Ex situ conservation methods

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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.

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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 threatened *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 established 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 domestication 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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