In situ and *ex situ* conservation methods and techniques

National Forest Inventories: how can they contribute to forest genetic conservation?

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Introduction

The history of humankind is one of the modification of forested environment, which by the degradation and fragmentation of forest ecosystems, their alteration through the harvesting of forest products, use of fire, or more general environmental alteration and by the introduction of pests, pathogens, or exotic species resulted in the erosion of biodiversity. Since we do not know the long-term, broad scale cumulative effects of past and current forestry practices, we cannot expect to achieve the long-term goal of an ecological sustainability without monitoring and modification of our forest management through time. Hence, we should treat forestry as adaptive management which must be accompanied with monitoring programmes to accumulate key information. In this context, the monitoring of forest genetic resources is one important component. But meaningful and operational feasible genetic monitoring is far from simple tasks. Because complete genetic inventories are neither possible nor practical, we are forced to use surrogate measures, each of which is adequate in its own right (LINDENMAYER *et al.* 2000). As biodiversity monitoring is a long-term process it only rarely provides instant results of direct value for political decisions makers. Hence, funding is often lacking. Therefore, we suggests that biodiversity monitoring may be combined with national forest inventories (NFI) or closely related forest resource assessments that are traditionally performed on a regular basis in several European countries (EC 1997).

Genetic monitoring can be defined as a goal-oriented assessment which is repeatedly performed in order to reveal temporal or spatial changes in genetic parameters (Box 1). Here we face the dilemma that in most cases the natural level of genetic variation of forest tree species must remain unknown. In many European regions, the 'natural' gauge against today's genetic variation can be compared with is not (longer) available. Especially for wide-spread tree species which has been impacted primarily by translocation of reproductive forest material this holds true. In certain eastern European countries there may be still virgin or close-to-virgin forests, however, data on genetic diversity cannot be simply used as a baseline for other regions. Forest tree species have

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 499–512, ISBN 80-967088-1-3

Box 1. Inventoring and monitoring – some definitions.

In a strict sense the two terms 'inventorying' and monitoring have to be differentiated. **Inventorying** in relation to the assessment of biodiversity can be defined as *'the surveying, sorting, cataloguing, quantifying and mapping of entities such as genes, individuals, populations, species, habitats, biotopes, ecosystems, landscapes or their components, and the synthesis of the presence/absence, relative abundance, and patterns of variation ...'* (STORK & SAMWAYS 1995). While inventories merely provide snapshots of the actual biodiversity status, **monitoring** can be defined as a goal-oriented assessment which is repeatedly performed in order to reveal temporal or spatial changes in particular parameters. For long-term monitoring of biodiversity change, information must be comparable over time and space. Hence it is recommended that the groups that are involved in monitoring activities should coordinate use of standard protocols in study design, sampling procedures, sample and data analysis and reporting methods. Biodiversity monitoring at the genetic or species level usually aims to develop a framework for predicting the development of the genetic or species composition in order to improve management or to serve as an early warning systems.

a natural geographic genetic pattern. Forests close to former ice-age refugia, for instance in the Balkans, generally harbour more genetic diversity at least at neutral or semi-neutral gene markers than virgin forests in central or northern Europe formerly did (*e.g.*, COMPS *et al.* 2001).

The objective of this paper is to describe briefly how aspects of a genetic diversity assessment may be incorporated into NFI or similar forest assessments. Emphasis is laid upon easy-to-use applications.

Indicators of genetic erosion

In principle, a successful biodiversity inventory (monitoring) should rely on two basic principles. Firstly, we need to identify the **criterion** which may be defined as 'the principal function and processes that genetic diversity is maintained'. Secondly, we then focus on those components that are most relevant achieving the criterion being either subject to major threats to be mitigated or in focus by the management targets. They are called **indicators**. Important and often completely missing is the establishment of threshold values (critical values) of a certain indicator (**verifier**). However, changes at the genetic level are extremely difficult to assess in practice. Mainly for the forest stand level those indicators tackling genetic drift, selection, gene flow or the reproductive system have been proposed (*e.g.*, BOYLE 2000). However, those genetic criteria and indicators are not always technically or operationally feasible and cannot be easily applied in NFIs. Following list of indicators that could be measured singly or in combinations on individuals and populations of a given species in a defined area as part of a systematic effort to monitor changes at the genetic level for the forest tree species may be helpful (cf. BROWN *et al.* 1997):

- Species distribution and number of sub-specific entities
  A useful genetic approximation is the assessment of formal taxa such as sub-species, varieties or entities such as ecotypes, chromosome races and landraces. The measurement of intraspecific morphological variation is an easily obtained indicator of genetic diversity. Morphological measurements can be obtained in the field or from field specimens, not requiring laboratory studies. Another advantage is that morphological characters may be ecologically adaptive (SCHAAL *et al.* 1991).
- Natural regeneration
  Mother Nature selects for reproductive success. That is why the occurrence of natural regeneration as part of forest stand structure is a suitable indicator whether a certain species is well thriving and its reproductive system is functioning.
- Population size, numbers and isolation
  Small populations are at relatively greater risk of loosing alleles, increased inbreeding and extinction due to stochastic events (see p. 413 ff., this volume). The number and isolation of populations in an area will reflect both the overall genetic diversity in the area and how this is structured.
- Environmental amplitude
  From provenance research is has been clearly shown that the climate is of major importance shaping the genetics of forest trees (*e.g.*, MÁTYÁS 1996, see p. 275 ff., this volume) while edaphic components are less important (*e.g.*, TEICH & HOLST 1974). The number of distinct habitats or environments in which a given forest tree species is found in a study area (for example based on climatic classifications or ecoregions) indicates adaptive variation.
- Genetic diversity at gene or genetic markers (generally less feasible for NFI, see below)
- Quantitative genetic variation (normally not applicable for NFI, separate specific surveys or field experiments needed)
- Inter-population genetic structure and mating pattern (normally not applicable for NFI, separate specific surveys or field experiments needed)

What can be realistically done through NFIs

The practical fieldwork to assess genetic resources within the framework of a NFI is confined due to the prime resource assessment (normally timber). Field plots are visited only once during an inventory cycle and field data of different inventory cycles are not always collected at the same time or season. Therefore, field data on phenological variation (*e.g.*, bud burst) cannot be recorded.

Species distribution

Knowledge of the abundance and distribution pattern of species is of great importance in its attempts to preserve and utilize (autochthonous) plant genetic resources and a NFI

can provide these data. Examples of actual tree species distribution may be found for Switzerland (BRÄNDLI 1996), Austria (SCHADAUER 1994) or many other countries. The actual distribution pattern can then be compared with the natural range of a species and an area outside this range can be identified as introduced. Knowledge where a forest tree species has been artificially introduced is an important piece of information for the management of genetic resources. Non-autochthonous populations can jeopardize autochthonous ones by hybridization and introgression either through purposeful introduction by humans or through habitat modification, bringing formerly reproductively isolated populations into contact (CARNEY *et al.* 2000). Especially species with small local population sizes are threatened such as *Populus nigra* L. by genetic swamping through widespread cultivation of *P.* × *euramericana* Guinier or ornamental clones (CAGELLI & LEFÈVRE 1995).

In determining the natural distribution of a tree species some prerequisites are essential. In an ideal case, the potential natural occurrence (potential natural vegetation, PNV) is at hand for each inventory plot allowing more subtle inferences. The PNV determines the climax vegetation assuming no human influence (TÜXEN 1958). For several European countries such maps are available (*e.g.*, Czech Republic – NEUHÄUSLOVÁ & MORAVEC (1997) (Fig. 1). However, often such maps developed by plant sociologists are not sufficiently fine-grained to be useful for a NFI. Therefore, an assessment on the plot level by the field staff may be needed. An operational derivation of the PNV applicable for field staff, which is usually not specialized in plant sociology premises could be: (1) aggregation of single PNVs to coherent groups of PNVs (*e.g.*, 126 PNVs are pooled to 26 PNV groups used for field assessment in the Austrian NFI), (2) an expert system or some kind of key that allows the derivation of these PNV groups from easily assessable parameters such as growth region, altitude, relief, soil type, geology, and regional climate. The field staff determines the mentioned parameters and uses them as input variables for the expert system, which suggests a PNV type that is checked for plausibility. For the Austrian NFI these techniques have been successfully used for many years.

With this information at hand the comparison between actual and natural forest communities becomes feasible in the field. There are two ways of possible comparison:

(1) Comparison of the two communities by means of the **quantitative** tree species' share; the actual proportions of trees species can be estimated easily, whereas estimation of potential natural proportions is rather sophisticated and not easy to obtain. For instance, the share of European beech (*Fagus sylvatica* L.) in the PNV type *Abieti fagetum* is variable according to special site conditions and developmental phases.

(2) Comparing the actual and potential occurrence of the **qualitative** tree species' share; focusing on the main tree species of a natural forest community this method is operational for NFI. The most relevant case is that a tree species is found at a plot, however, this species is not characteristic for the respective PNV and *vice versa*.

Subspecific entities

There are different subspecific entities: subspecies, varieties, races, *etc.* These taxa refer to populations restricted to geographical areas that differs from others in identifiable

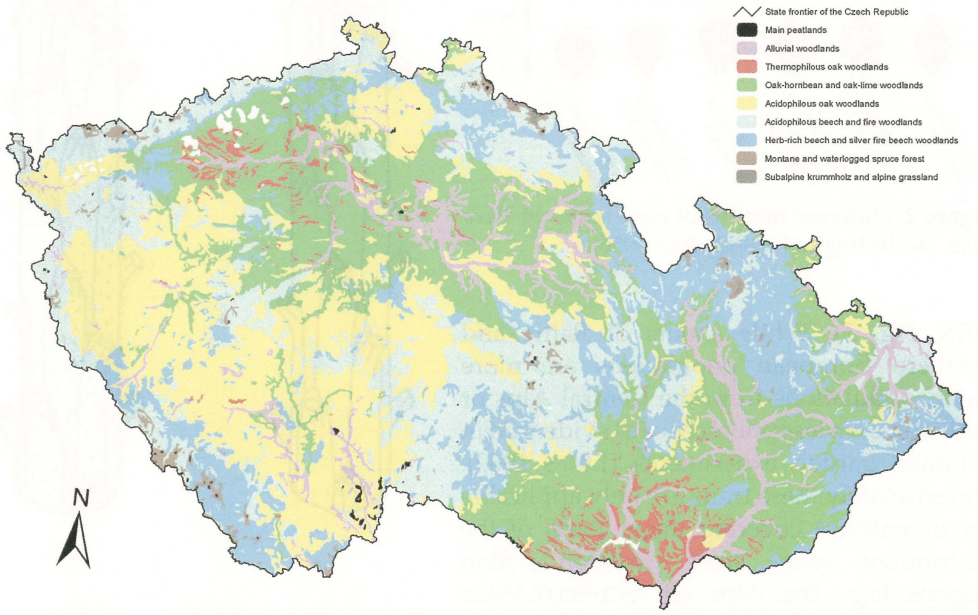

Figure 1. Map of the potential natural vegetation of the Czech Republic [simplified from NEUHÄUSLOVÁ & MORAVEC (1997), reproduced with friendly permission of authors and Institute of Botany, Academy of Sciences of the Czech Republic].

characteristics of the same forest tree species, but not to an extent of being classified as a separate species. Generally, emphasis should be laid on those characteristics that are predominantly genetically controlled (see p. 221 ff., this volume).

Since the infancy of forest genetics, certain morphological traits have been attractive to researchers. Thus, cone length, shape of cone scales, crown shape or other morphs have been studied across regions in many forest trees. Unfortunately, we often do not know how (strongly) those traits are genetically controlled. In spite of this limitation, data may be helpful. Morphological differences often used to identify subspecies or other subspecific entities may be applicable for surveys. For the genus *Quercus*, for instance, MAGIC (2002) provided a detailed (sub)species description which may be especially helpful in assessing genetic resources in the *Q. petraea-robur* complex in the Balkan Peninsula. Early attention of forest geneticists attracted *Picea abies* (L.) Karst. It has been known for a long time that cone length varies drastically with elevation (KIENITZ 1879). However, we can learn from seed orchards established with high elevation clones planted in low elevation, that cone length is only moderately genetically controlled while shape of the cone scales is not modified by the 'new' environment (Th. Geburek, unpublished data). However, different cone scales have been correctly used to identify varieties (PRIEHÄUSSER 1956, Fig. 2). Furthermore, differences of the indumenta (pubescence) of young spruce shoots vary within European populations (PRIEHÄUSSER

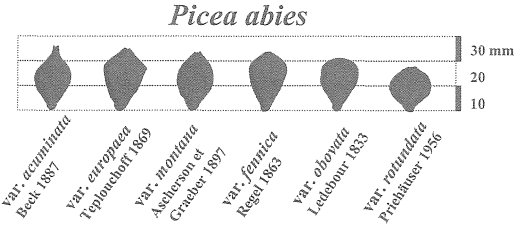

Figure 2. Different morphs of cone scales in *Picea abies* (according to PRIEHÄUSSER 1956).

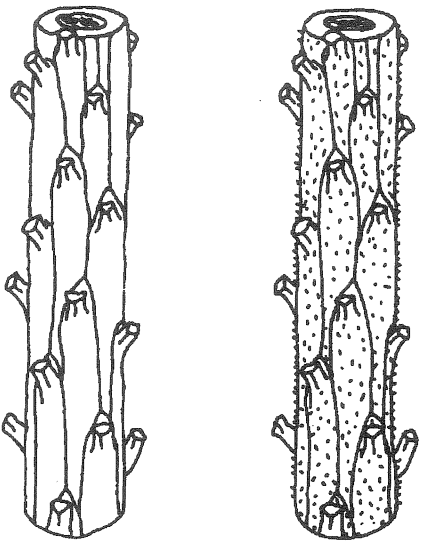

Figure 3. Different pubescence of young shoots in *Picea abies* (according to PRIEHÄUSSER 1958).

1958, Fig. 3). While shoots of Baltic and northern European populations are more hairy, populations from the Central Alps or Balkan shown less pubescence as evidenced in common garden experiments (LINES 1960). Unfortunately these traits are only applicable for inventories covering major parts of the distribution range since variation within regions [*e.g.*, the Alps (cf. SCHMIDT-VOGT 1972)] is normally missing. In this conifer, different crown shapes (branch habit types) differing with elevation are obvious in their native environment (SCHMIDT 1952) and the crown shape is maintained under different environmental condition (Th. Geburek, unpublished data). In Austria for instance, crown shape was surveyed for the first time during the period of 1971-80 and has been recorded again in order to detect differences which may have taken place in the last decades.

In *Larix decidua* Mill. the length of stomata rows on the upper surface of needles on short shoots relative to needle length may be assessed. This ratio increases with altitude and is largely under genetic control. Low-altitude provenances (*e.g.*, from Poland or Sudetes) can be clearly distinguished from the high-altitude populations in the Alps and Tatra Mountains) (MAIER 1992). Also in larch, cone size and shape vary considerably within the native range. More spherical forms are mostly found in the Carpathians (cone length approx. to 2 cm) while in the Alps more longitudinal stretched cones (cone length approx. to 3 cm) are typical (RUBNER & SVOBODA 1944). In areas where one suspects a heavy historic seed and plant transfer, this trait may be also applicable.

Other morphs such as spiral grain in *Fagus sylvatica* L. (TEISSIER DU CROS *et al.* 1980) or *Picea abies* (CAHALAN 2002) may also be also included in NFI.

Natural regeneration

Mother Nature selects for reproductive success. That is why the occurrence of natural regeneration is a suitable indicator whether a forest tree species is well thriving and the

reproductive system is functioning. However, the reverse is not necessarily true. First one has to assess whether in a certain forest the natural regeneration is 'necessary' at the time of field inspection and secondly if, where appropriate, the extent of the natural regeneration is to be evaluated with respect to the possibility whether a new stand can be established based on the current seedlings abundance and distribution.

Two key questions should be answered in this context:

(1) What is the current status of the area considered as natural regeneration?
- percentage and distribution pattern of the regenerated area;
- distribution of seedling height classes;
- existence of seed trees and small seedlings – 10 cm).

(2) What is the potential of the young plants?
- vitality;
- browsing characteristics and other damages.

There are several intricate problems linked to the inventory of natural regeneration. It is particularly important to determine the minimum number and minimum height of young plants per surface unit to define whether the existing regeneration should or should not be assessed. Identifying such threshold numbers is a difficult task. For the field staff the method must be operationable, but the minimum number of plants having a certain growth necessary for a successful regeneration differs from site to site and from species to species. Depending on the average height, different threshold numbers (minimum number of seedlings in order to identify an area as naturally regenerated) are used in the Austrian NFI , as illustrated in Fig. 4.

The final phase of regeneration has to be defined as well. In many European countries the natural regeneration is affected by heavy game browsing. The final phase may be defined when the seedlings have reached a certain height not longer accessible by game. For roe deer this height may be set as 130 cm. In many European countries browsing (game and domestic stock) threatens natural regeneration. A feasible procedure could be the combination of two sampling methods: the fixed area plot and the assessments on a subsample therein. Subsampling is appropriate because the number of damaged seedlings on the whole plot can be very high. For instance, in a fixed plot area plot of 300 m^2 a certain number of sample trees (*e.g.*, 5 individuals) are selected according to their height and their spatial distribution taking into account their significance for the further development of the regeneration. This can be done by selecting the highest seedling of each species, as long as they have a certain

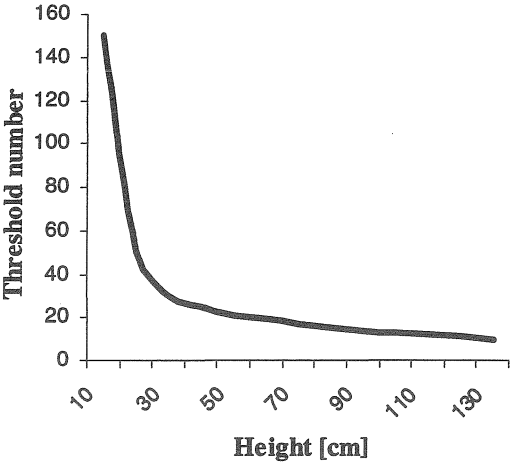

Figure 4. Threshold seedling numbers for the assessment of regeneration according to tree height, example from the Austrian Forest Inventory for 300 m^2 field plots.

minimum distance to each other. Only on these subsamples browsing damages are assessed. This approach will provide reliable estimates on the further development of the regeneration and is more efficient than an assessment of all seedling of the plot, because the majority of the plants will later die due to growth competition.

Proportion of natural versus *artificial regeneration*

Another important aspect which can be tackled by NFIs that include regeneration surveys is the proportion of natural versus artificial regeneration. There is one hurdle that has to be considered when the areas of natural and artificial regeneration are compared. The probability that a certain developmental phase will be detected is proportional to the duration of such a phase. As natural regenerations last normally much longer than artificial ones, detection probabilities differ. Therefore, area-related estimates of natural and artificial regeneration are not meaningful. The following example may further illustrate this. If the phase of a natural regeneration lasts 20 years and only 5 years for an artificial one, respectively, the estimate for the area of the natural regeneration will be fourfold biased assuming an identical proportion of the two regeneration regimes in reality. If the proportion of different time spans required can be estimated like in this example, this ratio can be used for a correction. However, this ratio often varies erratically or is unknown. Another possibility is to consider only those natural regeneration plots beyond that phase which mark the beginning of the artificial one.

Data collection for specific genes

Field data for specific genes can normally not be recorded through NFI. Of course, within a NFI plant material may be collected that is to be used in genetic surveys such as in *Quercus* spp. (PETIT *et al.* 2002) or *Picea abies* (SPERISSEN *et al.* 2001). However, morphs that are gene markers in forest trees are very scarce. A trait to be potentially used in conifers, is the colour of immature female cones which is considered to have adaptive significance. In *Pinus monticola* Douglas ex D. Don (STEINHOFF 1974) and *Picea glauca* (Moench) Voss (TEICH 1970) immature cone colour is encoded by a single gene, while both in *Pseudotsuga menziesii* (Mirb.) Franco (COPES 1972) and *Cryptomeria japonica* D. Don (TSUMURI *et al.* 1988) this morph is controlled by two or more genes. Adaptive coloration in animals has long received serious consideration (*e.g.*, HAMILTON 1973). In plants, discussion on colour pattern primarily stems from the attraction of pollinators to flowers and to dispersal of seeds (fruits) (*e.g.*, MURRAY *et al.* 1993). However, different coloration of female cones in conifers suggests a thermoregulatory function. The greenish-coned variant attains lower external temperatures than do reddish ones of comparable size (*e.g.*, *Abies concolor* (Gord. et Glend.] Lindl. – STURGEON & MITTON 1980). Such a thermoregulatory function is also suggested for *Pinus ponderosa* Dougl. (SMITH 1980), *Larix decidua* L. (GEBUREK, unpublished data) and *Picea abies* (Th. Geburek, unpublished data). In early studies it was presumed that greenish-coned variants in *Picea abies* are late-flushers, while reddish

-coned variants are early developers (SCHRÖTER 1898). A later more detailed study did not support this view (DENGLER 1955). It must be noted that in *Picea abies* only the immature cone colour is strongly correlated with elevation and not the colour of the full-sized cone (Th. Geburek, unpublished data). Coloration of male inflorescenes in conifers may have been similar adaptive function. However knowledge is much more limited and genetic control still remains presumptive (CARLISE & TEICH 1970).

In the most recent Austrian NFI, female cone colour polymorphisms in *Picea abies* and *Larix decidua* was used and was recorded in 1500 field plots for more than 4,000 sampled trees (Figs. 5 and 6). The field assessments were restricted to spring and early summer because later in the year different cone colours cannot be clearly distinguished.

Generally selective effects due to a global change are extremely difficult to quantify in natural forest stands. At this thermoregulative gene we expect changes in the genetic structure along elevations in the long-term and in future Austrian NFIs this trait will be recorded again.

Socio-economic and political aspects

Before commencing *in situ* conservation of forest genetic resources a sound plan including the reserve planning and establishment, future management and utilization should be clear for those mandated to carry out the conservation. Besides genetic aspects

Figure 5. Colour of female cones is an adaptive trait and differs in many conifers as exemplarily depicted in *Larix decidua*.

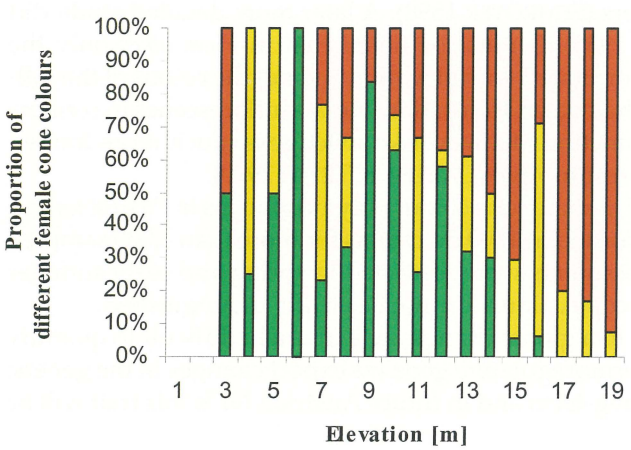

Figure 6. Proportion of different female cone colour (green, green-reddish, red) in *Larix decidua* along different elevations in the Austrian Alps (*e.g.*, 5 = 450–550 m, 17 = 1,650–1,750 m).

also non-genetical (socio-economic and political) ones are of high relevance that may be furnished by a NFI. Among others these include the continuity of the ownership, right of utilization of the genetic resource and access right. Generally in public forests action can be more easily taken than in private forests. Moreover, size of the private forest enterprise may further affect the *in situ* management. Goal conflicts that may arise from different ways of utilization such as area protection or potential forest clearance due to settlement and traffic routes have to be taken into account. Hence regional forest utilization plans and other land utilization plans are helpful instruments.

Forest history and management regime

For the conservation of forest genetic resources both the forest history and the current management regime are important component. For instance, in areas in which historically heavy seed and plant transfer occurred (see p. 437 ff., this volume) are to be excluded from *in situ* means when natural forest genetic resources are to be conserved. Furthermore, forests which were primarily artificially regenerated are less suited than areas in which forests were naturally regenerated.

Outlook: Remote sensing

Many different forms of remote sensing are available. While lately the emphasis on laser scanner and synthetic aperture radar data has increased, most work to date has used photographs and digital optical imagery, primarily from airborne and space borne platforms. Remote sensing provides one of the most efficient tools available for determining landscape-scale elements of forest biodiversity, such as the relative proportion of matrix and patches and their physical arrangement (for review see INNES & KOCH 1998). Remote sensing thus provides a means to make assessments across several different spatial scales, and is also critical for assessments of changes in ecosystem pattern over time. At intermediate scales, remote sensing provides an ideal tool for evaluating the presence of corridors and the nature of edges (forest

fragmentation) (RIITTERS *et al.* 2000). At the stand scale, remote sensing technologies are likely to deliver an increasing amount of information about the structural attributes of forest stands, such as the species composition and nature of the canopy surface the presence of layering within the canopy, the presence of (very) coarse woody debris on the forest floor (*e.g.*, GERYLO *et al.* 1998) and health status (ADAMS *et al.* 1999). So far remote sensing is still very limited as far as the assessment of genetic resources is concerned. For an early warning system for the genetic erosion of agricultural crops remote sensing has been already proposed in order to collect data on the introduction of crop varieties, reduction of habitat, socio-economic factors, environmental degradation and disasters (HUTCHINSON & WEISS 1999). For the assessment certain forest tree species that depend on the availability of certain habitats, such as *Populus nigra* L., to gather other area-related data for instance in the socio-economic and policy sector, or for an assessment of environmental threats, remote sensing is a very promising tool.

Conclusions

Any activity to conserve forest biodiversity must be based on reliable information about the current status of resources, their likely development and – of course – the conservation objective. Inventories and monitoring programmes can play a pivotal role but in order to optimize efforts it must be first decided which biodiversity level (genes, species), geographical scale, and time frame are aimed at. Secondly, availability of adequate resources and sound understanding of ecological dynamics in forests are cornerstones for an accurate evaluation and, thirdly, standardized protocols wherever possible make inventories and monitoring comparable and allow later the validation and calibration of findings. Therefore, a statistical framework is essential and should be planned ahead of any field work. While in many European countries an exclusive biodiversity assessment on a regional scale is not realistic, it may be – at least in part – combined with ongoing NFIs or related surveys. There are no general technical guidelines that have to be completely implemented at the national level. However, probably the most important information facing conservation and management efforts for forest tree species are adequate inventory data on their distribution and abundance and this type of information can be surely furnished. Forests of the former Soviet Union, for example, cover approximately 28 % of the global forests harbouring more than 570 species and subspecies of forest trees holding genetic resources of global importance. A first important step towards forest genetic conservation in this region would be the data collection on forest tree species abundance and distribution. Generally, information on the natural range and the potential natural vegetation also would come in handy to identify and declare *in situ* conservation areas (see p. 535 ff., this volume).

While it is already difficult to monitor species diversity, an evaluation of the genetic level is even more intricate and forest genetic conservation is partly suffering a backlash from unrealistic expectations from molecular genetics or conservation ecology. But certain morphological traits that are genetically controlled can be observed in the field and plant material used for genetic analyses may be also collected. Many scientists will argue that such information is of low practical value. Indeed, it is not possible yet to

assess the adaptive potential of forest tree species at a regional scale, but if we do not deal with genetic implications of conservation management explicitly, then they become simply buried in the assumptions of the decision-making progress. The advantage of a NFI accounting for genetic diversity is that it forces managers to explicate about genetic effects through the reproductive system, translocation or forest reproductive material and plausible interactions of demographic attribute and genetic compositions.

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Protected areas in Europe and their importance for conservation

G. Koch

Introduction

The most effective and efficient way of conserving biodiversity is to prevent the conversion or degradation of habitat to begin with. The measures must be complemented by a wide array of techniques to conserve individual species, populations and genes (MILLER *et al.* 1995). Maintenance of plant and animal genetic material in the wild (*in situ*) and outside their natural habitats, *e.g.* in plantations, seed storages, gene or pollen banks (*ex situ*) is essential of managing the human use of genetic diversity. These two strategies are defined and discussed in the Article 2 of the Convention on Biological Diversity (ANONYMOUS 1992a). *In situ* conservation maintains not only the genetic diversity of a population but also the evolutionary interactions that allow it to adapt continually to shifting environmental conditions, such as changes in pest populations or climate[1]. Of all the various categories for conservation of forest areas, 'Genetic Reserve Forests', 'National Parks', and 'Strict Forest Reserves' provide a high status for *in situ* conservation of forest genetic resources. This paper addresses protected areas in forests and their importance for scientific research with special focus on biodiversity and genetic conservation. Different categories of protection will be discussed in their international context – distinguishing between unmanaged and managed protected areas. Minimum standards that are briefly described for the design of reserves are needed to ensure international comparisons.

[1] World Resources Institute http://www.igc.org/wri/biodiv/in-situ.html

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 513–533, ISBN 80-967088-1-3

Protected forest areas

At the Fourth 'World Congress on National Parks and Protected Areas', held in Caracas, Venezuela, in 1992, protected areas were defined as *'areas of land or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means'* (IUCN 1994). Protected areas are important reservoirs for biodiversity and ensure that other benefits, such as soil and watershed protection, research and education, are secured. The long-term importance of protected areas is dependent on how they are chosen and managed.

The aims, definition, size and approaches to management of protected areas are becoming far more flexible. This broadening of scope means that land managers can use protected areas in a broader context than it was previously the case. Some of the uses of protected forests go beyond traditional conservation priorities and can include, for example watershed protection, soil protection and protection of all categories of biodiversity, *i.e.* also including the genetic level. The focus of protected area management is also shifting from individual protected areas towards protected area networks as part of a landscape or bioregional approach to planning.

Changing priorities have contributed to general confusion about the definition and purpose of protected areas. To address this, the World Commission on Protected Areas (WCPA) has drawn up a modified set of six IUCN (International Union of Conservation Networks) 'Protected Area Management Categories'. These were officially adopted by the IUCN in 1994.

To give greater coherence to the role and scope of protected areas within conservation planning and sustainable land use, the IUCN and its WCPA have expanded on this basic definition and developed the following categories of protected area:

- *Category Ia*: Strict nature reserve/wilderness protection area managed mainly for science or wilderness protection; an area of land and/or sea possessing some outstanding or representative ecosystems, geological or physiological features and/or species, available primarily for scientific research and/or environmental monitoring.
- *Category Ib*: Wilderness area; protected area managed mainly for wilderness protection; large area of unmodified or slightly modified land and/or sea, retaining its natural characteristics and influence, without permanent or significant habitation, which is protected and managed to preserve its natural condition.
- *Category II*: National park; protected area managed mainly for ecosystem protection and recreation; natural area of land and/or sea designated to (a) protect the ecological integrity of one or more ecosystems for present and future generations, (b) exclude exploitation or occupation inimical to the purposes of designation of the area and (c) provide a foundation for spiritual, scientific, educational, recreational and visitor opportunities, all of which must be environmentally and culturally compatible.

- *Category III*: Natural monument; protected area managed mainly for conservation of specific natural features; area containing specific natural or natural/cultural feature(s) of outstanding or unique value because of their inherent rarity, representativeness or aesthetic qualities or cultural significance.
- *Category IV*: Habitat/species management area; protected area managed mainly for conservation through management intervention; area of land and/or sea subject to active intervention for management purposes so as to ensure the maintenance of habitats to meet the requirements of specific species;
- *Category V*: Protected landscape/seascape; protected area managed mainly for landscape/seascape conservation or recreation; area of land, with coast or sea as appropriate, where the interaction of people and nature over time has produced an area of distinct character with significant aesthetic, ecological and/or cultural value, and often with high biological diversity. Safeguarding the integrity of this traditional interaction is vital to the protection, maintenance and evolution of such an area.
- *Category VI*: Managed resource protected area; protected area managed mainly for the sustainable use of natural resources; area containing predominantly unmodified natural systems, managed to ensure long-term protection and maintenance of biological diversity, while also providing a sustainable flow of natural products and services to meet community needs.

All six types of protected areas, as categorised by IUCN, provide distinct land management systems with the potential to conserve biodiversity while at the same time meeting other objectives. By this relatively universal approach, the Strict Reserve or Wilderness Area, for example, is employed where objectives focus upon the maintenance of relatively wild habitats and ecosystems, *e.g.* forest types or mountain ranges (MILLER *et al.* 1995).

In general, the maintenance of genetic diversity is possible in all these categories. However, specifically for *in situ* conservation of genetic resources, the categories I, II, IV and VI are most important. Category III (natural monument) includes mostly small landscape elements with aesthetic qualities which are generally too small for genetic conservation. Category V includes intensively used landscape with important cultural values, but which may show changes in the naturalness of the vegetation communities and taxa. Furthermore the main goals of 'Protected Landscapes' are different to the ones of genetic reserves. In protection of these genetic values, there is a clear distinction between 'protected forests without forest management' and 'protected forests with forest management'. A major advantage of *in situ* genetic conservation is that the genetic reserves are an inherent part of the forest, rather than requiring additional time and expense to establish.

Categories I and II from the IUCN definitions include semi-natural and virgin forests as well as forests in National Parks, which are relatively unmanaged. A common protection status of natural forests in Europe, being the basis for research, is the 'Strict Forest Reserve' (SFR) (SCHUCK *et al.* 1994, PARVIAINEN *et al.* 2000, EC 2000). This category is similar to the IUCN category I 'Strict Nature Reserve' and 'Wilderness Area'.

Protected forests without forest management: Strict Forest Reserves (SFRs)

The importance of nature conservation in forests has increased because of the impact of sustainability and forest certification issues. The Forest Steward Council (FSC) Certification process for example, defines in the principle 6.2 and 6.3, that protection areas should be established to protect threatened and endangered species and habitats. The ecological functions and values of certified forests should be maintained intact, enhanced, or restored, including (1) forest regeneration, (2) genetic, species, and ecosystem diversity and (3) the natural cycles that affect the productivity of the forest ecosystem.

SFRs are important protection sites in their own right and they provide the necessary reference data for nature-based silviculture in production forests. The term 'Strict Forest Reserve' is used very differently in Europe (PARVIAINEN *et al.* 1999). In many countries, wildlife control, fire suppression, and removal of invasive exotic species are permitted. The strict concept of no intervention is not realistic in all European forests. In these cases, 'Strict Forest Reserves' are best regarded as 'minimum intervention forests' with the details of intervention dictated by national legislation and other requirements (BÜCKING *et al.* 2000). The 'COST[2] Action E4 'Forest Reserves Research Network' stated, that the only feasible common requirement for SFR status is that no silvicultural intervention takes place[3]. A detailed comparison of SFRs and comparable categories in Europe is given by the European Commission (EC 2000, Appendix 1). The Austrian definition for 'Strict Forest Reserves' is as follows:

'Strict Forest Reserves are forest lands which are left for free development of the forest ecosystem where no direct human intervention takes place. Strict Forest Reserves are a contribution to the conservation of biological diversity. They are used for research, training and instruction purposes' (BMLF 1995).

There is some commonality but not complete coincidence between SFRs and *in situ* genetic reserves. The harvesting of seed, seedlings, saplings or grafts for commercial or regeneration purpose in SFRs is generally forbidden. There are two exceptions (1) needed for research and (2) where rare species or sub-populations are driven close to extinction (FRANK 1998). A useful combination of SFRs and genetic conservation is to use the close-to-nature managed buffer zones on the border of SFRs additionally as genetic reserves (see below). In contrast to the narrower focus of 'Genetic Reserve Forests', SFRs are oriented to maintain the all biodiversity of forest ecosystems and they are left to free development without silvicultural intervention (FRANK & KOCH 1999).

[2] COST (European Cooperation in the Field of Science and Technical Research) http://cost.cordis.lu/src/home.cfm

[3] http://www.efi.fi/Database _Gateway/FRRN/ frrnintro.html

Protected forests without forest management: National Parks

The interpretation of the term 'National Park' is even more confusing than that of SFRs. However, National Parks as interpreted by the IUCN (1994) generally include large landscape units and, in addition to forests, may include other natural formations or land-use categories. Some National Parks or parts of them are dedicated to scientific research, and this category is an important pool of forest areas where natural processes are not intentionally interrupted. Most large-scale reserves, such National Parks, are not dedicated entirely to maintaining natural processes, but include smaller, strictly protected 'non-intervention areas'. This is particularly true in the case of central Europe. Central areas in National Parks are effectively SFRs surrounded by managed protection areas, and therefore, well suited to protecting natural processes (BÜCKING *et al.* 2000). This is the case in many European countries (*e.g.*, Austria, France, Finland, Germany, Greece, Ireland, Norway, Portugal, Slovenia, Spain, Sweden). It is notable that in some countries, National Parks are considered to be of higher quality than SFRs. National Parks are legally protected by nature conservation acts and managed by 'State Forest Service' or 'State Nature Conservation Administrations'. In Austria, however, National Park legislation distinguishes between core area and peripheral zones. Any form of utilization is prohibited in the core area, whereas agricultural and silvicultural activities are generally allowed in most peripheral zones. Harvesting of seed, seedlings or saplings for research or commercial use is permitted in peripheral zones.

In addition to their conventional function, unmanaged protected areas support the survival of indigenous species and maintenance of ecosystems as well as habitat protection for endangered fauna and flora. Thus, they serve not only for conservation of within-species genetic diversity, but species-level biodiversity.

Recognising that forest management practices may have positive or negative impacts on genetic diversity and population viability (see p. 437 ff. and p. 651 ff., this volume), a research emphasis on the consequences of forest management practices is recom-mended (cf. MÜLLER-STARCK 1996, MÜLLER-STARCK *et al.* 2000, ROGERS 2000). Reference populations at long-term ecological research sites, 'model forests', and research natural areas for studies on effects of forest management are required.

Protected forests without forest management: Genetic Reserve Forests (GRFs)

GRFs as well as other conservation units play an important role for *in situ* conservation. There is a fundamental difference between the two strategies of *ex situ* and *in situ* genetic conservation. While *ex situ* conservation involves the sampling, transfer and storage of target taxa from target areas (see p. 567 ff., this volume), the *in situ* strategy involves the designation, management and monitoring of target taxa where they are encountered (MAXTED *et al.* 1997b, see p. 535 ff., this volume). *In situ* species conservation measures usually emphasize the protection and management of ecosystems and communities to avoid the loss of resident species. The key characteristic of *in situ* conservation is its dynamic nature allowing for continuous

evolution. While in agriculture *in situ* conservation may include 'On-Farm Conservation' or 'Home Garden Conservation', in forestry this conservation is commonly restricted to naturally regenerated forests of certain genetic value (ERIKSSON & EKBERG 2001, *loc. cit.* chapter 10). Generally, GRFs aim to preserve the genetic diversity of forest tree species in order to sustain the adaptive potential of forest tree populations, and thus to guarantee the long-term survival of tree species (LEFÉVRE 2000, FINKELDEY *et al.* 2000). Some of the advantages of this technique are to allow a dynamic conservation in relation to environmental changes, pests and diseases, to provide easy access for evolutionary and genetic studies and to permit multiple taxon conservation in a single reserve (MAXTED *et al.* 1997b). In Europe the definition of GRFs is a natural or semi-natural unit of conservation stands or populations where genetic conservation of forest trees is implemented. The forest has to be large enough to encompass sufficient genetic diversity, permit adequate internal pollination, and to allow the existence of several age classes (KOSKI *et al.* 1997, see p. 413 ff., this volume). To achieve genetic conservation objectives, such activities as facilitating natural regeneration, protection of individual trees, regulation of competition, *etc.*, are generally permitted, or even required, in genetic reserves. All management activities - including, for instance, collecting, regeneration - ensure the continuous existence, evolution and availability of genetic resources. The network of GRFs supplements the network of unmanaged protected areas, because in this unmanaged reserves active measures for genetic conservation may be limited or even prohibited (MÜLLER 2000). General guidelines for selecting and maintaining GFRs are as follows (MÜLLER 1993a, 1993b, KOSKI *et al.* 1997, FINKELDEY *et al.* 2000, see also p. 535 ff., this volume):

- The number of individual genotypes must be high enough to include most of the gene pool;
- The tree species in genetic conservation forests should be autochthonous. Exotic tree species are not desirable;
- Plantations of undesired origins should be removed;
- Natural regeneration must be possible;
- Areas of large clearcuts or other uses which reduce natural genetic variation are not allowed;
- The minimum size of genetic reserves should be at least 30 to 100 hectares, depending on the forest type. Smaller areas are accepted only for rare tree species and for small scale azonal forest types on specific sites.

The selection of *in situ* populations as genetic resources continues to be one of the problematic features. Different approaches based on an ecological approach, on fitness relevant traits, and on different types of genetic markers are reviewed in GEBUREK (2000). Like other requirements, the minimum size has changed over time. In Austria, for example, approximately 8,700 hectares are declared as GFRs. Most reserves are smaller than 30 hectares and 25 GFRs are even smaller than 3 hectares (Fig. 1).

A great potential for *in situ* conservation resides in protected areas set aside to conserve species that are difficult to be preserved *ex situ*. In addition, *in situ* conservation of forest species maintains not only the target species, but secure also a number of other species that share the same habitat. KEMP *et al.* (1993) point out that

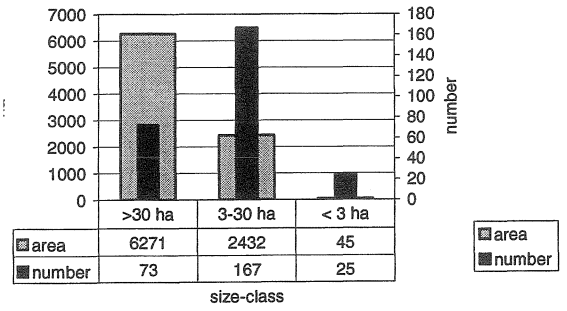

Figure 1. Number and area of Genetic Reserve Forests in Austria [from GEBUREK & MÜLLER, in press].

even when the objective is the conservation of a particular target species or population thereof, this objective may require the protection or management of whole communities – at least until there is a better understanding of the complexities and interactions between ecosystems and target species or populations. Even if they were more widely implemented, *in situ* programmes would not always be available or sufficient to maintain the diversity of species, populations and genetic resources. While *in situ* programmes are nearly always preferable when there is a choice, *ex situ* technologies have become increasingly useful as an adjunct to on-side conservation and restoration efforts (MILLER *et al.* 1995).

Protected forests with forest management: Biosphere Reserves

UNESCO has developed the concept of 'Biosphere Reserves' and defines them as a protected area including a core, a buffer and a transition zone (UNESCO 1995).

The idea of co-ordinating studies of natural systems at national, regional and international levels was inherent in the setting up of the International Biosphere Reserve Network, the backbone of the UNESCO's Man and Biosphere Program (EuroMAB 1993, Fig. 2). Biosphere Reserves are alternative types of protected areas with a combination of functions including *in situ* conservation of natural and semi-natural areas, sustainable management of natural resources, scientific research and monitoring, and environmental education and training (STORK & SAMWAYS 1995).

In combining the functions – conservation, socio-culturally and ecologically sustainable development, support for demonstration projects, support environmental education and training, research and monitoring – Biosphere Reserves should strive to be sites of excellence to explore and demonstrate approaches to conservation and sustainable development on a regional scale (UNESCO 2001).

Given the dual function of Biosphere Reserves, a system of zoning was developed to designate various levels of protection within the designated territory. Although the configuration may vary from the concentric rings envisioned by the original concept, Biosphere Reserves typically have three types of land-use zones:

(1) core zone: a strictly protected area where little human influence is permitted; this area is used to monitor natural changes in representative ecosystems and serve as conservation areas for biodiversity;

(2) buffer zone: an area surrounding the core zone where only low-impact activities are allowed, such as research, environmental education, and recreation;

Figure 2. Location of Biosphere Reserves in Europe [map compiled from data (dated 2004) from MABnet Web page http://www.unesco.org/mab].

(3) transitional zone: the outer zone where sustainable use of resources by local communities is encouraged and these impacts can be compared to zones of greater protection.

Many Biosphere Reserves simultaneously belong to other national systems of protected areas (*e.g.*, where the 'core' is a National Park). Generally, Biosphere Reserves are similar to National Parks in size, and may contain different protection zones (*i.e.*, from totally unmanaged areas separated from areas with a history of management). In contrast to the natural conditions within National Parks, areas that have been anthropogenically influenced often require some ongoing management.

The legal status of Biosphere Reserves is not clear and interpretations of the protection status vary from country to country. In some countries (*e.g.*, Germany), Biosphere Reserves do have a legal status, but in others this status may be absent or contingent on other factors or conditions (KLAFFL *et al.* 1999). Generally speaking,

Biosphere Reserves are suitable for the maintenance of genetic diversity. But, the management history – particularly any practices that could have influenced the native gene pool – should be reviewed in order to qualify the area also as a genetic reserve.

Not all managed protected areas can serve as a genetic reserves (depending on the national definition of the categories 'protected landscape' or 'nature reserve'). However, in those cases where Biosphere Reserves do qualify, there are some advantages:

- All relevant forest types can be selected. This is not always possible for legally protected areas, because many of them are established to specifically protect rare and endangered habitats;
- The preconditions are not as stringent as for unmanaged protected areas;
- Close-to-nature harvests are allowed.

Network of forest protection areas

Many institutions and governments have launched initiatives to slow down the depletion of the world's forests. Forest genetic resources networks, either operating on a regional level or focusing on a single forest tree species bring together partners with different interests and backgrounds. The European Forest Genetic Resources Programme [4] (EUFORGEN), coordinated by the International Plant Genetic Resources Institute, promotes both *in situ* and *ex situ* conservation, facilitates the exchange of reproductive material and information, and seeks to increase public awareness (see p. 63 ff., this volume).

The European 'Forest Reserves Research Network' is another good example for cooperation in research and capacity building (EC 2000). This programme was established in 1995 to promote the research of 'natural' forests. The objectives were to create a European network of forest reserves, to survey ongoing research, to standardise research methodology and to create an accessible central data bank. Results are important for the application of ecologically-oriented silviculture and for forest protection network planning. There are nearly 2.6 million hectares of 'natural' forests (*i.e.*, approximately 1.6 % of the total forest area) residing in 3,500 'Strict Forest Reserves' and other protection categories in Europe (PARVIAINEN *et al.* 1999). Most of these reserves are located in areas protected by law.

The goals of this network are as follows:

- Representativeness of all European forest types: A network of 'Strict Forest Reserves' should be established with complete representation of forest types in the different countries. The forest types should follow a classification at an international rather than national scale, and possibly to be linked to EU Habitats and Species Directives (ANONYMOUS 1979, ANONYMOUS 1992b).
- Each country should be responsible for establishing representative forest reserves.
- To qualify for a protected status, forests should be adequate in size, have minimal

[4] http://www.euforgen.org

border impacts, and have sufficient buffer zones.

Forest reserves are generally selected on the basis of their condition (*i.e.*, natural status) and type. Criteria to assess the representativeness are mostly based on forest vegetation types, but may include site types (*i.e.*, in Germany) and percentage of forest cover (*i.e.*, in Norway). Nature conservation aspects are often considered, and old forests are particularly preferred (for instance in Austria, France or Italy). However, forests affected by management may be included (BÜCKING *et al.* 2000). In Austria, there is special emphasis on the representativeness of reserves covering all forest communities in all forest eco-regions (Fig. 3). A network of standardised observation plots provides the basis for long-term documentation of natural development and of human impacts.

In addition to represent a range of forest types another selection criterion is the minimum percentage of the forest areas (FRANK & KOCH 1999). This percentage of protected forests recommended varies widely in the literature from 1 % to 30 % of the total forest area.

In general, legal protection is a requirement to assure long-term natural succession and undisturbed evolutionary processes, although currently a substantial and increasing proportion of non-classified protection areas are left unmanaged in Europe. 'Strict Forest Reserves' and 'Genetic Reserve Forests' may be protected by legislation. In some European countries (*e.g.*, Austria, France, Germany, Denmark, Italy, Netherlands, United Kingdom), the forest reserves are protected by administrative regulations or ministerial edicts. These include, without further differentiation, private contracts. There are also unmanaged areas without legal protection in various ownership categories, but these cannot be regarded as 'strictly protected'.

The long-term commitment to such a specialised (reserve) use of forests is normally restricted to public forests. The ownership pattern of SFRs and GRFs varies

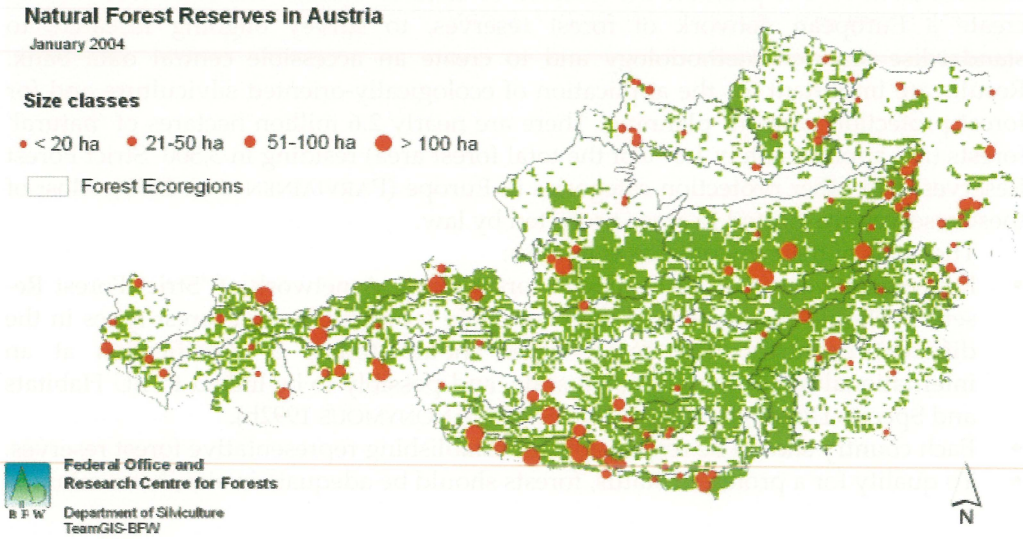

Figure 3. Location of 'Strict Forest Reserves' in Austria (dated 2004) .

considerably. In most countries, state authorities probably own the largest proportion of reserves.

Socio-economic and political factors affecting reserve networks

With the change of property rights a decrease of income is often observed. If reserves are established by law, preference could be given to forest types that have little current or potential economic value, thus neglecting some important (economically valuable) forest types in the reserve network.

Nature conservation by contract could provide an alternative providing a compensation for the income loss to forest owners. The establishment of SFRs and GRFs may be done on an individual and voluntary basis. However, to ensure the maintenance of biodiversity, certain standards have to be accepted.

Protected areas often result in a decreased public use of the forest. However, public education – as long as it does not directly or indirectly impact the reserve (*e.g.*, by tourism) – can be useful in garnering support for forest reserves and decreasing conflicts that could limit their establishment or security.

Research recommendations

Management of biodiversity and forest genetic resources can be effective only to the extent that it is supported by information and knowledge. A research and monitoring programme is an essential component of management and administration (MILLER *et al.* 1995). Protected forests serve as an important basis for close-to-nature silvicultural research, for evolutionary studies, and for providing a basis for 'naturalness inventories' (MAYER *et al.* 1987, GRABHERR *et al.* 1998, KOCH *et al.* 1999). Research in 'Strict Forest Reserves' requires sound conditions (minimum size, legal protection, time frame of protection) in order to fulfil long-term requirements. Multidisciplinary research should be promoted to understand natural forest ecosystems and their functions (EC 2000). Research activities in GFR should focus on the genetic structure of natural populations, the mating systems, and evolutionary processes including co-adaptation. These research subjects will gain increased importance also in view of a climate change (GREGORIUS & GEBUREK 1998). Results should be integrated into practical forest management through national and international training programmes and workshops. More interaction between interested and relevant stakeholders is required and a better dissemination of the results of research and monitoring is needed (PARVIAINEN *et al.* 2000).

Monitoring programmes should be established in as many forest reserves and protected areas as is required to determine changes in ecosystem condition from whatever source. Long-term monitoring and research should be co-ordinated at a national level, with EU and international linkage. There are many common linkages between European research projects and further tasks could be developed in collaboration. Some examples for research or technical programmes are:

- EUFORGEN[5] (European Forest Genetic Resources Programme) operates through networks in which forest geneticists and other forestry specialists meet and work together to analyse needs, exchange experiences and develop conservation methods for selected species. Activities of the networks focus on inventories of genetic resources, development of joint databases and lists of descriptors, identification of common research needs, efforts to submit joint project proposals, development of joint conservation strategies, and promotion of the establishment of national genetic reserve forests.
- BEAR (Indicators for forest biodiversity in Europe) is a European Concerted Action, which aims to develop a system of forest biodiversity indicators (LARSSON 2001).
- EFERN[6] (European Forest Ecosystem Research Network) has established an European forest ecology network and a comprehensive report containing current European forest ecosystem research requirements (ANDERSSON *et al.* 2000).
- PROFOR[7] (Protected Forest Areas) describes, analyses, and harmonises the wide-range of 'Protected Forest Areas'.

Socio-economic research should be integrated in conservation strategies (see , p. 13 ff. and p. 89 ff., this volume). Broader knowledge on biodiversity is needed in order to enhance the understanding of local and indigenous use of forest resources. Thus studies can teach us whether resources are being used sustainably and can help to identify incentives for conservation (OUEDRAOGO & RAYMOND 1996).

Design of reserves

The design of protected areas plays an important role not only in achieving the conservation objectives, but in allowing comparability of research results across reserves. A considerable body of literature related to reserve design is available but less has been written concerning *in situ* plant genetic resources conservation (BATISSE 1986, KOSKI *et al.* 1997, MAXTED *et al.* 1997a).

The first current consensus view of a reserve design is based on the Man and Biosphere programme (UNESCO) or on the guidelines for the establishment of strict forest reserves as discussed by MEYER *et al.* (2001) and HOCHBICHLER *et al.* (2000). All these design concepts include a central core area surrounded by a buffer zone. Standardised data collection procedures can enable comparisons of research data among reserves, provide comprehensive regional information and improve availability of information on distribution of tree species, dynamic of forest change under different conditions and effects of different environmental influences on tree species (KOCH & WALLNÖFER 1997). In particular, data collection procedures should describe the forest stand structure, shrub layer, regeneration layer and ground

[5] http://www.euforgen.org

[6] http://ifff.boku.ac.at/efern/

[7] http://www.efi.fi/projects/coste27/Introduction.html

vegetation to be able to repeat the measurements, and therefore to analyse regeneration and stand structure through time (PROJEKTGRUPPE NATURWALDRESERVATE 1993).

The forest vegetation type determines the physical structure of the forest, and has a critical influence on the energy balance and food chain within the forest ecosystem. The dynamics of the forest ecosystem are driven by the processes of regeneration, competition between individuals and senescence of tree species. In addition to the tree and shrub flora, the ground vegetation is also an important indicator of the forest condition. The condition can indicate the degree of human influence (GRABHERR *et al.* 1998) and regional patterns of variation. Furthermore, it is recognised that forest stand structure and vegetation are in close interaction with other components of the forest ecosystem.

Reserve size

Various recommendations are given for the minimum size for protected forest areas. The debate is often centred on the relative advantage of a single large versus several small reserves, the so-called SLOSS debate (HAWKES *et al.* 1997). The current consensus is that the optimal number and size of reserves depend on the characteristics of the target species or habitats. A common criterion for the size is the 'minimum structural area'. Defined by the area necessary for a certain forest community to be ecologically sustainable, this area determines the minimum size of a reserve. The minimum structural area varies with the forest type (KORPEĽ 1995). In Austria, the minimum size for a SFR should be 20–60 ha, depending on the forest community (FRANK & KOCH 1999). Other countries have also established minimum areas depending on the ecoregion and the forest type (*e.g.*, 30 ha in lowland areas and 50 ha in mountain areas). The size required for *in situ* genetic conservation will vary widely – depending on the species, its genetic structure, its density, *etc.* (*e.g.*, KOSKI *et al.* 1997, FINKELDEY *et al.* 2000). In some cases, the ideal reserve size for an *in situ* genetic reserve may be considerably larger than that estimated for a SFR. Such areas with more than 50 ha may be difficult to find or designate. An important question related to the minimum size of a reserve is, if the minimal or ideal number of individuals of the target taxon is sufficient for a viable population. Precise estimates of the minimum viable population (MVP) size varies and depends on the tree species, the lifeform, the breeding systems, the environmental factors and catastrophes (*e.g.*, fire, drought, pests) (LAWRENCE & MARSHALL 1997).

To be ecologically sustainable, the size of reserves should secure the diversity of the tree species, genetic diversity within the species, and the processes and conditions necessary for perpetual natural regeneration. The size and shape of a reserve should minimise the biotic and a-biotic disturbances (*e.g.*, windbreak, snowbreak) from outside (*e.g.*, NOSS & COOPERRIDER 1994). These disturbances potentially may be due to wildlife, livestock, pests and diseases and alien species. Problems are common in small size reserves, their location within commercial forests, or proximity to urban areas or tourism centres.

Buffer zones

Buffer zones around reserves can minimise unwanted impacts by providing habitats and safe passage for old-growth species. In Europe in many cases reserves are sheltered by buffer zones treated as non-intervention or are managed in a perpetually mature state (PETERKEN 1996). A refinement of the buffer concept was proposed by HARRIS (1984), who proposed the 'island archipelago approach' in order to improve the viability of Pacific Northwest old-growth reserves as wildlife habitat. The design of buffer zones is also intensively discussed by BATISSE (1986) based on the Man and Biosphere programme. He defines the buffer zone as an area, where research, educational activities, traditional subsistence activities and tourism are emphasized. Within selected conservation areas, designation of various zones can segregate management objectives and uses that may be incompatible and identify management activities by area. Areas of key significance for their genetic materials may well be zoned out of human visitation. Scientific research sites may warrant special protection (MILLER *et al.* 1995).

As forest conservation areas (SFRs, GRFs, Biosphere Reserves) are often 'islands' within larger commercial forests, it is important to establish buffer zones around the core – or strictly protected – area. Buffer zones should have a width of at least 1 to 3 times the height of the tree canopy from the border of the reserve (FRANK & KOCH 1999, Fig. 4).

Sample plot design

Sampling of vegetation and of plant populations are mainly done according to the plot method. Plot sampling involves observations at the sampling point within areas of standard size, usually called quadrats (MAXTED *et al.* 1997c). Most monitoring in GFR and in SFR will involve the sampling of temporary or permanent sample plots.

For SFR in Europe, HOCHBICHLER *et al.* (2000) recommended establishing a permanent network of sample plots and to supplement this with a number core areas, in which complete inventories should be made (Fig. 5). Two different levels of inventory within forest reserves are suggested: (1) a representative description of the entire SFR, and (2) a more detailed description of selected parts of the forest. The general description of the reserve should include:

(1) name and number of the reserve;
(2) area of forest;
(3) protection status;
(4) date of initial description;
(5) location, latitude /longitude, geographic-al region, *etc.*;
(6) mapping of forest vegetation communities (scale 1:5,000/10,000);
(7) mapping of site characteristics (scale 1:5,000/10,000).

The inventory should be designed to ensure that the range of present vegetation

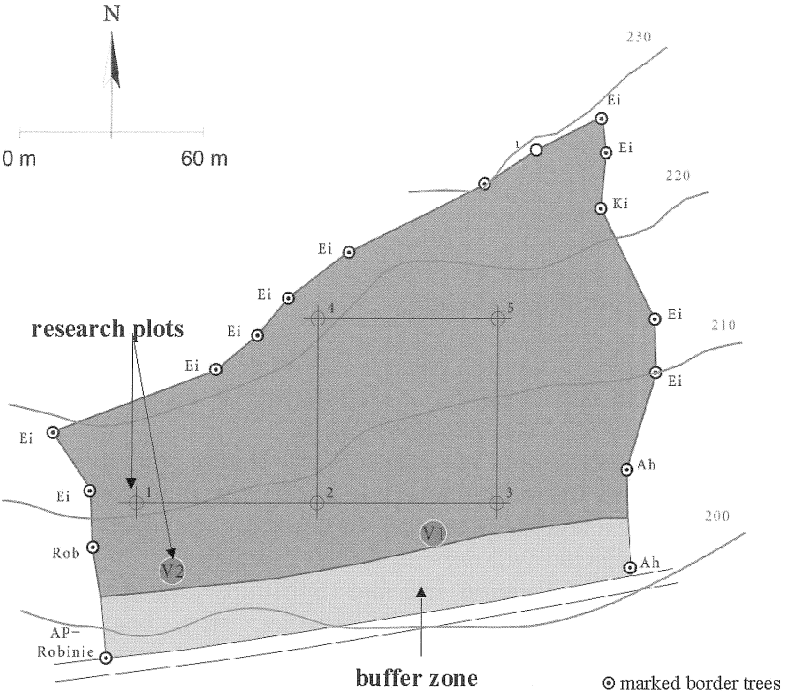

Figure 4. Buffer zone along the border of a 'Strict Forest Reserve' and grid of research plots.

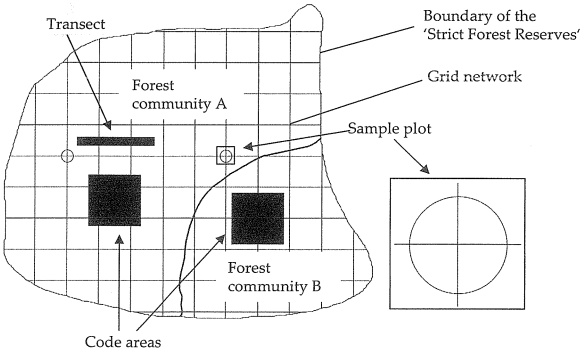

Figure 5. An example of a sample inventory design for 'Strict Forest Reserves' (HOCHBICHLER *et al.* 2000).

types are sampled, while taking into consideration the amount of funding and time available for the research. The basis of the recommended inventory design is the establishment of a systematic grid, which covers the entire forest reserve, and which is permanently marked out on the ground (Fig. 6). This is a fundamental element of the inventory design, and it ensures repeatability of the research. A recommended minimum sample plot density is 1 plot per hectare, with a plot size of 500–1,000 m². The density of sample plots on the grid can be increased within that particular area, to adequately sample the range of variation. In some cases, it may be necessary to choose smaller plots, for example, 250–300 m², on a denser grid,

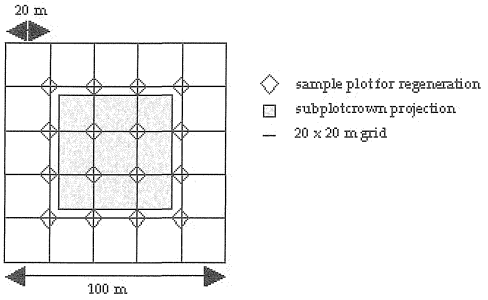

Figure 6. An example of a design of 'core area' in a German forest reserves (MEYER *et al.* 2001).

to gain representative samples. In contrast, for large, homogeneous areas, it may be possible to locate sample plots on the grid through a process of random selection.

For more intensive study of stand characteristics, including ecological or genetic studies, the establishment of core areas in selected parts of the reserve is recommended (KOOP 1991, MEYER *et al.* 2001). In general, squares are recommended over elongated transects. Inside the core areas the establishment of sub-plots is recommended for special investigations (*e.g.*, regeneration) (Fig. 6).

The purpose of permanent sample plots is to derive data on forest vegetation and stand structure over the entire forest reserve over time. A structured approach, describing each forest layer (*e.g.*, canopy, understorey) of vegetation, is recommended. The type of data collected will depend on the research but should minimally include plot characteristics (*e.g.*, location and site conditions) and description of each layer (species, height, diameter for woody species, *etc.*). For recording the forest regeneration or species diversity it is recommended to use permanently marked subplots. The size of sample plots and of subplots depends on stand density. Subplots may be circular plots or transects (Fig. 7). It is recognized that the research objectives will largely determine the design of sample plots. However, adoption of a minimum, standard dataset will facilitate the interpretation of results and the comparison of scientific data between different reserves or different countries. The process of management and monitoring is pivotal to the conservation of plant populations within a reserve. It is expensive in time and resources but is the only way to ensure that the target taxa or habitats are conserved effectively (MAXTED *et al.* 1997c).

Visitors' access

Four categories of people may use forest reserves: the local population (land-owners, local farmers, local communities), the general public, reserve visitors and the scientific community. If the access to the reserve is not restricted, the specific usage of visitors group must be considered when designing and managing the reserve (HAWKES *et al.* 1997).

If unmanaged forest reserves are to remain close to nature, direct and indirect impacts of visitors and other users must be minimized (PETERKEN 1996). In addition to scientific and educational activities there may often be some 'eco' (limited) tourism. In this case it is important that local use or visits by the public should be managed and controlled to ensure that there is no conflict with the goals of the reserve. Usually 'rights of access' are conceded, meaning that people may use a

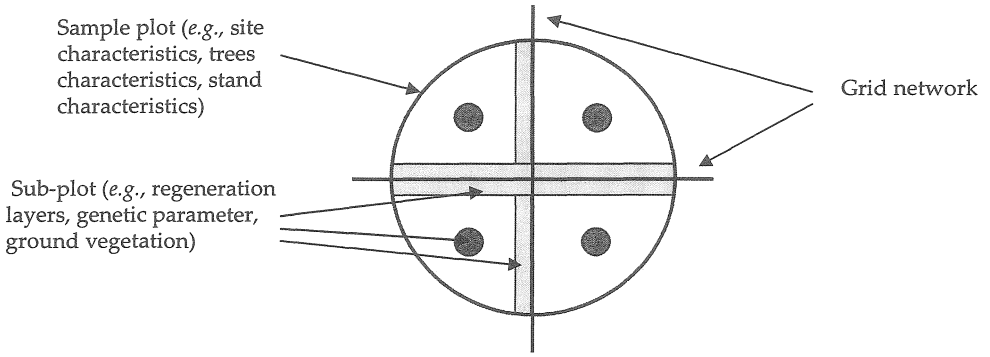

Figure 7. An example of a sample plot with supplementary subplots (HOCHBICHLER *et al.* 2000).

footpath/trail/way/forest road, but must stay on them. Unhindered access throughout the reserve is not encouraged, although this may be in conflict with the privilege to move freely in the countryside (*e.g.*, 'the right of common access' in the Nordic countries).

SFR offer a rare opportunity to observe structures and processes characteristic of natural forests. The support of a broad range of visitors is probably good for the long-term protection of strict forest areas. The modern conservation approach in densely populated countries is to restrict unlimited access and to route visitors by carefully designed trails (BÜCKING *et al.* 2000, CEBALLOS-LASCURAIN 1996).

Conclusions

Both strict protected forests and commercial forests have potential to maintain biodiversity. In both cases, a minimum size of forest areas, natural stand structures and adequate population sizes are required. The focus of genetic conservation is to maintain or enhance genetic diversity in defined target taxa.

The networks of 'Genetic Reserve Forests' and strictly protected areas (legally or voluntary protected) could complement one another and 'Strict Forest Reserves' can be useful in maintaining forest genetic resources (FRANK & KOCH 1999). If such reserves are selected considering also appropriate genetic criteria, it is possible to maintain tree species of the climax vegetation with adequate levels of genetic variation. Both categories of protected forests support the dynamic development of forest stands, either with a genetic or a forest community focus. When for genetic conservation a certain management is needed to maintain resource populations, conflicts with objective of nature protection may be envisaged (*e.g.*, SCHMIDT 1993, SCHMITT 1993). However, in many cases goals of nature protection and genetic conservation overlap considerably.

In general, additional effort has to be given to genetic monitoring, which is essential to follow the changes in a GFR (see p. 499 ff., this volume). Silvicultural

management is particularly needed for rare tree species and pioneer tree species. Therefore, this type of tree species will be best maintained in managed protected areas (*e.g.*, genetic reserves, buffer zones of National Parks, NATURA 2000 areas and Biosphere Reserves – depending on their legal status in each country).

Protected areas are particularly important for genetic research and monitoring evolutionary processes. In order to effectively manage protected areas to fulfil also genetic requirements, comprehensive information on the intraspecific (=genetic level) of biodiversity are required. There is still a deficit of such information. Despite aforementioned limitations, managed as well as unmanaged protected areas offer many possibilities for the maintenance of forest genetic resources. Silviculture, forest genetic conservation and nature conservation differ to some extant in their objectives but all are based, in the long-term, on maintenance of the gene pool. Enhanced co-operation and co-ordination among professionals in these fields, as well as the recognition of all potential genetic conservation areas, are recommended for realising a long-term conservation of forest biodiversity.

´We must make every effort to preserve, conserve, and manage biodiversity. Protected areas, from large wilderness reserves to small sites for particular species, and reserves for controlled uses, will all be part of this process. Such systems of protected areas must be managed to take account of a range of ecological and human-induced changes. This is no small task; yet humans must be equal to this challenge, or risk becoming irrelevant´ (Peter Bridgewater, National Parks and Wildlife Service, Australia)[8].

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[8] http://www.wri.org/biodiv/gbs-viii.html

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In situ conservation methods

P. Rotach

Introduction

Basic principles of conservation of genetic variation are essentially the same for all living organisms. The methods, however, may vary according to the specific objectives of conservation and the distribution and biological nature of the material to be conserved (FAO 1989). The term 'method' is often used to denote different concepts such as *in situ* conservation, *ex situ* conservation, ecosystem conservation, species conservation, static conservation, dynamic conservation and others. Here species, ecosystems, populations and individuals are considered objects of conservation and the term 'method' will be used to distinguish between basically different ways to conserve genetic resources like *ex situ* or *in situ*, dynamic or static, active or passive conservation.

Definition of *in situ* conservation is not very clear and the term has been used in different ways. The lack of clarity is in part due to *in situ* conservation being applied to wild species on the one hand and to domesticates on the other hand (MAXTED *et al.* 1997b). *In situ* conservation implies that a given population is maintained within the community of which it forms a part, in the environment in which it has developed (FRANKEL 1976). The term is frequently associated with wild, naturally regenerating populations in protected areas. However, *in situ* conservation has also been integrated into managed and multiple-use forests. In its essence, *in situ* conservation focuses on conserving a genetic resource in its original ecosystem, irrespective of whether such ecosystems have been subject to human interference. It simply means that the germplasm is conserved in the locality where it is currently found, either where it is naturally located or where it has developed distinctive traits under cultivation. *In situ* genetic conservation thus involves the saving of appropriate populations over generations, in order to maintain their potential for future evolution given by the adaptively or randomly developed genetic structures within the species (KOSKI *et al.* 1997).

The *in situ* method has several advantages. It is a dynamic method of conservation, allowing for natural selection processes, *i.e.* further evolutionary potential of the gene pool and the adaptive capacity of the population. This is important since no species is

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 535–565, ISBN 80-967088-1-3

static but is continually interacting with its physical environment and is competing with other species in the ecosystem. For a species to be viable in the future, it must be able to compete and it will only maintain its competitive ability if the evolutionary process is allowed to continue. This aspect is of particular importance today in the light of the worldwide climate changes which are taking place as a result of global warming. In addition, the *in situ* conservation method has the general advantage of conserving the functions of an ecosystem rather than a population or a species which means that it normally includes a great number of associated animal and plant species including all interactions among them. Finally, another advantage of *in situ* conservation, which is most important to the evolutionary development of a species, is that it is much easier, more secure and financially more efficient to conserve a viable population of a species in its natural habitat than in an *ex situ* situation. This is particularly true for tree species, since they require a lot of area to conserve thousands of individuals (MAXTED *et al.* 1997b).

It is commonly agreed today that the big challenge in using and developing *in situ* methods, however, is to expand our vision of protected areas to include multiple use reserves (see p. 513 ff., this volume) and even to integrate conservation of genetic resources into the production system of modern forestry (ALLEGRETTI *et al.* 1996, KANOWSKI & BOSHIER 1997). An integration of conservation and utilization would be highly effective both in terms of inputs and outputs. However, there may be important constraints to this goal. In forestry, uncontrolled and undocumented movement of forest reproductive material (see p. 75 ff., this volume) or the use of genetically modified material may pose a serious threat to the maintenance of genetic identity of local populations. Use in itself may therefore pose a threat to the possible future use of certain resources. Hence, for certain species it may be essential, independent of *in situ* conservation activities, to better control commercial use and movement of reproductive material. This may for example be the case for some economically important and common species such as *Picea abies* (L.) Karst. or *Fagus sylvatica L.* In spite of such constraints, conservation of genetic resources within protected areas need to be complemented by actions outside the reserves such as forests which are sustainably managed for multiple use. According to the World Conservation Union and the World Resources Institute, the total expanse of protected areas needs to be increased by a factor of three in order to maintain the earth's biotic resources (MCNEELY *et al.* 1990). The establishment or improvement of *in situ* conservation programmes thus will remain an important task in the future.

The following sections provide guidance in developing *in situ* conservation programmes. Since most of the theoretical aspects have been presented and discussed in this book, only practical aspects will be outlined. Furthermore, since objectives, conditions, prerequisites and many other factors vary for different species and situations, there exists not one but many different possible strategies for an *in situ* conservation. The following sections discuss the relevant criteria and principles which are important for developing species- and situation-specific *in situ* means. Even if *in situ* conservation of forest genetic resources should be integrated into the overall framework of sustainable forest management, this aspect will not be discussed here any further (readers are kindly referred to FAO, DFSC, IPGRI 2001 or ROTACH 1999, 2000).

Programmes to conserve genetic resources *in situ* are best undertaken and coordinated by a designated national agency, working in cooperation with regional and local agencies, landowners and other interested or concerned parties. Conserving and managing genetic resources in practice will also have to be incorporated into more general land use planning and management, because large reserves are unlikely to be designated only for the purpose of genetic resources conservation.

In summary, *in situ* conservation is a complex activity, requiring the integration of many disciplines and different groups of people. A good understanding of the different tasks to be done, and their necessary integration into a strategy are essential, calling for a systematic approach.

A systematic approach to develop *in situ* conservation programmes

Basically, a systematic approach requires an initial phase where necessary information is collected and priorities are discussed based on known and anticipated threats to the genetic resources in question. In a second phase, species and populations to be conserved are then selected, clear objectives are defined and management plans are drawn. Finally, a monitoring system needs to be put in place which will guarantee that objectives are reached and management activities are adapted in accordance with the observed development.

The process of developing and implementing an adapted, species- and situation-specific *in situ* conservation programme may thus be divided into the following seven activities which need to be accomplished:

- collection of relevant information;
- selection of target species and setting of priorities;
- establishment of basic conservation method (active, passive, dynamic, static);
- identification and selection of populations to be conserved;
- definition of conservation objectives and specific targets;
- definition of management guidelines (if any); and
- establishment of a monitoring system.

These activities will be outlined in the following sections. It has to be kept in mind, however, that this outline cannot serve as a recipe to simply go through step by step. It rather presents how such a complex tasks may be approached in a systematic way. It is far from being exhaustive and needs to be adapted to any given situation.

Collection of relevant information

Conserving species and their genetic resources *in situ* basically means maintaining their habitats and processes in the ecosystem as natural and functioning as possible. It is obvious that this can be accomplished only if all relevant information regarding the species and its natural environment is available. Species life history traits, important natural processes and their spatial and temporal dynamics need to be understood. De-

mography, eco-geography and genetic structure of the species should be ideally known as well as their habitat requirements. In order to be able to decide on conservation priorities and measures, threats and human impacts on the species or its natural environment need to be known. Finally, information on socio-economic values, current conservation status, existing relevant protected areas, ownership, stakeholders and many other practical or political factors are essential for efficient, well integrated, and realistic solutions.

In practice, however, very limited information is usually available, because resources for research are limited and the potential number of species to investigate is vast. Since threats to genetic resources may have severe, long-lasting and irreversible effects, it is unwise in most cases to delay conservation activities, although relevant information is incomplete. In such a situation, an approach based on systematic and robust principles and relying on best possible guesses may be more appropriate than waiting for elusive research data (FAO, DFSC, IPGRI 2001).

The overall objective of an *in situ* conservation programme is to ensure that the maximum possible range of genetic diversity is represented within the minimum number and size of reserves, established and run with a minimum of costs (MAXTED *et al.* 1997b). Since genetic conservation is a long term task for the benefit of future generations, reserve sites as well as site conditions should be sustainable for the foreseeable future. In order to minimize the need for interventions and thus running costs, populations selected as *in situ* reserves should possibly be growing under optimal habitat conditions, in sufficiently large, viable populations and in ecosystems with a maximum of intact natural processes and functions. In order to achieve these rather complex objectives, detailed information is required, especially on:

- population structure with its spatial and temporal dynamics,
- eco-geographic distribution of the species and its genetic structure;
- autochthony of populations, value and potential of the genetic resources;
- habitat requirements and habitat breadth of the species, availability and quality of habitats;
- life history traits – biological and ecological characteristics of the species;
- relevant biotic and abiotic factors of the natural ecosystem, including interactions and natural processes, dynamics of relevant processes, their sensibility to human impact and their actual status;
- threats to the species and its environment, causes and intensities, current conservation status;
- socio-economic value, importance of resources from an international perspective;
- existing protected areas, ownership, stakeholders, land use planning, legal and financial factors and other relevant information.

Table 1 presents an overview over this information, how it relates to the outlined activities in developing an *in situ* conservation programme and what sources may be used to collect the information. The details are discussed in the following sections.

Table 1. Basic information needed for the establishment of a network of *in situ* conservation areas.

Information on	Used for assessing	Source of information
Abundance Population structure Demography Dynamic	Endangerment: • demographic, environmental and genetic uncertainty, risks • natural vs. artificial distribution, fragmentation, declining populations, isolation, human impact • rarity, endemism (threat, priority species) Identification and selection of potential populations: • hot spots, core, outlier, peripheral populations • fragmentation, linkage, gene flow • GAP analysis Definition of conservation objectives	Inventories Inquiries (forest service, experts) Other sources of information (old distribution maps, flora's, vegetation databases, *etc.*) Combined GIS layers of distribution maps and maps of protected areas
Eco-geographic distribution Genetic structure	Identification and selection of potential populations: • differentiation, distinct populations • most diverse populations • range of environments to cover • number and distribution of reserves Design of reserve network Definition of conservation objectives	Combined GIS layers of distribution maps, vegetation maps, maps on geology and soil types, maps of eco-geographic zones, elevation models Genetic inventories or other genetic information (provenance trials)
Autochthony Value of genetic resources	Identification and selection of potential populations • valuable wild gene pools • valuable landraces • special resources of interest (morphotypes-genotypes, ecotypes) • exclusion criteria	Forest history Planning and management documents

Table 1. (continued).

Information on	Used for assessing	Source of information
Habitat requirements Habitat quality and availability	Identification and selection of potential populations: • viable populations with best ecological chances for future development Endangerment and risks: • human impacts • status of natural processes Definition of basic conservation methods Definition of management guidelines	Vegetation and soil maps Ecological, botanical, silvicultural literature Research results Disturbance indicators, present *versus* natural vegetation, history Field work
Life history traits Biological, ecological characteristics	Identification and selection of potential populations: • design of reserve network • viable, most self sustained populations Endangerment and risks: • breeding system, gene flow, migration • competitiveness • sustained regeneration • demographic and environmental uncertainty Definition of basic conservation methods Definition of conservation objectives Definition of management guidelines	Ecological, botanical, silvicultural literature Research results Observation Field work
Biotic factors, natural processes, actual status	Identification and selection of potential populations: • viable, most self sustained populations Definition of basic conservation methods Definition of conservation objectives Definition of management guidelines	Ecological, botanical, silvicultural literature Research results Observation Field work

Table 1. (continued).

Information on	Used for assessing	Source of information
Threats Conservation status	Endangerment and risks: • Selection target and priority species Definition of basic conservation method Definition of conservation objectives Definition of management guidelines	Compiled from different information above Map of protected areas
Socio-economic value International perspective	Selection of target and priority species	Markets, stakeholders, species distribution and density maps
Political, financial and practical frame	Identification and selection of potential populations: • coordination, integration in land use planning • sustainability and protection in the long run • exclusion of conflicts Selection of target and priority species Design of reserve network	Link with other governmental and non-governmental organizations Overall national conservation objectives Diverse sources

Selection of target species and setting of priorities

Setting priorities for forest genetic resources conservation and use is essential for the efficient allocation of limited resources of time, funds and personal (BAWA & KRUGMANN 1991, KEMP 1993). Therefore, in a first step, target species and their order of priority need to be carefully evaluated. The identification of genetic resources of priority on the species level is a cost/benefit consideration which may be based on the following criteria.

Current conservation status:

- number of populations and area which are already protected;
- range of eco-geographic distribution covered with protected areas;
- status and quality of protected areas, integrity;
- sustainability of target species within protected areas;
- *ex situ* measures.

Threatened species:

- rare species *i.e.* species occurring in highly fragmented populations, few locations only, small populations only, low density only;
- declining species (abundance, area, number of populations)[1];
- species with narrow habitat requirements (specialists);
- highly utilized or overexploited species;

[1] see also http://www.redlist.org/info/categories_criteria.html.

- species restricted to habitats strongly influenced by human activities;
- species which are experiencing other decisive negative impacts (biotic or abiotic factors, declining or insufficient habitat quantity or quality, grazing, burning ..).

Socio-economic value:
- economic value;
- potential value, intrinsic value;
- biological value (keystone-species, species supporting high biodiversity ..);
- cultural value.

Responsibility from an overall (international) perspective:
- endemic species;
- center of distribution;
- large remaining populations, important gene pools;
- high eco-geographic differentiation.

Distinctiveness of gene pools:
- gene pools existing under extreme situations, at the limit of distribution;
- different migration events, glacial refuges;
- old centers of diversity.

If sufficient genetic diversity is already safely and sustainably conserved from the full range of ecological habitats and geographical locations, then further active conservation may not be necessary. This may for example be the case for wide-spread, common species such as *Picea abies* or *Fagus sylvatica*. Care must be taken, however, when assessing information on current protected areas since in most cases these areas were not primarily dedicated to the objective of genetic conservation (see p. 513 ff., this volume). The fact, that a species is occurring within a reserve, does not necessarily mean that it is safely protected, since the number of individuals may be declining due to the natural dynamic, lack of management or inappropriate management for the sake of genetic conservation. The natural dynamics of the ecosystem in question thus need to be carefully analyzed, requirements and life history traits of the target species thoroughly reviewed, potential and likely developments of the system evaluated and compared to existing management plans. This will finally allow to decide if existing reserves are suitable for the sustainable protection of the genetic resources of the target species as such, with additional measures or with an adapted management plan. This will further be discussed in section 'Establishment of basic conservation method'. The contribution of protected areas to the conservation of genetic diversity also depends on some additional factors (BOYLE & SAWYER 1995, MACKINNON *et al.* 1986). Important factors that need to be considered when the conservation status of a species is evaluated are, for example, the optimal distribution of protected areas across the landscape and an adequate representation of eco-geographic zones, sufficient size and suitable design of the reserves (little edge effects, buffer zones) and sufficient integrity of the reserves, including levels of protection and extent of acceptance and respect of owners and other stakeholders.

Clearly, certain species are in more danger of genetic erosion, *i.e.* loss of genetic diversity or even of complete extinction than others. Evaluation of such risks is a rather

complex task, however, which requires a lot of profound information. This is especially true regarding the demography of the species and its dynamic over time and space since biological, demographic and genetic stochasticities and risks largely depend on the population structure and the changes made through human activities. Even if the relationship between rarity and endangerment is influenced by a lot of different factors (life history traits, mating system, natural *versus* artificial population structure, habitat availability and quality and many others), and rarity occurs in different forms (RABINOWITZ 1981), rare species clearly have a higher risk to face genetic erosion or even extinction than common and widespread species. The IUCN Red List categories (IUCN 2001) are based on important demographic indicators for different forms of rarity and may be useful to assess the threat. In order to qualify for a category of threat, one of the following evidence is needed: (a) population is seriously declining or is expected to decline at a specified rate, (b) population is localized, fragmented and declining at an unspecified rate, (c) population is small and declining and either fragmented or localized, (d) population is very small or localized, and (e) quantitative analysis shows a specific probability of extinction.

Habitat requirements are another decisive factor regarding endangerment. Species with narrow habitat requirements (specialists) are likely more threatened than generalists. Degradation of habitats through human impact may mean a high level of threat for some species while other species may profit from such a situation (for example pioneer species). A thorough evaluation of threats and their causes may not only help to select and prioritize species for conservation programmes; results already indicate reasonable conservation activities which need to be undertaken. As an illustration, consider the case of a species that is naturally restricted to narrow habitat conditions, like *Populus nigra* L. which is occurring naturally only in dynamic floodplains. In order to conserve black poplar, which is threatened in many European countries, it will not suffice to protect a number of *in situ* populations. In many cases the conditions of the river systems and its dynamic have been altered by human activities to such an extent that *P. nigra* is no longer regenerating naturally (LEFÈVRE *et al.* 2001). In such situation, conservation activities first need to improve or even restore the original habitat, *i.e.* to allow for flooding events or alternatively to create suitable conditions for natural regeneration by technical measures. Otherwise, conservation efforts will neither be efficient nor successful in the long run.

Assigning socio-economic values to species or its genetic resources is a complex, highly debatable and controversial task (see p. 89 ff., this volume). Depending on the perspective, value assigned to one and the same species will differ enormously. Defining socio-economic values is mainly a social or ethical problem. In spite of this, the choice of target species should be as objective as possible, based on logical scientific and economic principles related to the perceived values of the species (GIVEN 1994, MCNEELY 1988, PEARCE & TURNER 1990). Because perceptions and weights which are assigned to the different criteria generally are highly controversial, it is important that any selection based on socio-economic values of species is not only transparent but also widely supported by various stakeholders (GOs and NGOs). It also has to be kept in mind, that traditions, cultural importance, use and other factors (even mystic perception) associated with species may be equally important than economic or biological values; decisions and

activities may otherwise not be sufficiently accepted and respected by certain groups. Finally, it has been argued by several authors (GIVEN 1993, PEARCE & MORGAN 1994) that biodiversity is more prone to depletion if a species has little or no perceived value to humans because it is less likely to be given high conservation priority. From this it follows that each plant species is ascribed a comparative value and that the value given will have a marked effect on commitment of conservation; therefore it is important that overall value is ascribed as objectively as possible.

For highly valued, commercialized species, information on economic value is easily available. It is more difficult, however, to assess the potential of highly valuable species, which may contribute only little to overall economy simply because they are rare. The same is true for non wood products. Intrinsic values *i.e.* values that may arise in the future are another complex problem. Past experience has often shown that wild species once considered commercially 'useless' have proved on further examination to be 'useful', because they contain resistance genes (HAWKES 1990) or pharmaceutically active compounds. A nice example is Pacific yew (*Taxus brevifolia* Nutt.) which had no 'economic' interest (no value) until the substance *'taxol'* was discovered to be highly effective in cancer treatment (GOODMAN & WALSH 2001). All species certainly have intrinsic values that might become important in one or the other way for human benefits. Consequently, it could be argued that all species are equally important for conservation. However, as a selection of target species for a conservation programme is required, it is necessary to assign different weights to the different criteria and to rate existing socio-economic values higher than intrinsic values. Species value should be used with caution and may need to be revised periodically, since appreciations may change radically in the future, especially in the light of the drastic changes of the environment.

A species may be of little economic but high biological value if many associated species depend on it (CARTER *et al.* 1979, ROTACH 2003). For species occurring in sensitive, important ecosystems, or important flagship or keystone species, biological values may be given more weight than the other criteria.

Since value assignment is a complex task it may be useful to develop a system of points and weights to determine overall values of target species and in order to set priorities in an objective and as transparent way as possible.

Distribution of species does not respect national boundaries. Within the distribution area, the species and its genetic resources are not evenly distributed, due to both natural differences and human influence. Consequently, values, threats and priorities assigned to species are unequal within the distribution area. From this it clearly follows that different national *in situ* programmes do not need to select the same target species nor assign the same priorities. The responsibility for conserving any given species are surely not the same for all countries. Most countries have species for which they have a higher responsibility than other countries. National responsibilities from an overall perspective need to be defined through international cooperation between national programmes for conservation of genetic resources , such as the European Forest Genetic Resources Programme (EUFORGEN). At present, this task still has to be accomplished. It would be highly efficient in terms of costs and benefits, if the criteria of overall responsibility were considered when selecting target species and setting priorities for national conservation programmes.

It is rather evident that the highest responsibility and conservation priority for a national conservation programme has to be assigned to endemic species. High priorities and thus prime candidates for target species are also species which have (1) large remaining populations compared to other countries, (2) high environmental diversity which most likely translates into high adaptive diversity of the species, (3) their center of distribution *i.e.* their major occurrence in the country. As an illustration, let us consider the cases of stone pine (*Pinus cembra* L.) and yew (*Taxus baccata* L.) in Europe. Since stone pine is restricted to the Alpine arch, Austria, Switzerland and France clearly have a high responsibility for the conservation of this species. Yew, on the other hand, is rare and endangered in most European countries, except in Switzerland and in several East European countries. Even if the status of rarity and endangerment of yew makes it a prime target species for conservation programmes in many other countries, it may be more effective in terms of costs and benefits to concentrate conservation efforts in those countries with large remaining populations. However, such an approach faces diverse obstacles, the most important being a strong belief in the advantage of autochthonous genetic resources *i.e.* the opinion that local gene pools are both optimally adapted and adaptable to local conditions, consequently are the most valuable one and need to be conserved locally. Although there are strong arguments and results which question this view (HEYBROEK 1990, STETTLER 1986 and 1987, WILKINSON 2001). The importance of autochthonous populations for genetic conservation will further be discussed in the section on 'Identification and selection of populations to be conserved'. Local gene pools certainly may be valuable for conservation, but not because they are autochthonous but because they may be distinct compared to the overall genetic resources (see below). From this it follows, that in such situation, the species may be of lower priority, because not all the genetic resources need to be conserved through a national programme but only those parts which are distinct compared to the overall gene pool.

As already mentioned, distinctiveness of the gene pool is a criteria to be considered for the selection of target species or setting of priorities in certain situations. It may be an important criteria for borderline situations (limit of occurrence, edge of the distribution) where unique genetic resources exist that need to be conserved from both a national and overall perspective. This may be a reason to select a species for a national conservation programme, although it is both rare and has no economic nor other values. Distinct gene pools especially from the limit of the distribution area of a species (horizontally as well as vertically) most likely will play an important role in the future, given the environmental changes that are predicted.

Establishment of basic conservation method

MAXTED *et al.* (1997b) made the distinction between 'active' and 'passive' *in situ* conservation. Plant species are conserved in numerous environments unlikely to be considered genetic reserves, such as national parks, regional parks, natural reserves, landscape parks and many more; in each of these reserves the existence of particular species is coincidental, therefore passive, and not the result of active conservation management. These populations or particular species are not actively monitored and thus are more

vulnerable to loss or even extinction, because unfavorable environmental or biological trends would not be noted and measures to counter not adopted. In this sense, active conservation requires positive action to promote the conservation of the target species and the maintenance of the natural, semi-natural or artificially created ecosystems which contain them, including the need for associated habitat monitoring, restoration, management and protection. While conservation of genetic resources is a primary objective of most types of protected areas (MCKINNON *et al.* 1986), the general inadequacy of existing protected areas for genetic resource conservation is well recognized (IUCN 1993, *loc. cit.* pp. 175–176). Several reasons are responsible for this fact. Current protected areas commonly do not have an optimal location for conservation of genetic resources, because they do not sample all the species or the genetic variation within a target species. Moreover, the fact that an area is protected does not necessarily mean that a species occurring within the area is safely protected, since the population size may decline due to the natural dynamic, lack of management or inappropriate management for the species in question. Hence, for many target species additional conservation efforts *i.e.* active conservation in managed populations is required (FAO, DFSC, IPGRI 2001). Nevertheless, current protected areas do provide important conservation of many species, and thus are important pinpoints for the establishment of a network of *in situ* conservation areas. Their value for genetic conservation, however, needs to be carefully evaluated case by case.

For this reason, the basic conservation method *i.e.* active or passive *in situ* conservation needs to be established for each target species and given situation. While certain species may be conserved *in situ* without the necessity for active management, other species will need various protective and management measures to ensure the continued existence of the species, habitat conditions, ecosystem functions or associated species. Species thus generally differ in their basic conservation method. Highly competitive species such as the climax species *Picea abies* or *Fagus sylvatica* do not need active conservation in most cases because they are dominant and regenerate easily without any intervention. Other species such as pioneer species (*Betula* spp., *Salix* spp.) or specialists tolerating extreme site conditions (such as *Populus nigra* L., *Quercus pubescens* Willd., *Sorbus torminalis* [L.] Crantz) are week competitors; they either occupy a site only for a limited time, are restricted to very narrow habitat conditions, are highly dispersed or occur in highly influenced or man-made environments. Tree species with a metapopulation structure in which local subpopulations become periodically extinct with re-colonization from neighbouring subpopulations are at high risk of being permanently lost from small reserves. In strict reserves, such species are prone to disappear without active intervention in favor of them.

The basic method for a species may, however, partly depend on the environmental situation or the specific habitat conditions since both influence the competitive ability of the species and the natural dynamic of a given plant community. This means that, even if a general basic method applies to a given species, it may differ for each situation depending on the specific site conditions, the plant community and other factors such as former treatment or the naturalness of the ecosystem.

Consequently, for each case, the natural dynamic of the plant community in question needs to be carefully analyzed, requirements and life history traits of the target species

thoroughly studied, potential developments of the system evaluated and then compared to the objectives and targets of conservation. Results will help to decide if passive conservation is sufficient or if active conservation is needed and what kind of management will be suitable for the objectives of *in situ* conservation of the target species. Finally, findings will allow to decide if the existing protected areas are suitable for the continued conservation of the genetic resources of the target species, which areas are best suited for this purpose, what kind of interventions, if any, are indispensable and whether conflicts between the different objectives exist, in what form and if and how they can be resolved.

In situations where passive conservation is compatible with the conservation objectives and targets, existing reserves may be included in the network of *in situ* conservation areas, as long as they fulfill other important criteria such as sufficient size, adequate value of the genetic resource, suitable design and sufficient protection. It is obvious that the network of existing protected areas should form the core of any *in situ* conservation system since costs may be kept as low as possible; additional conservation areas need to be established only in locations or environmental conditions which are underrepresented in the network. Hence, to qualify as a potential new reserve, an area ideally should cover identified gaps and suffice other criteria such as sufficient size, adequate conservation value of the resource, the possibility for adequate long term protection or the lack of conflicts with other objectives. For certain species which allow for passive conservation such as the common widespread climax species *Picea*, *Fagus* or *Abies*, *in situ* conservation of genetic resources may in fact already exist to a large extent with only few new conservation units.

For species that ask for active conservation, long-term development of the stand and of its biotic and abiotic environment need to be analyzed in the light of the current management regime in order to project the likely development of the target species and on potential changes in the management.

For any given target species, a pragmatic approach could comprise the following steps:

(1) establish the basic conservation method based on relevant life history traits, silvicultural knowledge of the species and stands, the natural environment and dynamic of the system;

(2) review the network of existing reserves with respect to the occurrence (abundance, population structure) of the target species; collate all relevant information on the species in each reserve;

(3) in each reserve, reflect the basic conservation method of the target species with the specific habitat conditions, status of protection, recent and potential development, existing management regime, and determine the need for area specific protective and management measures;

(4) for each reserve, compare the new conservation objectives and protective and management measures with the already existing objectives and management regime, identify conflicts and evaluate possible solutions;

(5) retain reserves which are suitable for *in situ* conservation of target species; review them in the light of other important factors (size, restrictions, *etc.*) make final decision on suitability of existing reserves;

(6) establish, review or refine the distribution map of the target species and identify

'core populations' 'peripheral populations' or 'outlier populations';

(7) overlay distribution map of target species and map of selected reserves and identify potential gaps, if needed, list potential new areas or populations;

(8) if needed, rank potential new areas or populations according to other important factors such as size, ownership, distinctness of genetic resource, occurrence of other target species, naturalness, habitat conditions (see next section);

(9) if needed, select and establish additional conservation areas in order to complete the network of *in situ* conservation areas.

This pragmatic approach involves rather complex multi-criteria evaluations which ideally are supported by GIS (PRESSEY *et al.* 2000). Practically, the process involves finding criteria to evaluate existing protected areas (*e.g.*, the number and size of 'core',' outlying' or 'peripheral' populations it contains, the uniqueness or redundancy of genotypes it covers, its size and species composition) and potential new reserves. Selection criteria for the identification and location of new reserves are discussed in more detail in the following section.

Identification and selection of populations to be conserved

Many different criteria are associated with the selection of *in situ* conservation areas. In addition, weights and importance of the different criteria vary among species and specific situations and depend on the overall objectives of *in situ* conservation and the financial means which are available for conservation. Hence, selection of populations for *in situ* conservation is a rather complex task which is not only guided by pure scientific considerations but also by national and local priorities, strategic considerations and higher-level objectives of different kinds (*e.g.*, land use, conservation policy, forest policy, silvicultural management, legislation). For the identification and selection of populations as *in situ* conservation areas, the following criteria are useful:

- conservation value of resource population;
- distribution of genetic variation or eco-geographic distribution of target species;
- population structure of target species *i.e.* abundance, pattern of distribution, population size, core populations, outlier populations, peripheral populations;
- areas of special interest (*e.g.*, suitable existing reserves, areas with high species diversity, populations at risk in need of immediate attention);
- integrity of stand, ecosystem and habitat conditions, natural dynamic;
- land use planning, acceptance, ownership, conflicts with other land use, available finances.

Regarding the value of genetic resources, highly contrasting views exist. These questions, however, are very fundamental and should be answered and agreed upon from the very beginning of any conservation activity. It is rather astonishing to note, however, that in many existing conservation programmes it has not been clearly stated which genetic resources are valuable for what reasons and what priorities are applied when selecting them. Even on an international level, organizations dealing with genetic conservation such as EUFORGEN have not yet agreed upon criteria and priorities that may be used to assess the value of genetic resources.

The values of genetic resources depend on the objectives of genetic conservation and the priorities among them. There has however been considerable confusion over the issue of genetic conservation being for utilitarian purposes or to maintain natural evolutionary processes (YANCHUK 2001). In addition, there are opposing views regarding the question if conservation for most of the ecological concerns is met at the same time when objectives for utilitarian objectives are fulfilled. The different goals of *in situ* conservation that have been proposed and discussed in the literature (see for example KRUGMANN 1984, LEDIG 1986, ZIEHE *et al.* 1989) can be summarized in three major conservation objectives:

(1) conservation of economically important phenotypes or genes;
(2) conservation of adaptedness to given environments;
(3) conservation of genetic diversity and genetic adaptability.

Conservation of the genetic basis for certain desirable traits is the most common and traditional objective. Specifically, high frequencies of certain traits or certain trait combinations, *i.e.* the underlying genes or gene complexes are the object of conservation. Commonly, seed stands, plus tree collections, clonal archives, seed orchards, provenance trials or progeny tests serve as basic material for conservation. Neither origin nor integrity of a genetic resource is important; autochthonous populations, imported foreign provenances, landraces or selected and tested material from breeding programmes may serve as conservation populations (see p. 567 ff., this volume). According to NAMKOONG (1997), breeding populations are important components in conservation and, if properly structured, may be all that is needed.

Conservation of a population's adaptedness to a given environment is a common objective in nature protection. Because the genetic structure of a population is seen as the result of long lasting selection driven by environmental factors, local genetic resources are believed to be adapted to current habitat conditions and therefore are viewed as the most valuable resources. This may especially be the case for populations occurring under extreme habitat conditions. Object of conservation are therefore autochthonous gene pools while other genetic resources are of inferior value from this perspective.

A third approach is focussing on the conservation of genetic adaptability of a given species or the conservation of a maximum of genetic diversity within that species. Both objectives are largely identical, since genetic diversity is the basis for adaptation and evolution in a changing environment and an important buffer against pathogens and climatic extremes. Genetic diversity is thus highly valuable as such and needs to be conserved (LEDIG 1986). In addition, phenotypic (genetic) variation is also important for both improvement of economically important traits in the future and protection of these products by breeding for resistance traits against all kinds of pathogens. In order to capture as many genes as possible, especially rare or unique genes, populations to be conserved for this third objective are commonly selected among autochthonous gene pools which possibly sample a variety of different environments and have experienced little human influence. The conservation of rare genes requires large populations while conserving a maximum of genes and unique genes requires many populations from a maximum of different environments. Information on the genetic structure of the target species is needed to solve this dilemma of better selecting few but large populations or many but small populations.

Since target species differ with respect to their value for timber production, their range and pattern of distribution, their genetic structures, risks and threats to their gene pools and human impact on their gene pools, it follows that objectives of conservation differ considerably among species, especially regarding the priorities among the three major objectives. Hence, in a first step, objectives and priorities need to be clarified and decided on for each target species. Then, in a second step, values can be assigned to genetic resources and priorities among them can be defined. For species with a high economic importance, phenotypically selected and tested genetic resources certainly will have higher priority than autochthonous genetic resources, while it may be the opposite for species with little economic importance. Clearly defined priorities among the three major conservation objectives are a necessity for the establishment of any effective, cost efficient network of *in situ* conservation populations. Clear objectives and clearly assigned values to genetic resources are needed because number, size and distribution of the conservation populations depend on it. For the conservation of economically important genes, for example, a smaller population size is acceptable than for the conservation of genetic diversity or rare genes. In most cases, a combination of all three major objectives is needed, however, with different priorities among them, depending on the target species and specific situation. Assignment of values to genetic resources will help to come up with a suitable, cost efficient and highly effective network of different *in situ* genetic conservation areas.

Genetic conservation of forest tree species often concentrates on autochthonous resources (FINKELDEY *et al.* 2000, FRANK & MÜLLER 2003, KOSKI *et al.* 1997). In most cases, it remains unclear, however, why only autochthonous populations are selected as genetic reserves. The example of Norway spruce may serve as an illustration. The 'Technical guidelines for genetic conservation of Norway spruce (*Picea abies* (L.) Karst)' issued by EUFORGEN (KOSKI *et al.* 1997) does neither state conservation objectives and priorities nor does it assign values to the different genetic resources. Autochthony and an area greater than 100 ha are the only requirements for a genetic reserve. For an economically important species such as Norway spruce, one would however rather expect an emphasis on the conservation of the economically important genetic resources (including the results of breeding programmes) in combination with the preservation of the genetic diversity of the species. The importance for the conservation of genetic adaptability within the distribution area of Norway spruce differs of course; it clearly deserves a higher importance in areas with distinct environmental gradients as in Scandinavia or alpine regions, while it is less important in other areas of its natural range. Accordingly, objectives and values of genetic resources are expected to differ, and autochthony of populations is expected to be of more or less importance. In fact, rather different objectives and values for genetic resources than the ones recommended in the technical guidelines have been adopted by east European countries (PAULE *et al.* 2000) where seed stands and plus tree selections are considered as principle resources for genetic conservation. Accordingly, genetic reserves are established within the most valuable and approved seed stands which are used for production forestry and are more than 100 ha in size. A well balanced approach regarding the objectives and different values of genetic resources has been adopted by Germany (PAUL *et al.* 2000).

The selection of stands and populations for inclusion in a network of *in situ* conserva-

tion areas for a given target species ought to be based on the distribution of genetic variation, within and between geographic regions. All major gene pools should be conserved, but the number of conservation units on the other hand needs to be limited to a manageable, affordable level (GRAUDAL *et al.* 1997). Genetic variation can be assessed by different techniques. It is possible to study morphological and metric traits in field trials, biochemical and molecular markers or to guess on possible variation patterns from eco-geographic variation (see p. 275 ff. and p. 337, this volume). Unfortunately, genetic studies are only rarely or partially available, and even when data exist there are some difficulties in readily using such information for identifying conservation stands. However, populations of known superiority or distinctness (for example populations with high genetic diversity or differentiation, unique alleles, special traits, representing various migration routes) should be given special attention. The same holds true for any geographic variants or ecotypes (including subspecies) that may have been taxonomically identified.

In the absence of data on the distribution of genetic variation, a suitable approach would be to include different sites of the species biogeographic distribution area and selecting conservation areas more or less uniformly throughout the species range, together with any disjunct or unusual populations (LEDIG 1986). A somewhat more refined method is to apply a genecological[2] approach (GRAUDAL *et al.* 1995, 1997), which leads to the identification of different genecological zones. It is generally assumed that similarity of ecological conditions implies similarity of genetic constitution (FRANKEL 1970). This is based on the assumption that local adaptation through natural selection is the overriding force in the process of genetic differentiation between populations. In a landscape level analysis of genetic resources for *in situ* conservation we may then assume that genetic differentiation has tracked geographic and ecological variation (YANCHUCK & LESTER 1996) and that by providing spatial coverage for eco-geographic variation genetic variation will automatically be covered as well. Even if this may not be true, such an approach could provide an effective 'random' sample of populations across the species' range of distribution. Therefore, populations should be sampled in order to cover all genecological zones. However, unusual genotypes or rare genetic variants may be located in outlying populations or at the edge of the species range. Depending on the objectives of conservation, these populations may be of special interest. These populations are likely to fall through the 'coarse filter' based on eco-geographic zones and an additional effort must be made, if possible, to identify such genetic resources.

A comparison of the species distribution with well defined ecological zones will provide a good basis for the initial selection of conservation populations. Genecological zonation consists in identifying areas with uniform ecological conditions. Ecological zones can be derived from existing data and maps of vegetation, topography, climate and soil. Natural vegetation reflects the combined effects of the most important ecological factors and site conditions, topography or land form influences climate and soils and thereby vegetation while different aspects of climate are the most decisive factors for the distribution of plant communities. Fairly elaborate examples of practical

[2] Editors' remark: The word 'genecological' is here not used in its original meaning (see p. 279, this volume)

application of genecological zonation are found in GRAUDAL *et al.* (1995, 1997, 1999) and THEILADE *et al.* (2000, 2001).

Depending on the number and size of genecological zones, more than one population per zone should be selected, if possible. Especially for widespread, highly outcrossing species such as trees which often exhibit a semi-continuous pattern of genetic variation, more than one population may be necessary to sufficiently sample genetic structure (FAO, DFSC, IPGRI 2001). For species with scattered and disjunct distribution patterns many more perhaps smaller conservation areas are likely to be needed. In practice, the number of populations that needs to be selected as *in situ* conservation areas also depends on the levels of risks or threats at the population level, especially for rare and threatened species, the resources available to manage and maintain them, the values of existing genetic resources and the genetic distinctiveness found within the area and species. Hence, there does not exist such thing as a recommended number of *in situ* reserves; the number of reserves needs to be determined for each species and given situation separately.

The evaluation of potential populations based on genetic variation and/or eco-geographic distribution of the target species may result in a first overall idea for a network of *in situ* reserves. In several following steps, this 'backbone' of reserves needs to be modified and completed:

(1) existing suitable protected areas are designated and missing or under-represented areas (eco-geographic zones) are identified;

(2) potential populations occurring in the missing areas are identified and evaluated based on additional criteria such as population structure of target species, size of populations, type of populations (core, outlier, peripheral), populations of specials interests (threatened populations, important populations (gene flow, linkage, stepping stones), populations containing other target species, genetic or morphologic distinct population and possibly other criteria;

(3) the practical (ownership, legal status) and financial context needs to be reviewed because the final solution should be widely accepted and integrated in land use practices and be cost efficient and manageable.

Some of the mentioned criteria for the evaluation of *in situ* reserves will be discussed in more detail in the following paragraphs.

Knowledge about the population structure and demography of the target species is important in many different ways. From the pattern of species' distribution, risks and threatened populations may be inferred, core populations and outlier populations can be identified, and an adequate number of conservation areas (genecological zones, core and outlier populations, *etc.*) may be derived. Information on the demography of the species will help to identify areas of increased risks and threats that may need special attention. Moreover, isolated populations, gaps and existing barriers to gene flow which can be bridged or populations which are essential to link other populations need to be identified because such populations are of special interest as *in situ* reserves. Core and outlier populations are other focal points of special interest. Core populations are especially valuable for conservation, since they are the largest and most likely also the most viable populations that exist, growing under the best possible conditions. Outlier populations, on the other hand, may contain unique genes or different adaptive traits.

Empirical and theoretical studies show that peripheral populations are often genetically and morphologically different from more central populations, and that their conservation may be beneficial to the dynamic conservation of a target species (LESICA & ALLENDORF 1995). Peripheral populations, given their edge of range conditions and possibility of harboring rare genes, are of particular importance in providing the capacity to adapt to future climate change (*e.g.*, GUNTER *et al.* 2000). Hence, if maintaining genetic diversity and conserving adaptability and rare genes is an objective of *in situ* conservation, outlier and relict populations as well as populations at the edge of species distribution should even be over-represented in a network of *in situ* reserves because such populations not only have a higher chance of containing rare genes and gene combinations, but also may have an increased risk of losing them (FRANK & MÜLLER 2003).

As genetic diversity can be continuously eroded in small populations, conservation populations need to be large enough to conserve the existing genetic variation over generation. While low-frequency genes will be lost quite quickly from small populations, a large proportion of the genetic variation can be conserved by relatively few individuals, at least over few generations. If the objective of conservation is the maintenance of economically important genes, even rather small populations may be selected as conservation populations (NAMKOONG 1997, YANCHUK 2001). If, however, the major objective of conservation is the maintenance of genetic diversity and the conservation of low frequency genes, this leads to much larger population size requirements (see p. 413 ff., this volume). Again, the objectives of *in situ* conservation are decisive. In practice, the size of conservation stands will be highly variable, although too small populations are best avoided whenever possible.

For the maintenance of normal adaptive potential in quantitative traits (steady state of mutation and drift), LYNCH (1996) has suggested that 1,000 individuals would be an adequate effective populations size. For the conservation of genetic diversity 2,000 to 3,000 individuals are recommended (KRUSCHE & GEBUREK 1991), and for the maintenance of rare genes (< 1 %) a census number of 5,000 or more appears to be adequate for *in situ* populations in natural or wild situations for most types of low-frequency alleles (LANDE 1995, LAWRENCE & MARSHALL 1997). In the most ambitious case where recessive alleles at frequencies below 1 % should be conserved for future selection of traits, approximately 300,000 individuals are required (YANCHUK 2001). Of course, various other non-genetic considerations such as threats including the chance of catastrophic events, management requirements and others may necessitate larger populations than the 1,000 individuals. Rare species with low densities (25 individuals per100 ha) will require larger areas for *in situ* reserves than species with high densities (100 individuals per ha). Area requirements to capture the genetic variation of a 'population' may thus be in the range of 5 to 10,000 ha or more. The identification of core populations with high densities of the target species is thus important because higher effective populations sizes may be conserved on smaller areas.

It is obvious that the decision on reserve size is also linked to decisions of reserve design since a given number of 'conserved' individuals of a target species may be selected in few large populations or alternatively in many small ones. Large reserves are better able to maintain genetic and species and population diversity because of their greater species and population numbers and internal range of habitats (ABELE & CONNER

1979). Alternatively, a network of many reserves situated in distinct environments, *i.e.* many populations in different eco-geographic zones would better enable conservation of extreme ecotypes, unique genotypes and higher genetic and adaptive diversity. Hence, the conservation value of multiple reserves may be greater than the sum of its individual components (MAXTED *et al.* 1997b). However, if reserves are too small or too isolated the populations may become unviable in the long run. Smaller reserves will generally require more intensive management and monitoring to maintain the same population levels and diversity because of their inherently artificial nature (HAWKES *et al.* 1997). On the other hand, the extreme importance of the demography of populations in determining their minimum viable size has been emphasized by LANDE (1988). His point of view that environmental and demographic uncertainties may be of more immediate importance than genetic uncertainty suggest that it is wiser to 'replicate' conservation population, *i.e.* to have multiple conservation populations which consist of adequate reproductive and ecological units (GRAUDAL *et al.* 1995). Again, there is no optimal number and size for *in situ* reserves (NUNNEY & CAMPBELL 1993) because reserve design depends on the objectives of conservation and the target species. Factors such as the characteristic of the species, the population structure and demography, risks and threats, genetic structure or eco-geographic distribution need to be considered. Unmanaged populations may require larger numbers of individuals than managed, as the extrinsic factors in such populations will be under no control (SIEGISMUND 1994).

Establishing and managing an *in situ* reserve is expensive and therefore both the taxon and the reserve must be sustainable over an extended period of time or the investment will be forfeit. Therefore, the integrity of stand, ecosystem and habitat should be guaranteed for the time period. Human impact on the reserve and conflicts with other forms of land use should be as minimal as possible in the foreseeable future. Ownership and acceptance thus is an important factor. Legislation ensuring that once reserves are designated they are maintained and not developed for other uses may assist with the security and sustainability. In this sense, the selection of multiple reserves is advantageous since the eventual destruction of any one reserve will obviously have less overall impact. Moreover, if a species is extremely rare and restricted, *ex situ* techniques must have greater importance; they are in fact absolutely essential if the population size of the species has become so low that survival *in situ* cannot be guaranteed or where ecosystems in which the species occurs are so degraded that survival of the target species is doubtful. In any case, ecosystem and habitat conditions and natural processes should be as optimal as possible for *in situ* reserves in order to provide ideal conditions for the survival of the target species and to minimize the necessity for management interventions or other protective measures. This has of course a positive effect on running costs of conservation.

Definition of conservation objectives and specific targets

For each *in situ* reserve a practical management plan must be formulated and a monitoring system put in place to ensure that the objectives of conservation are met in the long run. Both successful management and monitoring require the formulation of

precise objectives and specific targets, especially regarding:

- major conservation objectives and priorities among them;
- target species and possible priorities among them in case of several species;
- precise boundaries and area of reserve where objectives and targets should be realized;
- if needed, zones with special objectives or priorities (*e.g.*, strictly reserved zone, managed zone, buffer zone), zones with special management regimes, zones with special priorities for certain target species, *etc.*;
- current and planned ideal population size of sexually reproductive individuals for each of the target species (or possibly zone);
- limits for populations size of sexually reproductive individuals (minimum) beyond which management regime needs to be revised and possibly changed, for each target species (or possibly zone);
- current and desired age class distribution of target species, family structure;
- current and desired area of regeneration needed for long-term sustainability (in a given period);
- special targets for regeneration *i.e.* conditions that allow artificial regeneration; in case of artificial regeneration, genetic resource used as planting material, minimal number of mother trees which need to be represented in planting material, *etc.*;
- detailed objectives and targets of tending interventions (minimum final stocking density, selection criteria, thinning from above or below, schematic thinning, *etc.*);
- isolation of reserve if needed , size of buffer zones and required distances;
- possibly additional objectives or targets (for example sex ratio for dioecious species).

Objectives and targets need to be as detailed and precise as possible such that criteria may be derived which can be used for monitoring purposes. For example, it is not sufficient to state that the target species should remain at the current frequency; a census number is needed. Because changes in population levels and density are a natural component of community dynamics, the objectives must allow for natural fluctuations due to stochastic, cyclical or successional changes as long as they do not threaten the long term viability of the target species. Hence, the objectives should rather define optimal and minimal census numbers, *i.e.* a frame the population of the target species is allowed to fluctuate in before other actions are taken. Regeneration is an essential prerequisite for the sustainability of the species and its gene pool over time. Hence, it is an important objective to ensure that the target species is continuously regenerating and that an adequate amount of regeneration (area, number) is constantly available either by way of natural regeneration or by way of planting. If artificial regeneration is indispensable, then the planting material needs to originate from within the reserve population and should be collected from a minimal number of individuals (50 or more trees). All these objectives and targets have to be precisely stated such that they can be checked periodically for success or failure. A monitoring system needs to be set up for this reason.

Definition of management guidelines

Once clear conservation objectives for a reserve have been formulated, a management plan for the selected reserve needs to be drawn. Management plans should be compre-

hensive but with all activities clearly documented, including timetables and responsibilities. It is preferable that management plans are kept simple with a minimum of technical jargon. Generally, a management plan should include:

- basic information on the conservation area, including maps, extent and boundaries, tenure status, ownership, history, forest inventory (species composition, volume, size classes, *etc.*);
- target species, conservation objectives and targets, reasons for selection of reserve, role of reserve in overall conservation strategy for target species;
- key reference documents on the area and target species, including any biological inventories, especially census, ecological or genetic studies of target species;
- description of target species (taxonomy (classification, identification aid, *etc.*), wider distribution, habitat preferences and limits, phenology, breeding system, phenotypic and genetic variation, biotic interactions (*e.g.*, pollinators, dispersal agents, herbivores, pests, pathogens, symbionts), local name, uses, overall threats, conservation status, *etc.*;
- evaluation of the site (reserve sustainability, factors influencing management such as legal constraints and access, obligations to local people or communities, *etc.*);
- description of the site (physical description such as geology, geomorphology, hydrology, climate, soils, land use and land tenure, biotic and abiotic description, vegetation, potential and limits of habitat related to target species, most critical factors and special risks for target species, site and population history, natural dynamic of the system, *etc.*);
- status of the target species in the reserve (distribution, abundance, demography, genetic structure and diversity – if known – autecology within reserve, interactions with associated fauna and flora, specific threats to populations, *etc.*);
- prescriptions (decisive factors that influence management, objectives of interventions and priorities among them, detailed management prescriptions with timing, frequency, duration, including selection criteria, volume, remaining cover, *etc.*, programme of interventions for planning period);
- organization (description of roles, responsibilities and rights of all those involved in management and use of the reserve, including permitted and prohibited activities and uses);
- monitoring including which criteria to be assessed and how to asses them, schedule;
- budget and human resources needed.

As the specific focus of establishing an *in situ* reserve is the maintenance of a specific target species or several target species, the management plan requires details associated with the target species being conserved, both at the general level describing the species (taxonomy, phenology, habitat preference, limits for habitat conditions, most decisive habitat conditions and factors, growth, competitive ability, breeding system, regenerative ability, conditions for regeneration, *etc.*) and the description of the target population at the given site (map of populations, density within site, autecology within reserve, synecology with associated fauna and flora, current situation of regeneration, age class distribution and others).

The objective of management is to ensure the continued existence of the target populations. Thus, firstly, management should aim to protect the population against risks and calamities. According to HELLAWELL (1991), communities are intrinsically dynamic with

basically three kinds of natural changes: stochastic, successional and cyclical. Stochastic changes are unpredictable; they result from natural catastrophes such as fire, windstorms, avalanches and others. Populations and communities have different levels of resilience to, or abilities to recover from, such events. In some cases a natural catastrophe may even be necessary for the maintenance of the population or community (*e.g.* need of fire as a breaker of seed dormancy). Management can only partly influence resilience against catastrophes (for example through improvement of individual tree stability); however it can mimic catastrophes in order to guarantee the continuance of the population, if needed. Successional change is directional and passes through predictable stages. It naturally involves the extinction of species. If the target species occurs only in certain stages of succession, successional change may have to be halted in a given stage by management interventions. Cyclical changes are usually associated with site dependent interactions such as competition. In the short term they may be quite dramatic but by there very nature, their effects do not persist, however. Therefore, extinction is unlikely, but genetic drift and founder effects may be important factors if populations persist at low levels for lengthy periods. If populations of the target species in the reserve are undergoing such cyclical change, intervention thus may or may not be necessary, depending on the level of fluctuation in population size. Hence, the management plan should include upper and lower limits for populations of the target species, beyond which management action is triggered. The formulation of a minimal census in the definitions of objectives clearly helps to decide if and when interventions are needed to counteract such cyclical changes.

Secondly, management should aim to create conditions which are favorable both for growth and vitality of the target species and for its natural regeneration. For this reason, in most cases thinning is not only permissible but necessary to ensure continued stability, vigor and regeneration of the stand. Although thinning is a form of unnatural selection which can modify the genetic composition of populations (see p. 651 ff., this volume), in most cases thinning is necessary to guarantee the continued existence of the target species. Of course the need for tending will depend on the species and site conditions and needs to be carefully evaluated. It is not possible to give detailed tending prescriptions for *in situ* reserves since the target species, the specific objectives and targets, possible risks and site conditions have a decisive influence. However, in order to avoid overstocked stands with all its negative effects on heath, vigor, stability and seed production, timely thinning is important. Usually, in order to maintain the genetic composition of the stand, either systematic thinning or thinning from below is recommended (KOSKI *et al.* 1997, GRAUDAL *et al.* 1997). Systematic thinning may however counteract natural selection while thinning from below may not lead to sufficient stability and vitality of remaining individuals, especially in older stands. Moreover, the initiation and development of natural regeneration may require stronger interventions with the removal of dominant trees in order to promote seed production and to create sufficient light conditions for the germination, installation and development of seedlings. Thinning does not have strong selective effects as long as selection criteria are similar to those of natural selection. For example, if trees are selected only based on their social status and not on quality traits, the selective effects of thinning are expected to be small (ROTACH 1994).

Regeneration is the most critical aspect of *in situ* conservation because the genetic structure of the next generation strongly depends on it. Natural regeneration is the preferred method. It is, however, not always reliable or possible. Natural regeneration certainly is the most desirable, efficient and economic method which commonly is also advantageous from a genetic point of view. However, even natural regeneration needs management expertise. The interventions need to be adapted to local circumstances, especially to the site and stand conditions. It is especially challenging and needs profound silvicultural knowledge to control the light conditions such that the seedlings of the target species will find optimal conditions while competitors and weeds are discriminated. Universally applicable instructions cannot be given, only few general principles. Since each generation has a specific genetic composition, it is good practice to continuously regenerate the stand on small patches and simultaneously in different areas. This kind of regeneration will allow multiple mating events to be transmitted to the next generation. Moreover, long regeneration periods should be used; individuals of the original stand should be removed gradually over time. The longer the regeneration period and the larger the proportion of trees that are involved in seeding, the higher is the probability of genetic information of the population to be sufficiently represented in the next generation. For this reason it is also advisable to keep a maximum of remaining trees during the phase of regeneration. Hence, it is advantageous (but more costly) to remove trees from the old stand in the course of several interventions in order to keep a maximum number of seeding trees while allowing for sufficient light conditions for the establishment and growth of the seedlings. With continuous small scale regeneration and long regeneration periods, a mosaic of stands with different age classes and genetic compositions is created and genetic diversity is best conserved and transmitted to the next generation (ROTACH 1994). The management plan thus should detail all the necessary intervention, including both temporal and spatial elements for the regeneration of the reserve during a given time period. For the planning period, detailed prescriptions should be given for each regeneration surface, especially on the location, size, number of interventions and duration, volumes to be removed or percentage of cover that remains, social status of trees to be cut and other important information (direction of cuts, risk to avoid, *etc.*).

Prior to the intervention, site preparation may be needed to favor natural regeneration. In addition, weed control frequently is necessary during the seedling stage. Later on, tending may be necessary to control for competition and to enhance abundance and vitality of the target species. In certain conditions, planting or direct seeding may be indispensable to guarantee sufficient regeneration of the target species. Planting is a fully acceptable method of regeneration for *in situ* reserves, provided that the reproductive materials used are of local origin and representative for the population *i.e.* that seed is collected within the reserve itself from a sufficient amount of individuals. Seed is to be collected from approximately 50 unrelated, widely spaced trees, preferably from the central parts of the reserve. If possible, bulked seed lots, representing as many trees in the stand as possible, should be used. To create high genetic diversity, mixing of different seed years from the stand is advisable. It is recommended to collect this kind of bulked seed during abundant seed years and to store it for future use if possible. The management plan should of course give all the necessary information and prescription

regarding all questions related to artificial regeneration and planting material.

Establishment of a monitoring system

It is unlikely that the ideal management regime will be known from the beginning. Objectives and targets thus may not be reached with the first management plan. Therefore, the population or populations of the target species in the reserve (and possibly also competitors and associated species) will need to be assessed regularly in terms of the objectives and targets in order to detect unwanted changes. If a change is indeed detected, the management prescriptions will need to be reviewed. Management may or may not be amended, depending on the nature of the change and the difference from the targets that were defined.

The monitoring process will likely involve the following decisions:

- key and associated taxa;
- method of sampling;
- observations and measurements (variables);
- periodicity of monitoring;
- data accumulation and statistics;
- feedback to management plan.

It is not possible (and not necessary) to record and monitor every species or individual occurring within the reserve. Monitoring thus involves the taking of samples of data that, if effectively selected, will reflect the overall picture of the reserve. Key species and sites within the reserve thus need to be selected for monitoring on a regular basis. The target species, which is the reason for establishing the reserve in the first place, will clearly need to be followed over time. It is likely that any taxonomically related species which may exchange genes with the target species will also be included in the monitoring programme. In addition, the abundance of other species may be directly related to or affect the abundance or diversity of the target species; these include parasites, pollinators, symbionts and competitors. Depending on the resources available, some of these associated species should also be included in the monitoring programme.

There are three main strategies for sampling a reserve: random, systematic or stratified random. In random sampling, every point in the reserve has an equal chance of being sampled. Locations may for example be determined using a random number generator to produce sets of coordinates. Random sampling is the most robust and statistically safe form of sampling. Systematic sampling means that samples are taken at regular intervals, for example along a transect or in a grid pattern. Because many biological phenomena are spatially auto-correlated, this has the advantage over random sampling of avoiding over sampling of 'uninteresting' areas at the expense of more interesting ones (MAXTED *et al.* 1997a). Stratified random sampling involves dividing the reserve into different but internally homogeneous zones (stratums) and taking samples at random independently within each zone in proportion to the areas of the zones. For example, zones could be areas of different vegetation or soil types. This would assure that all habitats are sampled and well represented. Stratified random is the most common sampling strategy applied in ecological research. However, in case of strong environmental gradients within the

reserve (elevation, temperature, water flow, *etc.*), sampling at regular intervals along a transects which parallels the gradients will more appropriate.

Numerous methods may be used for assessing species abundance or diversity for example density, frequency or cover. Density is the number of individuals per unit area. Frequency is the proportion of samples within which the target species occurs. Cover, finally, is the percentage of the ground occupied by the projection of the aerial parts (crown) of the species. Absolute measures of density may be assessed in the form of number of individuals, demographic structure, distribution pattern and biomass or volume. It may well be that abundance of different species will be recorded in different ways, depending on the accuracy required and the importance of the species to the conservation objectives of the reserve. The target species for which the reserve has been established most likely is assessed as density. In addition, estimates on age or vigor may be recorded for each individual that is counted (girth at breast height). Moreover, the area of existing regeneration may be recorded as it is encountered at or within a certain radius from the sampling point. For other species, however, a fairly rapid visual assessment of cover may be sufficient. Again for others, there presence or absence at or within a certain radius from the sampling point may be sufficient. If information on the genetic structure and diversity of the target species is wanted, genetic marker studies are required. There are various methods for sampling of individuals for genetic information (VON BOTHMER & SEBERG 1995). If all individuals within the sampling area have been tagged and labeled, numbers could be used to randomly select individuals for the genetic survey.

At a sampling point, two different ways of sampling may be applied: plot or intercept sampling: plot sampling involves taking observations at the sampling point within a usually circular or quadratic area of standardized size. Observation are made by systematically going through the area counting and perhaps measuring and even tagging each individual of the target species encountered. In the intercept sampling method, a measuring tape is laid out in a random direction at the sampling point and observations and measurements are taken on those individuals which intersect the tape.

If plot sampling is used, sampling may be done on temporary or permanent plots. Using temporary plots, *i.e.* sampling new plots each time is statistically more manageable since the assumption of observations being independent of each other is basic to most statistical procedures. However, ways exist of analyzing repeated observations such as time-series data from a set of fixed sampling units, and using permanent plots is certainly easier and more efficient. Today, it may be easy, accurate and efficient to map plot locations, boundaries and even individuals with the help of a Geographic Positioning System (GPS). Problems of accuracy and measurements in dense forests have been largely overcome today. GPS thus may be an excellent instrument for monitoring purposes.

There exist no simple rules regarding the number and size of sampling plots required, but generally the greater the number the more reliable the result (GOLDSMITH 1991). However, the information each new plot provides needs to be balanced against additional resources required to record the observations because the extra information gained from each newly recorded plot will diminish as the total number of plots rises. One way of finding the optimal number of plots is to graph the variance of the data against the

number of plots; a useful guide to find the minimum number of plots is the point where the oscillations of the graph damp down (GOLDSMITH *et al.* 1986). Another method for determining the appropriate number of samples was proposed by POOLE (1974). He suggests that variance (s^2), resolution (L) and number of samples (n) are related as:

$$n = 4\, s^2 / L^2.$$

In our case L would refer to the difference or the change in the numbers of individuals of the target species between years at a given site which the project is willing to consider unimportant (*e.g.*, the acceptable error rate). In most cases, size and number of samples taken from the reserve will be a practical compromise between the numbers that are required to produce statistically meaningful results with an acceptable error rate and the resources available for monitoring.

Regarding the periodicity, in case of long lived plants such as trees it will be in the range of several years, *i.e.* each 10 or even 20 years will be sufficient. However, in newly established reserves in which it is unlikely that the most appropriate management prescriptions are already known, intervals should likely be shorter than in well established reserves. As changes or adjustments to the management prescriptions become less necessary, intervals of monitoring may be extended.

After collecting the necessary observations in a monitoring event, results are compared to those of former events. Population characteristics are compared to those recorded in previous surveys to see if any significant changes have occurred in the intervening period. When interpreting the results of monitoring it is important to distinguish between effects due to management and effects caused by other, natural causes. Is the observed change the result of normal, natural cycles or processes, or an inappropriate management regime? The two causes are often difficult to separate. Natural factors influencing population characteristics may for example be climate change, long-term habitat alterations or successional or cyclical changes. In order to separate management related from natural causes, it may be necessary to monitor populations within the reserve, which are subjected to the management plan and populations that are left unmanaged. The establishment of different zones within the reserve may thus be advantageous from a monitoring perspective.

Monitoring should reveal trends in the observed population parameters for the target species. If these trends are interpreted as reflecting a deterioration of the conservation objectives for the target species, then the management plan and especially the prescriptions need to be reviewed and possibly altered. It is only by such a monitoring process that the need for changes in the management plan can be recognized. The monitoring process acts as a feedback mechanism, triggering changes in the management of the reserve if necessary and ensuring that the genetic resources of the target species are safely conserved.

Conclusions

At first glance, *in situ* conservation seems an easy way of conserving species and their genetic resources. This general overview should illustrate, however, that the establishment of effective and efficient networks of *in situ* reserves is far from being trivial. It is a highly complex and demanding task which needs a lot of knowledge, information or good guessing. Only a systematic approach will guarantee that all important genetic resources may be conserved in a minimum number of reserves and with a minimum of costs. Finally, two important things have to be kept in mind. Firstly, there does not exist an optimal standard solution for *in situ* conservation; solutions need always be related and adapted to the species, the demographic and eco-geographic situation, the conservation objectives and the national, social and political context. Secondly, this overview is not complete; there may be other important things that need to be included or considered which were not discussed here.

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Ex situ conservation methods

T. Skrøppa

Introduction

Conservation of genetic resources is performed *ex situ* when populations, individuals or reproductive material are maintained outside their original growth environment. *Ex situ* conservation in plants is usually applied in well-defined situations (FAO 1989):

- to safeguard populations or individuals that are in danger of physical destruction when protection *in situ* is not possible;
- to safeguard populations which are in danger of genetic deterioration;
- to ensure a readily available, continuous supply of reproductive material, either by creating a production source or through storage;
- to allow commercial improvement of a species through breeding activities and supply of genetically improved reproductive material.

Ex situ populations or collections with forest trees are established and maintained for a number of objectives, which most frequently can be classified into one or several of these four categories. Often *ex situ* conservation will be used as a complement to, or substitute for, *in situ* conservation of unique populations that are threatened in their natural habitat. The ideal approach of *in situ* conservation, or an integration of the two types of conservation methods, is often prohibited by increased pressure on land, change in land use or by economic constraints. *Ex situ* conservation becomes very useful when it is desirable to establish a well-defined seed source for commercial plantations, without doing expensive breeding operations, and at the same time assure that the reproductive materials produced are well adapted to the ecological conditions in the forest. This will be an evolutionary or dynamic approach to genetic conservation, if both natural processes and human management will determine the genetic structure of the next generation. Seed orchards and clone archives are examples of static *ex situ* conservation units as no change occurs in their genetic structure. However, they may be important populations of a breeding programme that will evolve on the basis of information from field tests where both natural and artificial selections occur. This information will be the basis for the selection of the parents producing genetically improved seeds for the establishment of forest plantations and of the next generation of the breeding population.

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 567–583, ISBN 80-967088-1-3

A general objective of genetic conservation of a forest tree species is to secure its ability to adapt to future environmental conditions and at the same time maintain the genetic basis for improved production and other benefits for human use both in short and long term (ERIKSSON *et al.* 1993). To achieve this it is necessary to save both multiple alleles at loci that have qualitative effects and maintain genetic variance due to variation in alleles that have quantitative effects (NAMKOONG 1997). It is of particular importance to conserve the additive genetic variation in adaptive traits, *i.e.* traits that respond to changes in the environment by the process of natural selection (ERIKSSON *et al.* 1993, LYNCH 1996). At the species level this can be best achieved by maintaining several populations that are genetically variable and are distributed across the different ecological zones where the species naturally occur or will be cultivated. The remarkable provenance variability found in adaptive traits of most tree species should be used to differentiate among populations (BROWN & HARDNER 2000).

Ex situ conservation populations are expensive to establish and maintain and will therefore generally be confined to species of high economic value. They will often have to serve multiple purposes, such as producing seeds for commercial forestry and at the same time secure the long-term maintenance of the genetic diversity of the seed source. However, sometimes plantings are established with the sole purpose of conservation, in particular when it is needed to safeguard endangered species or valuable populations that otherwise may be lost. Management practices and conservation efforts may vary for different conservation objectives, and may be very specific in different types of breeding populations and genetic tests.

A 'genetic resource' has been defined as a collection of biological material possessing genetic characteristics that might be of either highly specific or an extensive variable nature (ZIEHE *et al.* 1989, HATTEMER 1995). *Ex situ* conservation populations may be genetic resources of unrecognised genetic variability, but are frequently characterised genetically by phenotypic traits or genetic markers. Family relationships between individual trees may be known, and also family performance under different environmental conditions. In that case, a system of long-term field identifications, information storage and retrieval should be established and considered as an integral part of the *ex situ* conservation system (KLEINSCHMIT 1994).

This chapter discusses conservation of genetic resources in *ex situ* conservation stands and in different types of populations in tree breeding such as seed orchards, clonal archives and progeny tests. It will also treat research populations, such as provenance tests, and conservation of genetic resources in botanical gardens and arboreta. KLUMPP (p. 601 ff., this volume) treats *ex situ* conservation in seed banks and pollen and tissue storage.

Ex situ conservation stands

Ex situ conservation stands, in this paper, are defined as plantations established outside the original habitat of the genetic resources with one or several conservation objectives in mind, excluding populations in tree breeding and research. It may be to conserve the further existence or genetic integrity of one or more populations threat-

ened *in situ* and is the only solution for the conservation of the specific genetic resource. Frequently, the objective will be to establish populations that maintain as much as possible of the original genetic variability and allows for long-term adaptation to the local conditions at the planting site. In addition to conserving the original genetic variability, the established stands may be wanted as sources for reproductive materials for commercial forestry. It may then be important to integrate management for human needs with long-term evolutionary adaptation of the populations. Careful considerations, depending on the objectives, are therefore required both for the establishment and management of such stands. Establishment at several sites will be an insurance against unexpected losses, and will also allow further adaptation to a range of different environmental conditions.

Sampling the source population

Considerations of sample sizes of populations in dynamic genetic conservation should be based on genetic, demographic and environmental factors (GRAUDAL *et al.* 1997). From the genetic point of view the basic seed collection should sample the original population sufficiently well and provide enough additive genetic variance to allow natural selection to take place. It is also important that the established stands are large enough to reduce the risks of loosing genetic variability by random events (*e.g.*, drift) and to avoid the building-up of co-ancestry that causes negative effects of inbreeding in future generations. Population sizes recommended are based on calculations involving the effective population size (N_e), which refers to an idealised random mating population with equal number of males and females, equal fertility and number of offspring and addresses the loss of genetic variation (see p. 162 ff., this volume). These requirements are generally never fulfilled, and an effective population size of 50 individuals may correspond to at least 100–200 individuals in a natural stand (GRAUDAL *et al.* 1997, ERIKSSON 2001). The concept of minimum viable population size takes into consideration both the expected demographic development of the population and its genetic diversity over a number of generations (see p. 421 ff., this volume) and has been used to calculate population numbers. LANDE (1988) considers the demographic and environmental factors to be of more importance than genetics in determining minimum viable population sizes. In practical situations it is generally not possible to calculate any exact figure for such a number that takes all the different factors into account (GRAUDAL *et al.* 1997).

For sampling one source population, BROWN and HARDNER (2000) define an adequate sample as one that includes with 95 % certainty at least one copy of an allele with arbitrary frequency 0.05, see also ERIKSSON (p. 391 ff., this volume) and HATTEMER (see p. 413 ff., this volume). This requires seeds after open pollination from a minimum of 15 unrelated trees in natural forest stands. GRAUDAL *et al.* (1997) recommend that seeds should be collected from 25 trees sufficiently spaced to avoid relatedness. This will create a founder population for *ex situ* conservation with an effective population number of at least 100. The sample size should be increased if it is desirable to sample more rare genetic variants (KRUSCHE & GEBUREK 1991).

If the objective is to establish conservation stands representing the genetic variability in a larger area, a region of provenance, then seed samples should be collected from several, say 10-20, stands. The number of seed trees from each stand can then be reduced.

If the source population is very small and it is desirable to conserve the last remaining trees *ex situ*, vegetative propagation by rooted cuttings or by grafting may be necessary. In such cases, however, the remaining individuals are likely to be related if they are not widely dispersed and should be mixed with individuals from other populations in the established *ex situ* population where sexual reproduction is to take place. This will be further discussed under the concept conservation seed orchard.

Seeds should be obtained in a year with abundant flowering and good seed production. Care should be taken during seed collection and seed handling as genetic changes may be induced (HATTEMER 1995). Usually the identity of mother trees (seed donors) is not kept. After separate handling the individual seed lots are mixed in equal quantities to control as far as possible contributions from each mother.

Site selection and plantation size

The site selected should be representative for potential planting sites of the species, and the environmental conditions should make the stand able to produce reproductive materials for natural regeneration or seed collections. Isolation from other stands should be attempted to reduce seed and pollen contamination from outside sources. Isolation belts of 300-500 m are generally recommended. Such isolation belts also reduce the gene flow from the conservation stand into local populations, which in some cases may not be wanted. FAO (1992) recommends a minimum size of 5 hectares, preferably more than 10 hectares. Smaller areas may also be acceptable; in particular when each plantation belongs to a set of replicated plantations. GRAUDAL *et al.* (1997) recommend that the aim should be a final stand of size 500-1,500 mature trees or more. The shape of the plantation should be one that ensures adequate pollination within the stand and will depend on topography and the direction of prevailing winds. Other conditions, including land tenure, should as far as possible assure a healthy long-term existence of the established plantation.

Establishment

The *ex situ* conservation stand will generally be established by planting of seedlings, but may also be carried out by vegetative propagules or by direct sowing. The collected seed lots should be germinated and seedlings should be produced according to current practice. At planting, the conventional spacing for the species at the given site should be applied. HATTEMER (1995), however, recommends that conservation stands should be established by direct sowing or planted at narrow spacing to allow a fast adaptation to the new environment at the very beginning. This will require thinning once or several times at an early age.

Box 1. *Ex situ* conservation of *Pinus radiata* D. Don.

The widely planted tree species *Pinus radiata* originates from three isolated locations along the coast in mainland California and from two islands outside the Mexican coast, Guadalupe and Cedros Islands. The Guadalope Island pines, which carry resistance to important diseases of the species, are threatened by extinction, and the number of mature trees has decreased from 383 in 1964 to about 150 in 1992 (ELDRIDGE 1996, LEDIG *et al.* 1998). Grazing pressure of goats is the main reason for lack of natural regeneration. Seeds from several collections have been the basis for conservation plantations in several countries. One of these was established in Australia in 1994, when seedlings from 120 families collected after open pollination on Guadaloupe Island were planted on 23 hectares (ELDRIDGE 1996). Seeds for the next generation will be collected in the centre of this plantation and may be used to restore the original population in its natural habitat if the goat grazing can be controlled.

Management

It is important to secure the long-term existence of the genetic resource, and measures necessary to preserve the stability of the plantation should be taken. This will require a management system, which controls weed and other competitive species, animal grazing and fire. Sometimes it may be beneficial to establish conservation stands that mix two or more species, and special care must be taken in their tending as regards species composition. Thinning must be done sufficiently early and strong enough to reduce danger of windfall and snow breakage and should also promote flowering and seed production. The main objective of the conservation plantation will direct how thinning should be made. It should in principle support the natural selective forces and therefore not be purely systematic. However, if it is required that the plantation should produce seeds for commercial use, then compromises must be found.

Regeneration

A reproduction phase, which can either be natural or artificial, is required for the long-term existence of the genetic resource population. A slowly progressing natural regeneration has been recommended to secure the total genetic diversity of the stand (HATTEMER 1995) and should be encouraged if feasible. However, artificial regeneration by planting seedlings originating from the stand at the same or at another site having similar environmental conditions may also be applicable. The principles for selecting and harvesting trees should be similar to the first generation, but will also depend on the objectives of the *ex situ* conservation unit. However, if a directional selection is made for specific traits, then care should be taken to control the frequency of related individuals, *i.e.* half-sibs. Although costly, this can possibly be done by the use of molecular markers.

Box 2. *Ex situ* conservation stands of tropical pines.

Ex situ conservation stands of three Central American pine species, *Pinus caribaea* var. *hondurensis* Barr. et Golf., *Pinus oocarpa* Schiede and *Pinus tecunumanii* (Schw.) Eguiluz & Perry, were in the late 1970's and early 1980's established in Australia, Brazil, Côte d'Ivoire, India, Kenya, Tanzania, Zambia and Thailand (DFSC & FAO 2001). The programme had several purposes: genetic conservation of threatened populations, production of seeds that could be used for plantation establishment in these countries and generation of genetic materials for further breeding. An assessment of this programme was made in a study including 135 conservation stands of sizes 1 to 37 hectares planted at 39 different sites (DFSC & FAO 2001). The three pine species showed a remarkable adaptation to many different ecological conditions and had generally satisfactory growth. It was concluded that stands were in general well established and managed initially, but that active management, in particular thinnings, was neglected in later stages. Poor isolation from contaminating seed sources and limited cone setting restricted the use of the stands as seed sources and have made regeneration difficult. The local demand for seed of these species has also been much smaller than expected at the time of establishment.

The study (DFSC & FAO 2001) stresses several important considerations that should be made when establishing an *ex situ* conservation programme: the need for management and follow-up until generation turnover; seed production and conservation objectives may be difficult to combine in the same population; local interest in using the species and the provenances in question is important.

Effects of establishing several populations

The overall conservation of the genetic resources of a species is most efficiently done in a system with several rather small populations being established under different environmental conditions (NAMKOONG 1984, ERIKSSON *et al.* 1993, ERIKSSON & VARELA 1995, see also p. 585 ff., this volume). A set of 20 sub-populations, each with an effective population size of at least 50 individuals, has been suggested. The genetic resource of the *ex situ* populations established in this way from source populations will be conserved and developed further during the ongoing evolutionary processes at different sites. Due to the high costs involved in such a system it is most often done in a joint breeding and genetic conservation programme.

Ex situ *conservation stands of introduced species (exotics)*

Several conifer tree species have been planted outside its natural range in Europe. This is the case for *Picea abies* (L.) Karst. and in particular for species that were introduce from North America, *e.g.*, *Picea sitchensis* (Bong.) Carr., *Pseudotsuga menziesii* (Mirb.) Franco and several *Abies* spp. Both research and practical experience have shown that seedlings from seeds harvested in first generation plantations of introduced species frequently perform better in their new environment than seedlings from direct seed import (NIELSEN 1994, ENNOS *et al.* 1998). It therefore becomes important to conserve

the genetic resources of such 'landraces' and use them as a seed sources for commercial plantings. Older stands can be managed as conservation plantations and regenerate naturally, if feasible, or new conservation plantations can be established on the basis of seeds collected from a number of the trees in one or more stands. Such plantations should be established and managed in a similar way to the *ex situ* stands discussed earlier.

Box 3. *Ex situ* conservation of *Picea abies* in Sweden.

Seedlings from seed collections from at least 100 trees in 26 natural stands of *Picea abies* between latitudes 60 and 67° N in Sweden were, in 1987–90, planted in 67 plantations at 33 localities (SKOGSSTYRELSEN 1997). Each plantation was planted with seedlings from one natural population and the size varied between 1 and 6 ha. Most populations were replicated at two or more plantations located in the region were the seed was collected. The aim of the programme is a dynamic conservation of the genetic resources of spruce systematically sampled from a large area. The plantations will be managed as normal forest plantations.

In southern Sweden, where it is more difficult to identify autochthonous populations and to avoid pollen contamination from transferred provenances, grafted clonal archives were established at six localities. Altogether 586 spruce clones, originating from old stands that with high certainty were natural, were grafted in these archives.

Conservation seed orchards and clone collections

The term seed orchard is most frequently used in the breeding context to denote the mass production unit of genetically improved seeds. However, a type of seed orchards may also be established to fulfil specific genetic conservation objectives without being part of a breeding programme and will then be denoted conservation seed orchards. The aim may be to conserve or even increase the genetic variability of collections of single trees or small populations that have an endangered status, insufficient fructification or unwelcome pollination in their natural environment. This will in most cases relate to rare species or those with scattered distribution. The collection of a sufficient number of clones scattered from a large, but ecological similar area, will constitute a new interbreeding population that will produce seeds with a high genetic diversity. The offspring can be planted in the forest and be exposed to natural selection at different localities. Such conservation seed orchards have been established in Germany for the wild fruit trees *Malus sylvestris* Mill. and *Pyrus pyraster* (L.) Burgsd. (KLEINSCHMIT & STEPHAN 1998).

Clone collections may be established in cases when evacuation of single individuals or threatened populations is needed, without the intention of seed production. This method is discussed more in the section on clonal archives.

Conservation seed orchards will most frequently be established by vegetative propagation either as grafts or rooted cuttings and not so often by seedlings (seedling seed orchards). Techniques for establishment, choice of locality and management will

be similar to the same type of units in tree breeding and will be discussed in that section.

It is beneficial to include a large number of clones in a conservation seed orchard. The individuals will often come from small populations where chances are high that inbreeding has taken place. It is therefore important that they represent many stands or many trees with a scattered distribution within each ecological region to obtain a high outcrossing rate. It has been advised that at least 50 or preferably more clones should be included in the orchard to avoid loss of additive genetic variance and secure against loss of important alleles (KLEINSCHMIT & STEPHAN 1998). The size of this type of orchard should, from a management and seed production point of view, be no less than one hectare. An initial spacing of 5 × 5 m could later be increased to 5 × 10 m, if needed to obtain abundant flowering. With 50 clones and eight ramets per clone a seed orchard of this type will cover one hectare.

To prevent loss of genetic diversity the flowering and seed production should be observed in the conservation seed orchard and seeds should be collected in a year with abundant flowering. It is advisable that the bulked seed lot consists of approximately equal amount of seed from each parental clone from which seeds should be collected separately.

Ex situ conservation populations in tree breeding

The main purpose of forest tree breeding is to produce reproductive materials for the establishment of future forest stands with specific adaptive properties and qualities beneficial for human utilization. A typical breeding programme will consist of a hierarchy of planted populations: clonal archives, seed orchards, progeny tests and more advanced generation family tests (WHITE 1987, ZOBEL & TALBERT 1988, EL-KASSABY 2000). A subset of the materials in these populations is selected to be the parents for the next breeding cycle and constitutes the breeding population. The germplasm in these plantations originates from initial selections in natural stands or adapted plantations. The populations are established and maintained *ex situ* and are frequently only intended to be managed and kept for a short period of time. Their genetic structures may be fixed, apart from genetic changes due to population reductions caused by natural events or human management. The breeding population allows for planned changes in genetic structure, being composed of a selected set of genotypes that will be crossed according to some mating system to produce the next generation of the breeding cycle (NAMKOONG *et al.* 1988). It will be subject to ongoing breeding and selection for several generations and should adapt to changing environmental conditions. At each generation, a subset of its members will be selected to provide genetically improved materials for commercial use, *i.e.* by seed production in seed orchards.

The different populations in a tree breeding programme represent genetic resources of characterised genetic variability and offer a potential for the realisation of particular desired trait expressions, and are thus valuable genetic resources (ZIEHE *et al.* 1989). How well they conserve genetic variability depends on several factors, *e.g.*, size and procedure of the initial sampling, size of the breeding population, intensity of

selection, type of selection procedure, levels of inbreeding and long-term maintenance of the populations. Specification of levels for these factors will depend on objectives, genetic knowledge and the materials available. In the early days of breeding, conservation of genetic resources was not an important consideration. More recently it has been recommended that conservation and long-term breeding should be accomplished simultaneously in the same populations (*e.g.*, DANELL 1993a, ERIKSSON *et al.* 1993).

In the following a presentation is made of different types of populations in tree breeding programmes and the role they may have in the conservation of genetic resources. An evaluation of the influence of tree breeding on the gene pools of forest trees in general is also outside the scope of this chapter and has recently been done by EL-KASSABY (2000).

Genetic variability in breeding populations

Success of a breeding programme depends on the presence of additive genetic variance in the breeding population, both for the target trait(s) selected and for other traits of adaptive significance. In early generations alleles at intermediate frequencies ('common alleles') will provide most of the gain from selection (FALCONER & MACKAY 1996, NAMKOONG *et al.* 1988) and are therefore of main interest to the breeder. Less common alleles, occurring in a small proportion of individuals, contribute to potential variation that may be important for long-term breeding (DANELL 1993). For the conservation of rare alleles or low frequency recessive alleles, census population sizes of 5,000 or more are required. Such genes cannot be maintained in breeding populations and should be conserved in larger *in situ* reserves (YANCHUK 2001).

Relatively small initial sample sizes from the natural or planted forest are needed to establish a breeding population that will produce considerable genetic gain and at the same time fulfil some conservation objectives. Estimates of sample sizes needed vary, but it is suggested that the effective population size (N_e) should be in the range 30 to 80 (YANCHUK 2001). This sample size will provide genetic gain for the selected traits and will also be sufficient to keep with a high probability one copy of neutral alleles of frequencies higher than 0.05 in the population (YANCHUK 2001). More individuals are required to keep multiple copies of dominant alleles in the breeding population and very large sample sizes to keep recessive alleles (YANCHUK 2001). Population sizes as suggested above should be sufficient to provide gain from selection for at least ten generations of breeding. However, additive genetic variation will be lost at each generation in the traits that are under direct or indirect selection at rates that depend very much on the selection procedure. In other traits there will be a loss in additive genetic variance due to random sampling by a factor $1/2N_e$ per generation (BULMER 1980). For not too small N_e this will have limited negative effect on the short term or for the first ten breeding generations, which can be as long as 200 years with European conifers (DANELL 1993). As stated by ERIKSSON (2001), this is a random loss of genetic variance and not specifically related to adaptive traits.

Frequently tree breeding is done in several independent populations that may have different adaptive profiles. This is the case for breeding programmes of *Pinus sylvestris*

and *Picea abies* in Sweden, which are based on populations with effective population sizes between 50 and 100 and distributed along light and temperature climatic gradients (DANELL 1993b). This metapopulation conserves genetic variability combining both inter- and intrapopulation variation and secures at the same time the potential for adaptation to a wide range of environmental conditions. ERIKSSON (see p. 585 ff., this volume) discusses the multiple population breeding system, its use in genetic conservation and links to tree breeding.

Clonal archives

Clonal archives or clone banks are collections of individual trees, often of high age, that have been propagated vegetatively as grafts or rooted cuttings, or in more recent years, by micropropagation methods (see p. 623 ff., this volume). Such collections were in the early stages of tree improvement programmes used as an intermediate storage of plus trees selected in wild stands before they were further propagated in seed orchards or subject for further genetic testing. When the grafts start to flower, such collections can be used for making artificial crosses for progeny testing and for further crosses among selected parents when progeny test data become available.

Clonal archives should be established with several replicates of each clone, preferably in replicated contiguous plots. This will make thinning possible and reduce chances for loosing clones due to fire and other disasters. As an example, clonal archives with *Picea abies* in Norway were established in four-tree row plots at an initial spacing of 2.5 × 5.0 m with four replicates. Grafting is either done directly in the field on rootstocks planted earlier or on rootstocks growing in containers in the nursery that later will be planted in the field. Propagation procedures may vary between tree species, and grafting may for some species be problematic due to graft incompatibility. It may be necessary to fence the archives to avoid animal browsing, and weed control may initially be necessary. The clonal archive should be planted at a site that promotes flowering. It may be beneficial to stimulate flowering using hormone applications (PHARIS *et al.* 1987). Recently, clonal archives have been established by growing grafts in large containers in breeding orchards where they can be given optimal water and fertiliser treatments. This will facilitate both flower induction and the making of controlled crosses. However, grafts in potted breeding orchards can only be kept for a few years and do not play an important role for genetic conservation.

Clonal archives preserve specific genotypes and are static genetic conservation units. Often clones from different provenances are mixed in the same archive. No natural regeneration is intended at the archive site. The archives therefore have a limited lifetime, and it may be necessary to regraft the clones when the objective is long-term preservation of the specific genotypes. The clonal archives are reservoirs of genetic variability that to a large degree is characterised. This variability is in particular important in the early stages of a breeding programme, but can also be used to provide materials at more advanced stages when genotypes with specific genetic properties are wanted.

Seed orchards

Seed orchards are the mass production populations for seeds from a breeding program to commercial forestry. They should deliver consistent, abundant yields of genetically improved seeds for high yielding plantations within a specific ecological zone. The parents in the first-generation grafted seed orchards were phenotypically selected in forest stands. Studies have shown that the amount of genetic variation present in natural populations can be retained or even increased in the selected set of parents, as was demonstrated in a review of results from 12 orchards (EL-KASSABY 2000). However, somewhat reduced genetic diversity was found in the offspring from second-generation seed orchards based on a reduced and selected set of parents (EL-KASSABY 2000). These results show that in particular first-generation seed orchards can be valuable genetic resources even after they have terminated as seed production units.

Seed orchards are generally of two types, established either by grafts or rooted cuttings (clonal seed orchard) or by seedlings (seedling seed orchard). The type of orchard is to a large extent determined by the age to flowering and the generation in the breeding program. The first-generation seed orchards in species that take decades to the first flowering, such as *Picea abies* and *Pinus sylvestris*, were grafted with scions from trees selected in mature stands. In other species, *Picea mariana* (Mill.) B.S.P. and *Pinus contorta* Dougl. are examples; seedling seed orchards were established at dense spacing with seedlings from a large number of families. They were later thinned on the basis of information from family tests. Most advanced generation seed orchards are clonal, based on selections made in full-sib families after crosses among members of the breeding population.

Seed orchards require layouts different from the clonal archives. The ideal situation is when the seed orchard functions as a closed random mating population. This requires close to equal reproductive output from the different genetic units in the orchard. It is important to maximise the outcrossing rate and minimise inbreeding. Therefore, each clone in a clonal seed orchard should have every other clone as a neighbour and the average distance between members of the same clones should be maximised. GIERTYCH (1975) has reviewed different seed orchard designs. To achieve the predicted genetic gain from breeding it is also important that the orchard is located so far from local stands of the same species that outside pollen migration is minimised. This is in particular important when the orchard is producing seeds for an ecological zone different from where it is located. Seed orchards should be established at a site that promotes flowering.

Seedling seed orchards are established with members of half-sib or full-sib families, which may originate from parents selected in natural stands or from controlled crosses in clonal archives or clonal seed orchards. At establishment, a planting design must be chosen that allows for thinning based on genetic information and also maximises the distance between members of the same families. A large number of seedlings are planted per family at the same or even closer spacing than ordinary plantations. They are frequently designed to be thinned based on genetic information both from the orchard itself, if the site conditions are appropriate, and from progeny trials estab-

lished at several sites with sibs from the same families. The genetic diversity present in this type of seed orchard at reproductive age will therefore be influenced by several factors: the number and selection of the original parents, the way families are generated (*i.e.*, degree of relatedness), and the selection and thinning regime that is conducted in the orchard. In the second or later generations of breeding programme the genetic variability among families may be strongly influenced by methods and intensity of selection in the breeding population.

Seed orchards require more intensive management than clonal archives. At establishment, weed control is necessary and cultivation measures should maintain conditions favouring growth and flowering and reducing competition for water. Thinning is important to provide enough light to the crown to promote flowering and can also be an important component of the genetic management of seed orchards specifically when the thinning is based on genetic information from progeny trials. The planting design and the specific thinning regime, being random or selective within and between families, will influence the genetic diversity of the orchard when its starts to produce seeds.

Seed orchards are genetic resource populations that play an important role in producing seeds for production plantations. They will play a minor role in the breeding programme when breeding continues into the next generation and more advanced reproductive materials is available, either from new seed orchards or by vegetative reproduction. However, the first-generation grafted orchards can have a role in genetic conservation for a considerably longer period of time, in particular as clonal archives of progeny tested clones. It is in more rare cases that natural regeneration will take place at the seed orchard site so that the orchard population will develop into a new generation.

Genetic test plantations in tree breeding

Genetic field tests are established in tree breeding programmes for a variety of purposes. The most common field plantations are progeny tests that are planted to evaluate the breeding values of parents to be included in breeding populations or in seed orchards. They are established from seedlings from half-sib families from open pollination in stands or seed orchards or from controlled crosses, but may also be based on full-sib families from specific crossing designs (BRIDGWATER 1992). Progeny tests are planted in field designs with replicates and can be planted in single tree plots or with family members in multiple-tree plots (LOO-DINKINS 1992). Progeny tests should be planted at several sites with different environmental conditions. The number of seedlings per family and site may vary, but is most frequently between 15 and 40. Field tests may also be established as a source for selection of the next generation of the breeding population and may rarely be converted into seedling seed orchards. A third type of test plantations is clone tests that are planted to evaluate the performance of different clones for inclusion in clonal breeding programmes.

A large proportion of the trials are planned to provide genetic information during a short-term period, say 10-20 years, and neither experimental design nor site conditions will make them reliable for long-term genetic studies, nor to be regenerated naturally. However, even if these trials were not designed with genetic conservation in mind, they are valuable genetic resource populations due to the amount of genetic information available

of each individual, clone or family. It is of particular importance that clone or family performance is known from several sites and for traits of high adaptive value.

The field trials are particularly valuable for two purposes: screening for genetic resistance to insect, decease and mammals; and for studying the implications of global climate change and producing materials with specified and directed genetic variation in traits related to climatic adaptation (YING 1995, XU *et al.* 2000, LIPOW *et al.* 2002, 2003). The family relationships will be important for identifying low frequency or recessive resistance alleles. The available information in other traits makes it possible to estimate genetic relationships between traits.

The first-generation progeny tests often contain a large number of families from different populations in the same ecological zone. Selections are normally made before the plantations close (crown closure) and self-thinning starts, and most often plantations are then infrequently utilized. One exception is the collection of wood quality data, which has to be done at a later age. However, by systematic thinning some of these progeny tests will have the possibility to develop into mature stands that contain a large proportion of the original genetic diversity. Some of these tests should therefore be managed and maintained as genetic resources. The future use of the materials will require that field identifications are updated, and databases of genetic information should be kept.

Ex situ *conservation in research plantations*

Forest geneticists have established field experiments with provenances, families and clones for other purposes than tree breeding. Such trials have provided valuable knowledge about the distribution of genetic variation in phenotypic traits, which have been used in breeding and for planning and implementation of genetic conservation programmes. As in the breeding trials, a large proportion of these tests are planned to provide genetic information during a short-term period, say 10-20 years. Some of the trials with families and clones should be thinned and managed to develop into mature stands similarly to the first-generation progeny tests.

One particular type of research plantations is provenance trials, which contain smaller or larger samples of provenances from the whole or a subset of natural range of a species (see p. 275 ff., this volume). These trials are often planted both within and outside the natural range of the species and may involve institutions in several countries in an international co-operative effort (*e.g.*, IUFRO). Information is therefore available about the performance of provenances when growing in very different site conditions. The value of a provenance trial as a genetic resource will depend on the number of populations included, the number of individuals per population and on the geographic coverage of the distribution area. The experimental design used at planting will also have implications for how long the plantation can serve as a reliable genetic test. However, several of the provenance trials can be thinned and still represent a large part of the available genetic variability in a species. Due to effects of natural and artificial selection, trials may develop into local 'landraces', with very diverse genetic background. In some specific cases provenances trials have been used to restore a valuable seed source that has disappeared in its native environment, as described in Box 4.

The international series with North American conifer species planted in several European countries constitute one specific type of provenance trials. They represent valuable genetic resources of these species outside their natural range, which may be proven useful in their original environment (LIPOW *et al.* 2002).

Box 4. Restoring a seed source from a *Picea abies* provenance experiment.

The IUFRO 1964/68 provenance experiment with Norway spruce planted in 20 field trials in 13 countries (KRUTZSCH 1974) has been used to restore a Polish provenance that otherwise would have been lost. In 1979, scions were collected from individual trees of the Kolonowskie provenance from 14 field trials in 11 countries, and were grafted in what is called a 'reconstitution seed orchard' (GIERTYCH 1993). This provenance was proven to be a generalist in this large experiment, exhibiting good performance on all sites. The original seeds were collected from a wide spruce area with known geographic co-ordinates. The original population, however, is lost. Currently, 109 clones in the grafted seed orchard, which produces seed for commercial forestry, and established progeny tests, represent this population (CHALUPKA, pers. communication).

Ex situ conservation in arboreta or botanical gardens

In addition to their extensive collections of wild and cultivated plants, botanical gardens and arboreta maintain samples of individuals of different tree species (HAMANN 1992). Such collections played earlier a key role in plant introductions from one continent to the other and in taxonomic research. However, the many collections in botanical gardens have failed to fulfil basic requirements for the conservation of genetic resources due to several factors: the low number of individuals represented of each species, lack of knowledge of exact origin, improper labelling and problems to collect seeds due to inbreeding and interspecific hybridization (HURKA 1994). As a function of the small number of individuals, the within species genetic variability, in most cases, is totally underrepresented. At present, these institutions therefore play a minor role in the conservation of genetic resources of forest tree populations. They can contribute to the maintenance of unique and rare genotypes that in particular could be important when propagated for ornamental use. That will require long-term strategies for their maintenance and reproduction.

Collections of trees in arboreta often have a role as public parks and are important for rising public awareness. They are therefore valuable for demonstration and education. A careful design is required for *ex situ* collections that can serve this purpose and relevant information about the species and their genetic resources should be provided.

Conclusions

Ex situ conservation methods can be successfully applied in a variety of situations. They can be used when a genetic resource is threatened in its natural habitat and its further existence and development require a reestablishment at another location. In the early domesti-

cation of a tree species *ex situ* stands can function as seed sources allowing rapid procurement of seeds for commercial plantings. *Ex situ* populations are in particular important in tree breeding programmes when genetic management is required to enhance the gene pool simultaneously for both human utilization and adaptation to a variety of environmental conditions. This can best be achieved in a network of sub-populations that are allowed to develop in response to different conditions of growth or selection criteria.

Ex situ populations are generally too small to maintain all rare or low frequency alleles that may have potential future value. Large *in situ* reserves will contain such alleles in adequate numbers, and an integration of *ex situ* and *in situ* populations may therefore be necessary if new allelic variants must be sought for long-term breeding. The two types of methods should therefore be considered as complementary.

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Multiple population breeding system as a method to conserve genetic variation

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Introduction

One important issue in breeding, and to some extent in conservation, is that the utility of commercially important forest trees experience profound changes during their lifetime. Traits of high economic value today may be of limited value at harvest 50 –150 years later. For example, one type of wood may be highly appraised today but of low value at a later occasion. Most of us have noticed a rapid change in environmental conditions during the 1990s, which means that the growth conditions for our tree species have changed. We anticipate that the rapid development in most forestry disciplines will also extend to silviculture. A breeder of annual crops may easily cope with changed cultivation techniques by running progeny trials under the new cultivation regime. Even so, it will take several generations until the annual crop will be marketable as a new variety. It is obvious that a forest tree breeder cannot change silviculture regimes from one year to another, although it might be possible at times during a rotation. Similarly, tree breeders cannot change breeding goals too frequently since the long rotation times are constraints. Forest tree breeders must prepare for uncertain futures by developing a breeding strategy to mitigate the effects of changed breeding goals and changed environmental conditions. This was the reason for the development of the *Multiple Population Breeding System*, MPBS, for forest tree breeding during the mid-1970s by NAMKOONG (1976) and with its first operational suggestion by NAMKOONG *et al.* (1980). It was later extended to combined tree breeding and genetic conservation (NAMKOONG 1984). Some authors use the term metapopulation for the same concept as MPBS (*e.g.*, DANELL 1993, HATTEMER 1995). For a discussion of MPBS it is useful to examine the functional roles of different populations.

The functional roles of populations

The role of a production population is frequently confused with that of a genetic con-

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 585–599, ISBN 80-967088-1-3

Box 1. Functional types of populations.

Genetic conservation population: the seeds, acorns, nuts, plants, tissue cultures, or trees that are included in the genetic conservation activity
Breeding population: the collection of trees that will carry the advancement of breeding into future generations
Production population: the population that should produce human utilities
Propagule population: the trees or plants utilized in sexual or vegetative propagation

servation population. This was one of the reasons why KANG (1982) stressed that it is important to identify the functions of different types of 'physical' populations. Such a distinction of populations is of greatest significance since different populations require different amounts of genetic variation. The definitions of the different functional populations are given in Box 1 from ERIKSSON & EKBERG (2001).

As is evident from Box 1, not only trees but also various parts of trees belong to the genetic conservation population as long as they are aimed for the purpose of genetic conservation. Trees in all kinds of progeny trials belong to the breeding population as long as these trials are aimed at breeding. Similarly, the trees in seed orchards or clonal archives will also belong to the breeding population if these trees are used for mating to obtain seeds for establishment of a filial generation within the breeding population. If the trees in seed orchards or clonal archives are used to produce seeds or scions for vegetative propagation they belong to the propagule population. Finally, all forests that are used for production of any kind of human utilities belong to production populations.

As seen from the role of seed orchards and clonal archives, one physical population may serve more than one function. Similarly, the breeding population may under certain conditions also serve as a genetic conservation population (see below).

Since additive variance is the fuel for natural selection and for breeding, it is of greatest significance that there is a high additive variance in the genetic conservation and the breeding populations. If there is a large segregation in the breeding population, it is important that the variation is narrowed down in the production population. As one example, it is obviously useless to have early flushing Norway spruce (*Picea abies* [L.] Karst.) plants in the production population at sites where late spring frost will kill or severely damage the early flushers. Similarly, breeders will not select inferior growing genotypes for their seed orchards.

What is MPBS?

MPBS means that the genetic conservation population is split into subpopulations with each having an effective population size, N_e, of approximately 50 individuals. If the entire breeding population has 20 subpopulations, the census number of trees would be 1,000. With such a population size, few alleles would be lost for random reasons unless they are extremely rare. Each subpopulation has a separate breeding

goal, which could be the improvement of the same trait under a number of different site conditions or the improvement of a number of different traits. In genetic conservation the objective is related to adaptation to different environmental conditions of the different sublines. The split of the genetic conservation populations into several subpopulations allows for an increase of the total additive variance of the genetic conservation population with marginal loss of within-subpopulation variance (ERIKSSON *et al.* 1993).

It is worth pointing out that MPBS differs from *sublining* in that each subline has the same breeding goal. The objective of sublining is to avoid selection of related trees for seed orchards and thereby avoidance of inbreeding depression in the production population. These objectives are not mutually exclusive, however, and it is possible to have a few sublines within each subpopulation.

The MPBS is not hierarchical in contrast to genetic resources conserved for agricultural crops. In breeding of these species, there is mostly an elite population and several other populations with different levels of improvement. Genes from the lowest level can be introduced into the elite population stepwise via matings with plants in the intermediary populations.

Sampling of existing genetic variation and adaptedness

Owing to the long generation times of most forest trees, it is not meaningful to discuss forest tree genetic conservation in Europe beyond 20 generations. This means that discussion of losses of alleles and extinction of populations in the perspective of hundreds of generations such as done for short rotation species (LYNCH *et al.* 1995) will not be considered here.

It is intuitively understood in genetic conservation that the existing among-population variation should be encompassed in the sampling of genetic conservation populations (*e.g.*, ERIKSSON 2001). Of particular interest is the variation in adaptedness whereas genetic marker variation is generally not believed to reflect adaptive variation (*e.g.*, KARHU *et al.* 1996, LYNCH 1997). However, there are also reports claiming that isoenzyme variation reflects adaptive variation (BUSH & SMOUSE 1991, HATTEMER 1994). Except for the tree species included in intensive breeding there is usually limited or no information on the inter-population variation for the majority of tree species (ERIKSSON 2001). This means that maximum inter-population variation in the genetic conservation population can only be guaranteed for species included in intensive breeding. For other species, educated guesses must be used in selection of genetic conservation populations.

Many ecologists believe that natural selection is the sole evolutionary factor and that it leads to a continuously improved adaptedness. This is incorrect for several reasons as discussed in ERIKSSON *et al.* (1993) and more lately, the uncritical acceptance of the importance of local populations for conservation was discussed (*e.g.*, WILKINSON 2001). It is important to state that all evolutionary factors may be in operation simultaneously and that natural selection may have shifting directions over time. This means that the belief that natural selection occurring over enough number of generations

would result in the ultimate state of maximum adaptedness is false (ERIKSSON *et al.* 1993). For the reasons discussed above we must assume that the present genetic constitution is transient and one out of many possible. This further means that the present adaptedness should not be the target in genetic conservation; rather it should be the starting material for dynamic genetic conservation.

For any species to survive in a continuously changing environment it must be equipped with the potential for adaptation. *Safeguarding the potential for adaptation* therefore becomes the key objective in genetic conservation.

Effective population size

There are conflicting views on what size of the genetic conservation populations is required. On one hand, it is claimed that rarely occurring alleles should be encompassed in the populations (*e.g.*, HATTEMER 1994). On the other hand, it is claimed that rare alleles do not contribute to additive variance (FALCONER & MACKAY 1996; see also HOLSINGER 1993, ERIKSSON 2001) and for that reason rare alleles are of limited interest for genetic conservation. One of the reasons for this discrepancy is probably the difference in focus of population geneticists mainly working with genetic markers and quantitative geneticists focusing on phenotypic differences. Most quantitative geneticists have the opinion that the effect of any one allele on a quantitative trait is not large. This means that a particular phenotype may be the result of many different genotypes. HOLSINGER (1993) presented results from a simulation study with 16 polymorphic loci, which showed that no less than 13,000 different homozygous genotypes produced the optimum phenotype. Scientists working with markers such as isoenzymes are accustomed to the finding that each allele produces a unique phenotype, which differs from the phenotype produced by another allele. This may, at least, partly explain the difference in views on the significance of encompassing rare alleles in genetic conservation populations.

Even if rare alleles contribute to fitness they do not contribute much to additive variance since they – per definition – occur at low frequencies. For this reason they are not favoured by natural selection until they reach selectable frequencies. HOLSINGER (1993) gave the following reasons why rare alleles should not be targeted in genetic conservation populations:

- most rare alleles are detrimental and the results of recurrent mutations;
- a rare allele does not contribute to additive variance and its existence in a population is due to chance events and not to selection;
- even if a rare allele would be useful in a distant future it would be lost before needed;
- adaptive responses in the future might be obtained from other alleles with the same effect.

One extreme view was given by BROWN and BRIGGS (1991) in their discussion of conservation of rare plants. *'We contend that rare alleles have been of much greater concern than they deserve...'*. Although the objective of crop plant genetic resources conservation is different from the safeguarding of the potential for adaptation as the prime

objective of genetic conservation in the forest sector, it is interesting to note that MARSHALL and BROWN (1974) stressed that alleles in intermediate frequencies were of greater interest to include in the conservation than rare alleles. This was based on the assumption that the former had an adaptive value. Another conclusion from the study by MARSHALL and BROWN (1974) was that a sample size of 50 from each population would be satisfactory to capture most of the important hereditary variation.

Several authors have treated how to estimate the number of trees needed to capture alleles in low frequencies in individual or several loci (*e.g.*, BURLEY & NAMKOONG 1980, KRUSCHE & GEBUREK 1991, YANCHUK 2001). One conclusion is that the allele frequency is more important for the number of trees needed to capture low-frequency alleles than the number of loci with such alleles.

The rate of loss of additive variance in a population with N_e = 50 will be equal to approximately 1 % per generation ($1/2N_e$). The increase of the inbreeding coefficient is derived from the same formula and will thus be 1 % too (F = 0.01), assuming random mating. Most breeders (*e.g.*, DANELL 1993) agree that such a small loss of additive variance and small increase in the inbreeding coefficient, with accompanying inbreeding depression, will not be significant for breeding or genetic conservation. If the linear relationship between inbreeding depression and the coefficient of inbreeding is true; as the theory behind inbreeding depression predicts, then the observed inbreeding depression of 56 % in selfed Norway spruce (ERIKSSON *et al.* 1973) would lead to an inbreeding depression of approximately 1 % at F = 0.01.

There is a vast literature treating the genetic theory of the long-term response to selection (see BARKER 2001). Here it suffices to mention that experiments and breeding combined with selection over many generations (> 50) have shown that there is a steady response to selection even if the population size is rather small (< 50 individuals; *e.g.*, RASMUSSON & PHILIPS 1997). In such populations, the original variance is largely depleted after approximately 20 generations of selection (*e.g.*, KEIGHTLEY & HILL 1989, NAMKOONG *et al.* 1988). In spite of this no limit for response to selection appears. One possible explanation for the selection response is that the pooled mutation rate for quantitative traits may be rather high and that it generates new additive variance. Independently of the cause for the strong sustainability in response to selection in small populations, it is comforting to conservationists and breeders that their populations can be of manageable size.

Some gene conservationists (FRANKEL 1980, SOULÉ 1980) claimed that 500 individuals is a satisfactory number since the loss of additive variance will amount to approximately 0.1 % per generation. Thus after a random selection of 500 trees the remaining additive variance will amount to 99.9 %. If we increase the population to 1,000 trees, the remaining additive variance would be 99.95 %. It is obvious that such a small difference in remaining additive variance does not justify the extra cost in most cases. Five hundred has also been regarded as a magic number since the loss of additive variance may be compensated for by new mutations. The pooled mutation rate for quantitative traits has been estimated to 10^{-3} to 10^{-2} (LANDE & BARROWCLOUGH 1987). This means that the loss of additive variance in a population of 500 trees is balanced by new mutations. However, LANDE (1995) stressed that many of the mutants are detrimental mutants with large effects and in order to be on the safe side 5,000 trees

ought to be included in the genetic conservation population (see also YANCHUK 2001). This number of trees may correspond to N_e of 1,000 since all trees do not contribute to spontaneous regeneration of the filial population.

In spite of the different opinions about the usefulness of inclusion of rare alleles into the genetic conservation populations between quantitative geneticists and 'marker geneticists', there seems to be an agreement that *safeguarding of the potential for adaptation* is the prime objective of genetic conservation (ERIKSSON 2001, HATTEMER 1995), even if the objective is phrased in different ways.

Adaptive management of genetic conservation subpopulations

Theoretically, genetic conservation can be carried out either by one single large genetic conservation population or by several small populations. It is obvious that a species distributed over many selective environmental neighbourhoods in the sense of BRANDON (1990) has differentiated via adaptation to different environmental conditions. As described above, the existing populations, with their small or large imperfections in adaptedness, constitute a good platform for dynamic genetic conservation. Therefore, it is advantageous to split the genetic conservation population into smaller subpopulations to benefit from the adaptedness to different environmental conditions in the different subpopulations (ERIKSSON *et al.* 1993, HATTEMER 1995, NAMKOONG *et al.* 1980). According to BURLEY and NAMKOONG (1980), another advantage with a subdivided genetic conservation population is that the chance of capturing alleles in low frequencies, 0.005–0.05, with non-uniform or random distribution increases if several stands are included in the population.

For species such as *Fagus sylvatica* L., *Picea abies* (L.) Karst., *Pinus sylvestris* L., and *Pinus pinaster* Ait., the knowledge about among-population variation is substantial (*e.g.*, ALIA *et al.* 1995, EICHE 1966, PERSSON &, PERSSON 1992, VON WÜHLISCH *et al.* 1994). With this knowledge, it is easy to select genetic conservation subpopulations to encompass the existing adaptedness of these species. For many tree species there is also much information on among-population variation with respect to isoenzymes (COMPS *et al.* 1990, LAGERKRANTZ & RYMAN 1990, PETIT *et al.* 1995, VILLANI *et al.* 1994). In this connection, it is important to mention that genetic markers usually underestimate adaptive variation (LEWONTIN 1984). For a genetic marker to have the same discriminating power as a quantitative trait with a certain heritability, the number of loci involved in the regulation of the quantitative trait must not exceed $1/h^2$. Thus, for a heritability of 0.2, the number of loci regulating the quantitative trait must not exceed 5 (1/0.2); otherwise it will have stronger discrimination power than markers. Five loci is a fairly low number of loci involved in the regulation of a quantitative trait.

GEBUREK (2000) carried out a detailed analysis of criteria for selection of genetic conservation populations. He concluded that various types of markers may be used but they cannot fully substitute the time and cost demanding field-testing to estimate the genetic structure of adaptive traits. This agrees fully with the results obtained by REED and FRANKHAM (2001) in their analysis of 71 data sets containing both genetic markers and quantitative traits.

Twenty subpopulations were given as a recommendation for the application of MPBS genetic conservation (ERIKSSON *et al.*1993). This number is not a sacred number but could be substituted by a larger or smaller number of subpopulations dependent on the magnitude of variation in the traits used for the selection of subpopulations. A species such as the North American pine *Pinus resinosa* Ait. with a wide distribution but almost without variation in any trait requires theoretically just one genetic conservation population. However, for safety reasons it is not advisable to have just one genetic conservation population. A selection of a few populations is recommended, and preferably from different climatic conditions would be satisfactory for this species. The situation is very different in *P. sylvestris*, which also has a wide distribution and a *pronounced* ecoclinal variation for wood production per hectare in Sweden north of latitude 60° (ERIKSSON *et al.* 1980). Selection of an optimum population at one position along this cline will result in a gradually reduced production in the selected population with distance from its optimum position. The breeder has to compromise between many and optimally adapted breeding populations and the cost to manage many populations. Therefore, a drop in yield of 5 or 10 % in the margins of the breeding zones may be the price the breeder has to pay to make the breeding programme manageable. The Swedish breeding programme for this species has utilized the data from provenance research and identified 21 different breeding zones (DANELL 1993). Selection of subpopulations for genetic conservation will rarely be based on wood production per hectare rather it may be based on phenological traits, in cases when they are assumed to be significant for survival and development of healthy trees. A schematic illustration of how genetic conservation subpopulations may be selected is given in Fig. 1 for a situation when there is genetic knowledge about among-population variation. The circles represent populations along an ecological gradient. Populations 1–7 are typical of a species with ecoclinal variation while populations 8–10 represent a species with ecotypic variation. However, it should be noted that the author does not know of any clear-cut example of ecotypic differentiation in forest trees.

It is further assumed that the adaptive trait is of greatest significance for survival and wood production of the two species. With these assumptions, it is evident that populations 8–10 should be included as subpopulations in the genetic conservation of the ecotypic species. Populations 1, 3, 5 and 7 almost cover the range of variation of the adaptive trait and they should be selected since this adaptive trait was very significant for this species. However, populations 2, 4 and 6 have some genetic variation but it is assumed that they do not carry any unique alleles, which do not exist in any of the previous 4 populations. Should they also be included in the genetic conservation programme? If there were no funding limits, the answer would be yes. If the funding is restricted, their inclusion may be questioned. It is likely that genotypes represented in the gaps between populations 1, 3, 5 and 7 may occur in offspring from pairwise crosses among these four populations. If this possibility exists then the selection of populations 1, 3, 5 and 7 as genetic conservation subpopulations is satisfactory.

As discussed above, educated guesses have to be relied upon when selecting the subpopulations of species for which we have no information on among-population variation neither for adaptive traits nor for genetic markers (ERIKSSON 1998). If the subpopulations are kept separate without any intermating among subpopulations, it

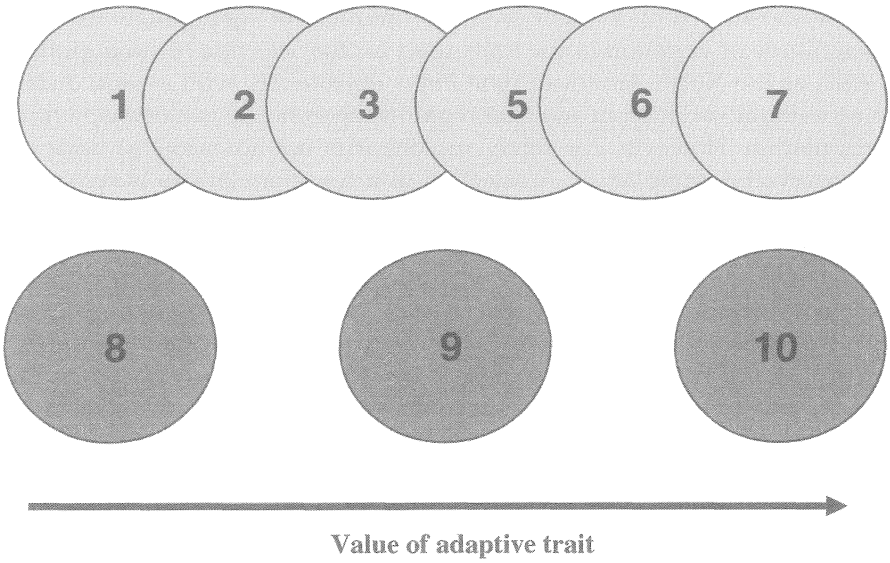

Figure 1. Schematic illustration of ecotypic (below) and ecoclinal (above) variation. The implication of the two different types of differentiation is discussed in the text.

means that a kind of assortative mating occurs within the individual subpopulations. This will also speed up the differentiation among the subpopulations. The development may be faster in small subpopulations than in one large population, especially if there are low-frequency recessive alleles that contribute to fitness (ENDLER & MACLELLAND 1988). If these types of alleles is present in a small population, then homozygosity for such alleles will occur at a higher frequency in a small than in one large population.

MPBS has different levels of intensity, varying from merely selection of *in situ* genetic conservation populations to artificial mating work and establishment of progeny trials. The latter is obviously more costly than the former but it has several merits. Even if we know that the environment is never constant, there may be a trend in the change of the environment. Today, climatic change due to greenhouse gases is much discussed and it is projected that the rate of change is now faster than ever before during postglacial time. Based on fossil data many forest ecologists, who have taken an interest in this issue, expect that most forest trees will not be able to migrate fast enough to cope with the expected change (DAVIS 1988). This means that it is important to be able to arrange the genetic conservation populations so that the process of adaptation can be managed appropriately. This is obviously most easily done by *ex situ* MPBS. Even plantations outside the present range of distribution of a species may be useful as a preparation for future environmental conditions.

ERIKSSON *et al.* (1993) discussed *in situ* MPBS and concluded that: 'In situ *conserva-*

tion can still be a significant part of any genetic management strategy. Economic constraints dictate its use for most species. In situ *conservation builds on what has proven to be evolutionary feasible, and provides for the continuing evolution of species under at least one set of environment and evolutionary conditions. It does not provide for all of the paths that evolution might have taken for a species in the past or for future paths other than that dictated by conditions experienced.'*

For the majority of tree species there is no alternative to *in situ* MPBS for economic reasons. As for *ex situ* MPBS, the subpopulations should be selected to cover the span of environmental conditions under which the species is growing. The need for regeneration of the *in situ* subpopulations is very important. In some instances, it may be necessary to promote regeneration either by plantation or by culling competitive tree species to obtain satisfactory flowering and seed formation. The lowest level of *in situ* MPBS is when there is no management of the populations at all but just the nomination of a forest stand as a genetic conservation population.

MPBS and conservation of associated species

Few forest geneticists have considered the genetic conservation of associated species in discussions of respective forest conservation programmes. VARELA and ERIKSSON (1995) suggested extension of a few of the MPBS subpopulations to a few hundred hectares covered by forest stands. These large subpopulations should be selected to encompass as representative a sample of site conditions of the species as possible. Since many flowering plants are very specific with respect to habitat demand one should try to get large variation in site conditions within each large subpopulation (BERG *et al.* 1994). One way to do this is to include different developmental stages of the ecosystem, from regeneration to over-mature, represented within an area occupied by a subpopulation. Another possibility is to include subpopulations with different degrees of human impact in the various subpopulations. If such an extension of some subpopulations can be carried out, the genetic conservation of a majority of associated plant species and smaller animals should be taken care of.

In nature, mutualism exists such that target species are dependent on associated species for pollination and seed dispersal. One example of such a mutualism is given by TOMBACK (1982) who used the following definition of mutualism: *'a mutually beneficial association between different kinds of organisms'*. She found that spreading of *Pinus albicaulis* Engelm. seeds was promoted by Clark's nutcracker (*Nucifraga columbiana* Wilson). Especially in tropical forests disruption of existing mutualism may occur following fragmentation of forests, which may be harmful for forest tree species (CORDEIRO & HOWE 2003). There are many such intertwined relations in nature, which also call for a greater emphasis on the associated species from forest tree gene conservationists.

Selection and evolution in various directions

The development of landraces in agricultural crops is a classical example of evolution

of a species in a variety of directions. Especially in the tropical part of the world, there are numerous landraces that have developed in conjunction with the variety of cultivation techniques and climatic conditions that exist (BERG *et al.* 1991). The selection taking place under these conditions is a combination of artificial and natural selection. Analogous to the agricultural crop situation the selection that breeders carry out in their breeding population can be regarded as a combination of artificial and natural selection. The artificial component originates when the breeder decides on which genetic entries should be culled and which should be left for future breeding. The environmental conditions at the progeny test sites will influence the performance of the different entries and in this way indirectly influence the selection that the breeder carries out.

If the breeder starts with several subpopulations that have reached some degree of adaptedness at their respective locations it is obvious that such subpopulations constitute a better starting material for future adaptation under these particular conditions than a randomly selected population (ERIKSSON *et al.* 1993, HATTEMER 1995, BARKER 2001). This is true as long as there is no major change of the environmental conditions and a thereby accompanied dramatic change of the direction of selection. It is important to stress that the existing adaptedness of a population is a reflection of its past evolution and does not constitute any preparedness for future environmental conditions. Even if our predictions for future conditions are not precise, they may give general directions for future climatic conditions. Such predictions may be used to design the selection of subpopulations for breeding and conservation as well as the location of plantations of these subpopulations as is aimed at in the Norway spruce and Scots pine breeding in Sweden (DANELL 1993).

In breeding according to the MPBS concept it is possible to have different breeding objectives in different subpopulations; one subpopulation could be designed for fiber production or even fiber farming, another for saw-logs, and still another for late flushing (to avoid exposure to late spring frosts). This means that each subpopulation may have different optima for their domestic fitness.

Most breeders of crop plants carry out the breeding in one breeding population for one type of site conditions. If breeding goals change then this change will affect the entire breeding population. The prime objective in genetic conservation of agricultural crops is to supply breeders with alleles that are not present in the breeding populations. A non-molecular genetics transfer of alleles into a highly bred variety can be carried out by 7–8 generations of back-crossing. The long generation times of most forest trees mean that it is impossible to switch from one breeding objective to another within a short period of time as may be done in agriculture. Therefore, preparedness for future changes in the environment is urgently required. One way to accomplish such preparedness is to split the breeding population and diversify the goals in the different subpopulations. The MPBS concept allows breeders and genetic conservationists to prepare for changes in future by having different objectives in different subpopulations. It seems a dynamic approach of genetic conservation is gaining terrain even for agricultural crops (BRETTING & DUVICK 1997).

Links to tree breeding programmes

The Swedish forest tree breeding of *Picea abies* and *Pinus sylvestris* (DANELL 1993) will be used to illustrate how combined breeding and genetic conservation according to the MPBS concept can be carried out. In both species, the breeding populations are split into approximately 20 subpopulations. These subpopulations are selected according to temperature and photoperiod, the latter of great importance for adaptation at high latitudes (EKBERG *et al.* 1979, ERIKSSON *et al.* 1980). These subpopulations will therefore match the genetic conservation objective of capturing the existing variation in adaptedness. The 20 subpopulations are planted so that different temperature regimes, both higher and lower than the present, are included at several latitudes (*i.e.*, photoperiodic conditions). This takes some account of preparation for evolution under possible future climatic conditions. These types of breeding programme, covering the entire range of the species distribution, and planned over many generations, removes the need for a separate genetic conservation programme. It may be complemented with marginal populations, not normally included in breeding programmes, to fulfil the function of a genetic conservation programme. For the operational tree breeding in British Columbia, YANCHUK (2001) carried out a comprehensive theoretical review on the requirements for combined tree breeding and genetic conservation. The MPBS concept complemented with a few sublines within subpopulations is an attractive alternative. ERIKSSON (2000) stressed the important role of tree breeding in genetic conservation. If genetic conservation is taken care of in breeding, it releases resources for genetic conservation of other species.

In Germany, a kind of joint breeding and genetic conservation of rare noble hardwoods is being carried out (KLEINSCHMIT 1994, see also ERIKSSON 2001). Scions are taken from trees within a climatic region and the grafts produced from them are planted in clonal archives, which should serve as seed production units. The seedlings raised from these archives can be used for progeny test plantations and for ordinary plantings. Whenever funding is available, *ex situ* MPBS is recommended for all species in active breeding as well as for species not included in breeding. In particular *keystone* species, *i.e.* species of great significance for the survival of other species, ought to be included in *ex situ* MPBS programmes.

Conclusions

MPBS is dynamic and allows for adaptation via natural selection or breeding. In contrast to hierarchical breeding, MPBS stresses among-population variation both in the traits targeted for improvement and in environmental adaptability. As a result we may obtain several adaptive peaks within one species. In contrast to hierarchical systems that require several generations of back-crossing to introduce genes into the breeding population immediate changes of breeding goals are possible in MPBS breeding.

A theoretical analysis during the mid-1970s by NAMKOONG (1976) showed that splitting the breeding population into several subpopulations should be more efficient

than breeding in one large breeding population. It has also been shown that hybridization between individuals from different subpopulations may result in increased additive variance in the F_2 generation, particularly useful for genetic conservation and breeding (NAMKOONG 1976).

In the majority of cases, MPBS will also be more genetically diverse than any hierarchical breeding system since matings will exclusively occur within each of the subpopulations. Thus there will be an increased uniformity within each subpopulation, which in turn leads to increased differentiation between subpopulations. The additive variance can be more or less maintained over generations in each subpopulation. Simultaneously, the among-population variance may be increased by applying different selection regimes in the different subpopulations either by using different breeding goals in the individual subpopulations or by exposing the different subpopulations to different site conditions. Progress may for any particular trait be faster in small populations than in large populations.

It will also be easier to include alleles that are rare at the species level but which are contributing to fitness under certain environmental conditions and therefore more common in some subpopulations.

Finally, the cost of running a MPBS programme is not expected to differ much from running a breeding programme with just one large breeding population.

Acknowledgements

I appreciate very much the discussions with and suggestions given by Inger Ekberg and Thomas Geburek during development of this paper.

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Seed and pollen storage: European focus

R. Th. Klumpp

Introduction

When seed trade was becoming a significant business about 200 years ago, during the time of the French revolution, seed storage issues were largely related to storage capacity and market competitiveness as entrepreneurs struggled to meet the demands of reforestation of devastated forests. One hundred years later, severe problems, arising from the use of unsuitable seed sources [*e.g.*, shrub forms of Scots pine (*Pinus sylvestris* L.)], led to the foundation of seed quality testing stations, forest genetics (*e.g.*, provenance) research and the development of science-based seed storage rules (ROHMEDER 1972). At the end of the 20th century, problems of forest dieback encouraged research into the improvement of long-term storage techniques in forest tree species, with the hope that biodiversity could be 'stored' until the pollution problem was solved. In addition, storage techniques for pollen were improved to provide alternative forms of genetic conservation for either breeding or micropropagation (*e.g.*, culture of anthers). Finally, molecular genetics provided tools for identifying and even manipulating single genes at the turn of the 21st century.

Within a time span of roughly 200 years, man succeeded to rebuilt the forests in Europe, timber was replaced by steel and plastic, and fuel wood was substituted by fossil energy. Society no longer respects the forest as a source of income, food and energy but more as source of recreation and 'nature', a feature which is often described as 'postmaterialistic change of values' (WEBER & MANN 1997). Hence, today's task is to provide instruments, methods and approaches in order to preserve biodiversity or perhaps to restore it. In spite of huge efforts for *in situ* conservation in forestry (see p. 535 ff. and 651 ff., this volume) as well as in nature protection (see p. 513 ff., this volume), there is an increasing need for *ex situ* conservation of propagules or any kind of reproductive particles from forest plants. The reason why is to be found not only in the rising awareness to care a broader set of forest species but also in the latest knowledge about the vulnerability of the concept of the gene bank: progenies from seed orchards may be affected by local site conditions (SKRØPPA & JOHNSEN 1999) or may even be contaminated with genetically modified material (ETC 2002). So the

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 601–622, ISBN 80-967088-1-3

question arises, what should be conserved instead of plant communities and plant collections? Should we store seeds or pollen or single genes? And how will the successful storage be achieved?

Storage of plant propagules is one of the basic concepts in the expanding field of conservation biology (HAWKES *et al.* 2000, *loc. cit.* p. 82) . Nevertheless, this technique is often criticized because of the 'freezing of evolution' (MAXTED *et al.* 1997), as the process of continuous adaptation to the changes in environment is interrupted for the stored germplasm. Numerous review papers have been published during recent years regarding the scope of germplasm storage from different view points, mostly concerning seed physiology and herbaceous species (*e.g.*, FARRANT *et al.* 1988, PAMMENTER & BERJAK 1999, PAMMENTER & BERJAK 2000), but also perennial woody plant species (WANG *et al.* 1993) as well as seeds of forest tree species (BONNER 1990) or cryopreservation (BONGA *et al.* 1997). Furthermore technical guidelines for model species have been published (*e.g.*, KOSKI *et al.* 1997). The following paragraph aims to provide a brief synopsis on the issue of storing germplasm with the emphasis on those tree species which are included in the European legislation.

Seeds, pollen or single genes?

In the absence of species-specific seed storage information for all forest tree species, some general guidance for appropriate storage strategies and techniques can be provided by considering three conditions:

(1) Species' (or population) status (*e.g.*, threatened or endangered);
(2) Species' reproductive biology;
(3) Mating system of the species.

Species' status

If the species is threatened, but not endangered, then there is less urgency for seed collection, perhaps affording time to wait for heavy seed crop years, when higher quality seeds are produced (SCHUBERT 1999). There may also be time for sufficient planning to maximize sampling of the gene pool in order to maximize the genetic contribution to the next generation (DEGEN & SCHOLZ 1996). In contrast, if the species is endangered, there is more urgency and plans should be developed that take advantage of available seed crops and have contingencies that recognize and allow for any destructive influences such as pollution.

Reproductive biology

Understanding the reproductive biology of the species can also assist in planning for seed collections and the most appropriate storage method. Species exhibiting regular

seed-crop intervals [(*e.g.*, birch (*Betula*) or ash (*Fraxinus*)] are easier to manage compared to some conifers such as Norway spruce (*Picea abies* [L.] Karst.) or European larch (*Larix decidua* Mill.) species, which have heavy cone crops only once every ten years (for review see p. 190 ff., this volume). Records on crop periodicity and the type of reproductive cycle (*e.g.*, Swiss stone pine (*Pinus cembra* L.) 3 years) assist in deciding whether to wait for a heavy seed crop or to collect and store seeds from a medium seed crop.

Apomixis is an example of a special reproductive strategy found in an endangered species of mountain ash (*Sorbus aria* [L.] Crantz.) JAKUBOWSKY & GUTERMANN 1996). Apomictic progenies are genetically identical to the maternal parent (*i.e.*, the seed tree). As such, it is important to have an appropriately large number of parent trees for seed collection as each seedlot will represent less diversity than in outcrossing sexually reproducing species.

Mating system

Finally, the mating system of a species can provide an insight into the most appropriate strategies for seed storage. Oak (*Quercus* spp.) and beech (*Fagus* spp.) are mainly outcrossing, whilst other broadleaved species are highly selfing such as *Magnolia* spp. Some conifers, such as fir (*Abies* spp.) or pine species (*Pinus* spp.), show intermediate or mixed mating systems. Some species have mechanisms that decrease the probability of selfing – represented in their phenology, flower development, or even the germination speed of the pollen. Embryo abortion (of selfed embryos) has been shown to be a powerful tool for reducing inbreeding rates in conifers. Finally, the distance to the mating partner plays a major role in determining seed parentage and hence, the mating system actually expressed in the seed (DEGEN & SCHOLZ 1996). For example, the reduction of the population size in the true service tree (*Sorbus domestica* L.; Fig. 1) in Bavaria/Germany during the second half of the 20th century, led to a dramatic reduction of seed number per fruit – an apparent result in the increase in selfing due to the decreased opportunity for outcrossing (HÜMMER 1990). In a well-intentioned effort to support regeneration in this species, seeds (with low genetic quality due to selfing) were regularly grown by non-governmental organizations (NGOs), resulting in more than 70 % seedling mortality in the second year. It required much effort on the part of forest geneticists to encourage a change in the approach of the NGO's to that which was more consistent with the genetic condition of the seeds, that is to collect seeds only from trees with near (within pollination distance) neighbours and that have on average more than 1.5 seeds per fruit. This is a prime example of the need to fully understand the biology of a species and the possible genetic implications of any actions taken upon the long-term conservation and restoration.

It is generally recognized that, historically and traditionally, storage of seeds, pollen or single genes has been mainly motivated by their commercial value. As such, the development of storage techniques has been in the context of focussing on particularly, high-value species or genotypes. In contrast, genetic conservation is done in the context of biodiversity values and aims to preserve the diversity of species, genotypes

Figure 1. The true service tree (*Sorbus domestica*) is one of the rare species in Europe. Seed collection should be limited to mother trees where the neighbouring trees are located within easy pollination distance (about 100 m). Photograph by R. Th. Klumpp.

and genes (MAXTED *et al.* 1997). Storage techniques and storage capacity problems can be quite different in the biodiversity conservation context, and in general, they are more challenging.

In addition to these biological, environmental and economic conditions, the decision of what type of germplasm to collect and store requires two further considerations depending on:

(1) the efficiency of collecting and conserving genetic diversity;

(2) the efficiency of managing the stored material.

The efficiency of collecting

Genetic diversity is not equally represented in different types of germplasm and it is also contingent on the means of genetic transmission within a particular species. The three organelles in plant cells that contain DNA – the nucleus, the mitochondria, and the chloroplast – are inherited differently in different species. For example, in many broadleaved species the genome of the chloroplast is inherited from the maternal parent however in many conifers, it is inherited from the male parent. Thus, pollen collection the preservation of genetic diversity of a certain genome to one parent, but its

genetic impact will vary depending on the species. In contrast, seed contains the germplasm of both parents. Moreover, for many plant species, the amount of pollen per flower is very small, not really sufficient for effective collecting and processing. Exceptions are collections in seed orchards using machines (BALDET 2002). Finally, the conservation of single genes in cell culture only conserves the variation of single traits.

The efficiency of managing

Compared with seed storage, pollen storage has several disadvantages. Under optimal conditions, seeds will germinate within a range of values that can be predicted and will produce plants immediately. Conversely the production of forest tree from pollen is still impossible and the storage of single genes requires sophisticated techniques (see this issue). Thus, more infrastructure – such as a seed orchard or a breeding station – is required to produce plants from stored pollen in traditional culture systems. This dependence on sophisticated infrastructure is an even stronger condition for storage of single genes using tissue culture (see p. 623 ff., this volume). In addition, there are potential hazards of age-induced mutations in long-term stored tissue. However, it is expected that such mutations – to the extent they are deleterious – will be largely eliminated by selection, so that the next generation will not be affected.

A further approach, which depends on a sophisticated infrastructure is the cryopreservation. The term cryopreservation commonly refers to the application of ultra-low temperatures (below –130 °C) for preserving biological material like seeds or clonally maintained germplasm. Under these conditions, metabolism is negligible, thus potentially extending storage time 'considerably, if not indefinitely' (TOWILL 2002). Advantages over conventional techniques are the absence of complicated humidity and temperature protocols, freedom from pathogen activity, and lower risk of genetic mutation (WANG *et al.* 1993 and JACKSON & FORD-LLOYD 1990). Theoretically, all parts of a plant (*e.g.*, protoplast, tissue, embryo, pollen and seed) can be cryopreserved. However, the practical value of this technique rests not only on viability after storage but also the competence of the cryopreserved material to regenerate plants. As pointed out by BAJAJ (1995), cryopreservation 'involving 'freezing-storage-thawing-culture' is a complicated, multiple event'. One and the same protocol may result in different response according to species, cultivars, clones, lines, hybrids, *etc.* Moreover the cryoability of cells or tissue greatly depends not only on the genotype, age and physiological state of the plant (cultures) but also on the growing conditions prior to freezing. Hence it should be avoided, to draw hasty conclusions from single results (BAJAJ 1995).

Finally it has to be stated, that besides all effort in developing efficient storage methods, the establishment of seed orchards (see p. 577 ff., this volume) is actually the alternative *ex situ* conservation method for species showing recalcitrant seeds, pollen or tissue, even as contamination with unsuitable pollen cannot be excluded in the seed orchards.

In summary, seed storage, particularly after heavy seed crop years, is the preferred conservation method. With the use of a suitable sampling technique, seed storage

ensures conservation of a considerable share of the genome. In particular, wind-pollinated species should be conserved *ex situ* as seeds. Pollen should be collected as a complementary approach for species that are endangered or have small populations, have isolated individuals, or are insect pollinators. Finally, conservation of single genes can be recommended in certain cases where the gene has particular value to the overall long-term survival and viability of the species or has a particular commercial value (*e.g.*, varieties). An additional indication for applying cryopreservation according to BONGA *et al.* 1997) is the storage of *in vitro* cultures, which should primarily be restricted to clonal lines of commercial value.

Pollen storage

The natural life span of pollen is considerably shorter than that of seed. Depending on the species, it ranges from a few hours [*e.g.*, wheat (*Triticum aestivum* L.) 24 h] to several months at room temperature. Conifers have some of the longest pollen survival rates. For example, the half-life (*i.e.*, half of the original viability remains) of pollen from Scots pine (*Pinus sylvestris*) may reach 279 days and of eastern white pine (*Pinus strobus* L.) 413 days (HOEKSTRA 1995 for review). High humidity, combined with a high temperature of more than 30 °C., leads to a decline in viability, which is preceded by delayed pollen tube emergence *in vitro*. However, dry pollen is surprisingly tolerant to hot and cold temperatures. Humid pollen, though, can be easily destroyed by ice chrystals if exposed to cold temperatures. Desiccation tolerance develops at the end of pollen maturation in the anthers. Premature collecting may result in reduced viability and vigour. Full maturity is assumed to be reached in the anthers under optimal weather conditions. Withstanding dehydration, as well as successful rehydration, are the important factors of desiccation tolerance. In general, storage of pollen much below 5 % moisture content (*i.e.*, in equilibrium with an atmosphere at less than 20 % relative humidity) should be avoided. A remarkable number of species possess recalcitrant (*i.e.*, cannot withstand desiccation) pollen (*e.g.*, the cucurbitaceous plants, Cucurbitaceae), a feature which may be due to adaptation to very humid climates and niches (HOEKSTRA 1995 for review).

Pollen is sensitive to water content and cannot be fully dried, even after a short exposure to moisture, without severe loss in viability. Furthermore, pollen is tolerant to desiccation because of its relatively small size (*i.e.*, diameter of pollen grain: 30–40 µm). This feature makes it very easy to store pollen below 0 °C. for a long time without using special equipment (HOEKSTRA 1995, RAZDAN & COCKING 1997).

Pollen collection should follow a protocol that recognizes the biological factors described earlier. For example, because of pollen sensitivity to moisture, pollen collection should not be done on rainy days. It may be necessary to study the rate of decline in pollen viability in the particular species before making the collection. Rapid drying of pollen can usually be done at 50 % relative humidity with gentle ventilation, preferably at room temperature (HOEKSTRA 1995). By contrast, freeze-drying, which is most widely used for desiccation, affects pollen viability (WANG *et al.* 1993). Viability as well

as vigour assessment can be done using staining tests (*e.g.*, tetrazolium salt) or as germination test *in vitro* as well as *in vivo.*

Pollen storage life will depend on the species' characteristics, pollen collection, handling methods, and storage conditions. The longevity of pollen is preconditioned by the levels of unsaturated linolic acids and antioxydants in the cell. The presence of oxygen has been shown to accelerate the aging process. Storage in vacuum or under nitrogen gas at room temperature has been shown to increase the life span of pollen (HOEKSTRA 1995 for review).

Under normal atmospheric oxygen pressure, desiccation-tolerant pollen generally will not survive longer than one year at room temperature at a reduced moisture content. For longer storage, the sample must be cooled or oxygen removed. Storage of dry samples at –20 °C. or lower can only be successful at below 20 % moisture content. Storage in organic solvents is reported to be a promising option. For long-term storage (*e.g.*, several decades), cryogenic storage is required. Storage in liquid nitrogen, after desiccation (*e.g.*, by freeze-drying), offers nearly infinite longevity (for review of pollen storage see WANG *et al.* 1993, *loc. cit.* chapter 3). Such apparent longevity has been demonstrated in Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco). In this case, Douglas ir pollen was stored at approximately 7 % moisture content at –196 °C. in liquid nitrogen (COPES 1985). After three years in storage, warming up was done in several steps: for 30 minutes at –20 °C in a conventional freezer and then gradually warmed to room temperature prior to pollination. After pollination, no significant differences in fertility were observed between the stored pollen and fresh, or one- or two-year-old pollen (COPES 1987).

Pollen extraction with the organic solvent toluene and storage at –20 °C is recommended in willow pollen management, whereby even the range of interspecific hybridization can be extended (KOPP *et al.* 2002). Meanwhile some few detailed protocols for the management of pollen are available, which offer well tested species specific solutions (*e.g.*, STANTON & VILLAR 1996).

Longevity and storage of seeds

The natural life span of seeds is best understood within the context of the reproductive strategy and successional status of the species. Deciduous pioneer species, in general, produce frequent and large seed crops, but their small seeds have a short life span (*e.g.*, *Salix* spp., several weeks; *Ulmus* spp., up to one year). Forest tree species in central Europe thus have seed characteristics suited for long-term storage (*e.g.*, *Pinus* spp.), medium-time storage (*e.g.*, *Abies* spp.) or immediate use (*e.g.*, *Ulmus* spp.). In contrast, species of the leguminous plant family (Leguminosae) are known to have a special seed coat (*i.e.*, testa) which enables their seed to live several decades even under difficult conditions (BEWLEY & BLACK 1994, *loc. cit.* 206–207). Furthermore, some pine species, such as knobcone pine (*Pinus attenuata* Lemmon), have serotinous cones in which seed are enclosed until certain conditions (*e.g.*, high temperature, fire) allow rapid opening (KEELEY & ZEDLER 1998).

The bigger the difference between the natural life span of seeds and the desired shelf life, the more knowledge and technological intervention are required, and probably the higher the storage costs. According to species-specific characteristics, seed storage conditions are generally designed to reduce metabolism. This allows the seeds to maintain viability while using little energy. Key factors for effective storage include moisture content, temperature, light, air composition and air pressure. Sealed containers with nitrogen, carbon dioxide and vacuum increase the germinability of seeds after storage in comparison with sealed containers of (ambient) air or even unsealed storage bottles. Light (*i.e.*, quantity, quality, duration) is known to be a species-specific stimulant for germination (BEWLEY & BLACK 1994, *loc. cit.* p. 274). Therefore, seed should be kept in the dark during storage. Recent studies on herbaceous species suggests that light response and seed mass co-evolved, as germination became less dependent on light with increasing seed mass (MILBERG *et al.* 2000). Temperature, as well as moisture content, directly influence metabolism (BEWLEY & BLACK 1994, *loc. cit.* p. 392). These factors are tightly linked. Seeds of most plant species can tolerate low temperatures as long as moisture content is reduced to a suitable extent. The lower the storage temperatures, the longer the potential seed storage life and the lower the risk of pathogen activity.

Seeds are usually classified according to their storage physiology into four categories (BONNER 1990):

(1) true orthodox;
(2) sub-orthodox;
(3) temperate recalcitrant;
(4) tropical recalcitrant.

True orthodox seeds tolerate desiccation and low temperatures. They retain viability for long periods under dry, cool storage. Examples of genera in this category include: the species of the genus *Abies* (firs), *Betula* (birches), *Picea* (spruces), *Acacia* (acacias), *Pinus* (pines), and *Platanus* (planes, sycamores). Seeds of this group exhibit a long storage life and can remain viable up to 50 years (WANG *et al.* 1993, *loc. cit.* p. 4).

Sub-orthodox seeds can be stored under the same conditions as true orthodox seeds, but not for as long (usually around six years). Loss of viability can reach 34 % when stored at subfreezing temperatures (–5 °C and –20 °C) and under moisture contents between 5 and 10 %. Seeds in this category may have high lipid content (*e.g.*, walnut (*Juglans*) species) or are small and have thin seed coats (*e.g.*, poplar (*Populus*) and willow (*Salix*) species) (see WANG *et al.* 1993, *loc. cit.* p. 7).

Temperate recalcitrant seeds are sensitive to desiccation but can be stored near freezing temperatures (*e.g.*, horse chestnut (*Aesculus*) and oak (*Quercus*) species, sugar maple (*Acer saccharum* Marsh.). They can be dried to relatively high seed moisture contents (35–50 % fresh weight) and stored safely at temperatures between +3 °C and –3 °C. If high moisture content and gas exchange are maintained, longevity may reach 12 or even 30 months (see WANG *et al.* 1993, *loc. cit.* p. 8).

Tropical recalcitrant seeds are sensitive to both desiccation and chilling. Examples includes Dipterocarpaceae, the spurge family (Euphorbiaceae, *e.g.*, Brazilian rubber tree, *Hevea brasiliensis* Müll. Arg.) and the araucaria family (Araucariaceae). Some in

this category cannot tolerate temperatures below +4 °C (*e.g.*, the Indian talura tree (*Shorea roxburghii* G. Don) or even 15 °C, in the case of the chocolate tree (*Theobroma cacao* L.) (WANG *et al.* 1993, *loc. cit.* p. 8).

Environmental conditions affect flowering and seed production, and vary over populations and from year to year. These varying conditions, in combination with differences in reproductive maturity among individual trees and non-random mating, results in different genetic composition in seed crops from year to year (*e.g.*, MÜLLER-STARCK 1985). The best opportunity for collecting high-quality seed with the widest genetic variation is during heavy seed crop years, when there may also be less selfed-seed. In addition, only fully ripened seeds are suitable for long-term storage. Some species even need post-harvest ripening (*e.g.*, Swiss stone pine; *Pinus cembra* L.). The treatment of cones and fruits after harvest will influence the quality of seeds and their long-term storage ability. For example, increasing water content of the cones will result in decreasing germination rate for the seeds. Control and correction of water content in cones is necessary soon after arrival at the seed processing facility.

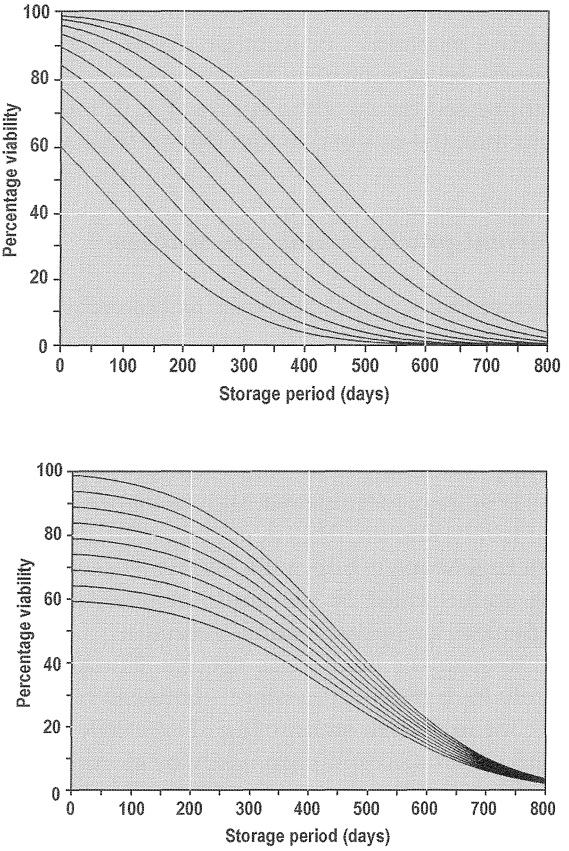

Figure 2. Sets of simulated viability curves. Schematic illustration of the model (above) of ELLIS and ROBERTS (1980) and the modified 'control viability' model (below) of MEAD and GRAY (1999). Both models describe the rate of viable seeds being left after some time, depending on the initial viability of the seed lot [from MEAD and GRAY (1999), modified].

Thus lastly, the storage potential of a seed lot depends on its initial condition before starting the storage process, which may be measured by viability parameters. ELLIS and ROBERTS (1980) developed a static model, to predict longevity of a certain seed lot. Fig. 2 (upper part) demonstrates, that the percentage of viability decreases very slowly with time in the case of high initial values, but rapidly in the case of low values. The validity of this model in North American forest tree species was tested by BONNER 1994, 1999). Nevertheless it should be realized, that seeds from wild populations will not fit to any model as well as seeds from crop varieties due to variability in seed maturity during collection as well as the variable degrees of dormancy (BONNER 1999).

MEAD and GRAY (1999) recently published an improved model, which assumes a ´control mortality´, or in other words, which underlays the fact that there is a species specific termination of the storage possibilities. This assumption leads to a more compressed mode of the curves (Fig. 2, lower part), which fits better to the experiment data than the original model (MEAD & GRAY 1999).

Current practices and case studies

Current practices with temperate and boreal species

Seeds of native European tree species, in general, have good potential for long-term storage. Most of the species are easily stored over several decades using common techniques. Suitable storage conditions are seed moisture content of 5 % and temperatures of approximately –10 °C, in sealed containers for coniferous species. Although there has been considerable research in this area, optimal species-specific storage conditions are rarely known. This is also true for broadleaved species, which are an even more heterogeneous group with respect to seed characteristics. Storage with constant cooling under sealed N_2 gas could be an economically interesting alternative to the more expensive cryopreservation technique.

Among the coniferous species, European silver fir (*Abies alba* Mill.) needs a relatively high moisture content – between 6 and 9 % – as well as low temperatures of –15 °C. for mid-term storage (*e.g.*, FOURNIER 1986). Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) can easily be stored for several decades with a moisture content of 4.5 % and temperatures between –5 and –15 °C. under sealed conditions (*e.g.*, MACHANÍČEK 1970). Larch seeds having a moisture content of 5 to 6 % can even be stored at temperatures of +1 to +5 °C. for ten years (VON SCHÖNBORN 1964). Table 1 gives a comprehensive overview on storage methods in European conifers.

Among the deciduous tree species, a moisture content of 8 to 10 % is suitable for storage temperatures between –5 and –10 °C in European beech (*Fagus sylvatica* L.), wild cherry (*Prunus avium* L.), as well as hornbeam (*Carpinus betulus* L.), common ash (*Fraxinus excelsior* L.), mountain ash (*Sorbus aucuparia* L.) and small leaved lime (*Tilia*) species for a storage period of five years (*e.g.*, WALKENHORST 1992). Norway maple (*Acer platanoides* L.) as well as sycamore (*Acer pseudoplatanus* L.) can be stored for more than three years if the moisture content is high (*i.e.* 30 %), storage temperature is between –3 and –5 °C. and the seeds are in plastic bags (WALKENHORST 1988). Other species of maple, such as silver maple (*Acer saccharinum* L.), are known to be recalcitrant (HONG & ELLIS 1996). Some technical guidelines for important broad-leaved tree species are given in Table 2.

Table 1. Storage conditions in coniferous tree species.

Species	Seed moisture content [%]	Storage temperature [°C]	Storage duration [years]	Remarks	Reference
A) true orthodox seeds					
Abies alba	12–13	–15	<3	MC not less than 7 %	v. SCHÖNBORN (1964)
	7–9	–20	>3		
	9–12	–8	2 ½	G % 72 → 44	MACHANÍČEK (1973)
	9–11	–7 to –15	2		MACHANÍČEK (1986)
	<9	–15	4-5	G % not affected	MÜLLER (1980)
	6	–15	4-5	G % not affected	FOURNIER (1986)
		–196		G % not affected	JÖRGENSEN (1990)
	8–15	–6 to –20	8-12	G % 55 → 30	BURKART (2000)
Larix decidua	4	4.4	>4	G % not affected	VLASE (1974)
	4.5–5	–10 to –15	10–20		GIRGIDOV & GUSEV (1977)
	5–6	–4 to –10			v. SCHÖNBORN (1964)
	6–8	0 to –10	10–15	G % 40 → 30	BURKART (2000)
	8–9	4	4	G % not affected	MACHANÍČEK (1970)
	8.4–10.9	4	16	G % 36 → 22	
		–196		G % not affected	JÖRGENSEN (1990)
Picea abies	4.5–6	2 to 4	>10	G % not affected	PAAVONEN (1983)
	4.5–7	0 to 5	5		GIRGIDOV & GUSEV (1977)
	4.5–5	–10 to –15	10–20		
	6–7	–5	24		SIMAK (1986)
	6–8	0 to –20	15–20	G % 95 → 85	BURKART (2000)
	7–8	4	16	G % reduced 18 %	MACHANÍČEK (1970)
Pinus nigra	5–6	2–4	3–6	G % not affected	GRADI (1983)
		5–7	10	G % not affected	BOYDAK (1984)
	8–10	0 to –10	8–15	G % 90 → 80	BURKART (2000)

Table 1. (continued).

Species	Seed moisture content [%]	Storage temperature [°C]	Storage duration [years]	Remarks	Reference
A) true orthodox seeds					
Pinus sylvestris	4.5–6	–5 to –15	>10		PAAVONEN (1983)
	5–6	–4 to –10		long term	V. SCHÖNBORN (1964)
	6–9	4	5–6	G % reduced per 10 %	MACHANÍČEK (1970)
	6–7	4	16	rapid reduction	
	6–8	0 to –10	10–20	G % 95 → 85	BURKART (2000)
B) sub-orthodox seeds					
Pinus cembra	8–9	–3 to –15	>10		LANG *et al.* (1994)
	8–20	0 to –6	3–5	sealed plastic bag	BURKART 2000

Explanations for tables 1 and 2: MC – moisture content, G% – germination rate [%].

Current practices with Mediterranean species

Similar to the situation with temperate tree species, although there are many publications related to storage conditions, optimal and species-specific storage conditions are not available for most Mediterranean species.

Among the coniferous species of the Mediterranean Basin, cypresses (*Cupressus* species) can be stored the longest (*i.e.*, more than ten years). For long-term storage, moisture content between 6 and 8 % and a storage temperature of –10 °C. are required. True cedars (*Cedrus* species), with a moisture content of less than 10 %, can be stored at temperatures between –2 and +3.5 °C. for up to six years (SCHOPMEYER 1974). Maritime pines (*Pinus halepensis* Mill., *P. pinea* L., and *P. pinaster* Ait. respectively) can be stored for up to six years with a moisture content of 6 to 7 % at temperatures between +2 and +4 °C (GRADI 1983).

Problems with recalcitrant and intermediate species

Seeds of Swiss stone pine have large edible nuts, which are difficult to handle. Seed collectors compete with animals that eat and disperse the species, such as the nut-cracker (*Nucifraga caryo-cactes* L.). Recent publications re-

Table 2. Storage conditions in deciduous tree species.

Species	Seed moisture content [%]	Storage temperature [°C]	Storage duration [years]	Remarks	Reference
A) true orthodox seeds					
Acer campestre	9–12	0 to –6	5–8	sealed plastic bag	BURKART (2000)
Acer platanoides	30	–5	3		LACROIX (1986a)
	30–45	–3 to –5	3		WALKENHORST (1988)
	20–30	0 to –6	2–3	sealed plastic bag	BURKART (2000)
Alnus glutinosa	4.2–6.3	4	9	G % not affected	MACHANÍČEK (1973)
	5–8	0 to –10	10–15	sealed plastic bag	BURKART (2000)
Betula pendula	4–7	4	3	G % not affected	MACHANÍČEK (1973)
	4–5	0 to –6	5–8	sealed plastic bag	BURKART (2000)
	6–10	0 to –10	5	G % reduced per 5 %	MACHANÍČEK (1986)
Fraxinus excelsior	6–10	0 to –6	5–7	sealed polyethylene	BURKART (2000)
		>–5	12	bag	SUSZKA (1982)
Prunus avium	8–12	0 to –6	1–5	sealed plastic bag	BURKART (2000)
	8–10	–5 to –10	5	sealed plastic bag	WALKENHORST 1992)
Tilia cordata	7–10	0 to –6	5–7	sealed plastic bag	BURKART (2000)
	8–10	–5 to –10	5	sealed plastic bag	WALKENHORST 1992)
B) sub-orthodox					
Acer pseudoplatanus	30	–1 to –5	3		NATHER (1988)
	30–45	–3 to –5	3		WALKENHORST (1988)
	15–25	0 to –6	2–4	sealed plastic bag	BURKART (2000)

Table 2. (continued).

Species	Seed moisture content [%]	Storage temperature [°C]	Storage duration [years]	Remarks	Reference
B) sub-orthodox seeds					
Fagus sylvatica	6–9	0 to –5	4–6	sealed plastic bag	BURKART (2000)
	8–10	–10	5	sealed plastic bag	WALKENHORST (1992)
			>5		SUSZKA (1993)
			8		LACROIX (1986b)
	4.7–6 and 8.4–10	–8	8		POULSEN & KNUDSEN (1999)
	7.8–11.5	–10 to –20	long term	optimum environment	LEÓN-LOBOS & ELLIS (2002)
Populus nigra	7–8	4	>60 days	vacuum	MULLER & TEISSIER DU CROS (1982)
C) temperate – recalcitrant seeds					
Quercus petraea	42–48	–3 to –4	0.5	204 → 147	DELFS-SIEMER (1993)
			1.5	embryo/kg	GILLE & NOWAK (1995)
				204 → 007 seedl./kg	
Quercus robur	42–48	–3 to –4	0.5	134 → 146	DELFS-SIEMER (1993)
			1.5	embryo/kg	GILLE & NOWAK (1995)
	40–41	2 to –2	1.5	134 → 052 seedl./kg	JANSON (1979)
				G % 68 → 63	

commend pretreatment of the seeds for after-ripening. Storage for up to three years is best done with a moisture content between 9 and 12 %, at temperatures between –4 and –15 °C., in sealed plastic bags (LANG *et al.* 1994). Thus, for two ecologically important European tree species, Swiss stone pine and European silver fir, storage is usually possible for only three and five years, respectively. To date, cryopreservation has been successfully applied only to European silver fir but this is not a common practice.

Among deciduous species, oak species present the greatest problems as large numbers of seeds are required to practice sustainable forestry, yet heavy seed crops happen only once every 15 years or so. In addition, there are serious pathogen problems associated with storage. Pretreatment in warm water (41 °C.) for two hours (Fig. 3) has been reported as a suitable approach for avoiding fungus infection during seed preparation for storage (DELFS-SIEMER 1993).

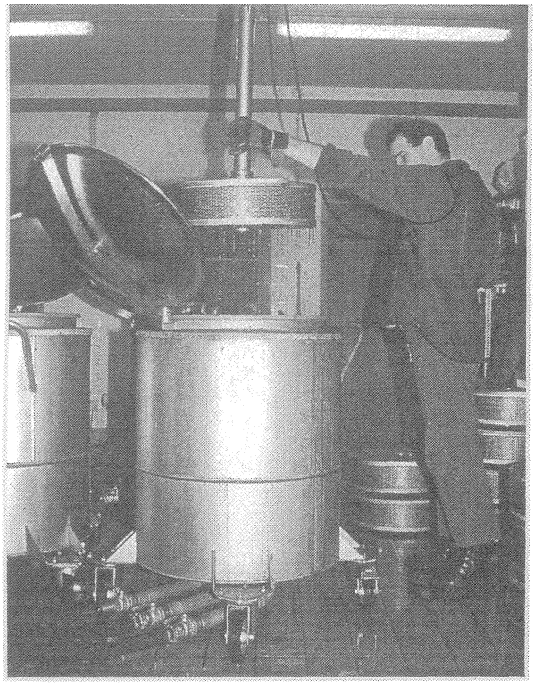

Figure 3. Seed lots of acorns are exposed to thermo-treatment in order to protect them against fungus infection during storage [from DELFS-SIEMER (1993); © BLV Publishers, Munich, reproduced with kind permission].

As drying of acorns results in severe loss of viability (*i.e.*, the critical moisture content is 35–50 %), a promising alternative technique has been developed involving frost hardening by applying temperature cycles over certain period. It was observed in a field experiment that the ability to tolerate low temperatures is highest during midwinter (Fig. 4, left), when acorns were stored in the upper layer of forest soil (GUTHKE 1993). This observation led to a climate chamber experiment in which these conditions were replicated (Fig. 4, right).

Acorns of *Quercus petraea* (Matt.) Liebl. were stored in climate chamber, applying a temperature cycle of 12 hours at +5 °C. and 12 hours at 0 °C. Seeds were regularly tested for different temperatures and analysed. It was shown, after 18 weeks treatment, that temperatures of –7 °C. resulted in the loss of only 10 % of the viable seeds (LT 10). The content of glucose and fructose in acorns was found to be responsible for the ability to survive frost. Hence, frost hardening provides an alternative technique to cryopreservation of excised embryos in large seeds. This technique has been developed for oak and horse chestnut species (GUTHKE 1993) and is one of the promising examples in solving the problems of storage in recalcitrant and intermediate tree spe-

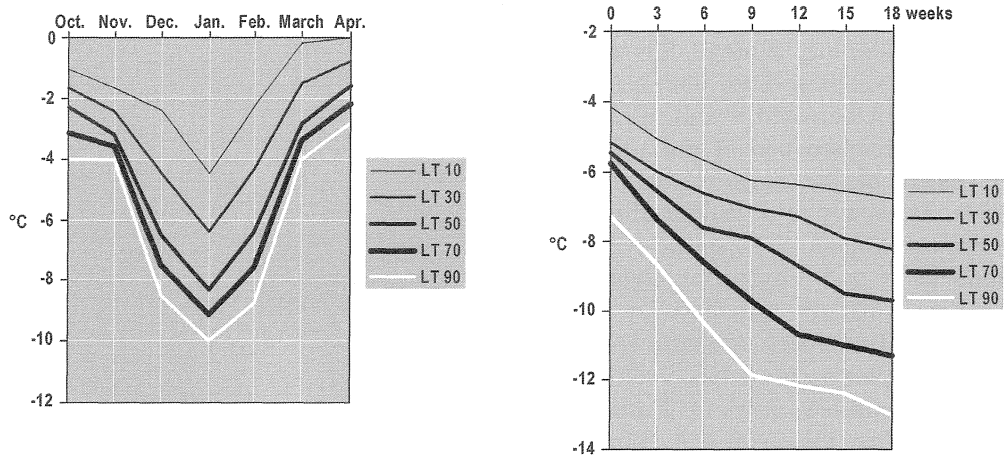

Figure 4. Development of lethal temperatures (LT) during a field experiment (left) and climate chamber experiment (right). The rhythm of changing temperatures provides an opportunity to develop frost hardening [from GUTHKE (1993), redrawn].

cies according to latest findings in seed physiology (PAMMENTER & BERJAK 1999, HONG *et al.* 2000).

Cryopreservation

Two general approaches have been applied for the cryopreservation of seeds and the tissue of seeds (RAZDAN & COCKING 1997):

- exposure to liquid nitrogen (LN_2) in a desiccated or partially desiccated state so that the tissues to be preserved are sufficiently dry to avoid freeze damage;
- using cryoprotectants and measures of controlled cooling to minimize freeze damage.

In order to have some guidance as to which species may respond well to various storage techniques, including cryopreservation, the following categories according to (WANG *et al.* 1993) may be used:

Group 1: Species with desiccation tolerant and LN_2-tolerant seeds
Group 2: Species with desiccation tolerant and LN_2-sensitive seeds
Group 3: Species with desiccation sensitive and LN_2-sensitive seeds

The most common species of conifers and small-seeded broadleaves belong to the first group. They can be characterized by their storage behaviour as true- and sub-orthodox seeds. Examples are species of fir, larch, spruce, pine, elm, hemlock and Douglas fir. The appropriate rate of cooling and rewarming, and the optimal moisture content for the seeds, is species-specific. Most of the species of this group exhibit high survival rates if moisture content is between 5 and 10 % (fresh weight), if application

of LN_2 (*i.e.*, liquid nitrogen) is done immediately and if the rewarming rate is at 30 °C. per minute (see WANG *et al.* 1993). In spite of the seed storage possibilities, more recent papers suggest the cryopreservation of embryonic cell lines in Norway spruce (*Picea abies*) and larch (*Larix* spp.) or the storage of bud tissue in Scots pine for future commercial purposes (CHAREST & KLIMASZEWSKA 1995, HOHTOLA 1995).

The second group can be described as species with fruit or nut crops such as hazels or filberts (*Corylus* spp.), walnut (*Juglans* spp.), beech (*Fagus* spp.) and cherry (*Prunus* spp.) species. The seeds of these species are known to be desiccation tolerant to moisture content below 10 %, but they are sensitive to temperatures lower than –40 °C (see WANG *et al.* 1993). The most recent review on storage methods in embryo axes, shoot tips as well as in somatic embryos of *Prunus* species are promising (DE BOUCAUD *et al.* 2002). These findings are of particular importance, as old fruit varities are endangered by pathogens. The need for more routinely applicable methods is stressed by MARINO (1997), who also warns that the effects of many of the cryoprotectants are not yet fully known. In contrast are earlier reports on the same cryopreservation techniques in *Juglans* species, where varying results were observed (DE BOUCAUD & BRISON 1995). In poplar species cryopreservation was successfully applied in seeds (AHUJA 1986), whilst additional approaches were not convincing, except the storage of shoot tips (LAMBARDI 2002).

Seeds of the third group represent both temperate and tropical recalcitrant seed. Among them are commercial species such as palm (*Phoenix* spp.), mango (*Mangifera indica* Lam.), and jack fruit (*Artocarpus heterophyllus* Lam.). Given the difficulty in storing seeds from species in this group, as well as their large size (storage capacity!), investigations have focused on storage of selected germplasm (*e.g.*, tissue culture callus, excised embryo axes, cell suspensions, *etc.*) at ultra-low temperatures. To complete the process, tissue culture is required to produce healthy plants from the stored tissue or isolated embryos (see WANG *et al.* 1993). Species which gave promising results were amongst others those from sessile oak (*Quercus petraea*), beech (*Fagus sylvatica*), and horse chestnut (*Aesculus hippocastanum* L.), for which species cryopreserved embryos exhibited good survival and growth (*e.g.*, JÖRGENSEN 1990). The latest review on the cryopreservation of oak species (*Quercus* spp.) recommends the storage of embryo axes (GONZÁLES-BENITO & MARTÍN 2002). For horse chestnut, tissue culture is recommended from different explants, where desiccation as well as heat stress induced increasing cryotolerance (JEKKEL *et al.* 2002).

Conclusions

Seed storage is not only a classical tool but also one of the most effective for the management and *ex situ* conservation of genetic resources. Pollen storage as well as cryopreservation are additional tools for special purposes. But all three tools should be used wisely based on holistic concepts for conservation and management. They should never be applied as an isolated tool but always as one from a suite of approaches. Recent research in seed physiology provided considerable improvement in ordinary seed storage techniques for a broad variety of species. Hence seed storage is

applicable even for long term periods – depending on the species. Cryopreservation is favourable in urgent cases of endangered species with orthodox or sub-orthodox seeds, especially when low rates of fertilization are to be expected. Establishment of seed orchards is the alternative conservation method for species showing recalcitrant seeds, even as contamination with unsuitable pollen cannot be excluded. There is an urgent need to foster the investigations in the physiology of the recalcitrant species in order to develop applicable storage techniques, which could include tissue culture and cryopreservation of embryos. The biggest challenge of the future may be to develop optimized protocols for the complete process of storage and germination, as it is already the case in agriculture. Only then, success in conservation and management of genetic resources by seed storage will be calculable.

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Micro- and macropropagation of forest trees

E. Wilhelm

Introduction

Global assessments (FAO 2001) have shown that the area of the world´s forests is shrinking. According to the current estimates, 0.38 % of the world´s forests were converted to other land uses (*i.e.* deforested) every year in the 1990s. At the same time, large areas reverted to forests, leaving a net annual loss of 0.22 %. The reduction was mostly in tropical countries, due to the rising human population. It is expected that the global demand for wood will increase sharply during the next few decades (FENNING & GERSHENZON 2002). In response, scientists have been working with forest industries to select, propagate and grow superior trees. One possibility for the future may be specialization of land use and resources into 'forest reserves' and 'plantation forestry'. In this way, genetic variation may be preserved within large areas of nature forest reserves. Additional areas may be designated for the application of new silvicultural concepts such as clonal forestry. Within the context of clonal forestry, the aim of vegetative propagation is to produce clones of elite trees that have been selected from populations of genetically variable siblings. When selection is made on the basis of exceptional growth rate, cloned trees would be expected to grow more rapidly than unselected seedlings. Vegetative propagation is essentially the reproduction of plant material so that the offspring will contain the characteristics of the parent material insofar as they are genetically determined.

The planting of cloned forest trees will require special care because, unlike most other plant species, trees live in one place over decades or even centuries. During these long periods, the successful trees must be able to endure every adverse physical and biotic component of their environments. Therefore, the relative risk of clonal plantations has been a topic of intensive discussions. Furthermore, field performance of plants produced via new technologies needs to be assessed. Field tests with trees require long-term experiments, which are often expensive (LIBBY 1988, 1991).

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 623–650, ISBN 80-967088-1-3

Micropropagation methods

Micropropagation is a special type of vegetative propagation in which small pieces of plant tissue are regenerated in an artificial medium under sterile conditions. This rapid plant propagation technique requires only little space and is independent of season. In addition, regeneration into complete plants via cell and tissue culture techniques also enables the genetic modification of plants. Nevertheless, micropropagation is labour-intensive and requires costly production facilities, making it expensive.

The success of tissue culture systems is generally dependent on the inclusion of plant growth substances (PGS) in the artificial media that regulate growth and the organized development of plants. PGSs are chemicals with the ability to modify plant growth, which are generally active at very low concentrations. Hormones are naturally occurring PGSs within plant tissues (endogenous). Plant growth regulators (PGR) are synthetic compounds with similar physiological activities (exogenous). PGSs are commonly divided into five major classes:

- auxins – cell growth and elongation, apical dominance, IAA, IBA, 2,4-D, NAA;
- cytokinins – cell division, release lateral buds from dormancy, Zeatin, 2-iP, BAP;
- gibberellins – stem length, flowering, GA3, GA4, GA7, GA80;
- ethylene – fruit ripening, senescence, abscission of leaves, wound hormone;
- abscisic acid: bud and seed dormancy, ABA.

The most important PGSs for micropropagation are auxins and cytokinins. In 1957, SKOOG and MILLER discovered that many aspects of cellular differentiation and organogenesis in tissue and organ cultures are controlled by an interaction between these two PGSs. High levels of auxin and low levels of cytokinins stimulate root formation on cuttings, whereas low levels of auxin and high levels of cytokinins induce axillar shoot proliferation in shoot cultures. In addition to these classical plant hormones, new natural growth substances with regulatory roles in tissue cultures have been discovered in recent years, such as polyamines, jasmonates, brassino-steroids, oligosaccharins, sterols, phosphoinositosides, salicylic acid, and systemins (GASPAR *et al.* 1996).

The first systematic work on culture of isolated plant cells and tissues in artificial nutrient solutions was attempted by HABERLANDT (1902) in Graz, Austria, more than 100 years ago and his experiments were fundamental for practical applications (HÖXTERMANN 1997). Research on tissue culture of forest tree species started in the early1930s, with callus and cell suspensions. Although there are some examples of successful regeneration of trees via protoplasts (reviewed by TIBOK *et al.* 1995), the importance of this technology is negligible. THORPE and co-workers (1991) counted the number of trees that can be micropropagated and found about 70 angiosperm species and 30 gymnosperm species. This is a small fraction, compared to the total number of 1,000 plant species accessible for micropropagation. Recently the tissue culture and biotechnology of conifers and broadleaved forest tree species have been reviewed (WILHELM 2003, ZOGLAUER *et al.* 2003).

Today, the most efficient culture techniques for trees are organogenesis – in form of axillary shoot regeneration – and somatic embryogenesis. In addition to the general

tissue culture-related problems (*e.g.*, production of chimeras, somaclonal variation or endogenous bacterial contamination), regeneration of woody plant species is still considered recalcitrant because of effects related to ontogenetic ageing.

Many reviews of forest tree biotechnology and micropropagation can be found in books edited by AHUJA (1988, 1993), BONGA & DURZAN (1982, 1987 a, b, c), BONGA & VON ADERKAS (1992), BAJAJ (1986, 1989, 1991) and JAIN *et al.* (1995 a, b, c and 1999).

Organogenesis

Experiments with tissue cultures of forest tree species have been ongoing for decades. The first successful callus proliferation and adventitious bud regeneration from cambial tissue was carried out by GAUTHERET in 1940 with English elm (*Ulmus procera* Salisb.). The first complete plants from tissue culture of a tree species were regenerated by WINTON in 1968 from leaf explants of *Populus trichocarpa* Torr. et Gray. Apical meristems, buds, callus, cell suspensions and protoplasts are normally used as the starting material for organogenesis.

The scheme for micropropagation by organogenesis goes through a series of steps including culture initiation, shoot multiplication, rooting and acclimatisation of plantlets. Several difficulties have been identified in the different steps. The morphogenetic responses of the different phases are regulated by addition of plant growth regulators.

Culture initiation starts with a decontamination or surface sterilization step, which is usually performed by dipping an explant into a chlorine-based solution. To minimize contamination, seedlings, grafted trees or branches are grown in the greenhouse. The basal medium normally consists of low salts, especially those of nitrate and ammonium, such as LM (LITVAY *et al.* 1981), MCM (BORNMAN 1983), GD (GRESSHOFF & DOY 1972) or WPM (Woody Plant Medium, after LLOYD & MCCOWN 1980). A high cytokinin level is frequently used to force the flushing of the axillary buds. In addition to the basal media, appropriate hormone levels and culture environment, the genotype and the physiological status of the explant are of key importance. If browning is not prevented then it may lead to the death of the explant.

Cytokinin induced microcutting propagation is the most frequently used method for ornamental plants including also woody species (PREIL 2003). The meristems are stimulated to develop and elongated shoots are either used for rooting or for subsequent multiplication via repeating the axillary branching. Multiplication rates of between five and ten per month can be achieved. The auxin-cytokinin balance allows the regulation of the multiplication process, *i.e.* high cytokinin levels usually induce high multiplication rates. The size of the shoots, however, can be drastically reduced by excessive cytokinin concentrations and the shoot anatomy can be negatively affected, such as vitrification (ZIV 1991). The leaves of vitrified plants appear glassy and water-soaked. This anomalous anatomy such as thin cell walls, high intercellular spaces and large vacuolated cells impedes acclimatisation of micropropagated plants in the greenhouse.

Rooting is normally achieved via adventitious rooting; auxins are applied as PGRs. The final step in all micropropagation systems is the acclimatisation phase, which governs the transition between *in vitro* and *in vivo* conditions. During this process, plants have to adapt to new environmental conditions such as lower relative humidity, higher light intensity and fluctuating temperatures. The intrinsic plant quality is important for acclimatisation. As such, excessive water loss by transpiration and an impaired photosynthetic apparatus are the two major problems.

For conifers, the most widely used procedures are either axillary shoot formation from existing meristems or adventitious formation of shoots via de novo formation of meristems. Both methods are closely related. The meristems are stimulated to develop by cytokinin application and the elongated shoots are rooted. Most published work on conifer propagation describes the initiation of cultures from juvenile explants. Chilling was applied as a dormancy breaking treatment for a 30-year-old tree of Sitka spruce (*Picea sitchensis* [Bong.] Carr.) (MAC AN T-SAOIR *et al.* 1991). Shoots can be induced to form directly on the zygotic embryos and on the organs of young seedlings, especially cotyledons, hypocotyls and shoot apices. The regenerative capacity of cotyledons has been found to be age dependent. Cotyledons from one-day-old radiata pine (*Pinus radiata* D. Don) seedlings were in the best physiological condition for shoot production (AITKEN-CHRISTIE *et al.* 1988). In New Zealand, a practical micropropagation system via adventitious shoot formation has been developed for radiatia pine (GLEED *et al.* 1995), in which micropropagated plants either go direxctly to the forest or are used as donor plants for cutting propagation (MENZIES *et al.* 2001). Dormancy breaking treatments such as stratification or treatment with H_2O_2 are used to increase the physiological response for shoot production. The capacity of conifers to form axillary buds varies not only between genera but also between genotypes of the same species. Where *in vitro* shoot multiplication has been possible, shoots have sometimes arisen from axillary buds, adventitious buds as well as from both sources. Continued subculturing as is practised for angiosperm species is often not possible in conifers. An intermediate step to elongate the shoot is frequently necessary before starting another cycle of shoot proliferation. Furthermore, bud multiplication has not been sustainable in many species. The most successful repetitive shoot production in conifers is the system for *Pinus radiata* (AITKEN-CHRISTIE & DAVIES 1988). This technique for radiata pine has been scaled up and was carried out in small fermentors (AITKEN-CHRISTIE 1991). Although axillary and adventitious shoot micropropagation has been reported for many conifers, even with mature trees, *e.g.*, larch (EWALD 1998), practical applications are hampered by low multiplication rates, difficulties in rooting and high production costs.

For angiosperm species, the most widely used procedures are either direct organogenesis (Fig. 1), by promoting pre-formed axillary buds, such as for chestnut, *Castanea sativa* Mill. (VIETEZ *et al.* 1980); or indirect organogenesis, by inducing adventitious shoot proliferation, such as for sycamore maple, *Acer pseudoplatanus* L. (WILHELM 1999), birch, *Betula* spp. (WELANDER 1993), apple, *Malus* spp. (JONES 1993), cherry, *Prunus* spp. (DRUART 1980) and species of poplar, *Populus* spp. (AHUJA *et al.* 1988). The latter are also examples of species in which nearly every genotype can be regenerated via tissue culture. These species are also capable of forming adventitious

shoots from leaf or internodal segments, which makes them very attractive for transformation studies. After initiation of the culture, the axillary buds are forced to flush, thus giving rise to new shoots. These shoots can be further multiplied by dividing them into apical and nodal segments. This shoot multiplication cycle can be maintained indefinitely. The multiplication capacity is characteristic of genus, genotype as well as by the physiological condition of the explants used to initiate the cultures. For axillary shoot multiplication, seedlings or trees up to an age of ten years generally causes no problems.

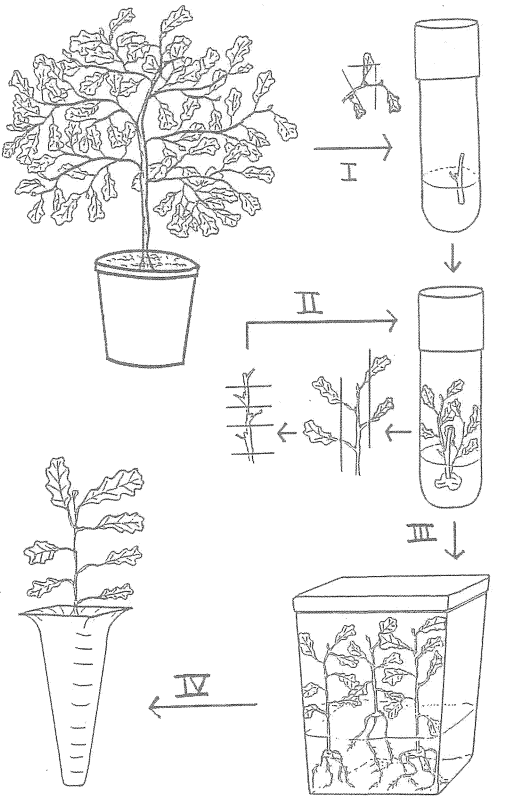

Figure 1. Micropropagation of pedunculate oak (*Quercus robur* L.) via nodal and shoot tip culture. I. Culture initiation; II. Multiplication; III. Rooting, IV. Acclimatisation.

Somatic embryogenesis

Somatic embryogenesis is the asexual development of embryos from vegetative (non-zygotic) cells experimentally induced *in vitro*. For some commercially important conifer species such as Norway spruce (*Picea abies* [L.] Karst.), white spruce (*Picea glauca* [Moench] Voss) and eastern white pine (*Pinus strobus* L.), somatic embryogenesis has become a well established technology. In the area of forest biotechnology, this propagation method is regarded as a system for genetic manipulations and as a system of choice for mass propagation of superior forest tree genotypes. The advantages include high multiplication rates, and the potential for scale-up in liquid culture (*e.g.*, bioreactors) and for direct delivery to the greenhouse or field as artificial seeds. Regeneration via somatic embryogenesis could provide the basis for cryopreservation and genetic improvement. Advances in conifer synthetic seed technology offer encouraging prospects for the use of somatic embryos for commercial plant production (ATTREE & FOWKE 1993, GROSSNICKLE *et al.* 1996, PARK 2002, SUTTON 2002, ZOGLAUER *et al.* 2003), but progress with broadleaved trees has not been as successful (MERKLE 1995). Much research has been given to the study of somatic embryos and their conversion into plants. The first reports on conifers were made for European larch (*Larix decidua* Mill.) (NAGMANI & BONGA 1985) and for Norway spruce (*Picea abies*) (CHALUPA 1985, HAKMAN & VON

ARNOLD 1985). Most published work on SE in conifers describes the initiation of embryogenic suspensor masses (ESM) from juvenile explants (female gametophytes or zygotic embryos). For broadleaved temperate forest tree species, oak (*Quercus* spp.) is probably the species with the best-developed system (WILHELM 2000).

The transition of a somatic cell into an embryo-forming cell is the first step in the development of a somatic embryo and is called the initiation phase. Applications of external stimuli on various explanted tissue can induce the process of SE. These stimuli include hormones, pH gradients, electrical fields and culture conditions such as light/darkness and the positioning of the explants. In summary, stress conditions provide factors that activate the signal transduction pathways. These pathways lead to unequal periclinal cell division, and then to the differentiation into a terminal and basal cell, which terminates the induction phase. The development of somatic embryos closely resembles that of zygotic embryos, both morphologically and temporally. It has been proposed that the development of embryos generally can be considered at three levels (LINDSAY & TOPPING 1993). The three levels are pattern in the organization of cells, pattern in protein accumulation and pattern in gene expression.

Pattern in the organization of cells. The stages in the development of SE in dicotyledons are usually described as follows:

- proembryos – small clusters of meristem cells from which somatic embryos will arise;
- globular stage – larger groups of cells not yet having a definite embryo-like shape;
- heart stage – cotyledon is specified from two lateral domains at the apical end, the hypocotyl region begins to elongate and the root meristem becomes differentiated; leads to the characteristic three-lobed stage;
- torpedo stage – elongated form of the heart-shaped embryo;
- plantlet – discernible, small, immature embryo with a primary root and shoot.

The stages in the development of SE in conifers can be divided into four distinct stages as suggested by VON ARNOLD and HAKMAN (1988 a, b):

- stage I – embryos consisting of small densely cytoplasmic cells subtended by a suspensor comprised of long, highly vacuolated cells;
- stage II – embryos with a more prominent and dense meristematic region, still attached to the callus by long suspensor cells;
- stage III – embryos with cotyledons;
- stage IV – germinating green somatic embryo.

Pattern in protein accumulation. At the biochemical level, there is a coordinated synthesis and accumulation of proteins, lipids and carbohydrates in different locations and during different times in the developing embryo. Extracellular proteins, which are secreted from the somatic embryo cultures, probably play a role in the regulation of cell expansion.

Pattern in gene expression. It is estimated (GOLDBERG *et al.* 1994) that approximately 20,000 to 30,000 genes are expressed during embryogenesis, many in multigene families. It is known from studies of *Arabidopsis*, as well as with pea and maize mutants, that the embryo is formed from modules that develop independently of each other.

The production of SE normally goes through the phases of initiation, multiplication, maturation and germination. Several problems have been identified in the different phases. The protocols for initiation differ widely in media formulations and the use of plant growth regulators. One major limitation was the inability to initiate embryogenic cultures from mature trees, which has been overcome during the last years for some species, including oaks, *Quercus suber* L. and *Q. robur* (CUENCA *et al.* 1999, TORIBIO *et al.* 1998, 2004). Developmental stages of the explant and genotype have been observed to influence the initiation frequency. In Fig. 2 somatic embryogenesis of Norway spruce is described.

Once the process of SE has been initiated, the multiplication cycle is started via repetitive or secondary embryogenesis that can be maintained for several cycles and theoretically indefinitely. In an optimized procedure, this step can be automated with the use of bioreactors. Problems are often reported that contribute to anomalous or deviant embryos. Long culture periods and use of high concentrations of plant growth regulators may cause somaclonal variation.

A major change occurs in embryonic development during the organ expansion and maturation phase. During the maturation phase, the embryos must accumulate nutrient reserves. Various types of stress, such as use of osmotic compounds (*e.g.*, high sugar content, sorbitol or PEG), and the application of ABA, desiccation or chilling treatments, have been investigated as switches for accumulation of storage products. In general, ABA is considered to be the most important factor in conifers. After maturation, the embryonic phase is terminated by germination, in which lipid and protein reserves are mobilized to enable root and shoot growth.

Maturation and low conversion frequencies are considered a major bottleneck for this micropropagation technique. Although there has been much progress and the technology is being used widely for conifers, there are little data available on field performance.

Haploid embryogenesis in trees

This topic has been thoroughly reviewed by VON ADERKAS and DAWKINS (1993). In nature, plants can be obtained naturally via parthenogenesis and apomixes (see p. 171 ff., this volume). Gynogenesis and androgenesis are the most commonly used gametic embryogenesis methods.

Gynogenesis is plantlet regeneration from cultured female reproductive tissues (*i.e.* egg, embryo sac or megagametophyte). Gynogenesis is of relatively lower importance because of its low success rate, limited application and labour intensive protocols. It has been quite difficult to achieve gynogenesis in angiosperms due to the inaccessibility of the female tissue and the complex nature of the embryo sac. In conifers, gynogenesis is similar to SE and the megagametophytes can be easily dissected from

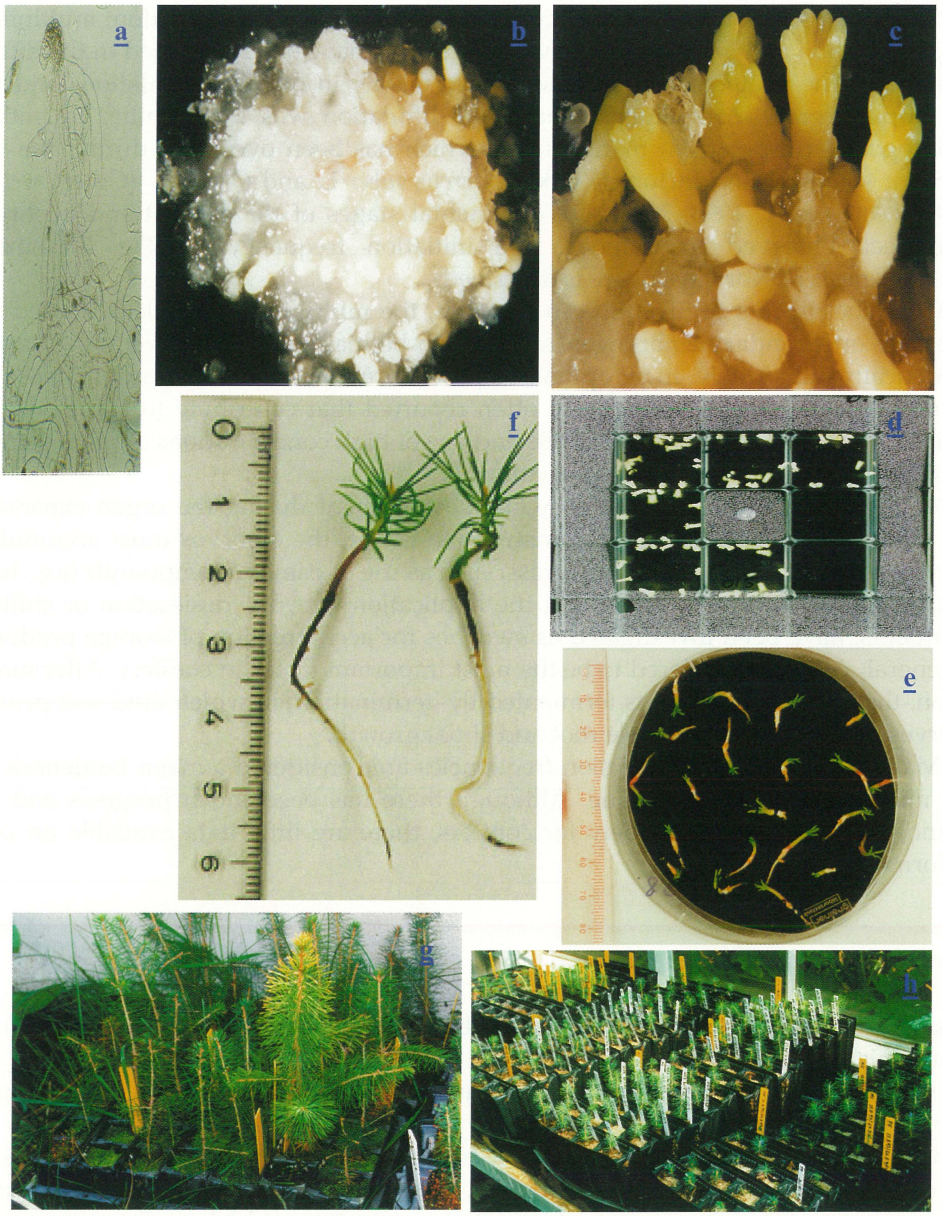

Figure 2. Somatic embryogenesis and plantlet production of Norway spruce (*Picea abies*). Stage I somatic embryo, consisting of an embryo head with cytoplasmic dense cells and the suspensor is comprised of long, highly vacuolated cells (a). Embryogenic suspensor masses with stage II embryos (b.) Organ formation of stage III embryos (c). Maturation of SE is achieved with partial desiccation under high humidity (d). Stage IV are converting into somatic seedlings on germination medium (e). Converted embryos with radicle growth after 30 days (f). Acclimatisation of somatic seedlings in the greenhouse (g). Growth of somatic seedlings from different cell lines in roottrainers (h).

the surrounding somatic tissue. The use as explants of megagametophytes in which the archegonia are not yet fertilized can result in the production of haploid somatic embryos, which has been demonstrated for the genus *Larix* by VON ADERKAS *et al.* (1987).

Androgenesis is plantlet regeneration from microspores *in vitro*. Androgenesis has been achieved in angiosperms, including several oak species, but not in conifers, although numerous attempts have been made. In angiosperms, following meiosis and further mitosis, the microspore mother cell divides into a tube cell and a generative cell. The generative cell eventually divides to form the two male gametes. The appropriate pollen stage seems to be important, but this may vary with different species. For example, sessile oak (*Quercus petraea* [Matt.] Liebl.) requires the early uninucleate pollen stage, whereas in others, pollen at the mid-late uninucleate stage was optimal. Androgenesis has a number of different developmental pathways. In some instances embryos are produced directly from the pollen or indirectly through a callus phase, which in turn produces embryos. For breeding purposes, androgenesis of broadleaved tree species has gained importance, because of production of haploid plants from microspores. Such plants can then be induced to produce homozygous diploids y chemical treatment. This offers the possibility of breeding pure lines (MINOCHA & - MINOCHA 1995). Successful androgenesis has been reported for several oak species (BUENO *et al.* 1997, JÖRGENSEN 1989).

Haploids are usually obtained by anther culture. Chromosome counts are used to confirm whether cultures are of diploid maternal origin or from haploid microspores within the anther. Counts may be unreliable because haploid cells frequently double spontaneously. A more reliable method to obtain haploid lines is by microspore culture. Successful examples of haploid culture in tree species include oak (*Quercus* spp.), apple (*Malus* spp.) and poplar (*Populus* spp.) (Fig. 3).

Production of haploid plants of tree species appears to be much more difficult than for herbaceous species. This is probably because most trees are highly heterozygous and contain large numbers of lethal and semi-lethal recessive genes. Therefore, homozygous plants produced from such species (*i.e.* where the recessive genes could be doubled) could be of low vigour and may not grow to reach sexual maturity. Furthermore, the importance of haploids for tree improvement has yet to be demonstrated.

Long-term storage of germplasm

Micropropagation offers opportunities for conservation and long-term storage of germplasm for endangered tree species. At the same time cryogenic storage of plant cells and tissue is recognized as a major method for long-term germplasm storage for species that are maintained clonally (WITHERS 1988). *In vitro* propagation is an important *ex situ* conservation method. *In vitro* storage techniques, including slow growth for the medium term and cryopreservation for the long term, offer advantages compared with traditional seed or pollen storage (see p. 601 ff., this volume). The use of *in vitro* culture techniques expands the options available for the collecting and exchange

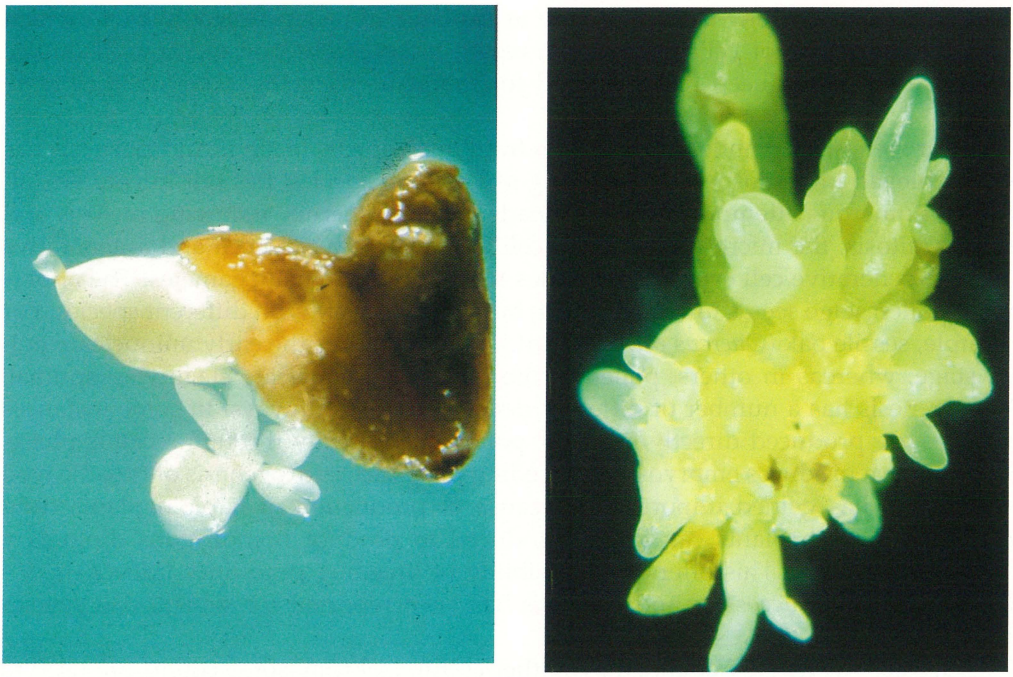

Figure 3. Anther culture of horse chestnut (*Aesculus hippocastanum* L.) (a); anther culture of sessile oak (*Quercus petraea*) (b), Photographs by J. Jörgensen.

of species that are only propagated vegetatively because of recalcitrant seeds (ASHMORE 1997, ENGELMANN 1997). Temperate recalcitrant seeds (*e.g.*, seeds from oak, chestnut and maple species) cannot be stored longer than three years. Furthermore, the use of cryopreservation with somatic embryogenesis in the context of breeding programmes allows the long term preservation of germplasm until their phenotypic traits can be evaluated in adult trees under field conditions (PARK *et al.* 1998, PARK 2002, SUTTON 2003).

Cryopreservation of zygotic embryos, somatic embryos, shoot tips or meristems has been identified as a promising method for germplasm storage, but the ability to regenerate this material into complete plants is required for the process to be useful.

Cryopreservation of biological material at ultra-low temperatures in liquid nitrogen (–196 °C) can be also useful in reducing the costs for maintaining plant material. At these temperatures, molecular motion is greatly reduced, and no liquid water phase exists. The main process of cell damage is the formation of intracellular ice crystals when approaching freezing point. Damage of this nature is a risk not only during freezing, but also when thawing. Once intracellular ice crystals are formed, they damage biomembranes as well as macromolecules, and thus disrupt the cell compartments and obstruct enzymatic reactions (ZACHARIASSEN & KRISTIANSEN 2000). Previous methods emphasized slow cooling (cooling rates of 1.0 to 0.1 °C·min^{-1}) or two-step cooling

techniques to attain cryopreservation. Strategies to prevent ice formation are to employ slight dehydration as well as use of cryoprotectives (*e.g.*, sorbitol, glycerol, dimethylsulfoxid [DMSO]). An alternative method is through vitrification, the transformation of a liquid to a glass. The advantages of vitrification are that it is a simple technique avoiding sophisticated equipment and it is also applicable to larger pieces of tissue (Fig. 4). In addition to the all the important physical parameters, the physiological state of the cell material, the relative cell volume as well as the thawing rate, are also important factors that determine a successful cryo-conservation regime. Embryogenic cultures of conifers should be in the early exponential growth phase and vacuolization of the cells low. DMSO is frequently used for conifers. Concentrations of 5 to 10 % have proved effecient for the cryopreservation of conifers and high regrowth rates after thawing were obtained (ARONEN *et al.* 1999, FORD *et al.* 2000, KLIMASZWESKA *et al.* 1992). CYR (1999) listed a considerable number of laboratories and their embryogenic germplasm collections. All important coniferous species are included in this list.

Several important broadleaved tree species have been cryopreserved (JÖRGENSEN, 1990). Recently the establishment of a cryopreserved gene bank of European elms has been reported (HARVENGT *et al.* 2004). Much progress has been made during the last decades and collections of several clonal genera are today stored in liquid nitrogen and more are in progress worldwide (REED 2001).

Optimally, cryopreservation is a method for long-term storage of germplasm without risk of somaclonal variation. To reduce the risk of somaclonal variation, the time span for the production of regenerated plantlets after the initial induction should be minimized as far as possible. Cryopreservation may be an alternative to subculturing

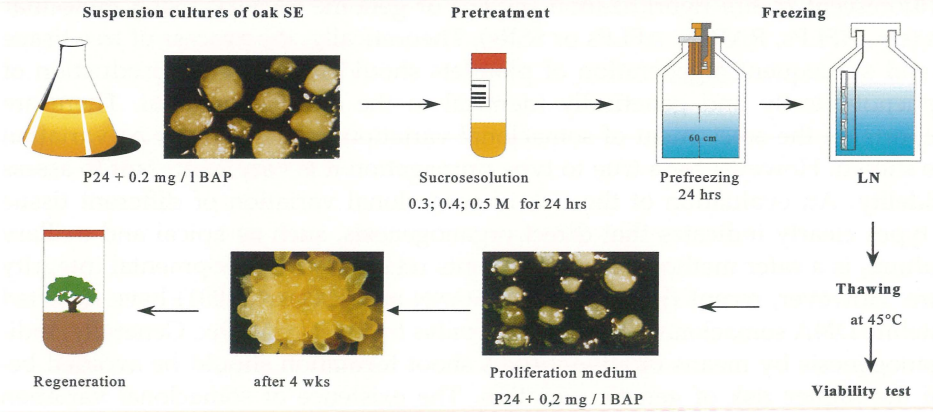

Figure 4. Cryopreservation of penduculate oak (*Quercus robur*) somatic embryos by means of vitrification.

over long periods. During the last years, cryopreservation became an indispensable part of any clonal breeding strategy based on somatic embryos (PARK 2002).

Somaclonal variation

LARKIN and SCOWCROFT (1981) proposed the term somaclonal variation for genetic instabilities detected in plants regenerated from any type of somatic cell culture. Plants regenerated from organ cultures, calli and protoplasts often show new phenotypic variability such as altered morphology, growth rate and resistance to toxic metabolites. Genetic variability in woody plants was first reported in *Citrus grandis* (L.) Osbeck by CHATURVEDI and MITRA (1975). The occurrence of somaclonal variation in woody plant species has been reviewed by DEVERNO (1995). Most somaclonal variations arise due to changes in the nuclear genome, but alterations in the mitochondrial genome and chloroplast genome have also been reported. Chromosomal instabilities are the most frequent forms of variations observed. There seems to be a close association between disorganized callus growth and chromosomal instability, as opposed to the relative stability of organized cultures derived from meristems. BAYLISS (1980) reviewed chromosomal variations in tissue culture and pointed out that they are probably the rule rather than the exception. Two theories have been proposed to explain the mechanisms leading to structural changes in chromosomes during cell culture (LEE & PHILLIPS 1988). The first mechanism is based on late replication of heterochromatin. The second mechanism is related to the consequences of nucleotide pool imbalance. Another potentially mutagenic event is the activation of transposable genetic elements in culture, which may be viewed as an adaptation to stress. Measuring somaclonal variation has been reviewed by JAIN and DEKLERK (1998). Variation may be evaluated with phenotypic and morphological markers (image analysis), biochemical markers (*e.g.*, isoenzymes, secondary metabolites), cytological markers (karyotype, flowcytometry, fluorescent *in situ* hybridization [FISH]) or genome analysis using molecular markers (*e.g.*, RFLPs, RAPDs, AFLPs or SSRs). Theoretically, the process of tree tissue culture and subsequent regeneration of plantlets should result in the production of plants phenotypically and genetically identical to the original material. There are many reports on the assessment of somaclonal variation, where little or no variation could be shown. However for a true to type propagation it is very important to assess clonal fidelity. An evaluation of the risk of somaclonal variation of different tissue culture types clearly indicates that direct organogenesis, such as apical and axillary shoot culture, is a safer method because explants retain their developmental integrity in culture. However, recent findings of RAHMANN and RAJORA (2001) have detected microsatellite DNA somaclonal variation in *Populus tremuloides* Michx. Generally, indirect organogenesis by means of adventitious shoot formation should be avoided because of the higher risk of genetic variation. The existence of somaclonal variation among micropropagated plants derived through the culture of organized meristems has been shown for many traits (RANI & RAINA 2000). Somatic embryogenesis had long been regarded as a stable system. However, assessment of genetic stability in conifer

SE such as *Abies alba* Mill. (ROTH *et al.* 1997) or *Picea abies* (BURG *et al.* 1999, FOURRE *et al.* 1997) have shown that mutations occur at various levels. Recent findings have demonstrated that several years of continuous *Quercus robur* SE subculture can result in tetraploidy (ENDEMANN *et al.* 2001). The risk of somaclonal variation may be decreased by use of low concentrations of PGSs systems. In addition, the time span for the production of regenerated plants after the initial induction should be minimized as far as possible. Cryopreservation is used routinely now as an alternative to long subculture periods for both organogenesis and somatic embryogenesis.

Field evaluation and performance of micropropagated trees

It is essential to evaluate performance of micropropagated trees for ecological and economical purposes. The final step in all micropropagation systems is the acclimatisation phase, which governs the transition between *in vitro* and *in vivo* conditions. During this process, plants have to adapt to new environmental conditions such as lower relative humidity, higher light intensity, and fluctuating temperatures. The intrinsic plant quality is important for acclimatisation. In this regard, excessive water loss by transpiration, an impaired photosynthetic apparatus and a poor root system may cause major problems.

Clonal performance of Norway spruce somatic embryos and the uniformity of clones propagated can be improved significantly by selecting plants with lateral roots at ex vitro transfer, whereas prolonged ABA-treatment displays a negative effect on subsequent plant growth of somatic seedlings (HÖGBERG 2003). Increasing data are now available on the field performance of *in vitro* propagated woody plants. Significant differences in height and groundline diameter were found in loblolly pine (*Pinus taeda* L.) favouring seedlings versus micropropagated plantlets (RAHMAN *et al.* 2003). In addition also survival rate, root/shoot ratio was better in seedlings, which was attributed to altered root system architecture. However BENOWICZ *et al.* 2002 showed similar pattern of physiological performances through 2 years of assessment for Douglas fir (*Pseudotsuga menziesii* var. menziesii [Mirb.] Franco) somatic and zygotic seedlings with respect to gas exchange, water relations, and frost hardiness.

Field performance of *in vitro* propagated hybrid curly birch (*Betula pendula* Roth × *B. pendula* var. *carelica* [Merckl.] Hämet-Ahti) showed that the tissue culture propagation method is a valuable tool for the 'true-to-type' propagation with regard to outstanding wood qualities. (EWALD *et al.* 2000). Micropropagated silver birches (*B. pendula*) and seed-born material did not differ from each other as regards survival and height growth. However between single clones large significant differences were detected (VIHERA-AARNIO & VELLING 2001). In addition two ornamental *Prunus* species were assessed in long term evaluation studies (JESCH & PLIETZSCH 2000, 2001). The plant material was propagated vegetatively via micropropagation, cuttings, cuttings of *in vitro* propagated plants and graftings. The morphological results indicate that the *in vitro* propagated plants had a more juvenile character and showed a more vigorous growth. Early differences between treatments decreased with the increasing age of the

plants. However, results on the phenological and physiological characteristics indicate that soil and climatic conditions had a greater influence than the propagation method. Microclones of mulberry (*Morus* spp.) genotypes were compared with cuttings derived from the same genotypes. Micropropagated plants showed significant morphogenic vigour when compared to cuttings (ZAMAN *et al.* 1997).

It could be demonstrated, that *in vitro* procedures affect the development of the resulting plants. *In vitro* treatments should therefore be balanced to obtain high quality plants. Besides these fundamental aspects, a wide array of practical problems need to be solved, such as mechanical handling and automated planting. In addition, it will be necessary to reduce productions costs for commercial application. Data on field performance of micropropagated trees are rare. Mostly reports are available which relate to growth characteristics measured 1–6 years after planting, but observations need to be continued over a longer period. This is especially important in cases where tissue-cultured trees have made a slow start. As a consequence of the limited data on field performance, many questions remain unanswered, such as: are plants derived from somatic embryos of mature trees completely rejuvenated? Field data on the performance of somatic embryo plants from mature tissue are rare and inconclusive. Generally, in order to implement this technology it will be necessary to produce plants using tissue culture, to establish norms for nursery tests and to design field trials for public demonstration.

Macrovegetative propagation methods

Grafting and budding

Grafting is a technique used to unite parts of different plants by bringing the cambium of each into contact and then creating a situation under which the cut surfaces can unite and continue to grow. These techniques involve two important stages: the cutting and preparation of the grafting surfaces, and the procedures for maintaining the graft.

Grafting has recently been suggested by LEV-YADUN *et al.* (2000) as a practical means of conserving genetic diversity in the wake of harvesting operations, particularly in the context of tropical forests in areas where labour costs are reasonably low. A team of grafters might follow the harvesting team and graft the cut trees with shoots from the canopy of the same tree or with selected material. Other advantages in addition to conserving genetic diversity potentially include preserving the mycorrhiza and other soil biota, keeping a large quantity of root biomass (on average 26 % of shoot mass) alive and active, preventing soil erosion, shortening the period of time for forest regeneration and increasing sustainability. However, grafting is very labour-intensive and must be done while the cambium is active during spring and early summer. It remains to be demonstrated in the future, how successful this strategy will be. However for testing a large numbers of clones and for mass propagation it is an unrealistic approach.

The most common and/or useful techniques are described as follows.

Open-ground budding. Bud grafting is a standard technique for propagating a number of plants in the open ground during the summer months. It is a more economical technique than grafting as only one bud is used, compared with an optimum of three to five for grafting. It is also normally carried out when the rootstock is in active growth with rapidly dividing cambial cells. The usefulness of this technique has improved with developments in propagation facilities, production systems, tying-in materials and the refinement and adaptation of the actual technique. Applications include uses in bench grafting, budding *in situ* on container-grown rootstocks and in certain instances, as an alternative to late winter/spring whip and tongue grafting. Budding is essentially the use of a single bud (scion) plus a portion of bark with or without a sliver of wood that is sited on the rootstock between two flaps of ring (*e.g.*, T-budding). Alternatively, the sliver of wood replaces a section of rind (patch budding) or replaces a pre-cut veneer of rind and woody tissue (chip-budding).

Open-ground grafting. Grafting outdoors in the open ground is a major propagation technique in the commercial nursery, mainly for fruit and ornamental trees. A multibudded dormant scion is grafted onto an established open-ground rootstock.

Bench grafting and top-working. Bench grafting includes grafting and budding techniques that are performed inside a covered structure, normally a shed or greenhouse. It is a standard method used to propagate a much wider range of plants than the other grafting and budding techniques, including many of the rare and high value ornamental trees. The economics of bench grafting need to be carefully considered. The decision will largely depend on the species of plant to be propagated, the cost of rootstocks, expertise in grafting technique and aftercare. Dormant bench grafting is performed during the winter, normally using dormant deciduous hardwood, evergreen hardwood or sometimes softwood scion material. Summer bench grafting is performed during late summer, normally using semi-ripe wood scion material.

Bottom-working. The graft is sited near ground level, the optimum height being 10 cm above the soil.

Top-working. The graft is sited at an optimum height of 0.5 to 1.8 m above ground level.

High-working. This is essentially a category of top-working in which the scion is placed high on the rootstock stem, sometimes as high as 2 to 2.5 m. It is commonly used to shorten the period between production and marketing for standard weeping trees.

Graft incompatibility is defined as the partial or complete failure of the union between scion and rootstock, which may be a result of genetic differences between the rootstock and scion, plant viruses, use of excessively vigorous rootstock with a very weak scion, formation of layers of cork tissue, abnormal distribution of starch at the actual graft union, differences in biochemical compounds or death of cambium and phloem tissues at the graft union (MACDONALD 1986).

The success of the union between the rootstock and scion is largely determined by the degree of incompatibility between them. Some plant families present few problems, even to the extent that different genera can be grafted together. Other species

Table 1. Recommendations for grafting conditions for some forest tree species.

Scion and rootstock species	Time to initiate graft	Type of graft recommended
Abies spp.	Dec–Feb	Side
Larix decidua	Jan–Feb	Side
Picea abies	July–Aug, Dec–Feb	Side
Acer spp.	Jan–Feb	Side or whip
Betula pendula	Jan–Feb	Side

require very specific matching between rootstock and scion. The incorrect choice of rootstock can lead to weak growth and poor quality. The time of the year the graft is performed is also important as it represents the physiological condition of the scion and rootstock. Recommended conditions for some commonly grafted forest tree species are presented in Table 1. In each case, pot-grown rootstock is recommended.

Cutting propagation

Cuttings are separated plant parts shoots with leaves, shoots with buds, roots with buds rooted and developed into whole plants. Propagation via cuttings is today the most frequently used method for vegetative multiplication. Generally, large-scale cutting propagation is only possible for some forest tree species, whereas in horticulture it is widely used and very important. In some regions of Japan, afforestation with rooted cuttings of sugi (*Cryptomeria japonica* D. Don) has been carried out for centuries. ISIKAWA (1987) reported that approximately 50 million rooted cuttings have been produced per year. The area reforested by cuttings is nearly 12,000 ha in total, while the area reforested by seedlings covers about 48,000 ha. Cutting propagation is a well-known method for vegetative propagation of many important tree species, *e.g.*, Norway spruce (BÄRTELS 1992). Considerable progress has been made with large-scale propagation of elite trees and the multiplication of seedlings arising from crosses (BORNMAN 1987). Several million trees of Norway spruce obtained from cuttings of selected clones are planted annually in Finland, Sweden and Germany (BRIX & VAN DEN DRIESSCHE 1977). Recently more than 15 million rooted cuttings were planted mainly in southern Sweden (SONESSON *et al.* 2001). However clonal forestry with Norway spruce based on cutting propagation has not been successful, regardless of the method used to maintain rooting ability (HÖGBERG *et al.* 1995). SONESSON (2003, cited in HÖGBERG 2003) presented an overview of the present situation in Sweden and concluded that the main reasons for the lack of success with clonal forestry were low rooting percentages and high degrees of plagiotropic growth. Producing a cutting plant of a tested clone was estimated to be 100 % more expensive than producing a seedling, and the cost for a bulk propagated cutting plants, with cuttings taken from a juvenile donor plant, was estimated to be 60 % higher than for a seedling. At present, bulk propagated cuttings are only produced commercially in Sweden in small numbers.

Significant bulk propagation programmes based on cuttings have been initiated for *Picea mariana* (Mill.) B. S. P. and *P. glauca* (Moench) Voss production in eastern Canada, and for *P. sitchensis* production both in Great Britain (LEE 2003, cited in HÖGBERG 2003) and Ireland (HARRINGTON 2003, cited in HÖGBERG 2003). Field plantings have also been made from cuttings of *Pinus taeda* L. (GUPTA *et al.* 1987) and *Pinus radiata* D. Don (HORGAN 1987). The main constraints generally are the low rooting capacity that is related to the phase change phenomenon and the high production costs.

There are three types of cuttings based on the stage of stem-growth development: softwood, semi-ripe and evergreen hardwood, and deciduous (leafless) hardwood cuttings.

Softwood cuttings are selected from the current season's growth before extensive lignification has occurred. They are normally taken from shoots soon after budbreak in late spring and early summer. At this time, shoots are in an active state of growth and therefore careful, rapid handling is required during their collection, preparation and aftercare. Examples of species that are well suited to this type of cutting include metasequoia (*Metasequoia glyptostroboides* Hu et Cheng.), white spruce (*Picea glauca*), European alder (*Alnus glutinosa* [L.] Gaertn.), European white birch (*Betula pendula*) and chestnut (*Castanea sativa* Mill.). For rooting, cuttings are quick dipped in 0.05–0.1 % IBA.

The shoot passes from the softwood phase into the semi-ripe wood phase as the tissues become lignified and summer dormancy commences. Semi-ripe wood and evergreen hardwood propagation is an important method for many conifers and broadleaved evergreen species. Cuttings can be taken from late August until February. The cuttings are then rooted in vapour pressure deficit situations such as mist, fogging or closed-case facilities. Disease problems may occur due to high humidity. More emphasis is placed on wounding because the stem has a greater physical resistance to the emergence of roots. Optimum concentrations for application of PGRs or rooting hormones using the quick dip method are 0.2–0.25 % IBA.

Deciduous hardwood cutting propagation is performed during fall and late winter/early spring. Cuttings are taken from stock plants from well-ripened, vigorous one-year-old shoots. The cutting is thus dormant and secondary thickening is complete. This is an important technique for ornamentals. The material is dormant and thus there is considerably more flexibility with its handling. For example, the unrooted material can be kept in cold storage. The basal cut surfaces of the cuttings are quickly dipped into a liquid rooting hormone containing 0.5–0.75 % IBA.

In addition to propagation by cuttings, other methods used include root cuttings and layering. Root cuttings are produced by severing roots into individual pieces, each of which is capable of developing adventitious buds and roots and, therefore, of regenerating into complete plants. The technique has been known since the 17th century. This method requires only limited skill and minimal propagation facilities, and is a fast way to multiply clonal material. Examples of species for which this method may be suitable include bottlebrush buckeye (*Aesculus parviflora* Walt.), black locust (*Robinia pseudoacacia* L.), European aspen (*Populus tremula* L.), trembling aspen (*Populus tremuloides*), and female trees-of-heaven (*Ailanthus altissima* [Mill.] Swingle).

Propagation by layering differs from other techniques. Adventitious roots develop from the stem while it is still attached to the parent plant. In practice, this shoot is severed from the parent plant at a time when sufficient roots have formed for it to successfully establish and grow when planted out or potted into a container. Layering is a very reliable propagation technique for some plants which have the ability to form adventitious buds adjacent to the cut stem and are thus able to produce a high quantity of layers over a given area. The physiological basis of layering includes two principles. First, a constriction is induced in the stem by bending, cutting or twisting, which restricts the flow of auxins and carbohydrates at the point of constrictions, thus promoting the initiation and subsequent development of roots. Second, light is restricted. A variation of this technique is known as stooling or mound layering, which involves the induction of adventitious roots at the base of stems by mounding up soil so as to exclude light by blanching. Layering can be an efficient, mechanized and economical propagation system. Examples of species for which layering may be appropriate include linden (*Tilia* spp.), apple (*Malus* spp.), hazel (*Corylus* spp.), cherry (*Prunus* spp.), chestnut (*Castanea* spp.) and maple (*Acer* spp.) species.

Factors affecting the rooting of cuttings can be categorized as occurring before (pre-removal) or after (post-removal) the cuttings are taken.

Pre-removal factors:

- Stock plants are important. Juvenile stock plants frequently result in higher rooting rates. In Norway spruce there is declining rooting capacity with the increasing age of the mother plant. One way to maintain the rooting ability is to use serial propagation (KLEINSCHMIT *et al.* 1973, *i.e.*, to take new cuttings from cutting plants in cycles of 3–4 years.
- Specific pruning treatments, so called hedging, where low hedges are forced by recurrent pruning of the donor plant, may be used to increase the vigour of the stock plants, thus improving rooting and increases the number of cuttings. A comparison of hedging and repeated cutting cycles for propagating clones of Sitka spruce has been performed recently and the success is encouraging for a clonal strategy with Sitka spruce (MASON *et al.* 2002).
- Carbohydrate and nitrogen levels within the stock plants (C:N ratio) or the condition of the wood are important. Balanced fertilizer and excess nitrogen fertilizers are not applied toward the end of the growing season. Potassium should be used towards the end of the growth cycle.
- Light is required for the manufacture of carbohydrates through the process of photosynthesis.
- The location from which the cuttings are taken can influence the overall quality of the cutting, its ability to root and the subsequent growth habit. The plant 'juvenility factor' is strongest towards the centre at the base of the stock plants. A cutting from a vertical shoot will grow horizontally after rooting. This horizontal habit gives a ground cover appearance. This positional effect is known as topophysis.
- Rooting of stem cuttings can be improved by taking them from shoots that have been grown in the darkness (*i.e.* etiolation).
- Time of the year that is optimal for propagation is dependent on genus, species

and cultivar (*e.g.*, some plants can be rooted virtually the year round), and also on the geographical location of the nursery, propagation facility and growing system.

Post-removal factors:

- Cuttings should be handled with care (*e.g.*, minimum water loss, tissues are not bruised). Softwood cuttings require particular care.
- Cuttings must be stored and planted in the correct direction (*i.e.* polarity).
- The type of cutting used is dependent on genus, species and cultivar; time of the year the cutting is taken; quantity of cuttings required and propagation facility available.
- Proper nutrition of the plants is critical. For example, rooting success with some root species has been doubled with appropriate fertilizer application.
- Appropriate container types, climatic conditions, humidity and temperature should be provided.
- Plant diseases should be controlled.
- Other post-removal factors that can affect the rooting and viability of the cuttings include: wounding of tissue during preparation of cuttings, removal of tissue or incisions at the bottom of the cutting (splitting of the stem base), removal of terminal flower buds or vegetative buds or shoot tips, reduction in size of the leaf lamina, removal of basal leaves etc.

The initiation of adventitious root formation is a complex morphogenetic phenomenon in which auxin has a central role. When a shoot is cut, auxins, which are produced in the shoot apex, accumulate at the shoot base to induce rooting. Generally, rooting is a developmental process consisting of four different phases (MONCOUSIN 1991a, b): the induction phase, when the capacity for root formation is determined; the initiation phase, when visible cytological changes occur; the organization phase, when root primordia can be seen to be produced histologically; and a growth phase, when primordia develop into roots. According to DEKLERK (1998), each phase has its own requirements.

Rooting is normally achieved by application of synthetic auxins, such as indolebutyrid acid (IBA) and naphthalenacetic acid (NAA). In contrast to the rapidly degrading natural auxin, indole-3-acetic acid (IAA), the synthetic auxins are more stable. A pulse treatment with high concentrations but short application times is better than continuous application of low auxin levels. Callus formation, which may be an impediment for later transfer to the soil, can be avoided by pulse treatments. Generally, many extrinsic and intrinsic factors are involved in rooting, and research in this area is very active. Low rooting capacity is associated with the ageing effects.

Auxins are applied to the base of cuttings to increase overall rooting percentages, hasten root initiation and increase the number and quality of roots and encourage uniformity of rooting. Concentrations between 0.5 and 1 % are applied either as a solution (alcohol or acetone) or a powder (talc). Fungicides are also usually included in these applications.

One of the most important criteria for the successful rooting of cuttings is a reliable rooting media. The percentage of rooting and the quality of roots can, in many in-

stances, be directly linked to the medium itself. Variation in root development increases costs as more grading is required to discard unrooted and poorly rooted cuttings. The components used to form rooting media are normally naturally occurring, but there has been increasing interest in artificial or manufactured substances. Rooting media are usually selected on the basis of quality and cost. Good quality is usually accomplished by the following factors: optimum pH between 5.5 and 6.5, without harmful salts, minimum of weed seeds and diseases, optimum air-filled porosity (approximately 30 %), an adequate supply of oxygen to the base of the cutting, and good drainage to allow the development of a fibrous root system. The major constituents for rooting media are peat, bark, sawdust, fine and coarse sands (grits), perlite, vermiculite, expanded polystyrene chips or beads and loam. Additional constituents incorporated into rooting media may be fungicides, fertilizers or mycorrhizal fungi.

Studies on the cutting propagation of pedunculate oak performed by SPETHMANN (1986) resulted in the following conclusions:

- Optimum rooting medium is a combination of 0.5 % IBA + fungicide Euparen 10%.
- A decrease in rooting success was noted in relation to the age of the mother tree (ortet). An ortet of 2 years provided 90 % rooting success of cuttings; an ortet of 20 years provided only 30 % rooting success of cuttings.
- Seasonal effects in transplant success were noted. Transplanting the cuttings after the middle of May was superior compared with early spring.

Topophysis

The term topophysis is related to differences in maturation or developmental potential among apical meristems of different branch hierarchical order, which may result in differences in performance among vegetative propagules taken from different parts of the same donor plant, such as plagiotrophy. The leading shoots of woody plants typically grow vertically upwards (negatively geotropic growth), while lateral branches tend to grow at a more horizontal angel (plagiotropic growth). Plagiotropic growth is suggested to be associated with the mature phase of conifer ontogenesis (MCKEAND 1985).

A major difficulty associated with the micropropagation of forest trees is the relatively poor success with adult trees. Most species can be easily propagated vegetatively only during their juvenile phase, but many desirable traits are only expressed in mature trees. As the tree ages, and at least until maturity is reached, the rooting ability of vegetative propagules declines. Maturation (ontogenetic ageing) is the ongoing process of phase change, which results in relatively permanent changes. GREENWOOD (1987) proposed four developmental phases for the maturation process, each characterized by a unique set of morphogenetic competencies: the embryogenetic phase (close to the mature embryo); the seedling phase (close to an ideal juvenile phase); the transition phase (including the acquisition of reproductive competence); and the mature phase (reached when reproductive competence is highest and the capacity for

height and diameter growth is lowest). Sometimes phase changes are accompanied by changes in such features as foliage (*e.g.*, *Hedera* spp., *Eucalyptus* spp.). Although phase changes have been widely studied, the mechanisms are not well understood. Alterations in DNA-methylation have been linked with ageing (RICHARDS 1997). Large differences in the extent of DNA methylation between meristematic areas of juvenile and mature *Pinus radiata* trees have been found, whereas differences in the extent of DNA methylation between differentiated tissues of juvenile and mature trees were small. In meristematic areas, there was a gradual decrease in extent of DNA methylation as the degree of reinvigoration increased (FRAGA *et al.* 2002a,b). Morphological, physiological, biochemical and molecular events that occur during maturation have been widely reviewed (HACKETT & MURRAY 1996, PEER & GREENWOOD 2001, RUAUD & PAQUES 1995, VON ADERKAS & BONGA 2000).

Rejuvenation is the return of mature tissues to the juvenile phase. This is of considerable interest because it would allow more successful propagation of proven mature genotypes. The position from which the explant is taken from the adult tree is important, because juvenility persists longer in some parts of the tree than in others (BONGA 1987). Juvenile characteristics may be preserved at the base of the tree in ontogenetically young tissue, whereas maturation occurs in the periphery of the plant in tissue that is ontogenetically older, but chronologically young, as a function of development and physiological gradients (HACKETT & MURRAY 1996). The use of tissues with juvenile characteristics facilitates propagation of mature trees. When this material is not available, some manipulations for reversal of ageing, or partial rejuvenation, are helpful. *In vivo* methods include pruning, hedging, the use of stool beds, grafting (serial grafting), the use of root suckers, spraying of plant growth regulators, etiolating and forcing of branch and stem segments for epicormic bud flushing (BALLESTER *et al.* 1996, EVERS *et al.* 1996). Additionally, *in vitro* methods have been developed, which include culture of selected explants such as epicormic buds, repeated subculturing, micrografting onto juvenile rootstocks, adventitious bud formation, somatic embryogenesis and horizontal subculturing (BALLESTER *et al.*1990).

Various markers on morphological, biochemical and molecular levels have been identified for characterization of different developmental phases. Molecular markers identified by mRNA-differential display technique have revealed several genes, which are involved in the maturation process of ivy (*Hedera helix* L.) such as dihydroflavonol reductase (MURRAY *et al.* 1994). Recently MADS-box genes, which are known to be involved in reproductive development through initiation of flowering, have been cloned from apple (*Malus* spp.) (VAN DER LINDEN *et al.* 2002) and a gene for DNA-methyltransferase has been cloned from peach (*Prunus persica* [L.] Batsch) (GIANNINO *et al.* 2003). These are promising candidates for phase change markers.

Conclusions

Micropropagation of forest tree species is a rather new technology in comparison to traditional propagation systems that have proven successful for centuries. Neverthe-

less, much progress has been achieved in improving this technology for forest trees by manipulating growth media and culture conditions, as well as by testing a variety of explant sources. However, the basic understanding of the physiological mechanisms involved has often been lacking. Today it is important to expand our knowledge in the area of developmental tree physiology in order to improve propagation systems as well as to overcome the main bottlenecks in particular ontogenetic ageing and low conversion frequencies in SE systems. With the new tools offered by molecular biology it is nowadays possible to analyze gene function and regulation even in forest trees. For these fundamental aspects, tissue culture of forest tree species are offering excellent model systems for studying the physiological, biochemical and molecular events related to morphological, developmental and pathological responses.

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How can silvicultural management contribute to genetic conservation?

Th. Geburek & F. Müller

Introduction

Since the first utilization of forests ecosystems, human activity has intentionally or unintentionally altered the gene pool of forest tree species. However, forest ecosystems will only persist if genetic diversity of forest trees is dynamically maintained. This especially holds true in view of environmental changes due to air pollution and global warming. Given the goal that genetic diversity is to be maintained for both the short-term (domestic fitness) and the long-term (Darwinian fitness), genetic aspects should also be embedded in silvicultural management in order to confer forest tree populations on the ability to keep an appropriate or natural level of genetic diversity. Even in high-intensive forest plantations this holds true. Intrinsically, all silvicultural means affect natural evolutionary processes. Moreover, it is very often intended to mimic natural successional effects and, concomitantly, genetic processes are also imitated. However, silviculture may also have a negative impact on forest genetic resources. General aspects of how forest management, including effects due to clonal forestry, is changing the gene pool of forest trees aspects have been described elsewhere (*e.g.*, SAVOLAINEN & KÄRKKÄINEN 1992, GEBUREK & THURNER 1993, HUSSENDÖRFER 1996, MÜLLER-STARCK *et al.* 2000, FINKELDEY & ZIEHE 2004, p. 437 ff., this volume) and will not be addressed here in detail.

Generally, genetic conservation is not an exclusive objective even in close-to-nature forestry (ecological forestry) (cf. SEYMOR & HUNTER 1999). In principle, forest management has to be assessed based on the impact of their methods on evolutionary processes, notably through

(1) genetic drift related processes (*e.g.*, limited number of seeders);
(2) mating system related processes (*e.g.*, reproductive isolation);
(3) fertility and viability selection related processes (*e.g.*, selection of plus trees); and
(4) migration related processes (*e.g.*, transfer of forest reproductive material).

Th. Geburek & J. Turok (eds.)
Conservation and Management of Forest Genetic Resources in Europe
Arbora Publishers, Zvolen, 2005, 651–669, ISBN 80-967088-1-3

Since not all forest geneticists are familiar with silvicultural methods, we briefly introduce common silvicultural types. After this, some technical guidelines in silvicultural management with emphasis on genetic conservation are provided.

Common silvicultural types

The utilization of forest trees through coppicing probably goes back to Neolithic times. Proper forest management started in the Middle Ages when agriculture was combined with (unsustainable) forestry in Europe. These early forms of forest management (custodial forestry) resulted in widespread forest exploitation and conversions to agricultural land, which were later limited by timber or forest ordinances. The significance of woodland achieved a new dimension. Close to dwellings, woodland was primarily utilized as wood pasture. As a consequence of pastoral forests, the area became sparsely stocked and was often used for fuel wood and less frequently for timber extraction. Finally, this utilization method led to 'sprout forests'. Woodland more distant from villages or hamlets – mainly noble property – was exploited by extracting single trees. Finally, the result of this utilization was 'selection forests'. Extensive cuts were necessary for the mercantile trade, with a high timber demand for noble residencies or bigger villages or cities. To better control clear-cuttings with succeeding single-cohort stands, the silvicultural type of a 'high forest' was born, strongly favoured by sovereign ordinances in Europe (MANTEL 1990).

Sprout forests

Coppice

Coppicing is a traditional, very old European practice of woodland management and wood production. Trees are felled in a short rotation, commonly between 15 and 30 years, and shoots are allowed to grow up from the base of a felled tree. Stooling (coppicing) is only suitable for broadleaved trees that readily shoot from the stump and include especially hornbeam (*Carpinus betulus* L.), oaks (*Quercus* spp.), sycamore (*Acer pseudoplatanus* L.), ash (*Fraxinus* spp.), black locust (*Robinia pseudoacacia* L.), chestnut (*Castanea sativa* Mill.) and common alder (*Alnus glutinosa* [L.] Gaertn.). A coppice is said to be even-aged when all stems harvested from a given stump in each felling operation are of the same age. In a mixed-aged coppice, the stems growing from each stool are of different ages: only one or two (the oldest) are felled at one time. Mixed-aged coppicing is an old and unfortunately declining technique in which light conditions need to be managed with a good deal of care (new shoots grow less vigorously when other stems remain). The woods are often divided into blocks called 'coupes' (compartments), which are harvested periodically, creating a continuous rotation of harvest and regrowth. The overall effect is to give rise to an irregular patchwork of coupes at different stages of growth. The technique helps to conserve soils and harvesting is less disruptive for the tree cover, therefore encouraging shade-loving tree

and shrub species and other woodland flora and fauna (MAYER 1977, *loc. cit.* p. 399, BUCKLEY 1992, ALLABY 1994).

Coppice with standards

A coppice with standards is a two-storey woodland management in which the overstorey is supposed to produce structural timber and, hence, is allowed to grow to their full height (standards), while the understorey is coppiced for fuel-wood within short rotations (approximately 15–30 years). For a yield-sustained management, standards must be uneven-aged, exhibiting a selection of forest-like structures. Main ly oaks (*Quercus* spp.), birch (*Betula* spp.), elm (*Ulmus* spp.), sycamore (*Acer pseudoplatanus*) and cherry (*Prunus* spp.) are used. If the shelter (standards) is dense, commonly hazel (*Corylus avellana* L.) is found in the understorey, otherwise more light demanding tree species are found (MAYER 1977, *loc. cit.* p. 403).

High forests

High forests are stands of trees that are generally of seed or seedling origin. This is the difference to a coppice. A high forest is said to be 'even-aged' when the stand, in which trees are mainly of the same age, is periodically regenerated on a large scale, and 'uneven-aged' when it contains cohorts of varying age and structure that are regenerated in small batches (called clumps). High forests may be the result of clear-cutting (even-aged), seed-tree methodology (clear-cutting with reservation of standards) (mainly even-aged), regular and irregular shelterwoods (uneven-aged), or selective forests (uneven-aged).

Silvicultural practices relying on artificial regeneration

The following recommendations are mainly related to the management of even-aged high forests that rely on artificial regeneration and underpin the importance of the selection of basic material (seed stands and seed orchards), the seed harvest and how forest reproductive material is processed.

Forest management system: clear-cutting with artificial regeneration (Fig. 1)

Characteristics: Clear-cutting is often an efficient and profitable harvest system. All the trees are removed on a given site at once or in short intervals. Historically, this management system is closely linked to artificial regeneration. In the 18th and early 19th centuries, European forests were clear-cut and then sites – partly after a short agricultural usage – were artificially regenerated by planting or seeding. Many autochtho-

nous deciduous forests (often broadleaves) were replaced by light-demanding conifers. In Europe, extensive clear-cuts (> 5 ha) are seldom found nowadays.

Genetic evaluation: From a genetic point of view, this management system is neither good nor bad. It affects genetic resources based on (1) the genetic 'value' of the previous stand (to be clear-cut), (2) on the forest reproductive material to be used, and finally (3) on the regeneration method (planting, seeding) itself. The selection of seed stands and seed trees, seed and plant processing, planting and seeding have different effects that are described in more detail in the following.

Selection of seeds stands

The environment of the seed stand should be similar to the site conditions in the case of afforestation. Forest genecology has shown without doubt that climatic conditions in particular shape the genetic makeup (*e.g.*, MÁTYÁS 1996), while edaphic conditions are less effective evolutionary factors (*e.g.*, TEICH & HOLST 1974). Predominantly indigenous or autochthonous, extended stands should be selected as the basic material for seed collection. Genetic risk may be reduced if several seed stands of a similar ecoregion are identified and used for seed supply. Seed stands should be adapted to ecological conditions. Hence, the reproductive system of the seed stand should be intact, as indicated by a high number of female/male flowering trees, availability of pollen and seed vectors, and seed production. If the stand is not affected by heavy browsing, the presence of natural regeneration is a good overall indicator for a well-functioning reproductive system. Species composition, horizontal and vertical stand structure should not impair the reproductive system. An important and easy-to-use criterion for an appropriate seed stand is its size. As a general rule: the bigger the seed stand the better. Isolated and small forest stands should be avoided. There is still very little information on this matter. Based on effective pollen flow, a varied sizes of 12–20 ha (1,200–4,000 trees) were estimated exemplarily for oaks (KREMER & MENOZZI 2001). There is certainly no magic minimum number of trees in seed stands applicable for all species (see p. 413 ff., this volume). The sexual system (dioecy *vs.* bisexuality) and, closely related to this, the mating pattern affect the ability of a species to cope with different effective population sizes (scattered *vs.* naturally widespread species, *etc.*). Besides these biological causes, different practical requirements on the genetic diversity of the seeds by the forest management (*e.g.*, less genetic diversity needed in short-rotation plantation) also make general rules unrealistic. This must be kept in mind when, as a scientific guess for seed stands, sizes not smaller than 5 ha are recom-

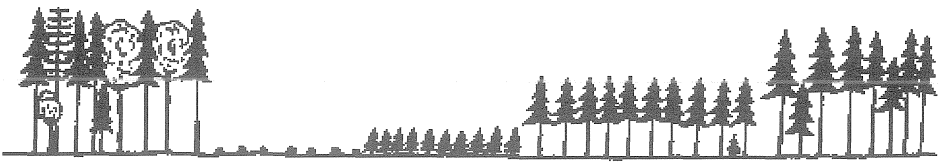

Figure 1. Front elevation of a clear-cut forest.

mended. Smaller sizes may be appropriate if pollen flow is desirable from neighbouring sources or bigger sizes are needed if pollen flow from adjacent stands of inferior quality is unwanted.

Selection of trees to be harvested

The number of harvested trees significantly affects the genetic diversity of the seeds. Theoretical and experimental studies, however, have shown that as a scientific guess at least 20 trees should be harvested (DEGEN & SCHOLZ 1996, HUSSENDÖRFER 1996, GEBUREK & MENGL 1998). Moreover, it must be clearly stated that there is no clear threshold. In addition, this number may also vary among different forest tree species. As already mentioned above, eventually the number of harvested trees is lower in naturally scattered species or in pioneers than in widely occurring forest tree species. Higher numbers are recommended in dioecious tree species such as *Populus* spp. Different aged cohorts (groups of trees with different age classes) within a formerly naturally regenerated stand may be the result of different ancestors, and/or different-size cohorts (groups of tree with different size classes) may be the result of natural viability selection or thinning (cf. HOSIUS 1993). Therefore, prolific seeders of different cohorts should be harvested in order to better mimic natural regeneration. Multi-stemmed trees or cohorts in formerly stooled stands as well as extended willow or popular stands comprising several hectares may be attributed to a single genet (*e.g.*, KEMPERMAN & BARNES 1976, CHANG *et al.* 1998). In apomictic tree species such as *Sorbus* spp. (JANKUN 1993) the number of trees harvested should also be increased. Within naturally regenerated stands, cohorts of related trees are to be expected due to limited seed dispersal. Heavy-fruited tree species such as *Quercus* spp. (GEBUREK & TRIPP-KNOWLES 1994, BERG & HAMRICK 1995) or *Fagus* spp. (*e.g.*, STARKE 1996, DOUNAVI 2000, HOSIUS *et al.* 2003) may be especially prone to this effect. To strengthen this view, it may be recalled that forked beech trees (HOSIUS *et al.* 2003) and trees with similar bud flushing (Th. Geburek, unpublished data) are found in clumps, indicating a non-random genetic pattern. Furthermore, single cohorts may originate from single seed catches of bird-dispersed tree species. In *Pinus albicaulis* Engelm., for instance, the multi-stemmed growth form is attributable to the seed dispersal by Clark's nutcracker (*Nucifraga columbiana* Wils.) (ROGERS *et al.* 1999) and it is very likely that a similar microspatial genetic pattern can be found in European bird-dispersed trees such as *Pinus cembra* L. Whenever seeds are collected in tree species that regenerate by natural or artificial vegetative means, special caution is advisable. It is recommended that seeds are harvested from (putatively) unrelated trees.

Practically, this can be achieved by sampling distant trees. As a rule of thumb, two or three tree heights may be a good guess. In *Robinia pseudoacacia*, typical distances between clones were from 90 to 190 m, but the range varied from several meters up to 300 m (CHANG *et al.* 1998). Furthermore, it may be added in this context that the genetic quality in mainly outcrossing forest species, *i.e.* the proportion of selfed seeds, may vary within different height or crown compartments (SHEN *et al.* 1981, OMI &

ADAMS 1986), although not always statistically significant differences were reported (BURCZYK *et al.* 1991).

Seed and plant processing

During seed storage, genetic changes are possible (ROOS 1980). Besides cytogenetic effects of seeds of forest trees (*e.g.*, VILLIERS 1974), which may be negligible in practice, genetic changes may be more relevant if the storage has different effects on the survival of certain families as shown in *Populus* spp. (GALLO 1991) and *Bertholletia excelsa* Humb. & Bondl. (KAINER *et al.* 1999). However, experimental data on selective seed storage effects are very limited for most European forest tree species.

Grading seed size may have selective effects. From field experiments established with seedlings derived from size-graded seeds, it is known that a higher growth during early juvenile stages can be expected with bigger seeds. However, this effect diminishes in conifers after 2–4 years and in broadleaves after 8–10 years (ROHMEDER & SCHÖNBACH 1959, *loc. cit.* p. 174). Similar observations are available for size-grade seedlings (ROHMEDER & SCHÖNBACH 1959, *loc. cit.* p. 171). It is tempting to speculate that smaller plants and smaller seeds resulting predominantly from consanguity and size grading would eliminate inbreds (SORENSEN & MILES 1974, CAMPBELL & SORENSEN 1984). However, in seed lots derived from sources along an elevational transect, a reduction of smaller grains (MÜLLER & SCHULTZE 1996) or smaller seedlings significantly reduced individuals that were well adapted to high elevations (HOLZER & MÜLLER 1985). Therefore, any size grading of forest reproductive material to be used in high-elevation afforestation is not recommended. It may be added in this context, that experimental evidence based on allozymes is controversial when plants are graded for height (MITTON & JEFFERS 1989, KONNERT & SCHMIDT 1996).

In forest trees, the reproductive pattern varies from year to year. Therefore, the genetic architecture of the seed crop harvested from the same seed stand cannot be identical, as seen in seed orchards (MÜLLER-STARCK *et al.* 1983) and in natural stands (EL-KASSABY 1993, KONNERT & BEHM 1999). To minimize these effects, different annual seed crops from single seed sources should be used.

Inbred seedlings are predominantly eliminated in a heterogeneous environment (MUONA *et al.* 1987). Therefore, homogeneous and favourable growing conditions in nurseries postpone selection processes after planting. In order to avoid this effect, seedlings may be grown in nurseries under sequential different environmental conditions.

To avoid negative selective effects, seedlings should be raised under climatic conditions that are similar to the future planting site. This should be taken into account when contract based cultivation is planned. For instance, surviving beech plants under more stressful environmental conditions in forests differed in their allele structures compared with the original seed lot, as well as with seedlings grown under less stressful nursery conditions (MÜLLER-STARCK & ZIEHE 1991).

Extraction of wild seedlings

In forest stands with a profusion of natural regeneration, wild seedlings are often manually extracted from a single patch for logistic reasons. Seedlings originating from this patch are often the progeny of a single maternal trees. In natural-regenerated forests, this patch ultimately provides only space for a very limited number of mature trees, which may share common ancestors. However, if large numbers were extracted from such a patch and were used exclusively as reproductive material planted from such patches and were planted, the genetic diversity of the afforestation would be reduced. Therefore, it is recommended that wild seedlings should be harvested from different patches.

Planting

Certain effects, previously mentio ned, may be later compensated if seedlings from different seed sources (different seed stands and/or seed harvests) are mixed. From a genetical point of view, a high number of seedlings should be used in order to provide a broad evolutionary basis (ZIEHE *et al.* 1995) (Fig. 2).

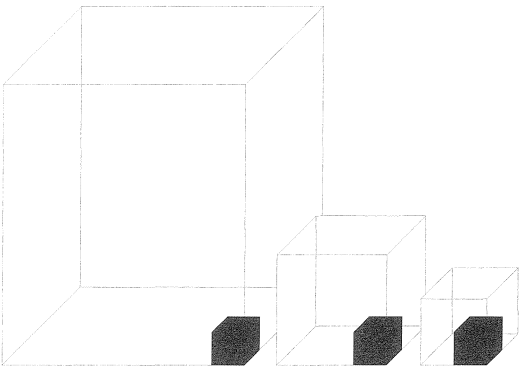

Figure 2. Initial and final population sizes in *Pinus sylvestris* stands established through natural regeneration, artificial seeding, and planting (from left to right). Cubes represent initial and final numbers of genotypes of the stands [from GEBUREK & THURNER (1993), © Cbl. ges. Forstw., reproduced with kind per-mission].

When sites are afforested with seedlings originating from a very small number of seed trees and forests suffer from biotic or abiotic impact, often inbreeding is referred to as the causal force by many forest practitioners. This is a common misunderstanding. A narrow genetic basis as such will not cause inbreeding depression. Inbreeding effects may occur only if related trees contribute to the next tree generation. Hence, the natural regeneration (filial generation) of a forest stand formerly established with such a genetic narrow basis will exhibit inbreeding depression at the earliest time. Of course, damages in the afforested stands may be due to the small genetic basis as such rather than to the co-ancestry of the reproductive material.

Artificial seeding

Acknowledging the guidelines for the seed collection and processing, artificial seeding will be a very effective means with regard to forest genetic conservation (Fig. 2). While seeding became out of fashion in the second part of the last century, European silviculturists attached great importance to this type of regeneration in the 19th century (cf. GAYER 1882, *loc. cit.* p. 287). In many cases, soil scarification with an excavator or by other technical means will be necessary to improve germination. While in nature most tree species shed their seeds in fall and many seeds provide food for animals often in surplus, artificial seeding is much more restricted in terms of numbers of seeds and, hence, should concentrate on spring or early summer.

Silvicultural practices relying on natural regeneration

Naturally occurring forest types

The natural regeneration in a managed forest mimics the reproduction of virgin forests. Without the knowledge of such natural self-regulating mechanisms it is difficult to assess whether a certain silvicultural practice is close-to-nature or not. Therefore, some of the typical natural European forest types are briefly described, following closely the definitions provided by LARSSON (2001).

Subalpine larch–stone pine forest

This type is maintained by frequent abiotic catastrophes (snow break, wind throw, avalanche, soil erosion) resulting in permanent regeneration. After a major disturbance (*e.g.*, avalanches) European larch (*Larix decidua* Mill.) naturally regenerates, building the pioneer stocking. Later, stone pine (*Pinus cembra*) trees will contribute to the regeneration that results in mixed conifer stands. During later development, stone pine will be the dominating species. Finally, on smaller patches, adult trees will collapse giving room again for larch regeneration on mineral soils or for stone pine regeneration on more mossy sites.

Subalpine and montane spruce forests

In subalpine elevations, the initial (pioneer) phase, which is dominated by *L. decidua* is succeeded by natural regeneration of Norway spruce (*Picea abies* [L.] Karst.), finally forming homogenous extended spruce forests. In this phase, small scale regeneration is permanent but scarce. Dead wood may play an important role in natural regeneration. Finally, the forests may be destroyed by abiotic catastrophes.

Boreal spruce forest

Snow-breaks, lethal frost and red fungi are the main disturbance factors resulting in small-scaled gap-dynamics with old trees, snags (standing dead trees) and dead wood. In the Scandinavian north, no large stand-replacing disturbances occur except for windfall events in the westernmost part. Extensive areas of fragmented old-growth forests are typical. Windfall and fire are the major natural disturbances towards the south-west and south-east, respectively. This type is dominated by Norway spruce (*P. abies*). Mixtures with Scots pine (*Pinus sylvestris L.*) and downy birch (*Betula pubescens* Ehrh.) are also common.

Boreal pine forest

Scots pine (*Pinus sylvestris*) dominates this boreal forest type. Sometimes downy birch (*Betula pubescens*) is found in the understorey. Natural regeneration is dependent on low-intensive fires that occur at short intervals. In Sweden, for instance, this forest type has burned with a mean fire interval of 46 years from approximately 1,100 A.D. to the present. In some areas, the mean fire interval is as short as 30 years, although the impact of fire has been greatly reduced in the last 100 years with fire suppression (ZACKRISSON 1980). While fire resistance of young Scots pine trees is rated as low, mature trees are better able to withstand fire. Tree density is generally low, which leads to ground fires causing great variation in size and age distribution within these forests.

Lowland and submontane beech forest

Apart from catastrophic wind throw, the main natural dynamics are caused by small gaps, but frequently these casual gaps will close, so that unevenly aged stands also show closed canopies and appear homogeneously structured. Due to the limited light supply resulting from the closed canopy created by common beech (*Fagus sylvatica* L.), the understorey is built up of very shade-tolerant species, such as English holly (*Ilex aquifolium* L.) or yew (*Taxus baccata* L.). Other tree species can only compete temporarily with the dominating beech trees. In its terminal phase, forests will disintegrate into small patches (cohorts) giving more light to the often already existing natural regeneration.

Mixed beech-fir-spruce forests

Large-scale disturbances do not occur in these mixed forests. Natural disturbances create small gaps in the order of magnitude of one-tree length in diameter. Locally regeneration is very similar to the selection forest. Common beech (*Fagus sylvatica*)

regenerates preferentially in patches, whereas Norway spruce (*Picea abies*) and silver-fir (*Abies alba*) are more likely to regenerate at the single specimen level.

Mixed oak-hornbeam forests

These mixed oak–hornbeam (*Quercus* spp. – *Carpinus betulus*) forests occur in regions with a sub-continental climate within the central European range of common beech (*Fagus sylvatica*), such as on the Upper Rhine plain, the rain shadows of the Harz, Rhön and Spessart, the Swabian-Franconian basin, the Bavarian plateau and Thuringia, the Austrian northern pre-Alps and the Vienna Woods, with the Polish central lowlands and adjacent hills of Silesia, Great Poland and Kujawy being dominated by oak (*Quercus* spp.) and associated with *Sorbus torminalis* (L.) Crantz, *Sorbus domestica* L. and *Acer campestre* L. Very little is known about natural regeneration. Presumably, small gap-dynamics are caused by tree-by-tree disturbances. Since oak trees may reach an age of 600 years, very few seedlings in these gaps are enough to sustain the existence of *Quercus* spp. over the very long life cycle. Regeneration of the secondary tree species is more spacious and continuous.

Mediterranean and submediterranean mixed oak forest

Very little is known about the natural regeneration in these forests due to the lack of remaining virgin forests. Wildfire, insect pests, fungal diseases and browsing are potential disturbances, but it is speculative how these forests naturally regenerated.

General genetic effects

In general, genetic effects may be summarized as follows: (1) short regeneration periods may cause incomplete transfer of the genetic information to the offspring, (2) certain natural regeneration systems reduce the effective number of reproductive trees, (3) filter effects combined with high pollen production in a dense stand may reduce the gene flow, (4) high-grading may reduce the population size, may reduce or modify genetic variation, and may influence evolutionary adaptability. And finally (5), one of the most important factors indirectly related to forest management is due to wild ungulate impact. The wildlife carrying capacity of many European forests has decreased over the years as a consequence of forest management. Furthermore, altered agricultural practices have led to higher game density in wooded areas. Besides other components, this development causes higher pressure on forests through tree browsing and bark stripping. WILDBURGER & LEBENITS (1996) described these effects for Austria, which are probably typical for many other European regions as well.

While, theoretically, virgin forests may provide the genetic baseline against which managed forests may be compared, we currently do not have sufficient data. So far, no essential differences in allozyme structures were found between six virgin forests, and

four natural stands of *Picea abies* populations in Slovakia (GÖMÖRY 1992) nor between 13 nature reserves and 110 naturally generated *Fagus sylvatica* stands in Germany (HUSSENDÖRFER & KONNERT 2000a). Certain forest management regimes change the population density, pollen and seed dispersal rates and dispersal mechanisms. However, these effects may be overestimated because different population densities did not considerably affect the pollen dispersal (see p. 188, this volume). To the best of the authors' knowledge there are no data available on whether natural sex ratios and demographically appropriate age-class structures are maintained in naturally regenerated forests.

Forest management systems

Although under several forest management systems, natural processes are mimicked, negative effects cannot be ruled out. In the following part, different systems relaying on natural regeneration will be described with special reference to genetic effects.

Forest management system: seed-tree stand (clear-cutting with reservation of standards) (Fig. 3)

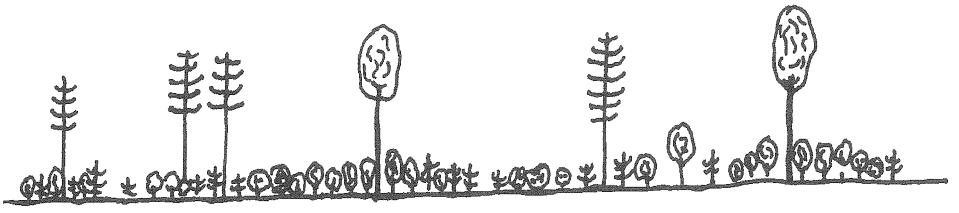

Figure 3. Front elevation of a seed-tree stand.

Characteristics: Usually, trees of seed-bearing age are evenly spaced to provide the seed supply required over the regeneration period. In central Europe, this method is mainly used for European larch (*Larix decidua*) and oaks (*Quercus* spp.), while in northern Europe, seed-tree stands are used as means of reforestation to a considerable degree. Although there may be visual similarities with shelterwood treatments, the difference is that the remaining crown cover provides an insignificant amount of shade following a clear-cut with seed trees. Managers must be aware that treatment failures usually result from inadequate numbers and fecundity of seed trees, or the failure to invest in required site preparation following harvest, and should modify their operations accordingly. The retention of residuals creates more structural diversity than that found in unmodified clear-cuts.

Genetic evaluation: Based on the relatively small number of adult trees, one may expect low estimates of genetic multiplicity or diversity in the next regeneration, mak-

ing seed-tree stands an inferior method from a genetical point of view. Among tree species, specific aspects, degree of isolation and size mainly determine its genetic usefulness. If the stand is well isolated from neighbouring pollen sources (< 1,500 m), the size should be as large as possible. Under less stringent isolation, seed trees catch considerable amounts of pollen from outside sources. In a small *Fagus sylvatica* stand with 71 seed trees/ha, genetic diversity in seeds was even higher than in the adult trees, indicating an effective pollen flux from other beech trees over a distance of at least 500 m (STARKE 1996). However, in a non-isolated *Pinus sylvestris* seed tree stand, YAZDANI *et al.* (1985) observed a small decrease in allelic richness harbored in the regenerated understorey compared with the seeders.

Forest management system: regular shelterwood (Figure 4)

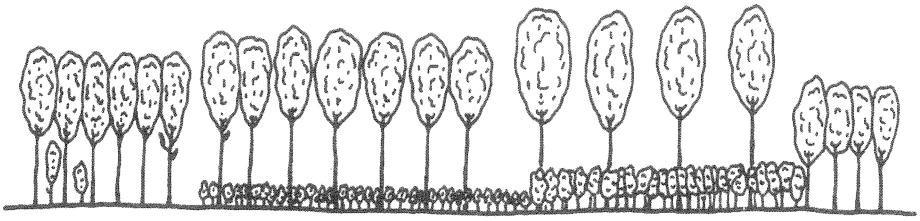

Figure 4. Front elevation of a regular beech shelterwood.

Characteristics: This silvicultural system is typical for pure beech forests, but it is also applicable for other species. Through simultaneous, regular and gradual opening of the canopy a natural regeneration is initiated. After a bumper crop, a seeding felling is performed extracting approximately 30 % of the adults. The emerging regeneration that results mainly from a single seed year is still sheltered by a canopy. Succeeding secondary fellings further open up the canopy over 10–15 years and finally all adult trees have been extracted. This system is also applicable for oak and pine forests. Of course, the higher light demand by oak and pine must be considered for the seeding felling and already approximately 40–50 % of the adults are removed in the first cut.

Genetic evaluation: The regeneration originates mainly from a single seed year. Therefore, it is likely that not all adult trees contribute to the next tree generation. However, this type mimics the dynamics of natural forest such as lowland and submontane beech forests (see above). Genetic differences between adult trees and natural regeneration are small, at least measured by genetic markers (*e.g.*, STARKE 1996).

Forest management system: strip shelterwood and wedge shelterwood (Fig. 5)

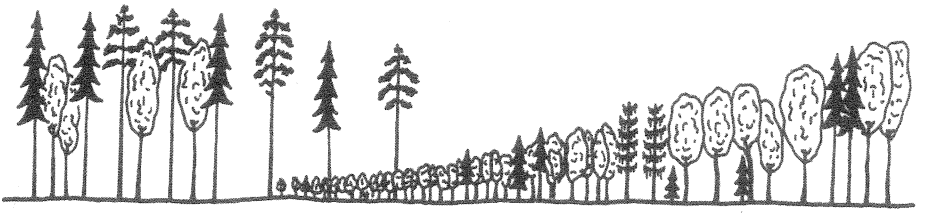

Figure 5. Front elevation of a strip shelterwood with different species.

Characteristics: Cutting starts at the edge of a forest stand. In a typical strip shelterwood, all trees are extracted along a strip. This type is not well suited for heavy-fruited trees such as *Fagus* spp. and *Quercus* spp. due to very limited seed flow. For linden (*Tilia* spp.), hornbeam (*Carpinus betulus*), ash (*Fraxinus* spp.), sycamore (*Acer* spp.) and fir (*Abies* spp.), a strip width of up to approximately 30 m is recommended, while this strip can be enlarged to 50 m for European larch (*Larix decidua*), pine (*Pinus* spp.), elm (*Ulmus* spp.) and alder (*Alnus* spp.). The optimal strip width also depends on the site conditions. A modification of the strip shelterwood system has cuttings that begin as narrow, interior, wedge-shaped strips with the apex into the prevailing wind, and are then successively enlarged and advanced. The regeneration is mainly natural and its interval is short. The young crop is fairly even-aged.

Genetic evaluation: Regeneration does not rely on a single seed year. Varying numbers of trees contribute their pollen and seeds to the regeneration. It is not likely that a major impact on the genetic structures – if any – exists through this system.

Forest management system: irregular (group) shelterwood (Fig. 6)

Characteristics: The irregular shelterwood results from group selection felling. Natural regeneration is initiated by irregular openings of the canopy. Gaps are enlarged by several peripheral fellings causing cone-shaped naturally regenerated cohorts. This system is mainly used for mixed forests [spruce, fir, beech, (pine)]. Although group selection and peripheral fellings are not limited in number or in time, normally the whole forest is regenerated after 20–40 years. Originally the irregular shelterwood system was used to naturally regenerate high forests. However, there are many modifications that partly form transitions to the selection forest.

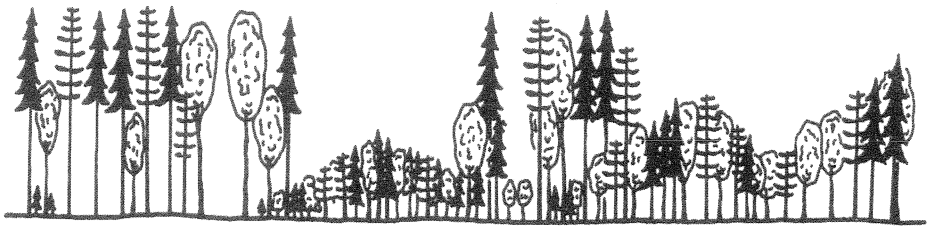

Figure 6. Front elevation of a group shelterwood with mixed forest tree species.

Genetic evaluation: Since this silvicultural system is very flexible it can take into account the seeding dynamics of the forest tree species. Relatively long regeneration cycles probably ensure that the genetic composition is not significantly altered among the parental trees and their offspring (*e.g.*, NEALE 1985).

Forest management system: selection forest ('Plenterwald') (Fig. 7)

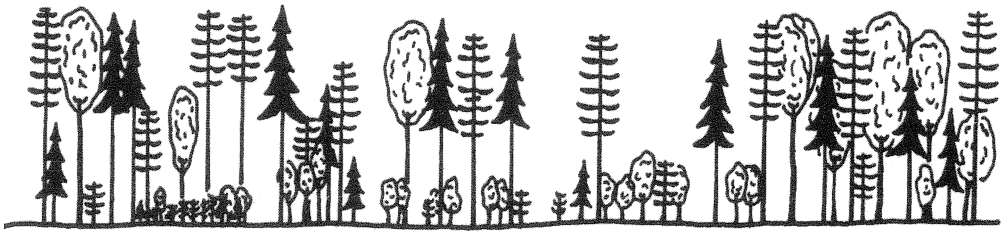

Figure 7. Front elevation of a selection forest.

Characteristics: The selection forest belongs to the 'Dauerwald' type. On more than 70 % of the area, several forest tree species (mainly silver fir, common beech, and Norway spruce) occur in a single-tree mixture. Improvement-tending, selective cuts or cuts to enhance natural regeneration are carried out annually or on a short-term basis. This management type relies heavily on the occurrence of shade tolerant silver fir.

Genetic evaluation: In many natural forest types such as mixed beech-fir-spruce forests (see above), selection forest like structures are temporarily typical. Since a relatively small number of trees belonging to a similar age or demographic cohort per area is characteristic, natural selection processes are less intensive, but persist over comparatively long times. The selection forest is the only silvicultural system for which natural regeneration is never interrupted on the whole area. It is plausible that this silvicultural system sustains genetic diversity for many mixed fir-spruce-(beech) forests at a very high level. However, it should be also stressed that a selection forest is not a natural forest but is man-made. It is likely that the selection intensity against fast growing seedlings is less intensive than in a regenerated stand established through

artificial seeding (or planting). In this context it is noteworthy that in an allozyme study, natural regeneration in a silver fir selection forest was characterized by fairly high positive fixation indices compared with natural regeneration in even-aged forests (HUSSENDÖRFER & KONNERT 2000b). This study did not indicate significant differences in genetic multiplicity between adult trees and their natural offspring.

Forest management systems: close-to-nature forestry – the concept of continuous cover forest ('Dauerwald')

Characteristics: In close-to-nature forestry, different aspects of several forest management regimes are combined mostly in an unconventional manner. Historically, close-to-nature forestry has its roots in the continuous cover forest ('Dauerwald'), which was firstly described by MÖLLER (1922) for *Pinus sylvestris* stands in northeastern Germany. It is not a silvicultural system in itself, but encompasses a range of silvicultural or management systems following the 'Dauerwald' principles (HELLIWELL 1997). Forests are generally uneven-aged. However, not all ages (or age-classes) must be present. There is no specific rule for wood extraction with the exception that clear-cuts are not allowed. The concept of a certain rotation time is not applicable. In order to balance the forest (species composition, demographic structure, *etc.*), game stocking must be appropriate. This aspect is very often ignored in many parts of Europe. Trees are individually selected and extracted annually. If artificial regeneration is needed to supplement natural regeneration, seeding instead of planting is preferred whenever possible. Hence, the 'Dauerwald' concept constitutes a proposal for a close-to-nature forest management, promising sustainable, productive, profitable, environmentally stable, biologically diverse, socially responsive forests, patterned after nature. Close-to-nature forestry has been recently mandated for the management of public forests in several central European countries and several silvicultural treatments used today implement this concept although with varied extent and intensity. In this context it may be added that a similar, if not to say identical, concept was developed by the Menominee Indians in the state of Wisconsin, U.S.A. (PECORE 1992). The 'Dauerwald' concept would be misinterpreted if one assumed that the result would be a natural or even virgin forest.

Genetic evaluation: The natural species concept, natural regeneration or the preferred seeding, and many seeders in a multi-layered stands with multiple demographic classes (age classes) are positive elements.

Conclusions

Forest management interferes to a varied extent with the objectives of genetic conservation. The even-aged plantation forests that rely on artificial regeneration often entail translocation of forest reproductive material. Although one can assume that this management probably has had the most important impact on natural forest genetic resources, all concomitants are difficult to assess (see p. 437 ff., this volume). The exclu-

cies composition and may have changed host-parasite interactions (*e.g.*, NAVEH 1974). Moreover, species composition was altered through the preference of even-aged forests, which are often dominated by conifers leading to unnaturally low proportions of certain deciduous tree species, in central Europe at least. Light-demanding, slow growing species have been especially driven to local extinction or at least have become very rare in conifer-dominated forests. Modifications of the natural genetic composition of forest tree species in man-made forests are inescapable although difficult or impossible to quantify.

Episodic disturbances, either natural or in the course of forest management, trigger the regeneration of forests (CRAWLEY 1990). Silvicultural management often mimics these natural processes as driving forces of regeneration. Hence, genetic processes specific to virgin or untouched forests are also unconsciously copied and a silviculture concept that relies on natural regeneration is conferring genetic diversity to the next tree generation, especially when regeneration cycles are long and trees of different ages or demographic groups are involved. Based on the experimental data available – even considering that most population genetic studies are based on non-adaptive genetic markers – close-to-nature silviculture probably offers the best guarantees for the conservation of genetic resources in managed forests. However, even highly artificial man-made forests can play a role in the conservation of genetic resources. In central-eastern and southern Europe, wooded land is often still synonymous with sprout forest (coppice or coppice with standards) and amounts to approximately 30 million ha, which equals 40.7 % of the forests[1] (Th. Geburek, R. Stephan and P. Bonfils, unpublished data). In more recent years, the conservation value of sprout forests has been recognized (BUCKLEY 1992, PETERKEN 1993) and many neglected coppiced woods have had their crop cycles reinstated, as proposed by SCHÜTZ & ROTACH (1993). From a genetical point of view, a coppiced forest is a clonal archive and genetic conservation is static. However, in sprout forests, competitive forest tree species can be grown in the overstorey, the density of which can be easily regulated. Thus, these ancient forest management systems can play also a major role in forest genetic conservation.

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[1] Data from the following countries only: Austria, Bulgaria, Croatia, France, Germany, Romania, Slovenia, Spain and Switzerland.

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