shows the distribution of *Fagus orientalis* in Turkey. Oriental beech in Turkey grows on northern mountain slopes towards the Black Sea. It occurs as pure stands in the Black Sea coastal mountains between 0 and 1500 m altitude, and as mixed forests with *Abies bornmuelleriana*, *A. nordmanniana*, *Pinus nigra* and *P. sylvestris* at 1500–1700 m. The optimum growing area is the Black Sea coastal region between 800 and 1200 m where dominant pure stands exist. In the Black Sea inland region it is found at 1000–1500 m, and in Marmara Region on the northern slopes, at 0–1000 m on the coast and at 1500–1800 m inland. It starts to appear on the northern slopes of the Strandja Mountains at 250–1000 m and ends at Istanbul. Demirköy, Kırklareli, Vize and Çatalca are the main regions of beech in Thrace. In southern Marmara, its lowest altitude is 500 m on the mountains of Kapıdağ and Karadağ, 1100 m at Uludağ, 1200 m at Alaçam. It is distributed between Yenice and Bilecik at 500–1300 m. Oriental beech is also found in the Gediz, Simav and Dursunbey districts at 1200–2000 m.

Beech occurs in enclaves outside the area of its usual distribution. A very striking example is the case of *Fagus orientalis*, which grows in forests on the famous Amanos mountain range and mountains in the Adana (Pozantı, Pos, Osmaniye, Sorgun Yaylası and Namrundağı) and Kahramanmaraş areas in eastern Mediterranean.

Fig. 1. Distribution of *Fagus orientalis* in Turkey



Common beech (*Fagus sylvatica* L.)

Common beech is found in the Strandja mountains (at Demirköy at 650 m) in Thrace, in Çanakkale (Bayramiç at 600 m), in Kazdağı (Gürgendağı district, 1300 m), in Edremit at 1500 m, and in Gediz at 1500 m and Simav at 1700 m.

***Quercus* spp.**

Eighteen species of this genus grow naturally in Turkey, two of which (*Q. aucheri* and *Q. vulcanica*) are endemic. It is often found in pure stands, but may also occur in mixed formations.

Oaks have the widest distribution area among deciduous trees in Turkey and cover approximately 6.5 million ha, 81% of which are coppice. Only 350 000 ha are productive high forest.

White oaks

- **Sessile oak (*Q. petraea* (Matt.) Liebl.)**

Q. petraea is the most economically important oak species in Turkey. It is found in pure and mixed forests. Three subspecies are found in Turkey:

- *Q. petraea* subsp. *petraea* has a limited distribution in Turkey (Thrace (Demirköy, Malkara, Keşan) and Mudurnu)
- *Q. petraea* subsp. *iberica* has pure and mixed stands in Marmara and the whole northern Anatolia (from 0 to 1300–1600 m)
- *Q. petraea* subsp. *pinnatifida* is located in East and Southwest Anatolia, mixed with other *Quercus* species, cedar and *Pinus nigra*.

- **Pedunculate oak (*Q. robur* L.)**

Pedunculate oak has two subspecies in Turkey:

- *Q. robur* subsp. *robur* has a wide distribution in Turkey including Northwest Anatolia, Thrace, Central Anatolia and South Anatolia. It has a good growth (height: 30 m; dbh: 1–2 m)
- *Q. robur* subsp. *pedunculiflora* occurs in East and Southeast Anatolia. It is found in the form of degraded coppice due to overgrazing and overexploitation.

- ***Q. hartwissiana* Steven. (syn: *Q. stranjensis* Turrill.)**

Strandja oak is found mainly in Thrace and northern Anatolia from 0 to 1300–1700 m, and locally in eastern Anatolia (Erzurum and Tunceli). It is found with other broadleaves in mixed forests of pine and fir. It also occurs in small pure stands.

- ***Quercus frainetto* Ten.**

This species is found in Thrace, Northwest Anatolia and Marmara regions reaching up to altitudes of 1000 m. It is found in mixed forests of beech, chestnut and oak. It is also found locally in Izmir (Kuşadası).

- ***Q. vulcanica* Boiss. Heldr. ex Kotschy**

Kasnak oak, an endemic species to Turkey, occurs at altitudes 1300–1800 m in Eğirdir (Yukarı Gökdere), Isparta (Şarkikaraağaç), Kütahya (Turkmen mountain), Konya (Sultan mountain), Afyon (Dereyaka yaylası). It is found in pure and mixed stands with Lebanon cedar, *Acer hyrcanum*, *Quercus cerris* and *Pinus*. A 1300 ha-area of kasnak oak forest has been designated as nature reserve, containing a 600-year old oak tree.

Other white oaks native to Turkey are:

- *Q. pontica* (eastern Black Sea)
- *Q. macranthera* (endemic to Turkey, occurs beginning from Mudurnu in western Black Sea to Erzurum in eastern Anatolia, at altitudes 1000–1900 m in mixed or pure stands)
- *Q. infectoria* Olivier (Aleppo oak) has a wide distribution mainly in the west, south, and southeast of Turkey. It also occurs in Marmara, Black Sea, and Central Anatolia regions. A pathologic formation of galls rich in tannic acid appear on Aleppo oak as a reaction to injuries caused by the egg-laying activities of a wasp (*Cynips gallae-tinctoriae* belonging to Hymenoptera: Cynipidae)
- *Q. virgiliana* Ten. is mainly found mixed with other oaks in dry areas of Thrace, western Black Sea (Bolu, Sinop), central Black Sea (Kastamonu, Sinop, Samsun, Çorum)
- *Q. pubescens* Willd. is mainly found mixed with other oaks in dry areas of Thrace, Marmara (Bursa), inner parts of western Black Sea (Bolu, Zonguldak, Sinop) and central Black Sea (Kastamonu, Sinop), Aegean (Afyon, Kütahya, Kuşadası, Manisa, Burdur) and Central Anatolia (Ankara, Kayseri, Sivas, Beyşehir).

Red oaks

Quercus libani, *Q. trojana*, *Q. cerris*, *Q. brantii* and *Q. ithaburensis* are native red oaks species in Turkey. *Q. cerris* is widespread in Turkey. It occurs in all regions, except for East and Northeast Anatolia, from sea level to altitudes of 1500 m (1900), mixed with other oaks (*Q. frainetto*, *Q. pubescens*, *Q. infectoria*, *Q. petraea*, *Q. libani*) and *Fagus*, *Carpinus*, *Castanea*, *Pinus nigra*, *P. brutia*, *P. pinea*, or in pure stands.

Evergreen oaks

Q. coccifera, *Q. ilex* and *Q. aucheri* are native green oak species in Turkey.

Current economic importance for the forestry sector

At present, conifer species are the primary species for industry. Oak is the most important firewood species in Turkey. Since *Fagus orientalis*, *Quercus petraea* and *Q. vulcanica* have high-quality timber, they are very important in veneering; they can be considered an important alternative to conifers.

Current silvicultural approaches

A great majority of forests in Turkey is natural or semi-natural. The general management policy is based on the close-to-nature approach. Clearcutting in large forest areas has been abandoned. Natural regeneration is the preferred method of regeneration. To guarantee successful regeneration, natural regeneration periods for the main forest tree species, except Turkish red pine (*Pinus brutia*), have been extended. At sites where natural regeneration is not possible, seed obtained from the nearest seed stand is used. The establishment and conservation of species diversity is assisted by the silvicultural practices. In all silvicultural practices, secondary or rare plant species found in association with the main forest tree species are protected. Some outstanding trees occurring in the site, which is subject to artificial regeneration, are also kept for conservation of locally adapted races.

The shelterwood method is applied for natural regeneration of beech forests in Turkey. Stand size is small for seeding cutting in regeneration plans. Replanting is usually suggested for understocked stands, which have less than 0.4 stand density. The situation of regeneration activities in beech and oak as of 1998 is given in Table 2.

Table 2. Regeneration status of beech and oak stands in Turkey (1998)

Species	Regeneration area (ha)	Regenerated area (ha)		Ongoing (ha)	Failed (ha)	No activity (ha)
		natural	artificial			
Beech	53 341	4522	6656	5418	273	36 473
Oak	7682	158	258	563	28	6675

Source: Department of Silviculture of General Directorate of Forestry of Turkey

Health state of the forest stands and threats to their genetic diversity

At present oak and beech forests in Turkey appear to be in good health, except for some local insect and fungi damage. But forests near industrial areas (especially the Marmara Region) are exposed to air pollution. Long-term effects of air pollution should be monitored through continuous observation.

Oak is the most damaged genus among forest trees owing to excessive firewood and animal fodder needs. A considerable portion of the population of Turkey still lives in rural areas close to forest or pasture lands, and they depend heavily on firewood for heating and cooking.

In general, anthropogenic factors such as agriculture, industrialization, urbanization, construction of highways and dams, illegal cutting, improper utilization of forest resources, fires and tourism play important roles in forest degradation. Particularly after the 1950s, these activities have increased the pressure on biological diversity that is beyond the carrying capacity of the lands. The most important consequences of these activities are the reduction and fragmentation of natural habitats. Also, with the development of 'nature tourism', untouched habitats are opened to local, national and international tourism.

Research activities and capacities related to genetic resources

Under the Ministry of Forestry (MOF), nine regional research institutes and two thematic research institutes conduct research in forestry. The Forest Tree Seeds and Tree Improvement Research Institute in Ankara carries out tree improvement, selection of gene conservation forests, seed stands and seed orchards. The Poplar and Fast-growing Forest Tree Species Research Institute in İzmit carries out tree genetic research, mainly on poplar and fast-growing species. Most of the regional research institutes have seed and tree improvement units.

There are two ongoing provenance trials for oriental beech carried out by the Eastern Black Sea Forest Research Institute in Trabzon and the Western Black Sea Forest Research Institute in Bolu.

In 1992, 15 provenances of sessile oak collected from different European countries were planted in seven locations of the northern part of Turkey in collaboration with the International Provenance Trials of Sessile Oak under the auspices of the Denmark Forest Research Institute.

Research is needed on population structure, seed orchard studies, and inheritance of special characteristics.

Current *in situ* gene conservation activities

Activities related to conservation of genetic diversity in forest areas are conducted by the Ministry of Forestry through programmes with various purposes and under different categories such as national parks, nature conservation areas, nature parks, natural monuments, seed stands and gene conservation forests.

To date, 32 national parks, 16 nature parks, 35 nature conservation areas and 56 nature monuments have been established and managed by the General Directorate of National Parks, Game and Wildlife, working directly under MOF. These areas are included in IUCN categories. Of these conservation programmes, nature conservation areas are considered the most suitable for *in situ* gene conservation purposes.

Table 3. Type, number and size of conservation areas in Turkey

Conservation type	Number	Total area (ha)	Forest area (ha)
National parks	32	630 246	298 923
Nature conservation areas	35	77 617	22 496
Nature parks	16	31 187	15 325
Nature monuments	56	1462	350
Protection forests	51	403 344	210 192
Forests under a special protection regime		3 185 684	3 185 684
Total		4 329 540	3 732 970

There are also other forest areas on steep slopes exposed to erosion. These are protection forests established under the Forest Law and managed by the General Directorate of Forestry (GDF) under MOF. They are not available for wood supply. These programmes are not

directly involved in *in situ* gene conservation but they could be, owing to their legislative status. Moreover a forest area of 3 185 684 ha is managed by GDF under a special regime, primarily for soil protection.

Gene conservation forests

In Turkey, in 1999, there were 163 registered gene conservation forests for 24 species with a total area of 23 408 ha, including a 3698 ha core area. Data for oak and beech are given in Table 4.

Table 4. Gene conservation forests for oak and beech in Turkey

Species	Number	Area (ha)	Core (ha)
<i>Fagus orientalis</i>	13	1893	471
<i>Quercus</i> sp.	10	1185	190

Seed stands

In Turkey, by 1999, there were 344 selected seed stands for 28 species with a total area of 46 345 ha, including a 19 572 ha core area. Data for oak and beech are given in Table 5.

Table 5. Seed stands for oak and beech in Turkey

Species	Number	Area (ha)	Core (ha)
<i>Fagus orientalis</i>	28	3697	1992
<i>Quercus</i> sp.	16	1629	899

Gene Management Zones (GMZ)

These are natural and semi-natural areas, protected with the purpose of maintaining the genetic diversity in target plant species. With the 'In situ Conservation of Plant Genetic Diversity in Turkey' Project, studies concerning the *in situ* gene conservation of important plant species have been started initially in selected sites in Kazdağı and Bolkar Mountains. Based on expected results of population biology and ecological studies, similar *in situ* conservation studies for the target species listed in the National Plan will be carried out in other places nationwide.

Current *ex situ* gene conservation activities

Currently there are 944 ha of seed orchards and clone banks of 24 species established in 149 locations. Currently, natural regeneration of the forest tree species remains the preferred method of regeneration and to date approximately 100 000 ha have been regenerated. Yet, not all woody species can be rapidly re-established following harvesting and problems still exist with fir, spruce and to some extent with beech.

Tree improvement

The Forest Tree Seeds and Tree Breeding Research Institute under MOF carries out tree improvement activities in Turkey.

Conifer species are in a leading position in tree breeding. Due to the large number of tree species and the limited resources for breeding, tree species have so far been divided into three categories based on the importance of the species in forestry and the intensity of breeding activities:

1. **Main autochthonous species:** they are the most economically important species for Turkey. Species in this category are *Pinus nigra*, *P. brutia*, *P. sylvestris*, *Cedrus libani*, and *Fagus orientalis*. All components of intensive breeding are applicable to this category.

2. **Autochthonous species of secondary importance:** the species in this category are treated with a lower level of selection and breeding intensity. This category consists of 28 species (some of them are endemic) including *Quercus robur*, *Q. petraea*, *Q. cerris* and *Q. frainetto*.
3. **Introduced species:** they cover poplar and fast-growing species, which are intensively studied and selected by the Poplar and Fast-growing Forest Tree Species Research Institute in İzmit.

Breeding zones for *Fagus orientalis*

1. Black Sea Coastal Region
 - 1.1 Middle (500–900 m)
 - 1.2 High (901–1300 m)
2. Black Sea Inland Region
 - 2.1 High (1100–1500 m)
3. Marmara Region excluding Thrace (seed production only)
 - 3.1 Low (0–500 m)
 - 3.2 Middle (501–1000 m)
 - 3.3 High (1001–1500 m)
4. Amanos Mountains Region (gene conservation only)
 - 4.1 High (1100–1500 m)

Use of reproductive material

Most of the seed used in afforestation and artificial regeneration activities is collected from natural seed stands. Records conveying the exact identity of the reproductive material used for planting and regeneration are kept from collection to planting. According to the seed source plan, the total annual seed demand for afforestation, artificial regeneration and erosion control is 341 875 kg for oak and 5243 kg for beech.

Existing beech stands produce enough seeds in all seed zones. The aim is to use improved seeds from seed orchards. Because of the difficulties in plus tree selection, progeny testing and grafting in beech, no seed orchards have been established for this species yet.

Institutions involved in genetic resources activities in Turkey

- Ministry of Forestry (General Directorate of National Parks, Game and Wildlife; General Directorate of Forestry; 11 Research Institutes)
- Ministry of Agriculture and Rural Affairs (General Directorate of Conservation and Control; General Directorate of Agricultural Research)
- Ministry of Environment (coordinating role)
- Turkish Scientific and Technical Research Agency (TUBITAK)
- Ministry of Culture (General Directorate of Protection of Culture and Natural Resources)
- municipalities and non-governmental organizations.

Priorities

There are many endemic or economically important plant species in Turkey. To set up GMZs for each of these species or a combination of these will be a costly programme, which cannot be funded with the present status of financial sources. Therefore, species with vital genetic

resources present in Turkey, globally and nationally important relatives of crops and forest tree species, or endangered rare plant species, have been listed as target species. *Fagus orientalis*, *Quercus aucheri*, *Q. hartwissiana* and *Q. vulcanica* are also included in the list. *In situ* gene conservation is focused on these target species. With accumulation of data and experiences new target species can be added to the *in situ* gene conservation programmes. The main priorities are as follows:

1. Establishment of GMZ;
2. Establishment of new gene conservation areas for target species;
3. Identification of the areas with important plant genetic resources and setting them aside as nature conservation areas or gene conservation forests as soon as possible;
4. Inventory of plant species and their densities in all protected lands;
5. Determining factors causing negative environmental changes in conservation areas;
6. Active participation and educational programmes for local people on *in situ* conservation;
7. Providing sustained political support and steady funding for effective *in situ* conservation of genetic resources of target plant species;
8. Professional and technical staff training;
9. Study of the reproductive biology, autoecology and density of target plant species in GMZs as well as outside GMZs;
10. Extensive information exchange;
11. Establishment of new seed orchards and clone banks for the other forest tree species;
12. Compiling information on existing provenance and progeny tests;
13. Legal and institutional strengthening;
14. Coordination of activities for implementation of the *National Plan for in situ Conservation of Plant Genetic Diversity in Turkey*;
15. Public awareness;
16. Establishment of Higher Board for Gene Conservation.

International collaboration

Topics for international collaboration are provenance trials, joint genetic research, establishment of databases, exchange of information, screening of genetic variation, long-term seed storage of beech and oak seeds, and strengthening of institutional capacity.

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Appendix I

Eight major forest areas with their dominant species and their ecological distribution are identified in Turkey:

1. Euxine–Colchis, *Fagus–Abies* forest area
 2. Mediterranean–Aegean forest area
 3. Mediterranean–South Anatolian forest area
 4. South Anatolian *Cedrus–Abies* mountain forest area
 5. Submediterranean *Pinus nigra* forest area
 6. Boreal *Picea orientalis–Abies nordmanniana–Pinus sylvestris* forest area
 7. East Anatolia *Quercus–Juniperus* forest area
 8. *Liquidambar orientalis* forest area
1. **Euxine–Colchis, *Fagus–Abies* forest area** extends along most of North Anatolia and the Black Sea Coast of Thrace (Istranca Mountains). On the slopes exposed to the Black Sea, dominantly mesophytic deciduous forest is formed in a belt from sea level up to 1200 m. The main species are beech (*Fagus orientalis*) often associated with several species of deciduous oaks (*Quercus dischorochensis*, *Q. pedunculiflora*, *Q. hartwissiana*, *Q. conferta*), hornbeam (*Carpinus betulus*), chestnut (*Castanea vesca*), maples (*Acer trautvetteri*, *A. planatoides*, *A. campestre*), alder (*Alnus glutinosa*, *A. barbata*—only in Northeast Anatolia), *Tilia rubra* and *T. tomentosa* (only in Northwest Anatolia). The following species may be considered characteristic for this belt: *Rhododendron ponticum*, *R. flavum*, *Prunus laurocerasus*, *Vaccinium aretos taphylos*, *Daphne pontica*, *Hedera colchica*, *Corylus avellana*, *Epimedium pubigerum*, *Ilex colchica*, *Frangula alnus*, *Helleborus orientalis*, *Lathyrus aureus*, *Staphylea pinnata*, *Trachystemen orientale*.
 2. **Mediterranean–Aegean forest** mainly consists of *Pinus brutia*, *P. pinea* and *Quercus aegilops* occurring as pure or mixed stands at lower altitudes (between 400 and 700 m). At higher altitudes they are replaced by a *Pinus nigra* forest. *Pinus pinea* is usually reported not to grow far inland. *Quercus aegilops*, which has a larger distribution area in Turkey than in other countries, appears as loose stands in all areas. Below 400 m along the coast and in depressions, maquis covers large areas. The influence of the sea penetrates deeply inland and makes it possible for maquis to spread widely. The leading woody species of the maquis include *Arbutus andrachne*, *Calycotome villosa*, *Cistus creticus*, *C. salviifolius*, *Cotinus coggygnia*, *Juniperus oxycedrus*, *Erica verticillata*, *E. arborea*, *Laurus nobilis*, *Olea europaea* var. *oleaster*, *Phillyrea latifolius*, *Pistachia lentiscus*, *P. terebinthus*, *Q. coccifera* (often a dominant species) and *Styrax officinalis*. In many places it is very obvious that the coniferous forest has been degraded by the influence of man and replaced by maquis.
 3. **Mediterranean–South Anatolian forest area:** *Pinus brutia* is the main forest tree species up to 1300 m on the southern slopes of the Taurus Mountains. Along the coast and up to 600–800 m maquis is dominant. *Pinus brutia* is seen occasionally in maquis, but after the maquis belt some deciduous trees and shrubs such as *Quercus infectoria* subsp. *boissieri*, *Cercis siliquastrum* and *Fraxinus ornus* subsp. *cilicica* associate with this pine species. It occurs in large pure stands above 1000 m. Above 1300 m it is replaced by *Cedrus libani*.
 4. **South Anatolian *Cedrus–Abies* mountain forest area:** large stands of *Cedrus libani* occur mostly from 1300 to 2000 m and even up to 2200 m. Its distribution extends from western Taurus to eastern Taurus. Besides its general distribution area, there are two local cedar forests in Turkey: one at Sultandagi (on the western border of Central Anatolia and Aegean Region) and the other at Erbaa in Çatalan (on the northern border of Central Anatolia and North Anatolia). The latter is very interesting from the

phytogeographical point of view. In its distribution area it forms pure or mixed stands with *Abies cilicica*, *Juniperus foetidissima* and *J. excelsa*, *Acer monspessulanum*, *A. hyrcanum*, *Fraxinus oxycarpa*. From the ecological point of view, *Cedrus libani* and *Abies cilicica* prefer southwest and west slopes exposed to the rain-bearing winds. Climbing over the ridges of the Taurus and moving inland, *Cedrus libani* and *Abies cilicica* disappear and are replaced by *Pinus nigra*.

5. **Submediterranean *Pinus nigra* forest area:** *Pinus nigra* var. *caramanica*, the Taurus pine, is autochthonous only in Anatolia, Thrace, Cyprus and Crimea. It forms pure or mixed natural forest over large areas in West, North and South Anatolia. In general these forests are found on the southern slopes of the mountains in North Anatolia, whereas they occur on the northern slopes of the Taurus mountains in South Anatolia, facing inland, and advance towards the steppe region, forming mixed stands with deciduous oak (*Quercus pubescens*) and *Juniperus* species (*J. oxycedrus*, *J. foetidissima*), *Pyrus eleagnifolius* and *Cistus laurifolius*. Further inland, *Pinus nigra* disappears and only wild pear (*Pyrus eleagnifolius*), *Quercus pubescens*, some *Juniperus* spp. and *Paliurus aculeatus* occur as the last remnants of forest before the steppe begins.
6. **Boreal *Picea orientalis*–*Abies nordmanniana*–*Pinus sylvestris* forest area:** *Picea orientalis* is one of the important trees in Turkey. It is distributed throughout the greater part of the Caucasus and Northeast Anatolia. In Turkey alone it forms about 200 000 ha of pure mixed natural forest. At high altitudes it sometimes forms pure stands over a considerable area; on the other hand, it is often accompanied by beech (*Fagus orientalis*), fir (*Abies nordmanniana*), and pine (*Pinus sylvestris*). The highest limit for oriental spruce forests is in northeast Turkey, at 2400 m in the Yalnızçam mountains. It ranges from sea level to 1800–2400 m in many districts but reaches its optimum development above 1200 m. The rarity of *Picea orientalis* in the lowlands of the area may be more connected with the influence of biotic factors than with climatic and edaphic factors. However, its general distribution is determined mainly by climatic factors. Where the climate is markedly dry, *Picea orientalis* diminishes. Its growth is restricted by deficient rainfall and low atmospheric humidity. The ground flora in the spruce forest is usually poor and consists largely of mosses, lichens, and liverworts. But where there is sufficient light and space, the ground is occupied by *Rhododendron* and *Vaccinium*.
7. **East Anatolia–*Quercus*–*Juniperus* forest area:** most of East Anatolia is covered by scrub, often dominated by Juniper and deciduous oaks. These remain from the old forests, which suffered much destruction by man. Leading species include *Juniperus excelsa*, *Quercus infectoria* subsp. *boissieri*, *Q. libani*, *Q. brantii*, *Q. pubescens*, *Acer monspessulanum* subsp. *cinerascens*, *Pistachia eurycarpa*, *P. khinjuk*, *Sorbus persica* etc. *Populus tremula* and *Betula eurycarpa* can be found in small groups or stands at high altitudes.
8. ***Liquidambar orientalis* forest area** occurs in southwest Anatolia in Muğla Province. Very recently some new areas were discovered in West Anatolia in Aydın (near Çine), in Burdur and Antalya Provinces. It covers the floodplain and is often associated with *Alnus orientalis*, *Platanus orientalis*, *Ulmus* spp., *Smilax excelsa*, *Periploca graea*, *Vitis vinifera*. Apart from its general distribution, it is recorded as growing by the Asi River near the south coast of Hatay Province. Many herbaceous species grow under this forest such as *Alisma plantago*, *Phragmites communis*, *Lysimachia vulgaris*, *Glycyrrhiza glabra*, etc. The total area of *Liquidambar* forest is about 6000 ha.

Endemics of forest tree species are scattered throughout Turkey, but are concentrated in several areas of phytogeographical regions. There are two native fir species in Turkey,

Cilican fir (*Abies cilicica*) and Caucasian fir (*Abies nordmanniana*), both of which are widely distributed. However, these species have unique subspecies that are endemic to Turkey. Because of their unique growth forms and their ability to survive in difficult environments, these subspecies represent valuable genetic resources for future breeding programmes. *A. cilicica* subsp. *isaurica* is found in South Anatolia, around Antalya and Konya-Bozkir; Uludag fir (*Abies nordmanniana* subsp. *bornmuelleriana*) grows in the north (around Samsun) and Mount Uludag; Kazdagi mountain fir (*Abies nordmanniana* subsp. *equitrojani*) grows on the Kazdagi Mountain.

In the western part of North Anatolia (Euro-Siberian Region, Euxine province), the leading species are *Fagus orientalis* and *Acer cappadocium*.

Broadleaved forests in Central Asia

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Owing to the aridity of the climate, the distribution of broadleaved forests in the five republics of Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan), is rather limited; they mostly occur in mountain areas. They are represented by small groves in areas where conditions are the most favourable (sites with sufficient rainfall and moderate air temperature). Plants forming these forests or participating in their development are typical mesophytes. Major tree species include oak, walnut, apple, maple and ash.

Oak

On the territories of the Republics of Central Asia, oak (*Quercus robur* L.) can be found in natural conditions only in the western part of **Kazakhstan**, where remnants of former deciduous forests are still conserved as small groves, coppices and brushwood, found on the slopes of river valleys (Ural, Ile, Emba and others), in floodplains and in river deltas. The total area of oak forest plantations amounts to about 1800 ha. In other Republics it was cultivated long ago and is rather widely used for gardening and in other types of forest plantations.

Walnut

Central Asia is one of the main centres of origin of walnut (*Juglans regia* L.). The greatest areas of walnut forest are concentrated in **Kyrgyzstan**, where they cover over 39 000 ha; they are distributed on the slopes of Fergana and Chatkal mountain chains. In spite of their value, the present condition of these forests is rather unsatisfactory. They suffer from uncontrolled grazing, resulting in reduction of their biological diversity and economical interest.

In **Uzbekistan**, forests with prevalence of walnut occupy about 4000 ha in the territories with the most favourable conditions on the slopes of Chatkal, Pskem, Ugam and Fergana mountain chains at altitudes 800–2300 m. Walnut is often mixed with other deciduous trees: apple, alycha (*Prunus vachuschtii*), maple, wild pear etc. The main areas of walnut forests are concentrated in Tashkent and Dzhizak regions. From the point of view of genetic resources conservation, the unique stands of Tashkent region (with a total area of about 1500 ha), formed by small groves of walnut are of special interest.

In **Tajikistan** walnut forests cover 6300 ha or 1.6% of the total forest area. They are distributed on mountain slopes of the Pamir–Alai system at altitudes 1300–2000 m and are characterized by a sharp fragmentation: small stands alternate with scrub or forestless spaces. The forests can grow in areas of difficult access, at sites receiving a great amount of rainfall, or at riversides and near springs. This high belt is formed by walnut mixed with ash or poplar, and the second belt is formed usually by *Acer turkestanicum* Pax and *Malus sieversii* (Lebed.) M. Roem.

Wild apple

Several wild apple species are found in the wild in Central Asia. *Malus sieversii* (Lebed.) M. Roem. and *M. kirghisorum* Al. et An. Theod. are the most widely distributed and the most important species. Wild apple grows in the belt of tree-brush vegetation in the mountains of Tarbagatay, Jungar Alatau, Tien-Shan, Pamir–Alai, at foothills and on the top belt of mountains. It participates in the formation of undergrowth in juniper forests, accompanies walnut and other deciduous and coniferous plants. It also forms pure stands at altitudes of 1000–2300 m, with an understorey of hawthorn, alycha, etc.

In **Kyrgyzstan** pure wild apple forests or forests including wild apple occupy 15 500 ha or 2.2% of the forest area. The main areas are concentrated in Fergana chain; *M. kirghisorum* Al. et An. Theod. is more widely distributed in southern Kyrgyzstan.

In **Kazakstan** wild apple occupies approximately 12 000 ha.

In **Tajikistan** wild apple plantations are less important; they occur after maple or walnut cutting, as a component of the upperstorey in broadleaved forests.

Maple

Maple forests in **Kyrgyzstan** occupy the second place among deciduous trees. In natural conditions two species grow here: *Acer turkestanicum* Pax and *A. Semenovii* Regel et Herd. The forests are formed mainly by *Acer turkestanicum* Pax, growing on an area of 26 600 ha. The main plantations are concentrated in southern Kyrgyzstan at altitudes 1100–2700 m, and about 70% are located on very steep slopes (21–40°).

Plantations with *Malus kirghisorum* Al. et An. Theod. are widely distributed in broadleaved forests in **Tajikistan**. Walnut often grows in association with maple in the understorey, and with *Juniperus seravschanica* Kom and *J. semidlobosa* Rdl along the upper tree limit of distribution. Maple plantations in Tadjikistan occupy about 44 000 ha, growing up to 1800–2000 m above the sea level.

In **Kazakstan** maple plantations, mainly of *A. Semenovii* Regel et Herd., are considerably less frequent. They occupy an area of about 2800 ha.

The area covered with maple forests in **Uzbekistan** amounts to 6400 ha. The main areas are in mountains of the Kashkadarya and Surkhan-Darya regions. Maple grows here together with juniper, walnut and other forest trees, but it can sometimes form small, rather dense pure stands at altitudes up to 2000–2500 m asl. The most widespread species in Uzbekistan are *Acer turkestanicum* Pax and *A. Semenovii* Regel et Herd. and, seldom, *A. rubescens* Franch.

In **Turkmenistan** maple plantations (*A. turcomanicum* Pojark ex Czerniak.) grow at Kopetdag, mainly in the zone of juniper stands. Maple does not form pure plantations. The total area of stands with maple amounts to about 6000 ha.

Ash

Ash (*Fraxinus* sp.) in Central Asia is distributed in forests along the rivers and on mountain slopes, in river valleys, plains and up to the mid-altitudes of mountains. The distribution of its plantations in the Republics is irregular.

In **Kazakstan** ash plantations (*F. sogdiana* Bge.) are distributed mainly in the south and in the valley of the Charyn river on an area of about 2000 ha. Ash plantations in Charyn are of the greatest interest, with ash the main forest-forming species. It grows here together with groves of *Populus ruinosa* Schrenk, rare trees of *Eleagnus* and numerous shrubs.

In **Kyrgyzstan** ash plantations have a limited distribution (400 ha), restricted to the southern part of the Republic in Chatkal and Fergana forest areas.

In **Tadjikistan** ash plantations (*F. sogdiana* Bge.) occupy small sites of 1–2 ha on the mountain chain of Peter First and on the southern slope of Gissar mountain chain at altitudes 100–1650 m, on riversides or near springs with constant and abundant humidity. *F. raibocarpa* Regel is also distributed here on small territories, in the low- and mid-altitude belts in Gissar mountain chains, low hills of southern Tadjikistan, etc.

In **Turkmenistan** ash grows in forests along the rivers and, where humidity is sufficient, on slopes and in gorges in the lower part of the mid-altitude of Kopetdag mountains.

In **Uzbekistan** ash grows mainly in mountains (Pskem, Zeravshan, Turkestan, Gissar mountain chains and others). *F. sogdiana* Bge. is the most widely distributed. *F. raibocarpa* Rgl is also distributed in the south, in Surkhan–Darya area. The area covered with ash forests amounts to about 470 000 ha. The greatest areas are concentrated in Tashkent and Dzhezak regions.

Plane

Plane forests (*Platanus orientalis* L.) can still be found in the mountains of the Pamir–Alai system, basically on the territory of **Tajikistan** (Zeravshan, Gissar, Dravaz, Karategin mountain chains). There are proofs of the presence of plantations in **Turkmenistan** (Kopetdag). They grow as narrow strips along mountain riversides at 1000–1300 m; they never grow on slopes without any irrigation. Plane also forms groves near springs. Other species cannot usually grow in such dense groves.

Persimmon

Persimmon plantations (*Diospyros lotus* L.) can be found as small stands (several hectares) at Pamir–Alai (Darvaz, Karategin mountain chains) within the territory of **Tajikistan**. They are distributed at 1200–1300 m at moist sites. Walnut, alycha and hawthorn grow together with this crop and on Darvaz chain, oriental plane (*Platanus orientalis* L.). Persimmon also grows in the south of **Uzbekistan** (on Tupolang and Sangardak riversides) as separate trees or small groups.

Conclusions and perspectives

Although the main territories of the Republics of Central Asia are characterized by arid conditions, broadleaved species requiring high moisture and moderate temperature are still represented quite significantly in the region.

The economic importance of these species for the forestry sector is rather high. They are integral components of the forest ecosystem, and consequently, of biosphere as a whole. Nut-bearing and fruit plantations are sources of valuable fruit. Broadleaved trees forests contain a rich diversity and are valuable sources of genetic material for forest tree breeding.

Numerous facts prove that in the past they occupied more extensive areas. The reduction of the distribution area of broadleaved forests is still ongoing, owing not only to natural conditions of Central Asia but also to anthropogenic factors. The equilibrium of the forest biogeocenose is broken; this threatens the existence of many plants and animals, leads to impoverishment of genepools, loss of useful properties of many species and, as a whole, decrease of functions of forests. After intensive cuts, broadleaved forests of Central Asia lose their ability to regenerate and disappear completely, and are replaced by shrubs.

Studies on *in situ* and *ex situ* conservation of broadleaved forests have been carried out, but insufficiently and irregularly in different regions. Mainly investigations on walnut and apple breeding have been carried out.

The territories under special State protection do not always cover areas of distribution of broadleaved forests and generally occupy small areas in Central Asia. There are only four reserves and one State Natural Park in Kyrgyzstan, i.e. 1.1% of the total area of the Republic. In Uzbekistan only 1.8% of the area is under strict or long-term protection (categories I and II of IUCN) and the strictest protection regime (reserves) concerns only 0.5% of the territory. The countries of Central Asia are distinguished by a great diversity of natural conditions, and, accordingly, great diversity of forest species, and it is therefore an absolute necessity to extend the network of protected areas.

Forestry organizations, botanical gardens and other botanical institutions, and some agricultural organizations are involved in activities related to forest genetic resources.

Needs for international cooperation on broadleaved species conservation are obvious. The geographical conditions of the countries of Central Asia are similar, and similar species grow in these countries. There is a need for coordination of activities at regional and international level, with other Asian countries and countries of southern and eastern Europe.

Overview of legislation and other regulations on oak and beech genetic resources

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The best way to preserve genetic diversity in the long term is to incorporate sensible actions into legislation and regulations. Already during the first meeting of the EUFORGEN Network on Social Broadleaves (France, October 1997) it turned out after a brief inquiry that many of the participating countries applied a different set of regulations concerning the conservation of Social Broadleaves in their own territories. It was decided that it would be useful for reasons of information exchange and improvement of the situation to compile a table containing all the different existing legislation, regulations, laws, rules and recommendations regarding oak and beech genetic resources in the participating countries. A synthesis was prepared on the basis of the country reports that have been presented during this first meeting. The synthesis was presented during the second meeting of the Network in the form of a table. It was further improved during the third meeting. The table was circulated among participants and they were asked to verify the given information.

To date, the overview covers the status of legislation and other regulations regarding oak and beech genetic resources in 27 European countries as of June 2000 (Table 1). During the next meetings of the Network the table will be updated according to the most recent information. Newly attending countries will also be added. After every meeting the updated table will replace the former one on the Web page of the Network (http://www.ipgri.cgiar.org/networks/euforgen/euf_home.htm). If at any time, a country wants to change some of the information currently provided, they should inform the EUFORGEN Secretariat in Rome (euf_secretariat@cgiar.org). Countries that are not yet included in the table could send their information directly to the Secretariat as well. Non-participating countries are also requested to send data on their current situation regarding the existing legislation and other regulations on oak and beech genetic resources in their country to the Secretariat.

Clarifications of the different possible situations (see Table 1)

The questions as well as the situations given in the table are set up in such a way that most of them could be answered with a simple 'yes' or 'no'. However, in one case they should be answered with 'N' (National), 'R' (Regional) or 'L' (Local) when it concerns the basis of the regulations, and in another case with 'r' (recommendations) or 'l' (legally binding regulations) when it concerns seed zones within one country.

(numbers 1–9 below refer to rows numbers in the tables)

- 1, 2 and 3 Countries participating in the OECD Scheme on Forest Reproductive Material and member countries of the European Union are of course implementing the given legislation and regulations. However, in some cases countries that are not a EU Member State nor participate in the OECD Scheme could still follow these regulations in their national laws. They could of course also implement another type of legislation regarding Forest Reproductive Material.
- 4 The questions about the Regulations on genetic resources are specified at the level of:
 - the species—oak and beech (red lists for instance);
 - the individual tree level (in some countries certain individual trees are protected);
 - the area or habitat level (which is more an indirect protection). This occurs mostly in Forest Reserves or Nature Conservation Areas;
 - the protection of genetic material within a certain area against undesired pollen or seed transfers (introgression) from planted trees of alien sources in the vicinity of these areas.
- 5 Sometimes certain regulations cannot be met if the desired material from a certain source is not mentioned in one of the categories of the OECD Scheme on Forest Reproductive Material or the EU Directive EEC 66/404. In these cases some countries have possibilities for the use of material with less stringent requirements by way of exception. This could be regulated for example in a Derogation.
- 6 Some countries are divided in two or more seed zones. Transport of material from one zone to another within the same country is subjected to either legally binding regulations or just non-legally binding recommendations.
- 7 When incentives such as subsidies, tax reduction facilities, or any other stimulus promote the use of certain genetic resources, this could be indicated here.
- 8 Regulations could be based on different levels of public services. These could be at the 'National', 'Regional' or 'Local' level.
- 9 This last question refers to the ratification (yes/no) of the Convention on Biological Diversity (CBD) held in Rio de Janeiro in 1992 by the participating country. So far, without any exception, all 27 countries have ratified and implement the CBD.

Table 1. Overview of legislation and other regulations on genetic resources of oak and beech (status June 2000)

Table 1: Overview of legislation and other regulations on genetic resources of oak and beech (status June 2009)		AUT	BEL	BGR	HRV	CZE	DNK	FIN	FRA	DEU	HUN	IRL	ITA	LTU	LUX	MDA	NLD	NOR	PRT	RUS	SVK	SVN	SPA	SWE	CHE	TUR	UKR	GBR	UZB	
1	Country participates or follows OECD Scheme on Forest Reproductive Material—yes (y)/no (n)	y	y	n	y	y	y	y	y	y	y	y	y	y	y	n	y	y	y	y	y	y	y	y	y	y	n	y	y	
2	Country follows EU Directives and implements them in national legislation—yes (y)/no (n)	y	y	n	y	y	y	y	y	y	y	y	y	y	y	y	y	n	y	y	n	y	y	y	n	nt	y	y	n	
3	Country implements national legislation different from the OECD Scheme and the EU Directives—yes (y)/no (n)	y	n	y	n	y	n	n	n	n	n	n	n	y	n	y	n	n	y	n	y	y	y	y	y	n	y	n	n	y
4	Regulations on genetic resources—yes (y)/no (n)																									y				
	—protection at tree species level																									y				
	oak	n	n	y	y	y	y	n	y	n	n	n	y	y	n	n	n	n		y	y	y	n	n	n	y	y	n	n	
	beech	n	n	y	y	y	y	n	y	n	n	n	y	n	n	n	n	n		y	y	y	n	n	n	y	y	n	n	
	—protection at individual tree level	y	n	y	y	y	y	y	n	y	y	n	y	y	y	y	n	y		y	n	y	y	n	n	y	y	n	y	
	—protection at area/habitat level	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	
	—planting in vicinity of protected areas allowed	y	n	n	y	y	n	y	y	y	y	y	y	y	y		y	y	y	y	n	y	y	y	y			y	y	
5	Are there exemptions to regulations (derogations)—yes (y)/no (n)	y	n	y	y		n	n	y	y	n	y		n	y	n	y	y		n	y	y	n	y	n	n	y	y	n	
6	Recommendations (r) or legally-binding regulations (l) related to seed zones:																													
	—general	–	r	r	?	l	–	l	r	r	r	–	l	–	–	–	–	–	l	l	r	l/r	l	r	–	r	l	r	r	
	—oak	–	–	r	?	r	r	–	r	r	r	–	l	l	–	r	–	–	–	l	l	–	l	–	r	r	l	r	r	
	—beech	–	–	r	?	r	r	–	r	r	r	–	l	–	–	r	–	–	–	l	l	–	l	–	l	r	l	r	r	
7	Are incentives applied in relation to genetic resources of oak and/or beech—yes (y)/no (n)	n	y	y		y		n	y	n	y	n	n	n	y	y	n	n	y	y	y	y	y	y	y	y	n	n	n	
8	Are existing regulations based on national (N), regional (R) or local (L) level?	N	N/R	N	N	N	N	N	N	N/R	N	N	N/R	N	N	N	N	N	N	N	N/R/L	N	N	N	N/R	N	N	N	N	N
9	Country ratified and implements the Convention on Biological Diversity—yes (y)/no (n)	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	y	

[†]Since Turkey is an EU candidate, the process for harmonization of the Turkish legislation with the EU legislation is ongoing.

Technical presentations on gene conservation and management of European white oaks

Genetic processes promoting genetic diversity in oak and beech forest

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Introduction

The objective of this contribution is to outline the features and characteristics of genetic processes that may modify and shape genetic diversity, especially in oak and beech. We will concentrate on the following topics that need to be addressed for conservation purposes:

- spatial level at which processes are acting: range vs. stand level
- type of genetic diversity that may be modified by the processes: neutral vs. adaptive
- time span at which the processes have a significant impact: several generations vs. one generation.

Most of these processes are well described in most textbooks of population or quantitative genetics. We will in each case recall the theoretical background, summarize experimental results available in oaks and beeches and evaluate the potential impact on genetic diversity.

Direct processes

Population size, neighbourhood size and genetic drift

Theoretical background

The population size N is an estimation of the number of individuals contributing to the next generation. The higher the population size, the less the population is prone to loss of genes due to genetic drift. The definition of N requires several assumptions, namely (i) that individuals mate at random with equal contribution of each breeding individual; (ii) that the number of progeny per parent follows a binomial distribution; (iii) that population size remains constant at each generation; (iv) that generations are discrete and not overlapping. In natural populations, these assumptions are quite often violated, thereby leading to the concept of effective population size N_e . N_e is the population size of a theoretical population fitting to the above-mentioned assumptions that would provide the same probability that two genes taken at random would come from the same individual as observed in the real population (inbreeding effective population size). N_e can be estimated by demographic data or neutral gene marker data. Different methods have been proposed in the literature, based either on linkage disequilibrium (Hill 1981), allelic distribution (Chakraborty and Neel 1989), or temporal changes of allelic frequencies (Waples 1989). Although estimates of N_e based on these assumptions may be of interest for comparisons with results of other species, they are hardly applicable to beech and oaks. Firstly, relevant demographic and genetic data are not available, and second the aforementioned definitions are restricted to a single population isolated from neighbouring populations. Wright (1969) proposed a continuous spatial model in which a neighbourhood population size is calculated. The 'neighbourhood' is an area in a

continuous forest from which the parents located in the central part may be drawn at random. The size of a neighbourhood depends upon the variance σ^2 of the parent-offspring dispersion distance. Considering a two-dimension space, if dispersal distances are normally distributed with equal variances along the two directions σ^2 , the neighbourhood is a circle of radius 2σ and has an area of A and 86.5% of the parents will lie within the neighbourhood.

$$A = 4\pi\sigma^2$$

(1)

This general definition has then been further extended to the case of monoecious dispersing genes by pollen and seed (Levin and Kester 1968). For outcrossing species like oaks and beeches, if the standard deviation of parent-offspring dispersion distances is σ_p^2 for pollen and σ_s^2 for seed, then A becomes:

$$A = 4\pi(\sigma_p^2/2 + \sigma_s^2)$$

(2)

Experimental results

There is scarce information about dispersal variances that could be used to provide approximated estimates of population parameters. The following estimates are derived either from experimental data obtained in gene diversity surveys, or from pollen dispersion studies made in oaks and in beech.

- Le Corre *et al.* (1998) provided estimates of σ^2 that were inferred from the spatial distribution of gene frequencies in *Quercus petraea*. The basic assumption of the calculation is that the spatial population differences are the result of a balance between migration, drift and mutation. Using a geostatistical method (Le Corre *et al.* 1998), the following estimates of 84, 93, 116 and 148 m were obtained for four alleles (*Aap4*, *Aa6*, *Dia2*, and *Pgi3*). By using formula (1) and a mean estimate of 110 m for the dispersion variance, a mean estimate of 15.20 ha is obtained for A for *Q. petraea*. This estimate should be seen as an overall mean value over several generations, because it derives from today's population differentiation that resulted from a balance between different evolutionary forces over several generations.
- Streiff (1998) and Streiff *et al.* (1999) have estimated pollen dispersion using parentage analysis that was implemented in an adult oak stand. They concluded on a standard deviation for dispersion distance of 175 m for *Q. robur* and 133 m for *Q. petraea*; these are extremely crude estimates based on strong hypothesis over long-distance dispersal (Streiff 1998). The resulting estimates for neighbourhood areas for *Q. petraea* and *Q. robur* are, respectively, 19.32 and 12.01 ha, using formula (2) and assuming that seed dispersal can be ignored when compared to pollen dispersal. In comparison to the previous estimate derived from Le Corre's study, the estimate obtained from the parentage analysis is an 'instantaneous' estimate corresponding to only two successive generations. Interestingly the two estimates amount to similar values.

Since the neighbourhood area varies between 12 and 20 ha, corresponding neighbourhood sizes vary between 1200 and 4000 trees, assuming that the average density of an adult (reaching the stage of seed cut before natural regeneration) stand varies between 100 and 200 trees/ha. Assuming that the neighbourhood sizes correspond to the effective population sizes, a straightforward expectation from these estimations is the erosion of gene diversity due to drift.

A large study of the spatial distribution of genetic variability at the within-population level confirmed limited structuring at the microscale level (Leonardi and Menozzi 1996). An autocorrelation study of 11 enzyme loci in 14 populations over the Italian biogeographical range for the species found significant spatial structuring for only 11.5% of all genotypes. No correlation between the amount of spatial structuring and environmental (latitude, longitude, altitude), structural (mean and standard deviation of tree size), and genetic characteristics (mean expected heterozygosity, mean F_{is}) of the population was found. No significant differences seem to exist among loci if low heterozygosity loci are excluded from the analysis.

In beech some information on this topic can be obtained by autocorrelation studies. An autocorrelation study of 11 isoenzyme loci in 14 populations over the Italian biogeographical range for the species found significant spatial structuring for only 11.5% of all genotypes. No correlation between the amount of spatial structuring and environmental (latitude, longitude, altitude), structural (mean and standard deviation of tree size), and genetic characteristics (mean expected heterozygosity, mean F_{is}) of the population was found. No significant differences seem to exist among loci if low heterozygosity loci are excluded from the analysis (Leonardi and Menozzi 1996).

Impact on genetic diversity

For a neutral marker, in the case of an isolated population the expected decrease of gene diversity per generation (H_t/H_{t-1} , where H refers to Nei's gene diversity and the subscript t to generation t) amounts to (Hartl and Clark 1989):

$\frac{H_t}{H_{t-1}} = (1 - \frac{1}{2N_e})$	(3)
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In other words, less than 5×10^{-4} of diversity is eroded by drift alone, ignoring diversity that may be regenerated by either mutation or migration.

For a quantitative trait, the rate of decay of the additive genetic variation V per generation is of similar amount, ignoring selection and mutation (Franklin 1980):

$\frac{V_t}{V_{t-1}} = (1 - \frac{1}{2N_e})$	(4)
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Assuming an adaptive trait subjected to weak stabilizing selection, as it may occur for most traits in natural conditions, the decay resulting (for traits of heritability values varying in the range of 0.3–0.6) is in the order of 10^{-3} (Lande 1980, Lande and Barrowclough 1987), close to the rate due to drift. The rate is even lower if heritability is lower as is usually the case in forest trees (Cornelius 1994). However losses due to drift and stabilizing selection are largely compensated by the rate of spontaneous mutations that accumulate in the different loci that contribute to a quantitative trait. Most experimental results that were obtained on short generation species show that the increase in genetic variation due to mutations is about 10^{-3} of the environmental variation (e.g. 3 to 9×10^{-3} of the additive genetic variance for traits with heritabilities varying between 0.1 and 0.3) (Lynch 1988, Houlié *et al.* 1996). Based on this reasoning, "a population with N_e larger than 500 can maintain nearly as much genetic variance in typical quantitative characters as an infinitely large population" (Lande and Barrowclough 1987).

Hybridization

Experimental results

Interspecific hybridization is a long recorded fact in oaks. Evidence of natural hybridization comes from observations made in natural populations and in controlled crosses (Ruhston 1993). Our interest here is the evaluation of the impact of hybridization on genetic diversity. If different species exchange genes at equal rates, then hybridization contributes to the sharing of genetic diversity among the different interfertile partners. However if there is an unbalanced exchange of genes, then species may benefit differently from hybridization. Recent data (summarized in Table 1) originating from mating system analysis and gene flow studies in a mixed *Q. petraea* and *Q. robur* stand, indicate that there might be an asymmetric exchange of genes.

Table 1. Comparison of inter- (t_b) and intra- (t_w) specific outcrossing rates in *Q. petraea* and *Q. robur* growing in a mixed stand

Molecular markers	<i>Q. robur</i>			<i>Q. petraea</i>			Reference
	S^+	t_w	t_b	S^+	t_w	t_b^+	
Isoenzymes	0.05	0.63	0.32	0	1.20	-0.20	Bacilieri <i>et al.</i> 1996
Microsatellites	0.03	0.94	0.03	0.01	0.89	0.11	Streiff 1998

⁺Selfing rate

⁺Negative values of hybridization rates indicate that *Q. petraea* was most likely pollinated by extreme *Q. petraea*-like trees.

These results obtained under natural pollination can be compared with those obtained in controlled crosses (Table 2). Combined results obtained over a three-year crossing campaign at the Forest Research Institute in Escherode (Lower Saxony, Germany) show that the success of the cross "*petraea* pollen to *robur* ovule" is much higher than the reciprocal cross (Kleinschmit and Kleinschmit 2000).

Table 2. Rates (in percentage) of intra- and interspecific crossing success in controlled crosses of oaks (Kleinschmit and Kleinschmit 2000)

Female species	<i>Q. petraea</i>	<i>Q. robur</i>
Male species		
<i>Q. petraea</i>	16.5	12.5
<i>Q. robur</i>	5.2	19.7

Both observations in natural conditions and results of controlled crosses support the conclusion that hybridization is likely to be preferentially unidirectional. If hybrids are fertile, and the asymmetry persists over successive generations, then there will be an increase of introgressed forms in the species of the pollen donor parent (*Q. petraea*), which should result in more diversity in *Q. petraea* than in *Q. robur*.

Impact on genetic diversity

For neutral markers, results stemming from different studies seem to indicate that *Q. petraea* tends to exhibit a slightly higher diversity than *Q. robur*, although the differences are quite small.

The impact of unidirectional gene flow on **adaptive traits** should be similar. However the trend may be counterbalanced by selection. There are only scarce results published that do compare the level of variation of quantitative traits with that of supposedly neutral molecular markers. Leaf morphological traits have been quite often compared among the two species *Q. petraea* and *Q. robur*. A larger morphological variation has been reported for *Q. petraea* (Dupouey and Badeau 1993, Kleinschmit *et al.* 1995, Bacilieri *et al.* 1995). No information exists for other traits.

Table 3. Comparison of gene diversity statistics between *Q. petraea* and *Q. robur*

Gene diversity statistic (number of loci and markers)	<i>Q. petraea</i>	<i>Q. robur</i>	References
H_e (13 isoenzymes)	0.219	0.213	Müller-Starck <i>et al.</i> 1993 Muller-Starck and Ziehe 1991
A (13, isoenzymes)	3.1	3.2	Müller-Starck <i>et al.</i> 1993 Muller-Starck and Ziehe 1991
H_o (13, isoenzymes)	0.222	0.184	Zanetto <i>et al.</i> 1994
H_e (13, isoenzymes)	0.245	0.252	Zanetto <i>et al.</i> 1994
A (13, isoenzymes)	2.73	2.70	Zanetto <i>et al.</i> 1994
H_e (9, SCARs)	0.389	0.391	Bodénès <i>et al.</i> 1997

H_o : within-population observed heterozygosity; H_e : within-population expected heterozygosity

A: within-population mean number of alleles

Intraspecific gene flow

Theoretical background

Gene flow is basically understood as gene migration between different populations, although it may as well include gene exchange between trees in a single population. Different theoretical schemes have been proposed to evaluate the impact of gene flow on the subdivision of genetic diversity to within- and between-population diversity. The most general is the island model of Wright (1969) in which a set of populations having the same population sizes (N) over successive generations exchange genes with the same rate m between all pairs of populations. A somewhat more realistic model is the stepping stone model (Kimura and Weiss 1964) where only neighbouring populations exchange genes at the same rate. More recently the concept of metapopulations was introduced, which includes the possibility of extinctions of populations. All these models refer to a set of populations that occupy a given limited spatial range. The expansion of the natural range has recently been investigated by considering colonization (Le Corre and Kremer 1998, Whitlock and Mc Cauley 1990). Because oaks and beeches occupy large areas since more than 10 000 years, with only limited expansion at the edges of the natural distribution, the spatial restricted models (island and stepping stone) may be more relevant than the others, although the situation may evolve if one considers the vegetation changes due to global climatic changes. These models predict that only a limited amount of gene flow among populations ($N_m > 1$, N_m being the number of genes migrating between populations) is necessary to achieve genetic homogenization among populations at equilibrium between the different evolutionary forces (migration, drift and mutation), as is shown by the general relationship between population differentiation and the number of migrants.

$G_{ST} = \frac{1}{1 + 4N_m}$	(5)
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The general island model has also been extended to uniparentally inherited genes, as for example chloroplast genes (Petit *et al.* 1993b, Birky *et al.* 1989). The equilibrium value of G_{ST} a haploid gene is (Birky1989):

$G_{ST} = \frac{1}{1 + 2N_m}$	(6)
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This extension allows the separation of gene flow for plants into its two components (pollen and seed) and to estimate the proportion of both components in the total gene flow in the frame of the island model (Ennos 1994, El Mousadik and Petit 1996).

$\frac{m_p}{m_s} = \frac{2 \left(\frac{1}{G_{STc}} - 1 \right) - \left(\frac{1}{G_{STn}} - 1 \right)}{\left(1 - \frac{1}{G_{STc}} \right)}$	(7)
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Where m_p and m_s are, respectively, the pollen and seed migration rates and G_{STc} and G_{STn} are the differentiation coefficients for cytoplasmic and nuclear genes.

Experimental results

Gene flow can be assessed in two different ways. In the indirect way we infer gene flow rates from information on population differentiation, based on a number of assumptions related to the theoretical model used (*island*, *stepping stone* etc.). The strongest assumption is that the estimate of the number of migrants is made at equilibrium between different evolutionary forces. This may take a few hundred generations, a time unlikely to have elapsed for extant oak and beech populations.

In the direct way pollen and seed dispersion are estimated by using markers. Originally, physical dispersion was investigated with tracing methods or dyes (Wright 1952). However these methods provided only measures of physical dispersion, ignoring post-migration pollen or seed viability. Recently the availability of hypervariable genetic markers such as microsatellites made it possible to estimate the effective dispersion distance, by assessing parentage relationships between offspring and parent trees.

Indirect estimates

Several range-wide gene diversity surveys were conducted, that provided estimates of population differentiation (G_{ST}). Assuming that differentiation is at equilibrium, inferences can be drawn to estimate the mean number of migrating genes among populations; the data were compiled separately for nuclear genes (Table 4) and for chloroplast genes (Table 5).

Table 4. Estimation of the total (by pollen and seed) number of migrating genes (N_m) between populations

Species	Geographic range	Marker	N_1	N_2	G_{ST}	N_m	References
<i>Q. petraea</i>	Whole range	isoenzymes	7	13	0.032	7.6	Zanetto <i>et al.</i> 1994
<i>Q. robur</i>	Whole range	isoenzymes	7	13	0.024	10.2	Zanetto <i>et al.</i> 1994
<i>Q. petraea</i>	Whole range	isoenzymes	81	13	0.025	9.7	Zanetto and Kremer 1995
<i>Q. petraea</i>	Western part	isoenzymes	32	11	0.023	10.6	Kremer <i>et al.</i> 1991
<i>Q. petraea</i>	Whole range	isoenzymes	21	8	0.027	9.0	Le Corre <i>et al.</i> 1997
<i>Q. petraea</i>	Whole range	RAPD	21	31	0.024	10.2	Le Corre <i>et al.</i> 1997

N_1 : number of populations analysed; N_2 : number of gene loci

Extensive gene flow among populations is clearly revealed by these data. In the frame of the island model, these results show that, on average, and over the whole range, populations behave as if they would exchange about 10 nuclear genes per generation in all pairwise associations. The second important result is the asymmetry between pollen and seed flow. The ratio of pollen to seed migration rates deduced from equation 7, varies from 200 to 300, which is higher than any estimate reported so far (Ennos 1994, El Mousadik and Petit 1996).

Table 5. Estimation of the number of seed-migrating genes (N_m) among populations

Species	Geographic range	N ₁	G _{ST}	N _m	References
<i>Q. petraea</i>	Western range	58	0.905	0.052	Petit <i>et al.</i> 1993a
<i>Q. robur</i>	Western range	22	0.925	0.041	Petit <i>et al.</i> 1993a
<i>Q. pubescens</i>	Western range	81	0.769	0.150	Petit <i>et al.</i> 1993a
<i>Q. petraea</i>	South of France	51	0.829	0.103	Dumolin-Lapègue <i>et al.</i> 1999
<i>Q. robur</i>	South of France	142	0.782	0.140	Dumolin-Lapègue <i>et al.</i> 1999
<i>Q. pubescens</i>	South of France	142	0.907	0.051	Dumolin-Lapègue <i>et al.</i> 1999
Eight European white oak species	Whole range	345	0.828	0.104	Dumolin-Lapègue <i>et al.</i> 1997
<i>F. sylvatica</i>	Whole range	74	0.83	0.102	Demesure <i>et al.</i> 1996

Direct estimates

Experimental studies using parentage analysis between offspring and mature trees have recently been used in oak species to investigate gene flow (Dow and Ashley 1996). The same method has been applied in a mixed *Q. robur*-*Q. petraea* stand located in the west of France (Streiff *et al.* 1999). The area of the study stand was 5.76 ha and it was located in the central part of a larger forest that covers approximately 700 ha. The study stand comprised 296 adult trees in equal proportions between the two species. 984 acorns were collected from 13 identified mother trees in the study stand, and their corresponding male parent was identified by paternity analysis. For 7 of the 13 maternal progeny arrays sampled, an excess of nearby matings and a preferential direction of pollination was found. For the remaining progeny arrays, no departure from random distribution of male parents was detected. A common trend among all families was a high percentage (averaging 65% for *Quercus robur* and 69% for *Q. petraea*) of offspring that were pollinated by male parents from outside the study site. By pooling the data over all families, the average pollen dispersal curve within the stand was inferred and fitted to a negative exponential distribution. This model extrapolated for distances over the spatial scale of the study stand was insufficient to explain the high level of gene flow detected by the paternity analysis, suggesting substantial level of long-distance pollination events. These results suggest that the total distribution of mating events according to distance is likely to result from the combination of two functions, one reflecting preferential mating with neighbouring trees due to local pollen dispersion, and a second one due to pollen transport over longer distance.

In beech only preliminary results are available. Paternity analysis of a set of open-pollinated sibships carried out using RAPD markers in a northern Apennines population allowed a preliminary estimate of pollen effective migration (Troggio *et al.* 1996). The mean average distance between sibships (mother plant) and potential father trees (31 m) was larger than the mean distance (29 m) from incompatible ones.

Impact on gene diversity

For neutral markers, gene flow is a strong genetic homogenizing force that tends to decrease population differentiation among populations, as in shown in Table 4. Since oaks and beeches are gregarious species that occupy almost continuous ranges, gene flow is quite efficient in maintaining low population differentiation.

At the stand level, pollen flow may be seen also as a force that erases any spatial genetic structure that may build up due to limited seed flow, preventing also from correlated matings that may increase the inbreeding level of the population. Since acorns migrate at short distances, one would expect that the repetition of natural regeneration would contribute to increase the possibility of matings between related individuals and install a strong spatial genetic structure at the stand level, and subsequently the inbreeding level of the population. Spatial analyses of genetic diversity at the stand level have indicated that this is not the case (Bacilieri *et al.* 1994, Streiff *et al.* 1998). Only adult trees that are separated by

less than 50 m tend to be genetically related, and this is only the case in *Q. petraea*. The lack of any strong spatial genetic structure is the result of the important pollen flow, including long-distance flow.

The impact of gene flow on **adaptive traits** is less obvious. The existence of important provenance differences detected in provenance tests suggest that gene flow, even if it is extremely important in oaks, is not sufficient to homogenize populations. This is clearly shown by the level of population differentiation for quantitative traits (Q_{ST}), that was calculated in provenance tests and that varies in the range of 0.40–0.60 (Kremer *et al.* 1997a). Q_{ST} values exceed for most traits assessed in provenance tests the G_{ST} values (Table 4) that were observed for molecular markers (Kremer *et al.* 1997a, Kremer *et al.* 2000). These conclusions should however be taken with caution, since Q_{ST} values are only available for those traits that were actually assessed in provenance tests (in general growth, flushing and form). Different results may be obtained with other traits not yet investigated.

Selection

Theoretical background and experimental results

There is a large body of theoretical work on the impact of natural selection on the gene frequencies in natural populations, whether the form of selection is directional, disruptive or stabilizing. At a single locus, even slight differences in fitness values can have dramatic consequences on the fixation of alleles. The rate of evolution of quantitative traits can also be extremely rapid, when subjected to selection. However, there are only limited experimental results available in oaks:

- Provenance tests of oak and beech that were established in various European countries all indicate important differences between provenances, which can be attributed to selection effects (Kremer and Muhs 1996, Kremer *et al.* 1993 for reviews). Several of the traits assessed show clear geographic trends, as bud burst and growth cessation, whereas others (growth and form) do not follow geographic patterns.
- There is some indication that oaks can respond quite rapidly to selection pressures, as is witnessed by the adaptation of exotic oak species in Europe. Northern red oak (*Q. rubra* L.) is a widely used species in Europe, whose natural distribution is the eastern United States and Canada. The introduction of the species is well documented, and started at the end of the 18th century. Only a few generations elapsed since the introduction. However the comparison of introduced and natural populations (Daubree and Kremer 1993, Ducousso *et al.* 1997) raised in comparative tests, has shown that introduced populations have systematically shifted from the natural ones. The changes were not only visible on genetic markers but also on adaptive traits such as bud burst and leaf coloration. As a result, in less than 10 generations introduced populations tended to become European landraces.

Impact on genetic diversity

The impact of selection on gene diversity is only relevant to adaptive traits since, since most gene markers behave as neutral genes. Selection is generally seen as a mechanism decreasing genetic diversity. The decline of diversity depends on the form and intensity of selection. As there is a lack of experimental data about these two features, one can only advocate general theoretical speculations. Most theoretical models predict that stabilizing, disruptive and directional selection decrease additive genetic variance (Roff 1997). However, in real situations referring to oak and beech forests, the decrease of genetic variation due to selection may be less than expected for a number of different reasons:

- The mutational variance on multilocus adaptive traits may generate enough variation to compensate (Lynch 1988).
- Gene flow, especially pollen dispersion over long distance may continuously restore

variation that is expected to decrease under the sole mutation-selection balance.

- Important ecological site variation within a given forest would result in some ecotype differentiation and can actually be seen as a cause of maintaining genetic diversity at the scale of a forest population.

Interaction between processes

Genetic processes that shape genetic diversity act as interactive processes contributing to the evolutionary history of populations. The quaternary history of oak and beech forest has recently been investigated by comparing both genetic data and information of fossil deposits (Huntley and Birks 1983, Dumolin-Lapègue *et al.* 1997, Demesure *et al.* 1996). This retrospective analysis contributed to the understanding of evolutionary processes on genetic diversity. Current human interferences in oak and beech forest is expected to modify as well diversity and it is the objective in this contribution to outline the human activities that are likely to decrease or restore diversity.

Evolutionary history of oak and beech forest

The evolutionary history since the last glaciation is now well understood in oaks. Based on pollen fossil data, oaks were restricted during the glacial periods in three main southern European refugia (Spain, Italy and the Balkans). Since the glacial periods lasted for more than 100 000 years and the refugia were partially isolated, it is likely that oaks were genetically differentiated between the three. This is clearly supported by the differences in chloroplast genes that can still be observed today (Dumolin-Lapègue *et al.* 1997). But it was probably the case for nuclear genes as well. Then as the climate warmed up, oaks migrated northwards. Did this history have an impact on the today's genetic diversity? The answer is quite different according to the type of trait, either gene markers or adaptive traits.

For **gene markers** including nuclear markers, there is clear longitudinal trend of variation as indicated for isoenzymes and RAPDs (Le Corre *et al.* 1997, Zanetto and Kremer 1995). Although the differences are small between populations (see G_{ST} values in Table 4), these differences follow a clear geographic trend.

For **phenotypic traits**, the evolutionary scenario resulted in different patterns on today's variability. It can be reconstructed in three successive steps. (i) As the recolonization began, oaks migrated northwards and quite often by long and rare dispersal of seed. Foundation effects that increased the differentiation between populations characterized this mechanism. (ii) As oaks progressively occupied the central and northern part of Europe, pollen flow established communication between stands originating from eastern and western refugia. Pollen migration increased as stands from different origins merged, and reduced the initial nuclear differentiation between the genepools of the different refugia. (iii) Finally local selection pressures acting on the established populations contributed to their genetic differentiation, which constantly increased with time. New patterns of differentiation were installed that are totally different from those left after colonization. As a result, today's pattern of geographic diversity of adaptive traits is in no way a consequence of the separation of oaks in three main refugia (Kremer *et al.* 1997b).

The picture beech is much less clear. Fossil pollen data indicate a major refuge area in the Carpathians and one in southern Italy. A vast (140 stands) survey of allozyme variability (Comps *et al.* 1990) revealed a larger variability in southern populations with a probable refugium in the Balkan peninsula. Chloroplast DNA data indicate that beeches recolonizing Europe from Southern Sicily seem to have been stopped by the very rapid progress of those coming from the Balkan peninsula (Demesure *et al.* 1996). Regional studies revealed the local details of the recolonization events. In eastern Europe Vysny *et al.* (1995) investigated the local migration pathways. In Italy the values of a synthetic multivariate statistic from Sicily to the Alps showed a gradient strikingly similar to the sequence of dates (obtained from

palynological records) tracking the post-glacial recolonization of the Italian peninsula (Leonardi and Menozzi 1995).

Human interferences

Oaks are among the forest species that have the longest domestication history. Introduction of silviculture is thought to have started as early as the 12th century in some oaks of the Loire River in France. Several human operations may have strong impacts on genetic diversity:

- Population transfer: long-distance vs. short-distance transfer and natural vs. artificial regeneration
- Silvicultural regimes: high forest vs. coppice regime.

There are no detailed experimental results on this topic. The whole field of the impact of silviculture and management regimes still needs to be investigated.

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***In situ* conservation**

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Introduction

In situ gene conservation is a dynamic approach that exploits the evolutionary processes in the natural habitat of a species. Its aims are to maintain populations of a species on their natural site and to safeguard their adaptability to changing environmental conditions over many generations. *In situ* gene conservation of forest trees may take place in both unmanaged and managed forests.

'Passive' conservation is possible in protected areas such as national parks and nature reserves. National and nature parks, nature reserves and wilderness areas often represent strictly protected areas of forest (no exploitation). Suitable oak stands protected by this means may be regarded as 'genetic resources'. The prerequisite, however, is the accessibility of the genetic resource (e.g. by means of seed harvesting). Managed forest areas selected for the purpose of conservation of genetic resources are usually embraced by the term "gene reserve". Seed production areas (seed stands) may be regarded as *in situ* gene conservation units even if their selection is based rather on economic and silvicultural than on genetic considerations. Corresponding to this, a well-balanced *in situ* gene conservation programme usually includes different kinds of gene conservation units complementing each other.

'Active' conservation is thus implemented in managed forests by adopting specific silvicultural measures to promote gene conservation, and in forests with a special function for gene conservation (e.g. 'gene reserves').

Silviculture

Silvicultural treatments can influence the genetic structures of populations (e.g. Ledig 1992). Thus, meeting the aims of gene conservation requires a suitable form of silviculture. The key aspects of *in situ* conservation are described below.

Regeneration

Regeneration is of crucial importance for gene conservation. Usually **natural regeneration** is preferable (Rotach 1994). Especially in mast years the large number of acorns produced represents an enormous pool of diversity and an important potential for adaptation. Ideally, the relatively rare mast years should be used for the enlargement of regeneration areas. Care should be taken to include many mother trees in order to increase the number of trees involved in seeding. The best possible environmental conditions for germination and survival of the seeds should be provided. The regeneration method using successive felling in different cuts (preparatory, seeding and final cut) is a flexible and suitable instrument in this regard. Furthermore, it can be adapted to the requirements of different oak species. If necessary, site preparation, game regulation or protection (e.g. fencing) has to be provided in order to guarantee the success of natural regeneration (Matić 1996). The regeneration of the stands should be conducted in a continuous way considering small regeneration areas and long regeneration periods.

Successful natural regeneration requires experienced foresters. Training and incentive of the forest service is essential to implementing *in situ* gene conservation.

Artificial regeneration is a silvicultural measure used for *in situ* gene conservation only as an exception. However, various situations justify the use of artificial regeneration: first, the insufficient natural regeneration due to poor or irregular seed production, damages caused by mice, game and concurrent growth of other vegetation (weeds); second, the enlargement of existing oak stands without a sufficient number of seed trees. Regardless of the different methods of sowing and planting, it is important that the number of plants constituting the artificial fraction of the founding stand be as great as possible (Müller-Starck 1996 loc. cit., p. 269). Only reproductive material of the local origin is allowed.

Thinnings

Tending and thinning usually aim at increasing stand stability and promoting economically superior phenotypes. Advanced growth (wolf trees) and ill-shaped individuals are removed as a kind of negative selection during the young growth and thicket stage. This may result in a concomitant genetic selection and potentially compromises the objectives of conservation of genetic diversity. In specific gene conservation stands, efforts should be made to conserve all phenotypes regardless of their economic value (see section below on "Other zones"). Unintentional changes of genetic structures due to thinning operations are of less concern in later stages of the life cycle of a stand (from polewood stage on).

Special silvicultural systems (e.g. coppice with standards)

Over hundreds of years, oak forests were of particular economic interest in large areas of Europe. Accordingly, special types of management, such as coppice with standards and coppice forestry, have been developed.

In coppices with standards a certain proportion of the trees originate from sexual reproduction (standards). Besides vegetative reproduction, there is probably enough natural regeneration through seeds to guarantee the onward transmission of genetic information to the descendants. In managed coppice with standards forests, regeneration processes are far less dynamic than in high forests. Nevertheless, they may be regarded as adequate for genetic conservation. An even higher quality of genetic conservation, however, can be achieved through transition to high forest.

Coppice forests are unsuitable for dynamic *in situ* gene conservation because of their lack of sexual reproduction.

***In situ* gene conservation areas**

Gene reserves

The following considerations mainly refer to forest areas specifically selected and managed for gene conservation purposes: the 'gene reserves'.

Requirements

Forest ownership

The establishment of gene reserves should be promoted wherever forests are identified as being of particular genetic interest. Preference should be given to public-owned forests (commune, state etc.), where long-term integrity of populations and access to genetic resources is most easily safeguarded.

Different zones

In order to meet the various requirements for gene conservation in gene reserves, different zones may be distinguished.

- **Core zone.** The core zone represents the main area of a gene reserve and contains the target population.
- **Buffer zone.** Stands of unknown or undesirable origin may exist in the surroundings of gene reserves. Creating a buffer zone can minimize gene flow from such stands into the core zone. The origin of the populations within the buffer zone is identical to the origin of the core zone population. Alternatively, the buffer zone may contain other species that do not hybridize with the target species. As a recommendation the width of this zone should be at least 500 m.
- **Other zones.** Establishing special protection zones can minimize the risk of strong selection due to management operations. The danger of particular genotypes disappearing through the systematic removal of advanced growth ('wolf trees') and ill-shaped individuals is especially great in the young growth and thicket stages. The establishment of a special zone in which inferior phenotypes are not removed can avoid this.

It is not mandatory to declare the various zones of a gene reserve. In some cases they are not necessary, in others they can be "tacitly" integrated in the total area of a reserve (e.g. buffer zones).

Size

Due to the lack of sound scientific information, the estimation of the minimum size of a core population of a gene reserve remains unsure. Considering the importance of a continuous regeneration process for the conservation of genetic resources (see above, 'Regeneration'), the estimated minimum area for a suitable management unit is approximately 20 ha. This size is assumed to be large enough to avoid random modification of the genetic structure of a population (genetic drift) and allow evolutionary changes of the original population. A single gene reserve can be composed of several gene conservation stands as long as the total size corresponds to the required minimum area of 20 ha. Smaller areas are acceptable (minimum 10 ha), if the specific situation allows the enlargement of the target population by artificial regeneration with local material.

Management and use

Gene reserves must be managed in order to maintain and promote genetic diversity. The following guidelines apply:

- The forest owner and/or manager is required to maintain the area and density of the oak population or to extend it.
- The introduction of reproductive material from outside is forbidden.
- Natural regeneration is given priority. In exceptional cases, artificial regeneration is permissible as long as the material originates from the same gene reserve.
- The recommendations for the regeneration are the same as for regularly managed forests (see section on 'Regeneration' above). Strong selective cuttings and other operations potentially reducing genetic variation must be avoided.

Registration

The declaration of genetic resources is an important aspect of the management of genetic resources. It records:

- The description of the genetic resources according to a standard form;
- The safeguard of the genetic resource, e.g. by a contract, an entry in the land register or any other suitable means.

The authorities responsible at national level keep a register of the genetic resources of oaks.

Selection of gene reserves

Different criteria must be taken into consideration for the selection of suitable sites for gene reserves. Some important points are listed below, though no claim is made that the list is either complete or definitive.

- **Distribution area.** Biogeographical regions can be defined on the basis of information on temperature, precipitation, soil conditions and plant communities. Due to different processes of natural selection these regions potentially indicate genetic differentiation within the natural distribution of a species. Typical oak stands representing biogeographical regions should therefore be considered as possible sites for gene reserves.
- **Plant sociology.** The site has an influence on the genetic structure of a population because of the natural process of adaptation through selection. Accordingly, phytosociological units can be employed as indicators for potential gene reserves. Describing 'typical' and 'special' occurrences of a species in terms of plant sociology makes it possible to include a wide spectrum of genetic variation in a network of gene reserves.
- **Site conditions.** Specific site conditions can have selective effects on oak populations. Thus, spatial changes in the site index or soil characteristics can be regarded as factors for promoting genetic diversity (Kremer and Menozzi, this volume, pp. 31–42) and therefore indicate interesting conservation sites. In general, gene reserves should be located on sites on which oaks are naturally strongly competitive.
- **Origin.** The conservation of autochthonous populations (of local origin) should have priority. Nevertheless, the conservation of well-adapted allochthonous populations ('landraces') can be also of interest and should not be excluded (Daubree and Kremer 1993). The origin of the basic material should be determined either by historical evidence or other appropriate means.
- **Isolation.** Gene flow in general may be considered as a mechanism for maintaining and enhancing genetic diversity. However, gene flow from stands of unknown or undesirable origin should be avoided in order to preserve the local character of the respective population. Gene flow from undesirable pollen sources can be reduced through the creation of a buffer zone, consideration of large conservation areas and/or isolated topographical units (e.g. valleys).
- **Mixed/pure stands.** Hybridization with other species is a mechanism for maintaining and enhancing genetic diversity (Kremer *et al.* 1998, loc. cit. p. 142). Thus, interspecific gene flow represents a valuable potential for increasing adaptability. Mixed stands of two or more interfertile oak species of local origin are at least as valuable as 'pure' stands and should be considered by any means for the registration of genetic resources (Finkeldey, in press).

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***Ex situ* conservation**

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Objectives

Ex situ conservation of genetic resources entails removal of individuals or reproductive material from their original environment. With forest trees, the term covers three different types of methods: (1) plantations (stands) that are either established to serve the purpose of gene conservation from the beginning, or converted for that purpose although originally intended for another use; (2) collections of trees including clone collections (clonal archives), seed orchards and arboreta; and (3) genebanks storing seed lots, pollen or tissue.

Planted *ex situ* gene conservation stands or populations of white oaks can be maintained complementarily to *in situ* conservation activities but are particularly important when *in situ* methods cannot be applied or are less suitable or less efficient. They become very useful when it is desirable to develop tree stands for increased timber production or to serve human needs for other products and benefits, but at the same time adaptation close to the natural conditions in the forest must be secured. This type of gene conservation provides for fast adaptation of a population to its environment, by combining natural processes with human management. Conservation of genetic resources in designated *ex situ* populations is, therefore, a dynamic approach.

Ex situ conservation methods can also be static in that they maintain a fixed genetic structure without any influence of evolutionary forces. This is often the case with collections of clones and seed and genebanks.

No gene conservation unit should be established without a clear objective in mind (Eriksson *et al.* 1993). All activities such as collecting, establishment of a plantation, its management and regeneration should follow the main objective, taking natural conditions and available institutional/financial resources into consideration. In general terms, the main aims for *ex situ* gene conservation stands will thus be to maintain and enhance the future adaptability of the populations combined with their enhanced value for human use.

The vegetative reproduction methods are recommended only for establishing *ex situ* gene collections, clonal archives or seed orchards. Using seeds or seedlings for *ex situ* gene conservation stands might ensure both higher genetic diversity for the conservation and less expensive establishment.

The relatively high genetic diversity of European white oaks should be preserved first of all by *in situ* methods. *Ex situ* conservation methods should be additionally used within the conservation strategy to ensure the preservation of rare or valuable genotypes, populations or taxa.

Main methods for *ex situ* conservation of genetic resources

***Ex situ* gene conservation populations**

Conservation of endangered populations

Within the distribution area of white oaks, valuable autochthonous populations (provenances) are currently surrounded by artificially established or strongly managed forests of non-local origin. The adaptive genetic variability of the local stands may not be conserved in the offspring resulting from mating events in case of natural regeneration (pollen gene flow). Sometimes the natural regeneration of these valuable stands is impossible

because of the changed environmental conditions (e.g. groundwater level decreasing through river regulations). To avoid these risks and to eliminate detrimental effects on genetic variation, a solution for the conservation of genetic resources will be to establish new populations at another location either using acorns or seedlings, if seeds are available.

Conservation of known genetic variation

Forest geneticists have established trials at multiple sites testing provenances, families or clones. Provenance trials have often been planted both within and outside the natural distribution area of the species and involve many countries. These trials are planted according to specific experimental designs with replications and the origin or family identity of each individual is known. The genetic variation of individuals belonging to the experimental populations has been characterized. Their value as genetic resources may depend on the number of populations included in a trial, the number of individuals per population, and the geographic coverage of the distribution area. At least for the first generation and as long as individual identity is kept on record, some of the research trials should be maintained as gene conservation units. Depending on the management and regeneration methods, the trials might continue to serve as genetic resources, but the occurrence of provenance hybrids in future generations must be taken into consideration. Due to the effects of natural and artificial selection, they may develop into local 'landraces', with known genetic structures. This characterized genetic variability can then be utilized for specific purposes.

Breeding and genetic resources conservation

Breeding tests are artificial populations established on the basis of specific selection criteria. Their main purpose is to produce afforestation material for generating future forest stands with specific adaptive properties and qualities beneficial for human use. Breeding populations are considered to have the largest possibilities for direct influence on the population genetic diversity through their initial composition, management and regeneration. The most targeted management of genetic diversity occurs in tree breeding programmes structured into multiple populations under different selection conditions.

A system of multiple populations was proposed by Namkoong (1976, 1984, 1986) to integrate tree breeding and gene conservation. According to this system, a large gene resource population is split into several smaller populations. The gene resource populations should be sampled to capture maximum genetic variability among populations, while maintaining a large genetic variation within the populations. In the most elaborate form, the populations should be established over a broad array of sites, thus sampling a wide range of environmental conditions. In managed populations, artificial selection is carried out to improve the adaptedness of each population and to increase genetic variability among them. The populations can also be left unmanaged. They can be established directly from natural populations and be actively selected and then either managed or unmanaged. Each population can be kept relatively small.

A set of multiple populations for joint breeding and gene conservation purposes may initially capture a large portion of the existing genetic adaptedness in the species. Natural and artificial selection will allow for a dynamic development both to changed environmental conditions and to human needs. A set of populations initially established in different ways and managed differently may thus be beneficial to match different breeding objectives.

Existing examples of the multiple population approach

A multiple gene conservation strategy has been adopted in forestry practice for *Quercus suber* in Portugal (Varela and Eriksson 1995). The plan involves both small, managed natural populations and large, managed and unmanaged natural populations covering most important environmental and management types. This approach will ensure that both the evolutionary and breeding objectives of gene conservation, as well as the conservation of accompanying species, are taken into account.

Changes in genetic structure

In dynamic conservation of genetic resources, changes in the genetic structure of populations occur during the adaptive process, which is intended as part of the conservation strategy. However, in some cases, such as evacuation or rescue of genetic material, the original genetic structures need to be maintained and adaptation to new site conditions may not be desirable. In fact, it might not be possible to achieve this purpose with *ex situ* populations, and it may be more appropriate to establish clonal collections in which seed can be produced through controlled crosses. Artificial regeneration by sowing or planting can then be carried out at the original sites, if required.

Sampling

The sampling of material to be conserved in *ex situ* populations depends on the conservation objective. Within a region of provenance, attention should be given to sampling of genetic variation both between and within stands. It is recommended that sampling 5-20 stands within a region of provenance would provide a very good and representative sample. Seeds should be collected preferably in a year with abundant seed crop and the seed-bearing trees should be well-distributed in the stand, at least 50 m apart. When a single stand (population) is the basic target unit of conservation, it is advisable to collect seeds or seedlings from a sample of a minimum of 100 trees.

When tree breeding and gene conservation purposes are combined, the specific breeding strategy will determine the number of genotypes and the type of genetic diversity needed in each population. In converted experimental populations, the original genetic composition is fixed. It can be changed by thinning based on directed, genotypic selection to obtain a more directed diversity, depending on the specific objective of the gene conservation unit.

Establishment

The establishment of an *ex situ* gene conservation stand or population can be carried out by sowing or planting. Direct sowing in the field will allow for adaptation to the new environment from the very beginning. The same can be achieved by planting at narrow spacing (Hattemer 1995). Both methods will require early thinning. Nursery treatment and planting methods for the establishment of *ex situ* gene conservation populations should follow local standards. The size of plantations will again depend on the objective of the conservation unit. If the aim is to conserve genetic resources in a single region of provenance, then 2 or 3 plantations of sizes 2–10 ha each are suggested.

During the establishment a randomized design should be applied. If possible, the identity (origin and position within the plot) of the planted trees (genotypes) should be kept on record. This is important if controlled crosses are to be made in the future. The design of combined breeding and gene conservation units will depend mainly on the specific breeding strategy.

Management

As it is important to secure the physical stability of the stand, careful tending is advisable. This should be done through the normal silvicultural practices used in the respective area. Thinning should be applied, initially systematic and later selective. Thinning should take into consideration the specific experimental design used in *ex situ* gene conservation populations, with individual identity kept if possible, and the overall objective of the gene conservation unit.

Regeneration

Gene conservation stands and populations established in *ex situ* conditions can be regenerated both naturally and artificially. Natural regeneration should be used, if applicable, and if maintenance of specific useful diversity is not the main objective. However, standard regeneration of an *ex situ* population will be by artificial means. The principles for selecting and harvesting trees should be similar to those used for the first generation. Equal amounts of plants should be used from each parent tree in order to keep a high effective population size.

General remarks

Ex situ gene conservation populations can belong to a variety of types: progeny test plantations from seed lots originating from one or several stands; populations for evacuation purposes; breeding populations in a multiple population system; converted field experiments or breeding tests. The latter type may be thinned provenance, family or clonal trials and also abandoned seedling seed or clonal orchards. The potential risks associated with the occurrence of provenance hybrids in future generations of trials should be carefully considered.

Ex situ conservation of genetic resources may be carried out at different levels of complexity. In its simplest form it means the establishment of a forest plantation based on the target genetic resource population, which was selected and designated for this purpose. The plantation can be managed as a traditional forest plantation to develop into a mature stand. It can be regenerated naturally or by reforestation. If regular interventions are needed to meet specific objectives, then the management becomes more complex and expensive. Such types of gene conservation stands will therefore be preferred as an alternative *ex situ* conservation method for species with high economic value (provenances of Spessart, Slavonia etc.) or rare, endangered oak taxa (ecotypes) such as *Quercus frainetto*, *Q. virgiliana*, etc.

Ex situ conservation of genetic resources in seed orchards and clone collections

Objectives

Genetic resources of white oaks can be conserved *ex situ* through the establishment of seed orchards and clone collections. These types of gene conservation units will be applied whenever the *in situ* and the other *ex situ* conservation methods described in these Guidelines are not successful or are very difficult to implement.

Seed orchards may be based on the material from vegetatively propagated trees (clonal seed orchards) or from sexually propagated trees (seedling seed orchards). Seed orchards established specifically for, or fulfilling gene conservation purposes, produce seeds of valuable autochthonous or non-autochthonous populations as well as single trees worthwhile to conserve owing to (i) their endangered status (including heavily air-polluted areas or areas affected by extensive human activities), (ii) insufficient fructification in their natural environment, (iii) unwelcome pollination in their natural environment or (iv) the need to produce seeds of good quality over longer periods of time than the lifespan of the basic material. If the number of clones from a certain population is not sufficient for the establishment of a gene conservation seed orchard, or only rare genotypes or single trees should be conserved, then the genetic information of these individuals can be conserved in clone collections for a longer time than the lifespan of the basic material. Additionally, the establishment of gene conservation seed orchards or clone collections is a necessary regeneration measure to complement the storage of pollen in genebanks.

The seedling seed orchards should also represent the genetic diversity of the population, region of provenance or taxon preserved. A special form of seedling seed orchards may be the pasture forest, which was traditionally planted and managed in many parts of Central Europe. The wide spacing of single trees and the purpose of acorn production for cattle were the main criteria to establish such pasture forests. This type of forests has both conserved rare genotypes or taxa for several centuries and ensured reproductive materials (acorn).

Establishment and utilization of seedling seed orchards are recommended in case of genotypes, populations or taxa that have problems with clonal regeneration. At the same time, it would be useful to maintain a minimal number of acorn-bearing individuals within the natural area (e.g. *Quercus frainetto*, *Q. virgiliana*, etc.).

Changes in genetic structure

The possible genetic implications of seed production in seed orchards were described elsewhere. According to Hattemer *et al.* (1982), there is a strong relationship between the

number of clones or single tree progenies included and the probability of allele loss. The genetic structure of progenies in a seed orchard may also be influenced by the reproductive phenology and capacity of individual clones to produce flowers.

It is recommended to include the highest possible number of clones in a gene conservation seed orchard. Loss of genetic information caused by insufficient pollination or self-pollination can further be minimized by suitable seed orchard design, as well as careful consideration of the flowering intensity and the distribution of flowering clones before seed collection. If the infrastructure is available, changes of genetic structure between the original stands, the clones used and the seeds produced in a seed orchard should be evaluated by genetic studies at marker gene loci, in order to assess the genetic implications of the conservation practices on the material conserved or produced.

Genetic comparisons of seed orchards with basic material

Studies comparing the genetic structure of Norway spruce seed orchards with the genetic structure of the natural stands where the clones or single trees were sampled are quite rare. In a study carried out at isoenzyme gene loci (Bergmann and Ruetz 1991), no substantial differences were found with regard to gene frequencies and genetic diversity between a seed orchard and the originally sampled Norway spruce populations. The differences were greater for heterozygosity. Unfortunately, there are no comparisons of the genetic structure of the seed orchard progenies.

Concerning the sex types of oaks, there are no typical male and female genotypes (unfixed sexual dimorphism). Within a population the number of bisexual genotypes is usually determinant, but there are genotypes bearing dominantly either male or female catkins, simultaneously (Bordács 1994, 1997). The presence of trees with insufficient flowering capability (sterile genotypes) can be also observed. For the practical purposes of seed production, the female and bisexual types should be preferred when establishing seed orchards. The sexual character and the blossoming capability of each individual seem to be significantly stable, independent from the annual cycles (Bordács 1997, Kanazashi and Kanazashi 1997). Phenological differences should be also considered. Within a population, three to seven weeks differences can be observed in bud bursting and blossoming depending on climatic parameters of each year (Gračan 1993, Bordács 1994). In a wide range comparison of provenances, the differences in bud bursting might be considerable (Ducosso *et al.* 1996, Deans and Harvey 1996, Kremer *et al.* 1997). Early flushing is more dominant in the southern regions of provenance and the populations in higher altitudes (Kremer *et al.* 1997). For the purposes of gene preservation all phenotypes, including the extremely early and late flushing (super *praecox* and super *tardiflora*), should be preserved and used in the seed orchards.

Legal regulations

In most European countries, the production and movement of oaks reproductive material is regulated by national legislation. Therefore, the establishment of clone collections and seed orchards should always be carried out according to the respective regulations.

Collecting and propagation

In order to avoid loss of genetic information in a seed orchard or clone collection, the number of clones or single tree progenies should be as high as possible and should in any case exceed

50. If the size of a valuable relic population that needs to be conserved is not large enough to achieve the minimum sufficient number of clones, then the establishment of a clone collection should have priority. If there are different autochthonous relic populations in one provenance region, clones from these populations should be collected and put together in one gene conservation seed orchard, in order to achieve the minimum sufficient number of clones.

If the designated population has a sufficient size, trees should be sampled at random. In order to avoid the selection of trees related to each other, a minimum distance of 50 m should be kept between the selected trees. For the establishment of seed orchards it is recommended to pre-select seed-bearing trees within the populations. The seed-bearing capability of the preserved genotypes is one of the determinative characters to ensure sufficient crop production in the seed orchards.

For the establishment of clone collections and clonal seed orchards, vegetative material collected from old trees (plus trees) is normally used. Taking into account local climatic peculiarities, the scions should be collected by tree climbers during winter (from December to March) in those parts of the crown capable of flowering. The number of scions per clone depends on the seed orchard design and on the number of orchards to be established. Generally, 10–20 normal scions/trees might be enough for grafting. During tree climbing, damage to the trees should be avoided by using sophisticated rope-climbing techniques and careful climbing.

In most cases, grafting of white oak species is complicated. The most common method, split bark or side grafting, can be used. It can be carried out in a greenhouse where the scions will be grafted on 2-year-old rootstock potted the previous year. It is recommended that rootstocks be originated from the same stands/region of provenance than the selected trees to minimize graft incompatibility. Collecting and grafting should then be organized simultaneously, so that storage of the scions is not necessary. Grafting can also be done on seedlings in the field. In this case, the scions should be covered with plastic film in cold storage at 0°C, until the rootstock has been prepared in the right condition for grafting. If no cold storage facilities are available, the scions may be stored on ice in a cellar. If scions are collected in winter at very low temperatures, they can be stored at temperatures as low as –10 to –20°C. The best results will be achieved if the scions are grafted on rootstock just before flushing. In most cases, despite a perfectly conducted grafting, the success rate of the grafting is very low (0–20%). A 50% or higher success rate should be accepted as sufficient result.

The success of grafting can be increased by using moderate soil heating (25–30°C) for 1–2 weeks during the bud-bursting period. The green budding or grafting carried out in June–July can also be successful in the case of some genotypes, which cannot be propagated by common grafting methods. Combining different grafting (budding) methods might be recommended considering the genetic (phenological) characters of each individual preserved to ensure higher success rate in vegetative reproduction.

If very old oak trees need be conserved, vegetative propagation by grafting may be difficult. In this case, if the number of single trees is sufficient, the establishment of seedling seed orchards can be a suitable alternative. For the establishment of seedling seed orchards, the progenies of the single trees are to be collected, handled, stored and raised separately.

For seedling seed orchards, acorns or seedlings collected from the natural offspring of the productive seed-bearing trees should be used in order to expect a sufficient quantity of crop in the seed orchard. If possible, only the productive, non-sterile genotypes should be selected for seed orchards. The lifted seedlings should be transplanted for at least one year before the establishment of seedling seed orchard.

Establishment, management and tending

Seed orchards should be at least 500 m away from the closest white oak trees or stands to reduce unwelcome pollination from outside sources (the unwelcome pollen gene flow can

never be completely excluded but it might be minimized.). Additionally, planting border trees or increasing the seed orchard size can minimize the possibility of pollen contamination. To ensure sufficient flowering and seed production, the site should be exposed south to southwest on medium to moderately wet soils. The management of the seed orchard may be facilitated if the site is accessible by machines.

The aim of the clonal or seedling seed orchard design should be to maximize the outcrossing rate and to minimize inbreeding. Therefore, each clone should have every other clone as a neighbour. At the same time, the distance between individuals of the same clone should be maximized. Giertych (1975), Nester (1994), Schmitz (1995) and others have described different methods for the mixture of clones in seed orchards. The different methods described might also be used for oak seed orchards. Finally, due and early consideration of future schematic thinning will help to avoid any possible disruption in the distribution scheme.

Each clone or single tree progeny should be represented by an equal number of individuals. Graftings or seedlings which reached the age of 2–5 years and a height of 0.4–2.0 m might be planted in a pattern of 8×8 m or larger. If necessary, the initial space of 64 m^2 per individual can then be extended to 128 m^2 , by selective thinning of trees. The spacing used might also be different depending on the actual oak taxon or provenance, site conditions or silvicultural tradition. A minimum of 60 m^2 spacing is advised because of the considerable demand of oaks for light.

Because of the high pressure of game on forest vegetation, the oak seed orchards should be protected and fenced. Fences should also protect the acorns produced. During the first years, normal weeding including replanting is recommended. After the establishment of the plants, weeding is required only once a year. Owing to the value of the material conserved, special attention should be paid to fertilization and plant protection. Before the canopy is closed, a selective thinning should be carried out to ensure the flowering of lower branches.

The plant protection of seed orchards should be focused on the prevention of abundant occurrence of acorn galls (e.g. *Andricus* species) and weevils (*Balaninus* species). Generally, the prevention period using chemicals should be from the end of the blossoming period (April–May depending on the latitudinal location) to late summer (August). Rodents (mice, voles) can cause damages in autumn by collecting acorns. The clonal archives should only be protected by chemicals in case of strong infection by insects or fungi.

Seed collection in gene conservation seed orchards

Sufficient flowering and seed production in a seed orchard can be expected 10–20 years after the establishment, at the earliest. To prevent unnecessary losses of genetic information, the flowering and seed production in gene conservation seed orchards should be carefully observed prior to seed collection. Seed collection should only be conducted after a regularly distributed flowering, both male and female, has been observed on a minimum of 70% of the clones or single tree progenies. The smaller the number of clones or single tree progenies, the more carefully the question of regular flowering and seed production has to be considered.

To ensure the broadest possible genetic diversity, all individuals of a clone or single tree progeny bearing sufficient seed crop should be harvested. Collecting acorns only from individuals with abundant seed crop should be avoided. Selection based on acorn size might cause loss of genetic diversity, therefore all sizes of acorns should be collected. Within the seed lot, each clone or each single tree progeny must be represented with the same amount of acorns.

Seed collection can be carried out by collecting both on the branches of young trees, and under the older trees. Most acorns infected by weevils can be successfully used for direct sowing (nursery use or afforestation), therefore the floating of acorns should be carried out only if the acorns are stored or marketed.

Ex situ conservation of genetic resources in genebanks

Approach

Genebanks can be viewed as a complementary method to both *in situ* and *ex situ* gene conservation. Irrespective of whether the materials for *ex situ* gene conservation are seeds, pollen, tissues or clonally-propagated material, they conserve genes in a static way, and except for the changes due to genotype-dependent differences in mortality (loss of viability), there are no other causes for changes in the genetic structures during storage.

It should be noted that the methods of long-term conservation of white oaks' seed (acorn) and tissues are not yet developed enough to ensure economical and safe gene conservation. A relatively high genetic diversity of European white oak populations was reported by genetic studies (Kremer *et al.* 1997). The traditional *in situ* and *ex situ* preservation methods (*in situ* and *ex situ* stands, seed orchards, clonal archives, etc.) might be more economical and effective to preserve the genetic resources of white oaks.

Objectives

The principal objective of *ex situ* conservation in genebanks is to ensure availability of genetic material at different localities and periods of time. In case of need, genebanks can also serve as a source of forest reproductive material with high quality genetic properties. Other objectives of genebanks include the establishment (regeneration) of gene conservation units and the provision of genetic material for breeding and evaluation programmes later on.

Requirements

Genebanks can conserve any type of forest reproductive material (seeds, pollen, tissues, vegetative parts of plants). The different types of material represent different options regarding storage technologies, time period and required number of stored samples.

A significant aspect is the hierarchical organization of a genebank. Genebanks can store samples mixed at the population (stand) level, i.e. bulk samples, or individual samples. In both cases, the number of individuals included should be defined according to the main principles of a gene conservation strategy. It should be noted that larger seed lots and sampling modes which enable keeping parts of a seed lot separate will be required in case of genetically heterogeneous populations. The amounts and organization of the stored samples should reflect stated conservation objectives, which will help determine whether samples covering the most representative genetic variation should be included, or whether sampling should be aimed at the capture of rare alleles. Sampling of populations should ideally be based on the knowledge of overall levels and patterns of genetic variation and the mating system. Whenever possible, the samples included and stored in a genebank for conservation purposes should possess a hierarchical structure, e.g. trees—stands—regions of provenance.

Collecting

Collecting of samples which are to be stored in the genebank should be well documented, following commonly agreed guidelines and covering all important aspects, e.g. location, geographical and ecological data. The selection of individual trees should be kept on record. Their position should preferably be marked on a map. Notes on the flowering intensity and seed crop should also accompany each sample included in the genebank. In order to take a representative sample, the seed-bearing trees should be well distributed in the stand, adjacent or related trees should be avoided and the minimum distance between two sampled individuals (minimum 50 m, see above) should be mentioned in the collecting protocol. If a genetic inventory of a gene conservation stand has been carried out, the material should be collected from the same trees, which were already characterized in the analyses.

A minimum of 100 trees per gene conservation stand should be sampled. This can provide insurance against sudden loss of the forest population, and offer a source of suitable

reproductive material if gradual artificial regeneration is needed. Most representative material is to be collected in a mast year, when the highest number of trees can carry acorns. In most white oak species, abundant seed crops occur at irregular intervals (4–8 years). It is recommended to collect seeds during the first mast year after the designation of the gene reserve forest, and regularly afterwards. Recent experimental results show that seeds collected in the same stand in different years will probably not have the same genetic properties. Additional sampling for gene conservation purposes should therefore be carried out also between the mast years, wherever possible.

Seed storage

The long-term seed storage of white oaks has not been perfectly solved yet. Seed storage of *Quercus robur* for 1–3 years can be carried out at -1°C without significant loss of viability. The water content of acorns should also be set to $40(\pm 2)\%$ to store the acorn on the long term (Suszka and Tylkowsky 1980, Steinhoff 1993). Acorns of *Quercus petraea* or *Q. pubescens* can hardly be stored longer than one year because of their fast germination and high germination power. Fungi (mostly *Ciboria batschiana*) will infect germinated acorns more easily (Middelmann 1997). The different methods of thermotherapy used in genebanks cannot ensure long-term seed storage of white oaks comparable to that of conifer species, which might be stored for 10–15 years without significant viability loss. Experimental studies were carried out on the decrease of storage temperature (-3°C) and water content, combined with thermotherapy (Suszka *et al.* 1996, Middelmann 1997, Gille 1999, Suszka 1999, Schmalen and Herget 1999), or the increase of sugar content (Žitník *et al.* 2000) in order to ensure a longer storage term and better viability of acorns. The term of the storage could be increased up to 2–4 years, but no longer, and this period seems to be short given the high risk of the storage and the relatively expensive costs. Despite the promising preliminary results of the experiments, it is unlikely that the problem of long-term storage of oak acorns will be solved in the near future.

For gene conservation purposes, storage of bulk seed lots with a defined number of sampled individuals will generally be sufficient. If the seeds (also pollen and tissue material) are to be used for further breeding purposes or evaluation, storage of individual tree samples is preferred. Bulk samples can easily be created afterwards by mixing equal proportions of viable seeds. The decision on storage of individual or bulk samples thus depends on their future use. Seeds always need to be kept and handled under the best possible conditions, in order to maintain high levels of viability. For example, floating of acorns should be used to separate empty or infected acorns.

Procedures for seed testing in genebanks will follow in detail the international rules described in ISTA (1996). Germination losses are considered acceptable up to 20–25% for gene conservation purposes, even though classical commercial seed storage facilities might frequently be aiming at lower rates. As far as possible, all sources of selection pressure should be removed and all possible care taken to minimize changes of the genetic structures in the seed lots during the process. It is essential that genebanks have, or have access to, sufficient laboratory equipment to enable viability monitoring tests to be carried out in a regulated, uniform and timely manner.

Amounts

The amounts of seed material stored for gene conservation purposes vary according to the conservation objectives and depend on the practice applied in individual countries and regions. If 500–1000 individuals are considered a sufficient population size of the regenerated population in reproductive age, then the estimated size of representatively sampled seed lots given as 10–100 kg (1–5 kg/1000 pieces) per stand (population) seems safe. It provides a sufficiently large basis for the different types of selection that occur during storage and regeneration, and is also applicable from the point of view of practical storage capacities.

Numbers of seeds or plants per hectare used for the establishment of the regenerated population will follow local forestry requirements and practices.

Storage of pollen

With modern freeze-drying techniques, tree pollen can be stored at very low moisture content and at subfreezing temperatures. For regeneration purposes, however, this technique requires complementary female structures to enable use of the pollen for seed production. Strategies for the use of pollen, including sampling, storage and regeneration, in the conservation of white oaks' genetic resources still need to be defined and implemented. New developments and further testing of experimental material can be expected in this area in the near future.

Storage of tissues for *in vitro* propagation

Storage of tissue cultures also has the potential to provide a secure static *ex situ* conservation method. The technique involves micropropagation of meristems, embryos, or other types of tissues. It requires large investments in development, but if advanced cryogenic storage is used it will offer a secure conservation method. Conservation through tissue culture is still in the experimental stages for most forest tree species.

In vitro propagation can also be used if the traditional vegetative propagation methods (grafting, cutting) cannot be successfully used (e.g. individuals unable to be grafted). In most cases the *in vitro* clones can be easily transformed to normal plant phases placed in an *ex situ* clone collection or conservation stand and used for further propagation.

Deep-freezing in liquid nitrogen and similar techniques of cryopreservation hold promises for storing vegetative plant material and embryos of plants whose seeds are difficult to store in a desiccated state. Undifferentiated tissues, somatic embryos and organized meristems are suitable subjects to be kept safely for long periods. Cryogenic storage, the preservation of biological material suspended above or in liquid nitrogen at temperatures from -150 to -196°C , has been used for many years as a means of keeping animal semen for breeding purposes. This technology is relatively new to tree seed storage and hence the optimum moisture content levels, treatment conditions and time limits for storage of orthodox seeds still have to be determined. Cryogenic storage in the case of species with small seeds may be very cost-effective and the technology promises fewer concerns from the genetic viewpoint than conventional seed storage. There are many reports that describe the regeneration of plants from a proportion of explants after a brief period at -196°C , but few describe successful storage for longer intervals. Some success has been reported with the freeze preservation of callus cells, for instance in the case of *Prunus cerasus*, *Rubus idaeus*, or the hybrid poplar *Populus \times euramericana*. Callus cells of *Picea glauca* stored one year in liquid nitrogen gave rise to whole spruce plantlets. Although a considerable number of experiments have investigated cryopreservation techniques, methods are not yet sufficiently reliable to be used widely for storing the genetic material of major woody plants. New advances in this field still remain to be made.

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Programme

Wednesday 21 June

Arrival of participants to Sofia and transfer to Borovets

Thursday 22 June

Opening of the meeting

- 09:00 Welcome (Host country and Chair of the Social Broadleaves Network)
- 09:15 Introduction (IPGRI)
- 09:30 Adoption of the agenda and nomination of rapporteurs

Research

- 09:45 Genetic resources of social broadleaves in southeastern Europe—Joint session with the ongoing project (Luxembourg, Romania, Bulgaria and Moldova)
- 10:30 *Coffee break*
- 11:00 Joint session continued
- 11:45 Other ongoing research projects (overview)
 - FAIR OAK (A. Kremer)
 - OAKFLOW (A. Kremer)
 - FAIR BEECH (R. Stephan)
 - DYNABEECH (T. Geburek)
- 13:00 *Lunch*

Progress made in countries

- 14:30 Introductory country reports from newly attending countries (Turkey and Uzbekistan)
- 15:00 Update on progress made by countries (round table)
- 16:00 *Coffee break*

Legislation

- 16:30 Overview of legislation related to genetic resources of Social Broadleaves (S. de Vries)

Development of joint gene conservation strategies

- 17:00 Results of the survey and discussion (T. Geburek)

Friday 23 June

- 9:00 Current state of knowledge of biology and genetics of Social Broadleaves (A. Kremer)
- 10:00 Introduction to the working groups (J. Turok)
- 10:30 *Coffee break*
- 11:00 Development of technical guidelines for the sampling, design and management of gene conservation units (participants will be divided into three working groups for discussion)
- 13:00 *Lunch*
- 14:30 Presentation of results of the working groups and discussion
- 16:00 *Coffee break*

Documentation

- 16:30 EUFORGEN Database/Information platform
- 16:45 Web page
- 17:00 Bibliography

Public awareness

- 17:15 Leaflet
- 17:30 Slide collection (D. Jacques)
- 18:00 Other public awareness initiatives

Saturday 24 June**Morning:**

- 7:30 Field trip**

Afternoon:

- 14:30 Adoption of the report
- 16:00 Date and place of next meeting
- 16:30 Any other business

Sunday 25 June

Transfer to Sofia and departure of participants

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