Forest genetic resources conservation and management:

In plantations and genebanks *(ex situ)*

3

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This volume is one in a set of three guides to the conservation and management of forest genetic resources. These include:

Volume 1. Forest genetic resources conservation and management: Overview, concepts and some systematic approaches

Volume 2. Forest genetic resources conservation and management: In managed natural forests and protected areas (*in situ*)

Volume 3. Forest genetic resources conservation and management: In plantations and genebanks (*ex situ*)

The document has been prepared as a common effort between the Food and Agriculture Organization of the United Nations (FAO), the Danida Forest Tree Seed Centre (DFSC) and the International Plant Genetic Resources Institute (IPGRI), and draws on inputs of a great number of national, regional and international partner institutions throughout the world.

On 1 January 2004, Danida Forest Seed Centre (DFSC) became part of the Danish Centre for Forest, Landscape and Planning, KVL. The new centre, to be known as *Forest & Landscape Denmark* (FLD), is an independent centre for research, education, advice and information concerning forest, landscape and planning at the Royal Veterinary and Agricultural University (KVL). The development objective of FLD's international activities is to contribute to the increased welfare of present and coming generations, with particular emphasis on poor people, through improved planning, sustainable management and utilization of trees, forests, landscapes and other natural resources. The international activities are in part financed by Danish International Development Assistance. Contact: Forest & Landscape, Hørsholm, Kongevej 11, DK-2970, Hørsholm, Denmark.

The Food and Agriculture Organization of the United Nations (FAO) is the specialized UN agency in agriculture, forestry, fisheries and rural development. FAO provides information and technical support to member countries, covering all aspects of the conservation, sustainable use and management of forest genetic resources. Contact: FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy.

The International Plant Genetic Resources Institute (IPGRI) is an independent international scientific organization that seeks to advance the conservation and use of plant genetic diversity for the well-being of present and future generations. It is one of 15 Future Harvest Centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. IPGRI has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme and (3) the International Network for the Improvement of Banana and Plantain (INIBAP). Contact: IPGRI, via dei Tre Denari 472/a, 00057, Maccarese, Rome, Italy.

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While staff of all three main partner institutions have been involved in each of the chapters in the three volumes, taking full institutional responsibility for the contents, lead authors are shown against each of the chapters. These lead authors are responsible for the final contents, and provide focal points for those readers seeking additional information or clarification of topics discussed.

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Cover photo: *Dipterocarpus alatus* from Thailand. *Dipterocarpaceae*, which make up the major component of the South East Asian tropical rain forests, is an example of a family where recalcitrant seeds are a major constraint in cultivation and also circumvent *ex situ* storage of seeds. (Ida Theilade/FLD)

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Contents

PREFACE

Forests are the single most important repositories of terrestrial biological diversity. They provide a wide range of products and services to people throughout the world. Forest trees and other woody plants help support many other organisms, and have developed complex mechanisms to maintain high levels of genetic diversity. This genetic variation, both inter- and intraspecific, serves a number of fundamentally important purposes. It allows trees and shrubs to react against changes in the environment, including those brought about by pests, diseases and climatic change. It provides the building blocks for future evolution, selection and human use in breeding for a wide range of sites and uses. And, at different levels, it supports the aesthetic, ethical and spiritual values of humans.

Forest management for productive and protective purposes can and should be rendered compatible with conservation through sound planning and coordination of activities at national, local and ecoregional levels. Conservation of forest biological diversity, which includes forest genetic resources, is essential for sustaining the productive value of forests, and for maintaining the health and vitality of forest ecosystems and thereby maintaining their protective, environmental and cultural roles.

A major threat to forest ecosystems is the conversion of forest land to other uses. Increasing pressure from human populations who aspire to higher standards of living, without due concern for the sustainability of resource utilization underpinning such developments, raises concerns in this regard. It is inevitable that changes of land use will occur in the future, but such changes should be planned to help ensure that the complementary goals of conservation and development are achieved. This can be done by including concerns for conservation as a major component in land-use planning and resource management strategies.

Currently, the main problem in achieving conservation goals is the lack of adequate institutional and political frameworks that make it possible to consider choices about land-use and operational management that are fair to all stakeholders and can be efficiently implemented, monitored and regularly adjusted to meet new and emerging needs. Decisions on the conservation of forest genetic resources should be made not in isolation but as an integral component of national development plans and national conservation programmes.

The key to success therefore lies in the development of programmes that harmonize conservation and sustainable utilization of biological diversity and forest genetic resources within a mosaic of land-use options. Sustainability of action over time will be based on genuine efforts to meet the needs and aspirations of all interested parties. It will require close and continuing collaboration, dialogue and involvement of stakeholders in the planning and execution of related programmes.

In principle, there are no fundamental, insoluble technical obstacles to meeting conservation objectives. In recent years, a number of activities have been initiated to further conservation and the sustainable use of genetic resources. However, practical experience of these activities has been insufficiently documented, and the lessons learned have received little attention and have rarely been applied on a larger scale. The evidence of experience is that prudent and timely measures and programmes based on the best available knowledge can make a vital contribution to the conservation of forest genetic resources. It is therefore considered of the utmost importance that this experience, coupled with current knowledge of conservation theory, is made widely available in the form of generalized guidelines and procedures to serve as inspiration for others engaged in such conservation activities.

This guide is the third volume in a series of three that deal with the conservation of forest (trees and shrubs) genetic resources. This volume addresses technical requirements, and some applied approaches and experiences with the *ex situ* conservation and management of forest genetic resources. It outlines the role of *ex situ* conservation and reviews some of the strategies that may be employed: the managed development of *ex situ* populations, as well as storage methods, in the field and in seed banks, for *ex situ* genetic resources. With Volumes 1 and 2, and with other publications that are also addressing forest gene conservation issues at regional levels (such as ITTO 2000), we hope we have been able to provide the users of these manuals with an integrated view of the conservation approaches that can be used for the management of forest genetic resources around the world.

M. Hosny El-Lakany
Assistant Director-General
Forestry Department
FAO

Niels Ehlers Koch
Director
Forest & Landscape
Denmark

Emile Frison
Director General
IPGRI

INTEGRATED APPROACHES FOR *EX SITU* CONSERVATION AND USE OF FOREST GENETIC DIVERSITY

by Weber Amaral and Alvin Yanchuk

Ex situ conservation of forest tree genetic resources is mainly concerned with sampling and maintaining as much of the genetic variation as possible that resides within and among populations of selected target species. *Ex situ* conservation requires substantial levels of human intervention, in the form either of simple seed collections, storage and field plantings or of more intensive plant breeding and improvement approaches. Unlike breeders of agricultural crops, forest tree breeders cannot rapidly produce new varieties, nor can they quickly breed for new variations among populations. Therefore, the existing genetic diversity among populations is important and fundamental to the conservation of forest genetic resources, particularly as it may relate to maintaining genetic diversity in viable populations in the long term. This also suggests that special attention must be given to conserving intraspecific genetic variation in peripheral or isolated populations, as they could possess higher levels of characteristics such as drought resistance, tolerance to various soil conditions (Sterne and Roche 1974), or features that will help to protect them from future climate change (Muller-Starck and Schubert 2001).

As discussed in Vols. 1 and 2, a comprehensive genetic conservation programme will ultimately require some combination of *in situ* and *ex situ* conservation. However, there are many situations where *ex situ* conservation becomes the focal point of a gene conservation programme, and this volume addresses these objectives, issues and approaches. It is a critical component of many of the gene conservation plans and networks in most crop species, but can also have an important role with forest trees. The important features of an *ex situ* conservation programme for any particular species are:

- to be an important backup measure should other *in situ* conservation means be unworkable or unavailable
- to ensure that a wide range of the diversity (phenotypic and genotypic) available in a species is conserved
- to manage the regeneration of the species outside its original natural range **(provenance)** in a more controlled way (which is likely to further develop the population(s) for use or conservation).

As with all gene conservation efforts, available resources play a key role in determining which particular strategy can be developed for any particular *ex situ* gene conservation programme. With lack of sufficient technology and resources to assess the array of genetic variation available within or among populations, most often the *ex situ* collections are based on collections representing populations from different ecological zones or areas, and individuals within these populations which may exhibit typical or special phenotypic variations in morphological traits. Although it is desirable to sample populations with genetic knowledge of a particular species, many of the principles in genetic sampling are robust enough that it is still possible to do a reasonable job of capturing genetic diversity without such knowledge. Furthermore, the various types of tissues or seeds that can be collected, and then stored, archived or outplanted, play an important role in the ability to maintain and conserve genetic diversity *ex situ*.

In Chap. 2, aspects of static versus dynamic *ex situ* conservation are further explored (see Vol. 1, Chap. 2.4), and in Chap. 3 important aspects of sampling populations are discussed. The development of populations through various means traditionally viewed as 'tree improvement' is addressed in Chap. 4, which expands our previous discussion on aspects of 'dynamic' genetic conservation. In Chap. 5 some important principles and experiences are presented which have the specific objective of conserving genetic resources in *ex situ* plantations, and Chap. 6 examines many of the approaches for *ex situ* conservation in various storage facilities, treatments and use.

Chapter 2

METHODOLOGIES FOR *EX SITU* CONSERVATION

by Weber Amaral, Alvin Yanchuk and Erik Kjær

2.1 Introduction

Decisions on which strategies and methods of *ex situ* conservation to apply for a particular species will depend not only on its biological characteristics, patterns of genetic variation and present conservation status, but also on how much is known about its basic silviculture and management. *Ex situ* conservation must also consider which part of the plant will be sampled and conserved—whole trees, seed, tissues or genetic material in culture—and for how long. Perhaps even more important is the institutional capacity of the organizations concerned, and the availability of medium- and long-term funding to carry out successful *ex situ* conservation.

2.2 Which issues need to be considered?

In Box 2.1 we illustrate and characterize various levels of the genetic hierarchy that can be considered, and some options available for each. It must be emphasized, however, that it is difficult to develop a general set of guidelines that can be applied to all situations. In order to develop an efficient *ex situ* conservation strategy, a number of key questions regarding the conservation objective, the origin of the material to be conserved, the present use and the conservation status have to be addressed.

Box 2.1 Levels of the genetic hierarchy and their relation to *ex situ* conservation

- **Genes:** Important functional genes—for disease resistance, for example—could be mapped using molecular markers (Harkins *et al.* 1998). With new molecular technologies, the gene could be sequenced to examine the possible molecular basis of the resistance, and even cloned and introduced in the same or other species. However, conservation of such genes typically means conserving the actual genotypes in which they occurred.
- **Genotypes:** These are individuals that appear to be representative of the population, or for some trait of interest (either phenotypically or from clonal replicates). In order to maintain the genotype, material must be captured by vegetative reproduction. Collection of seed from an individual does not generally preserve the genotype of that individual.
- **Populations (provenances):** Although we are usually interested in conserving genetic variation which is representative of populations, we are ultimately required to sample a sufficient number of individuals that represent the gene

continued

frequencies in that population. The question then arises of how to allocate sampling resources such as numbers of trees, type of tissue, e.g. seed, cost of sampling, within and among populations.

- **Ecosystems:** We cannot measure the genetic variation of an ecosystem, because intraspecific genetic variation is the property of a species, and representative populations may transcend several different types of ecosystems (dry or moist, warm or cool, assemblages and competition with different species, etc.). The only way we can say we are 'sampling ecosystems' for genetic variation is to sample populations, in an attempt to capture genetic variation that has developed among populations as a result of historical colonization events, different selection pressures, or gene flow.

2.2.1 Objectives of conservation

When defining the objectives of *ex situ* collections or a conservation programme, the first step is to set some approximate targets for sampling or capturing genetic diversity. In other words, are we aiming to conserve a representative local sample of a species, a representative sample of geographic or provenance variation, representative genetic variation of a provenance or a stand, or simply a single genotype? (Genetic issues relating to sample sizes are discussed in more detail in Chap. 3.) The second step is to determine what may be the future use of the material. Is it to be included in tree breeding activities, directly into planting programmes, or for rehabilitation of the species in the natural habitat? In order to be effective, *ex situ* conservation will require continuous input in terms of funds and personnel. Experience has generally shown that such long-term commitment will exist only if objectives have been clearly defined and if there is some interest in utilizing the species or provenances in question in the future. If such interest does not exist, the efforts may be seen as conservation for its own sake with little long-term value, and programmes that depend totally on external funding are likely to be abandoned (Theilade *et al*. 2001).

2.2.2 Is the species presently being planted or not?

The next factor to consider is whether the material is presently used for planting or reforestation. Most valuable tropical climax species are presently not targeted for planting programmes. This group of species is particularly difficult to conserve *ex situ*, since little may be known about planting and silvicultural management. In most cases there is likely to be little available information on their biology, regeneration, seed behaviour (for example longevity on the tree, collection and storage techniques), pollination, pollinators, etc. Obviously, for species where little management information is available, *in situ* conservation may be the only realistic option. However, if this is not possible, because of threats to the natural habitat or limited control over management of the *in situ* populations, then conservation *ex situ*, particularly in **gene conservation stands** that mimic the natural conditions, may offer the best form of *ex situ* conservation (also see Chap. 5 in this volume and Chap. 2.4 in Vol. 1). Different tools to mimic natural conditions include planting of nurse crops, planting of multiple-species stands, and steps to support future natural regeneration. Since it is unlikely that much information will exist for many of these species, such stands will often be highly experimental. This represents a challenge to the conservation forester, but if the stands are designed properly, they may yield valuable information about the performance of the species or provenance and more generally on how to manage, utilize or perhaps rehabilitate the species in the future.

2.2.3 Evolutionary versus static conservation

The question of whether or not to protect genetic processes is important when developing an appropriate strategy for *ex situ* conservation. As previously described in Vol. 1 (see Chap. 2.4), genetic processes include all processes that lead to changes in gene frequencies, for example natural selection (adaptation to the prevailing environment) and intended or unintended selection in response to management practices. The conservation officer will have to decide whether the conservation strategy will aim to maintain gene frequencies of the original population and thus avoid any effects of genetic processes—**static conservation**, or whether the focus should be on supporting current adaptation and allowing gene frequencies to change according to local and relevant selective forces—**evolutionary conservation** (Guldager 1975).

Evolutionary conservation

Conservation of the species within its natural habitat (*in situ* conservation) is the most typical example of evolutionary or 'dynamic' conservation, but it ultimately attempts to preserve the adaptation potential of the species over the long term. Evolutionary conservation can, however, also take place *ex situ* in planted stands, where selective forces are allowed to work and where the planted trees can be regenerated from seeds rather than from vegetative propagation at generation turnover. Evolutionary conservation methods can vary but typically fall into two general categories:

(a) situations where the species, provenance or families can and will undergo some selection to current and future environments in their test locations (rather than focusing on conservation of specific genotypes)

(b) situations where the conservation planner deliberately wants to conserve characteristics of importance for the use of the species, such as straight stems and high growth potential.

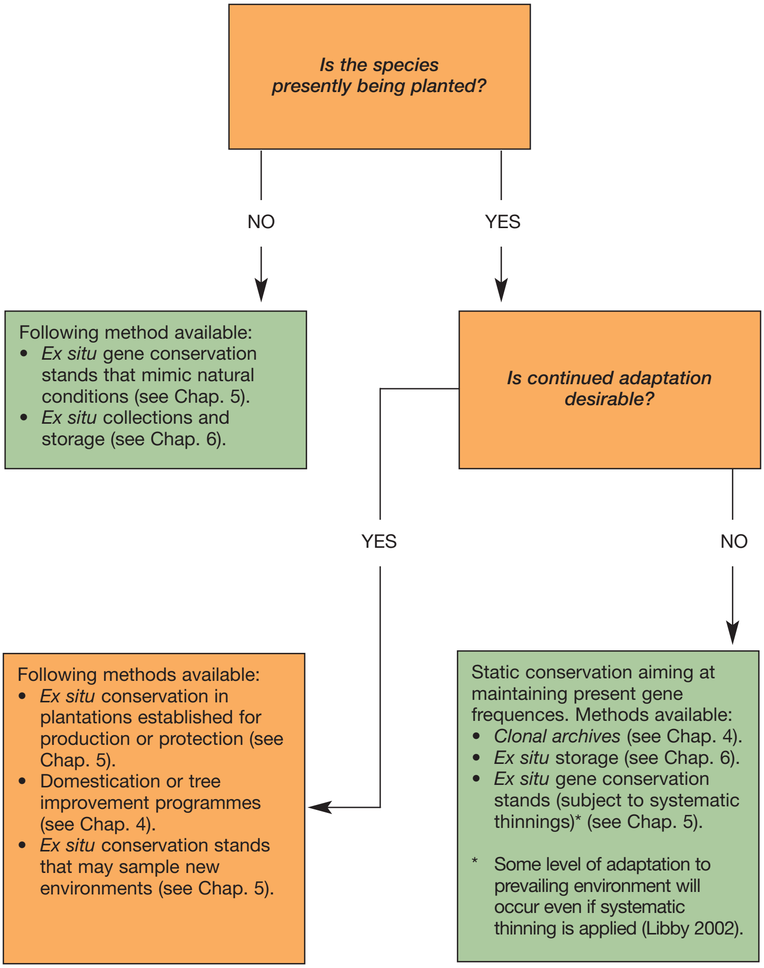
Method (a) can be referred to as **strict evolutionary conservation**, with the aim of facilitating and supporting natural selection for fitness. The method mentioned above where conservation *ex situ* is carried out in stands that mimic the natural condition of the species is an example of strict evolutionary conservation. Method (b) can be referred to as **evolutionary conservation in use**, and can be obtained in conservation stands growing under conditions similar to managed plantings. In general, the most efficient *ex situ* conservation is obtained by combining the conservation activity with active utilization of the species (see Chap. 4).

Existing planting programmes could serve as valuable vehicles for *ex situ* conservation, as long as there is strict control and detailed knowledge of plantation origins (for example seed sources, effective population sizes, etc.). Tree improvement programmes can serve as efficient conservation programmes as well, particularly those with large provenance and family testing components (see Chap. 4). Finally, in cases where a species is considered valuable, but is not at present widely used, domestication and increased use of the species may improve its conservation status (see for example Vol. 1, Box 2.7 on *Prunus africana*). When species or provenances are conserved through controlled use, this should be across multiple sites representing a range of different conditions. This is to ensure that genotype x environment interaction does not cause decreases in genetic potential for future use in different environments (Guldager 1975). Different approaches in traditional tree improvement programmes that consider highly directed evolutionary conservation are discussed in more detail in Chap. 4.

Static conservation

Static conservation (previously addressed in Vol. 1, Chap. 2.4) is characterized by the fact that a group of genotypes are typically the targets of conservation. This approach is most relevant when the objective is to conserve identified and tested genotypes of proven value, or when it is not possible to establish *ex situ* plantations with evolutionary conservation options.

For the maintenance of genotypes, vegetatively propagated 'clones' can be kept and protected in clone banks or clonal archives. For some species static conservation can also be applied by conserving seed lots in seed banks or by cryopreservation. These methods are described in more detail in Chap. 6. Finally, some degree of static conservation may also be obtained in conservation stands based on seedlings where management aims to eliminate effects of local 'natural selection', for instance by applying systematic thinning. It must be emphasized, however, that some degree of adaptation to local conditions will always occur, especially in connection with generation turnover, and because only individuals reasonably well adapted to the prevailing environment will survive. A general approach for making decisions when developing specific criteria on *ex situ* conservation is presented in Figure 2.1.



2.2.4 Conservation status

The current status of the species or provenance of interest needs to be considered, as it will affect the possibilities and approaches used for sampling (see Chap. 3). If the species or provenance is in immediate danger of extinction, and only a few reproductively fit individuals remain, then immediate action is the most important criterion almost irrespective of the conservation methodology. In this case, it is likely that all remaining material should be sampled, but the methods to apply for critically endangered species will depend on the status and the biology of the species—the selected conservation measure should be established quickly, in order to avoid further loss of genetic diversity.

In many cases, a combination of *ex situ* storage and establishment in stands will offer the most secure conservation of highly endangered species or provenances. In many situations it is likely that flowering and seed production will be limited in highly threatened species or provenances. In these cases, establishment of conservation stands based on vegetatively propagated material may be the only feasible option. By bringing the remaining material together in one or more stands, subsequent outcrossing of individuals and seed production may be secured.

2.2.5 Institutional capacity

In developing the conservation strategy, the capacity of the organizations involved should be considered carefully. The very nature of the activities involved with *ex situ* conservation—germplasm collections, storage, propagation, seed and seedling handling, nurseries, planting, establishment of secure field tests, clone banks, reserve plantations—makes it important for institutional capabilities to be in place over a long period of time. *Ex situ* stands depend on the continued survival and efficiency not only of their host institutions, but also of appropriate specialized staff who establish them and may not be present throughout the entire time span during which *ex situ* stands must be maintained. Although these stands are often of great value, if they are not used in some way, they may come to be viewed as a drain on the limited resources of the institutions where they are housed (Raven 1981).

As mentioned earlier, many uncertainties of continued institutional commitment can be mitigated if *ex situ* conservation is closely linked to use. *Ex situ* programmes are typically successful if seed harvest or production of other reproductive material is economical, or if *ex situ* conservation is integrated into active breeding and/or research programmes. In any case, continued institutional capability remains one of the main criteria for *ex situ* conservation and is an aspect to be seriously confronted at an early stage in planning.

Where institutional capabilities are weak, it may be desirable to pursue national and international coordination with other government and research institutions (see Chap. 5). It will probably be necessary to develop an integrated *ex situ* conservation strategy for a species that may cross national or state borders, as species distribution and therefore genetic variations do not consider political borders. Institutional aspects and regional coordination are discussed in more detail in Vol. 1, Chaps. 4 and 5.

SAMPLING STRATEGIES FOR *EX SITU* CONSERVATION

by Alvin Yanchuk and Søren Hald

3.1 Introduction

In principle, the information requirements for efficient sampling schemes for *ex situ* conservation are much the same as those for *in situ* conservation programmes (see Vol. 2, Chap. 2). However, as we shall see, the physical and financial limitations of most *ex situ* conservation methods will determine what type and amount of genetic variation can be sampled and therefore conserved. Although it is always desirable to capture as much genetic variation as possible, the total number of trees sampled must be manageable.

A representative sample of trees from each stand must be determined, then sampled, in order to adequately represent the population; however, the number of populations from which to sample is also important, and perhaps more important, as briefly mentioned in Chap. 1. Therefore, genetic conservation by *ex situ* methods requires some consideration of how to allocate sampling efforts among and within populations, or even from individual trees (numbers of seeds per tree, for example).

Priorities for which stands and which trees should be sampled need to be based on information about the likely genetic differences or potential value of the material (for example outlying populations, or populations that are known to have special attributes) and the level of risk to populations, as well as the ability to actually collect germplasm and store it. As previously mentioned, it may be the case that the threats to the remaining gene pool are so great that the decisions become relatively easy to make: all remaining material ought to be sampled and represented.

3.2 The population concept for *ex situ* conservation

Geographic representation of tree populations, or tree provenance, is a central concept that needs to be considered when designing sampling strategies for *ex situ* conservation. **Ecotypic variation** (specific habitat or adaptations to specific environments) among populations, and differentiation, may manifest distinctive genetic differences in many attributes, given enough time. **Clinal** variation is expected when genetic difference among populations is more gradual, reflecting less selective pressures over the species' distribution. Regardless of the pattern of variation in the species, these are common considerations in setting out sampling objectives, as ecological representative sampling of provenances can be an important component for future links between *in situ* and *ex situ* conservation in the field; namely, the potential to restore local forest tree populations after a loss or a major disturbance (reforestation efforts should use the local provenances, if they are available). Of course, *ex situ* collections are also made in relation to the development of future genetic testing and breeding (see Chap. 4).

Collecting strategies should therefore recognize the centrality of the provenance as the unit of genetic resources (Brown and Hardner 2000). Although the concept of provenance does not have a precise definition (exact boundaries of interbreeding populations are difficult to define in the presence of what is typically a genetic continuum), it has important practical implications and should be considered in documenting collections of seed and vegetative material.

Barner (1975) suggested the following characteristics that describe a provenance:

- It can be defined by means of physical or geographic boundaries, which can be identified in the field.
- It is composed of a community of potentially interbreeding trees (probably differing in genetic constitution from other provenances, by distance, physiographic separation or ecological habitat).
- It is sufficiently large to allow the collection of enough reproductive material to represent what we usually consider as a population (enough individuals, with enough material available from each tree).

In summary, the provenance concept helps meet the objectives of sampling plant gene pools for *ex situ* conservation by partitioning sampling among populations, to optimize the amount of genetic variation while keeping the number and size of samples within practical limits.

3.3 Sample sizes in *ex situ* collections

There are many estimation methods, and therefore a wide range of recommendations for the sample size required to conserve some level of genetic variation. Values in the literature range from 50 to 5 000, but the number depends upon the biology, the current genetic structure of the species, and many other factors related to the conservation objectives. Below are some examples of issues and sampling approaches that may be useful in gene conservation sampling:

- It is not particularly useful to suggest that genetic variation 'will' or 'will not' be conserved, or likewise, 'adequately' or 'not adequately' sampled—the sampling approaches used must be related to the genetic conservation objectives. With all sampling methods, there will be diminishing returns with increasing sample sizes, but we can approximate what proportions or types of genetic variations are expected to be captured. What may at present be more important in *ex situ* conservation is the ability of the conservation officer to quantify what is being operationally and practically conserved in terms of genetic variation.
- As noted in Vol. 2, *in situ* gene conservation focuses largely on (1) conserving genes at low frequencies that may be of some value in the future, and (2) conserving large enough population sizes to maintain a balance between mutation and genetic drift for quantitative (adaptive) genetic variation. Hence, *in situ* populations must be relatively large in order to continue to evolve under current and future environmental pressures; target numbers range from 1 000 to 5 000 depending upon many demographic factors (Lynch 1996) (see Vol. 1, Chap. 6). As this relates to *ex situ* conservation sampling, we can see that substantial collections would have to be made and stored in order to meet some of the objectives of *in situ* conservation. However, some species have biological characteristics that make such collections possible, such as some conifer species with large open-pollinated seed crops. Nevertheless, one should distinguish or document whether the *ex situ* sampling objectives are to conserve genes at lower frequencies (along with adaptive genetic variation), or focus exclusively on adaptive genetic variation.
- Brown and Hardner (2000) suggest that the region or area where the species occurs should be divided up into distinct ecogeographical zones and a limited number of individuals (around 50 per population) be collected at random (and, if possible, ideally from about 50 populations). This sampling frequency is considered sufficient to reach the minimum benchmark of 59 unrelated gametes, which allows the inclusion of at least one copy of 95% of the alleles that occur in the target population with frequency

>0.05. (For species with completely random mating this target equates to about 30 individuals, whereas in a completely selfing species it requires 60 individuals—for convenience we can round this to 50 random unrelated individuals, to allow for some of these variances in breeding systems among plants.) Collecting samples from around 50 sites will ensure the sampling of at least one copy of all common alleles (P > 0.05) that occur in more than 5% of populations, with about 90% probability.

- The well-known formula $1 - [1/(2N_e)]x100$, where N_e is the effective population size of the sample, or the number of trees sampled if they can be assumed to be unrelated (Lande and Barrowclough 1987), predicts the approximate proportion of heterozygosity or additive genetic variation captured in the sample (depicted in Figure 3.1). Once again, this type of adaptive (additive) genetic variation is the most important variation for genetic improvement in growth and evolutionary adaptive characteristics. It is apparent, then, that only a few dozen genotypes are required to capture a good percentage of the important genetic variation in the population.

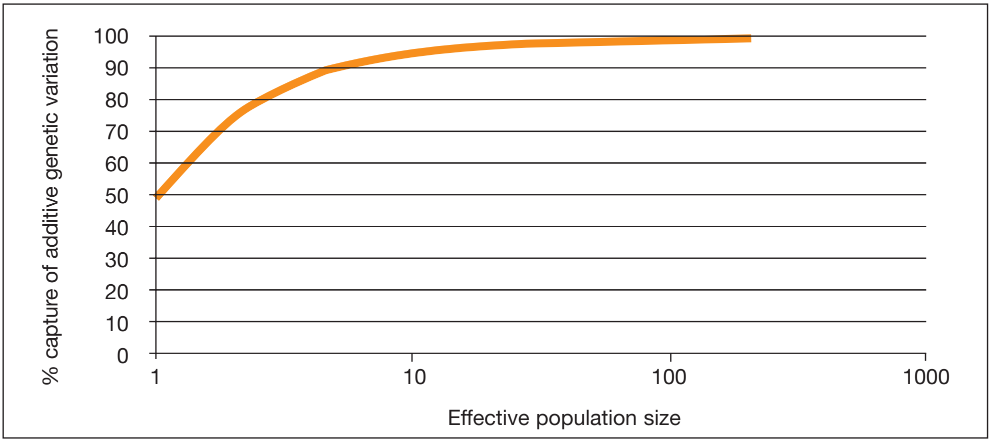


Fig. 3.1. *Relationship between population size and percentage of additive genetic variation conserved (heterozygosity) in diploid organisms, based on the approximation of* $1 - [1/(2N_e)]x100$

Note that the approximation of capture of quantitative genetic variation shown in the graph is for diploid plants. If the species or population is known to be polyploid, then this approximation is likely to be conservative, and the higher the ploidy level the more conservative it becomes.
For instance, if a conservation officer could collect from or capture 20–50 unrelated individuals (through vegetative propagation, for example), from the pattern shown in the graph in Figure 3.1 we would expect to have lost only 2.5% to 1% of the additive genetic variation in that population. However, a population of 50 individuals will have only a few copies (perhaps 5) of dominant alleles originally at frequencies of 10% in the population (Yanchuk 2001a).

- Loss of individual genotypes over time in both *in situ* and *ex situ* conservation programmes also needs to be considered. For instance, if an *ex situ* plantation is established with 1 000 trees per hectare, it is expected that the number of surviving adult plants will decrease over time (perhaps from 1 000 seedlings to 400 mature trees) as a result of competition or other factors. The number of surviving trees might need to be considered, and increased, in the planning and documentation of conservation targets over time, and ultimately determine how successful conservation may be. Similarly, in *ex situ* seed storage arrangements, there may be loss due to differential storage or longevity of seed accessions (seeds collected from different trees, for example), so initial values may need to be higher to allow for such losses. However, it is important to note that the loss in genetic variation in going from something like 1000 to 400 genotypes is very small (see Fig. 3.1).

These 'discrepancies' in sampling numbers between what *ex situ* conservation collections can typically provide and what *in situ* conservation can provide are essentially local issues that will be determined mainly by the conservation objectives, and the institutional and funding capabilities.

For some of the major commercial species, *ex situ* collections of thousands of samples can be made and are considered an integral part of the gene conservation strategy. In *Pinus radiata*, around 2 000 trees per population have been established as part of the strategy (see Box 3.1). For loblolly pine (*Pinus taeda*), in the Western Gulf Tree Improvement Cooperative, approximately 3000 first-generation selections are being conserved indefinitely in clone banks (Byram *et al*. 1998), as they represent a large investment in selecting parent trees, and valuable test information has been accumulated over time. In smaller breeding programmes with poorer institutional capabilities, fewer trees can be maintained and yet still conserve high levels of genetic variation; nevertheless, it is important to attempt to document what levels of gene conservation may be maintained by the sampling and conservation programme.

Box 3.1 *Ex situ* conservation of *Pinus radiata*

Plantation managers and researchers from Australia and New Zealand have long known that *in situ* conservation of the small native stands of *Pinus radiata* in California and Mexico would be desirable. However, these isolated populations were also well known to be under threat from increasing urbanization, grazing and disease. A workshop was convened in 1998 to discuss alternatives for *ex situ* conservation of these valuable genetic resources. Genes of intermediate and high frequency (therefore, most of the quantitative genetic variation of interest) are expected to be well conserved in breeding and research programmes throughout Australia and New Zealand, so the challenge was to develop a programme to conserve low-frequency genes in a feasible *ex situ* programme. Outcomes of the workshop were:

- Effective population sizes of 2 000 would be the target conservation size for each of the threatened populations. This should maintain alleles at a current frequency of 0.01 in the population.
- The rotation of population blocks that exist in Australia and New Zealand would be prolonged for as long as possible, and seed collected before felling.
- Existing population blocks of less than 20 ha should be replicated where possible, and new 20-ha blocks established when needed.
- Populations other than Cedros Island are largely secure, particularly mainland populations, which were planted in August 2004. Cedros Island seed was sown in 2003, but poor germination will likely entail that the few additional seed lots

continued

R. Burdon and C. Low (Forest Research Institute, Rotorua, New Zealand), in front of an ex situ *conservation stand of* Pinus radiata*. (Deborah Rogers/UC Davis)*

that are available will also have to be sown (K. Eldridge, *pers. comm.*).

Additional details, such as pollen contamination and costs of alternative seed production systems, were evaluated and further research identified. The cost of controlled pollination for conservation purposes was seen by industry participants as being too high to be practical, so open pollination will be used. In this case, contamination of the gene pools of populations in the conservation blocks by surrounding pollen becomes an important issue. It is hoped to manage conservation blocks with the surrounding plantations so that relatively less-contaminated seed may be collected up to 7 years after the harvest of surrounding plantations. *P. radiata* is one of the most important exotic planted species in the world, and it is probably worthy of such conservation efforts. Because of its extreme importance to Australia, New Zealand and Chile, the institutional capabilities are likely to be in place now and in the future to carry the necessary efforts effectively. For other species where *ex situ* conservation is also necessary but less support may be available, good conservation measures could still be put into place using the example of *P. radiata*.

Summarized from Matheson *et al*. (1999)

For many of the main commercial species, such as *P. radiata* or *P. taeda*, the conservation objectives are somewhat broader and easier to accomplish because of the economic support, the biology of pines and the amount of genetic information available for the species. However, for many tropical or semi-arid species that are under human pressure, only a few dozen populations or even individuals are available and conservation measures by *ex situ* collections are likely to be critical for the future of the species (see Box 3.2).

All of the above discussion on methods and sampling strategies assumes that genes and genetic variation are a static resource. For example, we often consider them as 'coloured marbles in a jar' and we are interested in capturing a representative sample of 'all colours from the jar'. As discussed in Vol. 1, genes and genetic variation are not truly static and can be created and altered by many factors such as mutation and recombination. Rasmussen and Phillips (1997) further discuss the various mechanisms that can alter genetic variation *de novo*. In developing gene conservation strategies we are obliged to use rather straightforward sampling approaches, based on assumptions of genetic variation being to some extent static, even if we realize that this is rather a simplistic notion.

3.4 Sampling among different populations

As mentioned in Box 2.1, ultimately we must sample individuals in order to survey or capture genetic variation; however, how we spread the sampling of individuals across the range of the species of interest—as a hierarchy of ecosystems, populations within ecosystems, or

Box 3.2 Some species for which *ex situ* conservation is considered the only option

Vateriopsis seychellarum
This dipterocarp species is found in the Seychelles. The species produces a high-quality timber, which makes it an interesting candidate for reforestation programmes. Today the species is confined to a few very small populations on Mahé. Populations on the neighbouring islands of Praslin and Silhouette are now extinct, probably because of early deforestation. The remaining small populations on Mahé are under pressure from increasing agriculture, invasive plants and possibly the absence of dispersal agents. However, the capacity of the species to regenerate appears to be good and a conservation programme involving planting of seedlings on other sites in the Seychelles is being initiated.

Cupressus dupreziana
This species is found in an area of 200 km^2 on the Edehi plateau in eastern Algeria, where it is in immediate danger of disappearing completely. According to studies of fossil pollen the species, which shows promising potential for afforestation in arid regions, was once widespread in the Sahara. A survey has reported that presently only 153 living specimens are left (FAO 1986). All remaining trees are old (the oldest being at least 2 000 years) and all regeneration is destroyed by grazing animals. In addition, it is believed that the water table has sunk to such an extent that regeneration is impeded. The species has been successfully regenerated in botanical gardens and, since there seems little prospect for conserving the remaining natural population, *ex situ* conservation is considered the only option.

Source: WCMC Tree Conservation Database

Monopetalanthus hedinii
This leguminous species is found in Cameroon, where it is reported to be the most endangered member of the genus. It is closely related to *Monopetalum heitzii* but appears to grow to larger proportions with relatively high growth rate. There is considerable interest in the species as a potential plantation tree. The species is recorded from small localities at Muyuka, Eseka, Kribi, Maleke and Lolodorf. It is under pressure from agriculture and may already be extinct from some of the sites above.

Source: Gérard *et al*. (1998)

representative types and numbers of individuals within each population—is an important consideration in efficient testing. This has been also discussed in Vol. 1, Sections 3.6, 3.7, with respect to defining priorities of representative samples from ecosystems and populations within an ecosystem, if the species is distributed as such. In Vol. 2, Section 2.2, the ecological zonation for *Pinus merkusii* was presented as another example of how stratification among populations, based on ecological areas, can be used. Also discussed previously (see Vol. 1, Chap. 6), is how the genetic variation within and among populations can be helpful in designing a sampling approach on the ground. Box 3.3 describes how molecular markers can be used to increase the design of sampling within and even among populations, to capture similar levels of genetic diversity from core and peripheral populations (i.e. stands on the edge of the distribution) in *Picea sitchensis* (Sitka spruce).

As shown in Box 3.3, if multiple populations can be collected for *ex situ* purposes, it is important to consider the characteristics of the stands from which the collection can be made. In summary:

- Are the collections being made from a widely dispersed species?
- Are individuals of the species growing in patches, and would these patches be likely to contain related individuals?

Box 3.3 Sampling and design for *ex situ* and *in situ* gene conservation in *Picea sitchensis*

Plant species, and populations within species, vary greatly in levels and patterns of genetic variation, which suggests that different sampling strategies are needed in order to capture maximum genetic diversity. However, the genetic population structure for many species will be unknown in advance of sampling, and sampling has generally relied on predictions from theoretical models, but can be refined with knowledge of breeding system and population distribution. *P. sitchensis* is a wide-ranging species with different population structures. In a study on *P. sitchensis* in the Pacific Northwest of North America, Gapare (2003) examined the specific effects of varying the area sampled, given a fixed population sample size, on capture of neutral genetic variation (using STS genetic markers) in core versus peripheral populations. Peripheral populations can be ecologically or geographically separate from core populations, and are often found at the margins of the species' range. Historical migration events, proximity to neighbouring populations and the resulting levels of gene flow can have an effect on the genetic structure and patterns of variation of both core and peripheral populations.

One of the most northern and peripheral populations of Sitka spruce on Kodiak Island, Alaska. (D. Piggot/Yellow Point Propagation)

The results confirmed that the two key components of a sampling strategy are (1) the size of the area sampled and (2) the number of individuals sampled. Specifically, larger population sample sizes and area sampled are needed to capture comparable amounts of 'allelic richness' (the average number of alleles per locus) in peripheral populations than in core populations, because of more spatial structure in peripheral populations (individuals grow in clumps, which are likely to contain related individuals). Sampling should be carried out over larger areas in peripheral populations in order to break up this neighbourhood structure. For example, for core *P. sitchensis* populations a sample of 150 trees, interspaced at least 30–50 m apart, sampled from at least 225–325 ha would be adequate to capture 95% of the allelic richness, but for peripheral populations the area sampled would have to be enlarged to at least 400 ha. These results show that molecular studies can provide important information on sampling strategies, or even the design of reserves, for both *ex situ* and *in situ* conservation.

Summarized by A. Yanchuk, from Gapare (2003)

- Are the collections to be made from the core of the population, or from the peripheral individuals or populations?

3.5 Planning and documentation of seed sources and maps

Once the populations have been chosen, and collections have been made, it is important that detailed notes of each collection, including collection methods and accurate maps, are duplicated and safely stored or archived. Accurate records have in the past been invaluable in re-establishing origins of important populations many years after collections. Many types of information have shown to be useful later on, including:

- geographic information system (GIS) coordinates of collection sites (even by mother tree if possible), and of course location of *ex situ* conservation arboretum or plantation
- road maps for access
- latitude, longitude and elevation (particularly if GIS coordinates are not available)
- detailed site maps of stand boundaries (if it is possible to delineate them)
- tree locations within stands (if GIS coordinates are not available), depending on the level of accuracy needed; detailed records will be useful when there is a limited number of trees to sample from
- approximate numbers of cones, counts or estimates of numbers of seeds, or pieces of scion material from each tree, as well as tree size, age and general form
- general characteristics of the stand (expected average age, density in stems per hectare, etc.).

The accurate collection and reporting of forest genetic material of all sorts is also becoming an important topic at the international level. To ensure the integrity of genetic samples being made, and for accurate records of where material comes from and may be moved to, the Organisation for Economic Co-operation and Development (OECD 2001) has developed a scheme for the control of forest reproductive material moving in international trade. This attempts to ensure that certain standards, specifications and terminology are used when collecting seed for movement to other jurisdictions. Currently 22 countries participate in the scheme, including tropical countries which are developing their seed exchange for reforestation purposes. Seeds and plants are produced and officially controlled according to common harmonized procedures. The OECD Forest Seed and Plant Scheme defines four broad categories of forest reproductive material recognized for certification:

- source-identified material (minimum standard)
- material from selected stands located in well-delimited regions of provenance
- material from untested seed orchards which can produce seed of improved quality
- tested material that is genetically improved.

Adherence to a formal system of documentation helps to provide confidence and clarity to others who may also be involved in the conservation and use of the material.

EX SITU CONSERVATION THROUGH SELECTION AND BREEDING

by *Alvin Yanchuk*

4.1 Introduction

Compared to genetic improvement in agricultural species, genetic improvement programmes for forest trees are relatively new. However, we now know quite a lot about how to genetically manage and breed many types of forest tree species. Although it is theoretically possible that we can maintain and develop the genetic resources of most tree species, in practice we can work intensively with only a few, and most of our experience so far is limited to pioneer and temperate zone species and their ecological conditions. For example, industrial forestry concentrates on probably less than 50 species worldwide.

Nevertheless, there is a hierarchy of levels of improvement that can address a range of possible economic investments in a tree species, or its populations, and may provide a valuable means of not only conserving but further developing the genetic resource of a species. Various methods of constructing a programme can therefore be considered, and the appropriate use of breeding and intensive genetic interventions evaluated for each (Namkoong 2003). This chapter outlines the role that various aspects of traditional tree selection, breeding and testing can play in developing some species, and thereby creating conditions highly suitable for *ex situ* conservation. Many topics are only briefly covered, and the reader should refer to classical textbooks on tree improvement (such as Zobel and Talbert 1984 or Namkoong *et al.* 1988) for further information. However, we attempt here to show some of the ways in which important genetic collections may not only be protected, but may also be enhanced with respect to the genetic variations that may be relevant for current and future human and ecological needs.

4.2 Choice of species

It may be desirable or even necessary to develop a breeding programme for a particular species, perhaps because of its local value or risk of extinction; however, the species must lend itself to relatively easy manipulation for collections (rooted cuttings, rooting, grafting, flowering, making controlled crosses among trees, seed collection, seed storage, growing seedlings), as well as for field testing and breeding. For example, although many species of *Abies* appear to be good candidates for traditional breeding programmes, it is extremely difficult to obtain flowering on young trees (Owens and Morris 1998). Even with adequate seed collected from parent trees in the wild, and with open-pollinated progeny-testing programmes to screen for the best parents, selected and grafted parents have not yielded seed in traditional seed orchard systems. Similarly, with many tropical species, which may have short-lived seed or recalcitrant seed, genetic testing and breeding may be complicated by seed storage and germination problems. Research into flowering control, pollinator mechanisms and factors, vegetative reproduction potential and seed production potential in different environmental conditions may well determine whether any further investments in breeding should or can be made.

Moreover, there must be a reasonable ability to provide a flow of material from the donor plants to the nurseries and then to the field, so that plantations (either field tests or operational outplantings) will survive and grow to provide the next generation of trees. In other words, good nursery and silvicultural systems need to be in place. Sometimes the need of some immediate attention for a species can help move along the research to make this situation possible, or improve it. For example, the discovery of only a few dozen individuals of *Wollemia nobilis* in Australia has led to several research initiatives to study a number of aspects of the seed biology and growth of seedlings (Offord and Meagher 2001) as well as the genetics of the species (Hanson 2001) (see Vol. 2, Box 4.4, Case 2). However, organizational capabilities must be realistically considered before any type of breeding programme is started.

4.3 Genetic processes

Young Pinus radiata *plantation in New Zealand. (Deborah Rogers/UC Davis)*

Old-growth stand of Pseudotsuga menziesii *in Vallombrosa, Italy. (Alvin Yanchuk/B.C. Ministry of Forests)*

The genetic forces in populations that can be affected by humans are traditionally considered to be selection, migration and population size. Although mutation is important in longer-term breeding programmes, say over 10 generations, it is not usually considered. Even though it is now possible with new genetic technology to locate or engineer genes with certain specifications, and to insert them in genomes to obtain large effects, the individual genotypes will still need to be managed within traditional programmes that manage (1) individual genotypes, (2) populations for many traits in (3) many different environmental conditions (Namkoong *et al*. 2004).

Artificial selection involves choosing individuals for reproducing the next generation, which has the effect of changing the average phenotype for the selected inherited characteristics. For many traits that seem to be affected by many genes, the process has worked well and is in accordance with common plant breeding experience. For traits with lower heritability, such as those relating to growth and adaptation, large immediate improvement is generally not possible (i.e. in one or two generations of phenotypic selection—selection based on the observed trait on an individual tree). Traits with higher heritability—such as straightness, form characteristics, some disease resistance traits and even some wood property characteristics—can be moderately improved by phenotypic selection. Therefore, in some cases, if the trait is of a higher heritability, phenotypic selection may be a better method as the farmer, forester or tree breeder can select directly on the observed characteristics of the tree at the expected age. This may be a very reasonable strategy, particularly if few resources are available for more sophisticated genetic testing.

Artificial selection programmes are sometimes considered by critics as 'unnatural', and selecting for some traits of immediate economic importance will ultimately prove to be a mistake. However, it is also important to note that all individual genotypes in the next generation are still subject to the many forces of 'natural selection'. By whatever mechanism humans select individual trees of interest, breed them and replant the next generation for selection, the human-directed changes must still function well in what is mostly still relevant to the immediate environmental challenges (Libby 2002). All exotic species that have been 'naturalized' to their new geographic areas have been, to a large extent, subjected to these pressures of natural selection and have nevertheless become important commercial species (such as *Pinus radiata* and *Pseudotsuga menziesii*) and have, in fact, broadened the genetic resource itself. The other two genetic processes that affect the conservation and breeding of forest trees—gene migration and population size—are discussed in Section 4.6.

4.4 Genetic surveys, selection in natural or naturalized populations, testing and breeding

Provenance test of interior Douglas fir in British Columbia. (Alvin Yanchuk/B.C. Ministry of Forests)

As mentioned earlier, relatively few tree species have undergone range-wide genetic surveys (provenance testing) using common garden experimental principles. However, these studies have shown that, in general, most forest tree species have patterns of adaptation in relation to the climate from which they originated. However, it is also known that adaptations cannot be perfect (Namkoong 1969), as a result of lag effects in adaptation and typically extensive pollen flow among populations. Furthermore, different species have evolved different strategies to deal with variable environments and climates and can be categorized as generalists, intermediates or specialists. Also, some species, such as many of the *Thuja* species, have levels of genetic variation which are substantially lower than most other conifers (Yeh 1988, Xie *et al*. 1992), and *Pinus resinosa* has shown almost no genetic variation (DeVerno and Mossler 1997). Therefore, generalizations cannot be made across all species.

A specimen of Pinus resinosa*, probably one of the oldest in North America, about 360 years old. (Alex Mossler/NRCan, Canadian Forest Service)*

When prior knowledge of genetic variation across the species' distribution is lacking, sampling strategies for new breeding programmes have tended to assume (1) that there are genetic differences among populations, and (2) that they follow environmental, ecological, climatic or even edaphic gradients across the distribution. As discussed in Vol. 1, Chap. 2, this is a conservative assumption but one that should be made in most cases, with respect to 'ecological representation' in sampling. For exotic species that have become naturalized in a country (sometimes referred to as **landraces**), it may also be important to consider provenance effects. If the various introductions can be traced back to their origins, or if a considerable length of time has elapsed since they were introduced, differences may have developed among populations. These different landraces could be an important approach for including some interpopulation variations in the initial sampling. For example, *Pinus radiata* breeding programmes have been able to take advantage of interpopulation differences in both the wild and introduced populations, and especially for those introductions from Guadalupe Island, which have been shown to have a much higher wood density (Rogers *et al*. 2002). Species or provenance tests that allow for a screening of the potentially valuable populations are typically the best approach to starting a base population for further breeding and development. Many programmes around the world have started with such studies, which range from introductions of related conifers to entirely new species that might have similar habitats. For instance, in several species of *Acacia* and *Prosopis*, introductions into several countries to test for populations that could be suitable for fodder, fuelwood, etc., may now be able to provide a base population to further select and breed species or provenances that performed well in various local conditions (see Box 4.1).

Box 4.1 International species and provenance trials of *Acacia* and *Prosopis* species

Background

In 1983–1987 seeds of 281 provenances of 43 species, mainly of *Acacia* and *Prosopis*, were collected under the coordination of FAO in 11 arid and semi-arid countries: Argentina, Chile, India, Israel, Mexico, Niger, Pakistan, Peru, Senegal, Sudan and Yemen. Seed storage and distribution were handled by Danida Forest Seed Centre, Denmark (DFSC), and seeds were distributed for evaluation from 1983 to 1989. Field trials of subsets of the seedlots were established by 40 institutes and projects in 22 countries. Although the project was officially terminated in 1987, an overall global evaluation of a selection of trials with countries concerned was thought necessary in order to gain some initial knowledge of the productivity of the species and provenances included.

Tapping Acacia senegal *var.* senegal *using a sunki (stick-shaped tool) in a 12-year-old plantation in El Obeid, Sudan. (FAO)*

Measurements

The assessments were carried out in collaboration between national institutes, FAO and DFSC, who also provided financial assistance for the assessment of the trials. During 1990–1994, 26 trials in 6 countries (Brazil, Burkina Faso, India, Pakistan, Senegal and Sudan) were assessed.

Results

Replication of provenances across trials was limited, and as a result, the extent to which performance can be compared in different trials and countries is also restricted. However, in Senegal the results suggested that some provenances of *A. tortilis* and the provenance of *A. senegal* had a large number of stems, which may be an advantage in the production of livestock fodder. For dry weight production, the best provenances were found in *P. juliflora*, *A. nilotica* and *A. tortilis*. In some trials no species seemed to do well, and this

continued

is in itself good information with respect to suitability of these species to new types of environments. Species with a low production were *A. aneura*, *A. holoserica*, *A. senegal* and perhaps *P. chilensis*, even though many of these species were represented by only a few provenances that may not be representative of the full potential of the species.

Although there may always be limitations to introduction of suitable species or provenances, these can be valuable starting points for further selection and breeding if organizationally possible, and the needs are present.

A series of field assessment reports has been produced for each of the 26 trials, and can be viewed at: http://www.dfsc.dk/pdf/Aridzone%20trials/index.html

Source: Raebild *et al*. (2003a-u, 2004a-e)

Humans have introduced hundreds if not thousands of tree species to new environments throughout history. Many of these introductions have failed, but many have succeeded. However, the introduction of exotic species must be carefully considered in terms of both the positive and the negative potential they may have in new ecosystems. For example, *Prosopis* has become 'weed like' in its behaviour in some African countries, so the biology of the species and its invasive characteristics also need to be considered. In other words, although there are thousands of species that may require our attention, ranging from simple collections and field plantations, not all of the introductions and follow-up breeding and improvement efforts can be successful.

4.4.1 Selecting a base population

If it has been deemed appropriate to move past a simple population level of selection outplantings for *ex situ* conservation purposes (see Chap. 5), the establishment of a base population to move to further selection and genetic testing is of course required. Many of the genetic sampling issues that were discussed in Chap. 3 are still appropriate here; the initial 'wild' or semi-wild unimproved population needs to be composed of a few to several hundred trees in order to make it possible to select enough superior individuals. For example, selecting 50 trees out of 5 000 represents a proportion of 1%, which would be in the desirable range. A relatively high selection intensity is required in order to make genetic improvements for the trait or traits of interest, particularly for traits with low heritability.

4.4.2 Progeny testing and field test designs

Over the past 30 years tree breeders have increasingly used techniques that more accurately identify and predict the genetic value or worth of selected parent trees (genotypic selection). The use of a mother tree's offspring, whether they are cuttings (clones) or seedlings, is a powerful technique for examining the genetic value of any parent tree. With many offspring trees per parent, planted across the test site(s) in a randomized pattern, some trees will sample better than average micro-environments and some worse, but on average the statistical average or mean of the parental selection or family will reflect the 'true' genetic mean (breeding value). It is generally a common practice to use about 20–30 offspring per family per test site. Including more observations per family tends to be inefficient with respect to the aim of being able to compare more families (Cotterill and James 1984; Magnussen 1993). Except for some very high-heritability traits, some form of progeny testing will always be required to select a population of 'winners' for further breeding and selection, or for seed production purposes.

For conservation purposes, progeny test plantations are also being considered as another form of *ex situ* gene conservation planting: they are typically planted in environments, climates or habitats of current or future interest, they contain progeny from a few dozen to even hundreds of parental selections, and there is an increased level of information (typically from measurements of the progenies). Several programmes in the Pacific Northwest of North America now consider particular progeny test populations as another important vehicle in *ex situ* conservation (Yanchuk 2001a; Lipow *et al*. 2002).

A young progeny test of coastal Douglas fir, established with an incomplete block design. (Michael Stoehr/B.C. Ministry of Forests)

In some of the earlier testing schemes used in tree improvement, genetic entries were planted as single seed sources in large blocks or row plots. However, forest tree testing environments are typically too patchy with respect to moisture or soil conditions that can confound growth or survival of genetic entries planted in groups, so various forms of randomized complete block (RCB) designs have been developed. With RCB designs, as blocks or replications are laid out with fewer numbers of trees per provenance, family, or clone in each block (with more blocks or replications on the test site), the efficiency of the design becomes greater. A typical and relatively robust row-plot experiment would be something like four-tree row plots, with eight replications or blocks. The most efficient design is one which uses single-tree plots: this would be an experiment with 25–30 progeny per genetic entry, which means there are 25–30 replications or blocks. Recently, many breeding programmes have considered new designs, such as incomplete block (ICB) designs (Fu 2003). Although these can increase efficiency of genetic testing, they are more complicated to install and the statistical analysis is more involved, so they should only be attempted if resources allow it. The most important criterion is to have enough healthy trees per genetic entry growing in a good representative test environment.

4.4.3 Selection at ages earlier than the typical harvest age

Except in the case of some fast growing species, such as hybrid poplar or *Eucalyptus* spp., it is rare in forestry for the selection age to be the same as the final harvest age. Measurements or selections are usually from juvenile trees, whereas we want to increase stand-level productivity from mature trees. This is particularly true for many timber species, fruit trees, or trees grown for by-products (such as resin production) where final harvest or final production estimates may be in the range of 20–80 years. If the genes that affect the trait at an early age change their expression at later ages, then selection and prediction of genetic improvement will be inaccurate, and genetic gain will be overestimated. Fortunately, however, in terms of achieving the maximum gain per unit time, the optimum age for forms of genotypic selection such as family selection is 6–12 years for most growth traits, even those that become important at age 25 or later (Lambeth 1980). Although there is little that can be done to improve this situation, it is an important concept that the breeder or forester should consider when presenting the possible benefits from selection and breeding.

4.5 Genotype x environment interactions

We also know that particular populations, families, or genotypes (such as clones) may perform differently in different environments, because genes express themselves differently under various environmental conditions. This **genotype x environment (G x E) interaction** is an important issue that has to be considered in all plant breeding programmes. Therefore, it is important that in initial testing schemes, multiple-site field testing is used in order to establish the magnitude of G x E interaction in the population or specific families of interest. In order to minimize the biases that can occur in selecting desirable genotypes, or families, they must be established on more than one test site, and preferably a minimum of three. More than three sites is useful if the range of target environments or habitats is large, but tends not to provide much further advantage when looking for genotypes or families which can be considered stable within an environmental range that is considered appropriate for one or more groups of genotypes (Johnson 1997). Establishing progeny test populations on more than one site also allows greater insurance of *ex situ* conservation in different test environments. The quantitative genetic analyses required to interpret G x E interactions correctly can be complicated and cannot be fully discussed here. Procedures such as those outlined by White and Hodge (1989) should be considered during data analysis.

4.6 Advanced generation breeding and testing

Once initial screening of a base population (from the wild, or from naturalized plantings such as the landraces discussed above) with progeny or clonal testing has been carried out (as described in Section 4.4), adaptive genetic variation in this selected 'breeding population' can generally be maintained with as few as 20–50 individuals. The basis for these relatively small numbers has been discussed in Chap. 3, in terms of how much genetic variation can be captured in populations of this size. Although a relatively small number of parents, perhaps 30, can contribute and develop a particular population of interest over several generations, 3–5 populations of this size, with different deployment objectives (climatic, habitat, or even trait differences), can cover a wide range of genetic potential, and will allow for many options in the future, if objectives change. This is the foundation of the multiple population breeding system (MPBS) that has been found practical and applicable in many breeding programmes around the world (Namkoong 1976, 1984). The MPBS can even take the form of several independent and partly advanced test stands for species that are not yet intensively under breeding and selection (see Chap. 5 for some examples). For many species that are currently of secondary value, this may be a more useful means of storing variation, e.g. a series of provenance tests may be considered a part of such a system of established 'pre-breeding' populations.

If it has been shown to be economically and biologically feasible to carry on with improving a particular trait or group of traits, the selected best parents identified through the progeny testing (parent testing and selection) need to be mated together to form recombination populations. With controlled crossing schemes, the breeder is essentially creating new allelic combinations in the array of new progenies from which to further select. This approach allows the breeder to effectively change the frequency of genes for the trait of interest. The change in gene frequencies for traits not selected, or not correlated to the traits under selection, will be governed by the sampling theory discussed in Chap. 3 and Fig. 3.1.

4.6.1 Mating designs

There are several ways in which trees can be mated together to form families for the next generation. Complete mating of all possible crosses is the most desirable situation, but generally not practical and not economically or biologically necessary. Making a few crosses in as short a time as possible (as long as each parent is crossed a few times, say 2–4) tends to be a more cost-effective approach.

The three main approaches for mating forest tree species, is usually dictated by the flowering biology of the species (monoecious, dioecious, age of the tree, pollen or female flower availability and receptivity, etc.). They are:

- **Nested design:** An individual tree is chosen as the pollen parent and crossed to two or more trees that act only as females, and exclusively to that one pollen parent. This design has been used primarily in animal breeding.
- **Factorial design:** A tree serves exclusively as a male or a female in some small mating groups, but is mated to more than one male or female acting parent. This is one distinct advantage of the factorial design; for example, if the breeder chooses a breeding group of eight unrelated trees, and mates them in the factorial design, four parents only need to provide pollen, and the other four only need to provide female cones for isolation and breeding. The total number of crosses in this situation is therefore 16.
- **Diallel design:** Trees can be used as both males or females at the same time; for example if a group of eight parents is assigned to one diallel, there can be up to 64 crosses as shown in Figure 4.1, presenting a diallel mating design. However, this would involve selfed progenies and reciprocal crosses which are generally not needed for most practical purposes in breeding programmes. A breeding approach within a diallel crossing scheme, such as by making only the ***d*** crosses in the design shown in Figure 4.1, still provides the breeder or tree improvement forester with 16 full-sibling cross families to select from.

		MALE							
		1	2	3	4	5	6	7	8
FEMALE	1	*s*	*d*	*d*	*c*	*c*	*c*	*d*	*d*
	2	*r*	*s*	*d*	*d*	*c*	*c*	*c*	*d*
	3	*r*	*r*	*s*	*d*	*d*	*c*	*c*	*c*
	4	*r*	*r*	*r*	*s*	*d*	*d*	*c*	*c*
	5	*r*	*r*	*r*	*r*	*s*	*d*	*d*	*c*
	6	*r*	*r*	*r*	*r*	*r*	*s*	*d*	*d*
	7	*r*	*r*	*r*	*r*	*r*	*r*	*s*	*d*
	8	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*s*

s= selfing cross ***d*** = double pair mating (each parent crossed four times)
c = crosses to complete the half diallel ***r*** = reciprocal crosses for the full diallel

Fig. 4.1. *A hypothetical diallel mating design for eight selected trees*

Irrespective of the mating design chosen, after 3–5 crosses per tree there is a reduced efficiency with respect to family and within-family selection, if a fixed number of trees can be planted in a test (King and Johnson 1993). For example, if the breeder selected a population of 50 trees, divided up the population into sets of 10, and applied a factorial mating of 5 females and 5 males to each factorial, there would be 25 new full-sibling families in each factorial, and a total of 250 new families from the original 50. Although it would be an optimal approach, it would involve a substantial amount of work, and about two-thirds of this number would be adequate, especially if it would allow the breeder to plant them out sooner. How the trees should be planted out is the topic of the next section.

4.6.2 Tests and selecting the next generation of trees

Although there has been substantial debate about which planting designs are best for advanced generation field tests and selections, the most effective method will probably be determined by local operational and practical matters rather than by theory. For instance, if a large homogenous single-family block (for example 100 full-sibling trees in each block with no replication) has been established, it will allow the breeder a much better visual comparison of all cohorts in that family. With homogeneous test environments, this method can be very effective. On the other hand, breeders are usually still interested in applying selection at the family level, before selecting a few individual trees within a group of the best families. In such cases, RCB designs (as discussed above) should be used, although selecting individual trees for the next generation is still possible from single-tree plot or ICB designs. The efficiency of these tests for selecting the next generation of the best trees from the best families is largely determined by the heritability of the traits under selection. For traits with lower heritability, such as growth, single-tree or small plot designs will usually be more efficient than large block plantings.

4.6.3 Breeding population structure

As discussed in Chapter 3, many of the lower-frequency genes, which may not have strong effects on traits that are currently of interest, will be lost by genetic sampling effects in small breeding populations, such as those discussed above. However, including dozens more individuals, or even a hundred, will only slightly reduce the chance of these losses, which are inevitable and unavoidable in most *ex situ* conservation programmes. Fortunately, these low-frequency genes that we expect to lose in smaller breeding or development populations do not have much effect on the genetic variation for traits under selection. As discussed in Vol. 1, Section 2.3, other approaches such as *in situ* and *ex situ* gene conservation programmes should be developed to accommodate these genes. Most conifer breeding programmes around the world now work with relatively small population sizes in the F2 and F3 generations but maintain larger genetic reserves by means of some of the other *in situ* and *ex situ* conservation approaches (see for example Byram *et al*. 1998, Yanchuk 2001a).

Once a population size has been chosen, it is important to consider issues of relatedness that will be built up over time in the breeding population, i.e. inbreeding. This is generally undesirable because it tends to fix unfavourable recessive genes in a population. Inbreeding is inevitable in any medium- to long-term breeding programme, so tree breeders have to design methods to minimize its accumulated effects. Subdividing the breeding population into groups that remain separate from intercrossing, sometimes referred to as **sublines**, is one such approach. It allows the breeder to make crosses among selected trees within sublines over generations, without crossing trees among sublines. However, if a programme is for a species of lesser commercial value, or one that cannot be intensively bred for many generations, inbreeding should not be a problem and more complex population structures need not be considered.

The above discussion on selecting, breeding and testing for another generation of trees, either to develop the genetic resources for better use, or to conserve available genetic variation for new conditions, has only been applied in very few situations, as it is costly and requires substantial expertise. However, in some cases, such as in a 'rescue breeding' effort, where the continued existence of a species may be in danger, different breeding strategies may need to be developed. For example, the American chestnut (*Castanea dentata*) is reduced to non-reproductive stump sprouts in its former ecosystems; only recovered hybrids (developed from crossing with a few genotypes from a resistant species) that can be bred to re-segregate for a high percentage of the American chestnut genome may save local gene pools (see Box 4.2). Although this procedure is more commonly known in captive animals, it can be done for trees as well. Breeding in these cases can involve reconstructing genomes from hybrid backcrosses, increasing the species' pure genetic background of variation by increasing the representation of original hybrid parents.

Box 4.2 Hybrid and backcross breeding to re-establish a component of the American chestnut in eastern North America

Castanea dentata (American chestnut) was once one of the dominant and most coveted tree species in the hardwood forests of eastern North America. However, around 1904 a blight, *Cryphonectria parasitica*, was introduced into the United States from the Orient, probably unintentionally from imported Asian chestnut trees. The blight, which killed trees to ground level, rapidly spread throughout the eastern states of the US and within 40 years most of the *C. dentata* trees in the eastern states were destroyed, although hundreds of thousands of trees remain as stump sprouts that rarely reach the flowering stage. Dead chestnut trees killed by the blight comprised 50% of the overall value of the eastern hardwood timber stands.

Native American chestnut in the wild, a survivor or an escape tree from the chestnut blight disease. (Paul Sisco/ACF)

Initial attempts to breed blight-resistant chestnut, initially by generating hybrids between more resistant *C. dentata* individuals and blight-resistant oriental chestnut parents, produced oriental chestnut phenotypes, short and branching, which were not competitive in eastern forests (Schlarbaum *et al*. 1994). However, a number of programmes are breeding blight-resistant *C. dentata* trees with a backcross method that will transfer blight

continued

resistance from Chinese chestnut to American chestnut, while retaining the desirable growth, form, and adaptability of the American chestnut (Burnham 1990). Highly blight-resistant progeny were recovered after intercrossing first hybrids between Chinese and American chestnut, and although it is expected that the entire breeding project will take decades, it is expected that within 5 years there may be a few lines of highly blight-resistant trees which are on average 15/16 American chestnut and 1/16 Chinese chestnut. A longer period of time, 30–50 years, will be needed to avoid inbreeding, by transferring the blight resistance of several different Chinese chestnut trees to a total of at least 100 lines of American chestnut. In addition, the use of molecular techniques to accelerate the breeding process is now considered to be feasible. A genetic map of chestnut, with regions associated with blight resistance identified, could be used to screen newly germinated nuts for blight resistance. This may enable several generations of backcrossing to be bypassed, yet still produce trees that have proportions of American parentage similar to those of trees bred using conventional backcrossing.

Summarized by A. Yanchuk, from Burnham (1990) and the American Chestnut Foundation (www.acf.org)

4.7 Traits of interest and the costs of assessment

Irrespective of the traits that it may be desirable to select for and ultimately improve, the forester or breeder must be able to evaluate them accurately and economically. Traits that are expensive to measure, such as wood properties, resin characteristics or some types of disease and pest resistance, may make a programme relatively ineffective if the costs are high, and might make it financially difficult to measure enough individuals from enough families in a progeny test to actually apply an adequate selection intensity. Although traits such as growth potential have low heritability, large gains have usually been made because many trees can be assessed, thereby increasing the selection intensity. Wood property traits (such as wood density; see Zobel and van Buijtenen 1989) have been studied and have been shown to have higher heritability than growth rate, typically twice as high. However, there also tends to be lower phenotypic variation on top of the additional costs of measuring most wood property traits, so trade-offs in gains must always be balanced by these three factors (that is, heritability, phenotypic variation, and cost of assessment) as they can affect the number of trees assessed and the subsequent selection intensity that can be applied.

In addition, there can be genetic relationships between two traits of interest. Many other traits are also important in selection, breeding and conservation, such as pest and disease resistance, tree form (which affects log quality) and many other wood properties or fruiting characteristics. Whether these are being directly selected for economic reasons or not, breeders have investigated these traits to establish the genetic and phenotypic relationships that could affect selection on other ‘primary’ traits (typically growth). For instance, in many conifer breeding programmes, it is well known there is a strong negative genetic relationship between diameter growth and wood density (Zobel and van Buijtenen 1989), so genetic gains in growth potential will be reduced if wood density is not to be reduced as well.

4.8 Biotechnology and its role in breeding

As mentioned at the beginning of this chapter, there have been substantial technical developments in plant genetics, not only in cloning technologies for many forest tree species, but also in changing the genetics of plants by using transgenic technologies. However, it is also well known that good silvicultural and breeding programmes must be in place first, before these have any potential to add additional value (Yanchuk 2001b). Biotechnological tools can, however, contribute to the management of *ex situ* collections (such as clone banks and other germplasm banks), by providing better tools to assess the levels of genetic diversity of tree species, or storing germplasm.

New molecular marker technology, as we have seen in Box 3.3, can be helpful with genetic diversity studies that can support the design of sampling strategies for *ex situ* collections, which also may reduce redundancy and duplication within collections. Markers can also be used in molecular fingerprinting for clonal identification purposes, and have the potential to be used to reveal the origin of timber and defeat illegal logging practices (White *et al*. 2000). Another widely used biotechnology tool, cryopreservation, aids the long-term storage possibilities of plant accessions where traditional seed storage is a problem (see for example Krishnapillay 2000). The new era of genomics is developing many molecular tools that may assist in the identification of potentially useful genes in genebank accessions, and the incorporation of these genes into breeding programmes. The example of backcrossing resistant genes into American chestnut, discussed in Box 4.2, is another case. Selection for resistance could be enhanced by the selection of progeny with markers associated to resistance genes from the Chinese chestnut (Kubisiak *et al*. 1997), or, on the contrary, by selecting resistant trees with more American chestnut type genes. Although marker-assisted selection is becoming more common in plant breeding, for example in soybeans (Arahana *et al*. 2001) and many other crop plants, its use in tree improvement programmes to date has been limited by costs, length of time for trees to reach maturity of assessment age, and the difficulty of locating the relatively large number of genes that affect most traits of interest (Johnson *et al*. 2000). Nevertheless, the information that is being generated from molecular genetics or genomics research is uncovering many interesting aspects of gene evolution and function, and will continue to provide useful tools and information to tree breeders as genomes become better understood.

4.9 Conclusions

Tree breeding techniques such as modern selection, breeding and testing methods are of course important in the pursuit of improving the economic or ecological traits of interest in a forest tree species. Important technical developments in tree breeding, which have been developed over recent years, mainly revolve around efficient field-test designs, efficient mating designs for advanced generation breeding, and many specialized techniques and tools for assessing traits of economic or ecological interest. Of utmost importance, however, is that selection, breeding and testing programmes consider the simplest and most effective methods that can be handled by the organization concerned in realistic time frames and meet the conservation or breeding objectives. In many situations only one generation of selection and testing may meet most of the objectives, and the programmes can move on to the next most important species.

With many types of species and many problems to resolve, not all techniques of genetic management can be applied to all species with the same management objectives. The management objectives may vary from saving the most threatened, to preventing the most loss, to maximizing economic gain; each of these would obviously require different techniques and levels of investment. In addition, the value of specific traits and environmental challenges can change over a period of time that is relatively short with respect to the generation length of most trees, so new selection criteria may be required in each generation. As mentioned in Vol. 1 (Chaps. 5 and 6), international or interagency programmes for priority species are likely to be necessary for more advanced selection, testing and breeding programmes, if they should advance beyond the more simple species and provenance trials. However, it is also likely that the participation of local people, for traits of interest to them, will likely be critical for any long-term success, and this has largely been shown to be the case even in developed countries.

ESTABLISHMENT AND MANAGEMENT OF *EX SITU* CONSERVATION STANDS

by Ida Theilade, Alvin Yanchuk and Søren Hald

5.1 Introduction

As we have discussed throughout these three volumes, existing protected areas make important contributions to forest conservation, protect many forest values, and represent very considerable achievements towards conserving forest genetic resources. It is also clear, however, that existing protected areas are not in themselves sufficient to achieve or sustain all forest conservation goals. Many do not represent important populations, are of inadequate size, are too disconnected from their surrounding environment and are inadequately protected from pressures which compromise their conservation value (Kanowski 2001). The World Conservation and Monitoring Centre estimates that more than 8 000 tree species are endangered (WCMC 2001). Only about 12% of these are recorded in protected areas and only 8% are known to be in cultivation (IUCN 1999). Therefore, thousands of tree species will depend on conservation outside protected areas: in managed forests, agricultural landscapes, or *ex situ* in botanical gardens, arboreta, seed banks or gene field banks. This chapter presents experiences with *ex situ* conservation of tropical trees in living stands (gene field banks) and discusses some of the inherent opportunities and drawbacks.

5.2 The aim of *ex situ* conservation stands

The purpose of *ex situ* conservation stands is to keep genetic resources in a secure area for future utilization. However, *ex situ* conservation is rarely found in this pure form. Some botanical gardens and arboreta have collections of threatened species, but these collections often consist of only a very few individuals. In forestry, *ex situ* conservation stands often consist of a larger number of individuals, but the long-term objective is most often, if not always, combined with an immediate and far more utilitarian purpose.

Humans have always taken or moved valuable plant material with them whenever they have migrated to new areas. The colonial period between 1850 and 1950 was an era of unprecedented plant exploration and introductions of exotic plants around the globe. During colonial times numerous *ex situ* populations of tropical trees were established to test promising exotic or indigenous species. The first rubber trees (*Hevea brasiliensis*) in Singapore and the teak (*Tectona grandis*) and *Cinchona* plantations in India were all *ex situ* plantings, but established at a time when no one even knew much about conservation genetics, let alone had to worry about it. This contrasts with today, where natural forests are diminishing, and often it is uncertain whether a given natural stand will still be available in a decade or two. Consequently, most *ex situ* plantings of trees, in particular tropical trees, could be said to be functioning as 'gene conservation stands' or 'gene field banks' as well.

It is estimated that only about 100 tree species are adequately conserved *ex situ*. These are almost exclusively species whose genetic resources have been assembled for domestication programmes, with which almost all substantive *ex situ* forest conservation activities are associated (NRC 1991). The purpose(s) of a particular *ex situ* conservation stand is of course all-important for decisions related to silvicultural management of the stand, which again will determine how many and which alleles are conserved and which may, intentionally or unintentionally, be lost.

5.3 The materials and methods for establishing *ex situ* conservation stands

Either vegetatively produced material or plants grown from seed can be established in living stands. The idea is that when these are established in a secure location they will experience a new environment while maintaining gene frequencies close to the original frequencies in the original populations. Conservation stands have to be large enough to (1) maintain the genetic integrity of the original population, and (2) generate large enough numbers of seed cones/flowers, a large enough pollen cloud, insect population(s), etc., for outcrossing in order to adequately maintain the population size in a seed crop.

5.3.1 Planning *ex situ* conservation stands

Site selection

Two things must be considered in the planning of conservation stands: (a) the area must be generally suitable with respect to climate, weather and soil conditions, and (b) it must be able to produce seed crops at some point in time for reproduction of the next generation. Both of these factors will contribute to good growth and good flowering, but it may not always be possible, and in fact it is probably rare, to be able to ensure both. In relation to the conservation objectives of *ex situ* stands, it is probably best to err on the side of good general adaptation and growth, and to assume that some reproduction by way of seed will occur at some point. Flower enhancement treatments could be applied in situations where trees are healthy, but are not producing good flower crops.

Site matching techniques can range from simple guesses based on broad ecological similarity, to sophisticated record comparisons of similar climatic conditions and patterns. However, it is not always possible to predict how an exotic species will perform in a new area, or—without previous provenance testing information—how populations will perform in new environments. Clearly, it is important to take into consideration climatic matches such as winter and summer lows and highs, precipitation levels and patterns throughout the year, growing degree days and general soil characteristics, to mention a few. Since good growth is the prime objective, and without prior knowledge, it is generally appropriate to establish *ex situ* stands in climatic zones similar to where they originated from. Since obtaining commercial quantities of seed from conservation blocks is not always something that can be relied upon (see Section 5.5.3), candidate trees from the conservation block could be grafted into a special orchard and managed for seed production (on a good flowering site). These seeds could then be used to regenerate the conservation block. The time and cost of such an operation would only be possible in rare situations, but nevertheless it is an option.

Isolation for minimizing contamination

Adequate isolation from contaminating pollen sources needs to be considered, since gene flow into the stand will seriously reduce its conservation value. The buffer zone required to eliminate contamination depends on the pollination pattern of the species. So far most experience is with wind-pollinated species, particularly pine species. For pines, a stand size of 10–20 ha is likely to generate an adequate pollen cloud within the stand for effective cross-pollination. However, the degree of pollen flow into the conservation block could have a substantial effect on the genetic integrity of seeds if the block is too close to other related species or different populations (see Section 5.5.2). FAO (1992) recommended minimum buffer distances of 330 m from populations with the potential to cross with the conservation block. These isolation distances may need to be greater or smaller, depending on the final size of the conservation blocks, and the characteristics of flowering and pollen flight in the species. However, as a rule of thumb, a distance of 330 m may be adequate to isolate most stands from significant effects of gene flow into the stand.

Size of the stand

At an early stage the conservation planner will have to consider how large conservation stands need to be in order to be able to meet the conservation objectives. The sampling strategy applied (see Chap. 3) is one important factor to consider when determining the size of the stands. In addition, each family (i.e. each sampled mother tree) should be represented by an approximately equal number of progeny in the conservation stand. Since natural or artificial thinning will occur in the stand, the level of expected mortality or the degree of thinning should be factored in as well. Indeed, this requires more planning before the stand is established, as it is the final numbers of stems at or near rotation that are important rather than the initial number planted. The assumption here is that at some point seeds or vegetative samples would be collected, the stand harvested and replanted to maintain the genetic constitution of original population. For instance, if the conservation planner is aiming at 1 000 trees at rotation age and the expected stocking at this age is 150 stems per hectare, the minimum stand size will be 6.7 ha.

In general the conservation officer also needs to consider some additional factors that could affect the possibilities of a generation turnover. One of the key issues, related to those mentioned above, is how to maintain the effective population size of the stand; in other words, how genetic variation might be reduced in the conservation block due to genetic drift (i.e. the effects of small sampling sizes) over time. Although one may be collecting from progressively fewer trees in each generation, it is likely that effective population sizes will remain similar after a few generations if adequate pollinations and seed collections have been made. Guldager (1975) estimates that in most cases genetic drift and inbreeding will be of little importance in well-managed stands of 10–30 ha. While it is likely these types of populations cannot maintain, over time, the exact gene frequencies that would be maintained *in situ,* it is important to recall they would retain high levels of adaptive genetic variation; both *in situ* and *ex situ* are live archives, and they both experience evolutionary events in different environments over time.

In addition to these genetic considerations, stand sizes must be kept at a manageable level such that the burden of establishment and future management is within the capacity of the institution in charge. For the establishment of *ex situ* conservation stands under the UNEP/FAO project on the conservation of forest genetic resources, a minimum stand size of 10 ha was recommended (FAO 1985). Species targeted under this project included *Pinus* spp. and *Eucalyptus* spp. In 1998 participants at the 'Conserve' workshop held in Canberra, Australia recommended a minimum population size of 2 000 individuals or 20 ha for *ex situ* stands of Monterey (radiate) pine (Matheson *et al*. 1999). Again, although the size of various *ex situ* conservation stands has been quite variable, it is important to remember that a minimum size of even a few hectares can be effective as long as it is combined with some management follow-up by the appropriate institution.

Access to land

Isolation and matching of site to seed sources presents a silvicultural and management challenge to the successful establishment and management of *ex situ* conservation stands. The conservation of multiple populations (i.e. several *ex situ* stands), requires several separate areas to be considered. This may present problems with access, maintenance, protection and security, and—not least—cost. However, there will probably be losses due to many unforeseen circumstances, so replications of the *ex situ* stands are important, and this is where collaborative projects across provincial or country borders can be of most benefit. Protection capabilities within the organization need to be assessed with respect to day-to-day management of roads, fencing, thinning and security over a substantial length of time. It is also important to consider land tenure and any rights over the land in order to avoid any future conflicts with ownership issues.

Species composition

- **Pure stands:** so far almost all experience in establishment of *ex situ* conservation stands has been with monocultures of relatively well-known plantation species. For these species, of which many are pioneer species, the establishment of monocultures based on well-known plantation technology probably represents the most efficient *ex situ* conservation method.
- **Mixed-composition stands:** many tropical climax species are considered valuable and are under some kind of threat. Only a few of these species are presently being planted, and for most of them little information on their management is available. Establishment of monocultures of these species may prove difficult or impossible, and in this case establishment of mixed-composition stands that mimic natural conditions may be the best option. Multiple-species stands would have the objective of conserving the prime species of interest, and perhaps others, which are part of normal ecological associations (such as climax species). Alternatively, species of no current conservation value or interest could be used in order to provide an ecological service to the prime species (nitrogen-fixing plants, for example), or to the local community to provide some economic benefits to maintaining the area as is. Systems such as these must have meaningful attributes, such as natural regeneration being relied upon as a cost-saving measure. As these areas become managed for more of the attributes typically considered with *in situ* conservation, more of the management approaches described in Vol. 2 become appropriate. In any event, fewer individuals of the prime species, or larger areas, will have to be considered as part of some trade-off. Because of the lack of information on the silvicultural management of the species these *ex situ* conservation stands will often be very experimental, which of course increases the risks of failure.
- **Planting of nurse crops:** another alternative to establishment of monocultures is the use of nurse crops. Nurse crops are fast-growing pioneer tree species which are established at a wider spacing together with the target species in order to quickly provide the necessary protection and the necessary microclimate for the target species to survive. For many late-successional species, problems of seedling establishment on open land have been encountered and the use of nurse crops may be necessary in order to successfully conserve sensitive species *ex situ*. The nurse crops can then be gradually removed as the target species are established and start to dominate the canopy. Good examples exist from Sinharaja, Sri Lanka where *Pinus caribaea* has successfully been used as a nurse, facilitating the establishment of site-sensitive tropical forest tree species that are late-successional (Ashton *et al*. 1997). As an alternative to simultaneous planting of nurse crops, the site-sensitive species may be planted beneath the canopy of existing plantations or stands. In conclusion, nurse crops should be used according to local experience and based on knowledge of the characteristics of the target species.

Ownership of genetic material in* ex situ *populations

Ownership of stands is likely to be straightforward in countries that have collected from native populations and established *ex situ* conservation stands within their country.

However, where germplasm for conservation has been collected or established outside a country, issues of access, use and perhaps ownership of important genotypes identified later on could be complicated. As is the case with exchanges of many plant and animal genetic material, agreements need to be developed between cooperating countries. The use of so-called Material Transfer Agreements (MTAs) between donor and recipient of genetic material is becoming more common. Baron and Siebeck (1994) describe in detail the aspects related to formulation of MTAs. The importance of MTAs and fair access and benefit sharing of forest genetic resources was reiterated in Vol. 1, Chap. 6, as it is now formally incorporated into the Bonn Guidelines on Access to Genetic Resources and Fair and Equitable Sharing of the Benefits Arising out of their Utilization, as adopted by the Conference of the Parties at its sixth meeting of the CBD (CBD 2001). The International Treaty on Plant Genetic Resources for Food and Agriculture adopted by the FAO Conference at the end of 2001 entered into force in 2004. The treaty's objectives relate to "the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of benefits derived from their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security". The treaty establishes a Multilateral System of Access and Benefit-sharing applied to a list of crops. The focus has been on facilitating access to plant genetic resources, because, as the Treaty recognizes, access itself is a major good. Provisions regarding access and benefit-sharing will be contained in a standard MTA that is currently being discussed and developed. One forestry genus, *Prosopis*, is included, as well as several woody species such as *Artocarpus, Citrus, Cocos* and *Malus*.

5.4 Establishment of *ex situ* stands

Of course, the prime objective of tree-planting activities is the establishment of a healthy, vigorous and fully stocked stand. This is especially true in the establishment of *ex situ* conservation stands, as the planted material needs to represent the same gene pool as the source population from which it was derived. Consequently, the best locally proven practices should be adopted in the raising of the seedlings for these stands (FAO 1992). Often it will be advisable to adopt methods that are more meticulous and therefore more expensive than normal standard routines.

5.4.1 Nursery techniques

In general, nursery procedures should follow the best locally proven practices; however, there are a few aspects which should be given special attention when dealing with material for *ex situ* conservation. All through the nursery system, seed lots need to be carefully labelled in order to avoid any possibility of confusion or mistake in genotype or population identification. In most cases seed from a given source will, for practical reasons, be bulked and treated as one seed lot. Retention of single-tree offspring identity may, however, be beneficial in the following cases:

- When the conservation stand will form the immediate basis of a tree improvement programme (see Chap. 4).
- Where seed behaviour is problematic in the sense that pre-treatment may affect germination and survival differently for the individual genotypes. Unintended selection is likely to happen as a result of various seed handling methods (El-Kassaby 2000). By following single-tree offspring separately, the conservation manager may ensure that all mother trees are equally represented in the material to be conserved *ex situ*. The question of whether to retain single-tree offspring identity after

establishment of the stand is addressed in Section 5.4.5.

- Nursery plants should be rejected if they are dead or obviously diseased or if they show morphological disorder (hybrids, albinos, mutants), but not because of delayed germination or slower than average growth since differences in these traits may represent genetic variation which should be retained in the established *ex situ* stand. Therefore the practice of grading nursery stock and planting only the larger size classes should generally be avoided.

The Tanzania Tree Seed Agency conducts pioneering work on indigenous species, while popular trees attract customers to the nursery and increase public awareness. (Ida Theilade/FLD)

5.4.2 Site preparation

In general, preparation of the site by complete cultivation is the best way of ensuring high survival—especially in climates with a low rainfall and a long dry season. However, the risk of erosion should always be carefully considered and as a consequence stands should, whenever possible, be established on gentle slopes. Site preparation techniques and approaches, as mentioned earlier, should be based on the best available knowledge of how to establish a particular species in a plantation setting, and typically site preparation questions are an integral part of that silvicultural knowledge.

The Tanzania Tree Seed Agency has established ex situ *plots of indigenous trees. A young stand of baobab, a valued species rarely planted by local people. (Ida Theilade/FLD)*

5.4.3 Layout

The design of individual stands should not follow any particular 'standard' layout but simply be adjusted to the characteristics of species and to local conditions. Some general considerations and recommendations which may serve as a basis for decision by the conservation manager include the following:

- The selected spacing should first of all be adjusted to allow for cultivation, weeding and thinning by hand or possible mechanical methods (FAO 1992). Again, the number of individuals planted should be large enough to ensure a fully stocked mature stand, even with some level of dieback and mortality due to competition.
- Under special circumstances, where the resources for future thinning seem unlikely to be available, a wider spacing may be considered. This could eventually result in an incomplete mature stand but the requirements for consistent and timely management will be lower, and perhaps ironically, could increase the chances for a successful mature stand.

- The shape of the plot will depend on topography and on the direction of prevailing winds. Generally, however, to ensure adequate pollination within the stands, the shortest diameter should not be less than 150 m (FAO 1992), and square plots are the most robust layout.

5.4.4 Demarcation

Permanent and efficient demarcation of the stands is a precondition for successful conservation. This may seem an obvious point, but nevertheless there are examples of conservation stands being abandoned as a result of insufficient demarcation. For example, of 135 *ex situ* conservation stands assessed 20 years after establishment, 13% had insufficient demarcation which meant that their conservation value was either completely lost or severely reduced (Theilade *et al.* 2001). Permanent demarcation, or tie points, can be obtained by using forest roads or wide fire lines as borders of the stands. Alternatively, poles of concrete, metal or wood may serve as markers and should be considered as the most trustworthy approach to demarcation. Regular inspection of the demarcation must be carried out, as well as ensuring that maps are correct and updated. Whenever possible, physical demarcation in the field should be complemented by global positioning system (GPS) coordinates.

5.4.5 Single-tree offspring identity

As mentioned earlier, it may be beneficial to keep track of single-tree offspring through the nursery, and in some cases even throughout the life of the stand. When tree improvement activities are to be based on these conserved populations, information on the performance of different families will be very useful. In cases where there is a strong genotype x environment interaction, thinning of the stand may accidentally lead to reduction of the genetic base. By keeping track of single-tree offspring, the conservation officer may ensure that no entire families are lost through the process of thinning (Guldager 1975). It must be emphasized that this kind of control is only feasible with a limited number of mother trees, and that it requires mapping of each individual tree in the stand. However, *ex situ* conservation without information on single-tree offspring is still very effective and meets most of our objectives. Considering the amount of time and resources involved in this kind of control and documentation, keeping track of single-tree offspring should only be considered when there is a clear indication that information gained from the effort will be of high value.

5.4.6 Early management

Weeding
Local conditions will dictate the need for removal of competitive vegetation throughout the life of the stand. Complete removal of weeds in the early stages is strongly recommended to optimize growth and survival of the target species during the establishment phase and, even more importantly, as a precaution against fire damage (FAO 1992). Weeding ceases at the time of canopy closure, but occasional climber cutting may be required thereafter.

Replacing of dead or missing plants
In situations where substantial mortality has occurred after the first one or two years after planting, it should be discussed whether fill-in planting is needed. There is no threshold percentage that determines whether fill-in planting should be initiated. In each individual

case the conservation planner will have to consider whether fill-in planted trees are likely to be able to catch up with the rest of the stand and contribute to the final stand structure. In most cases, however, it is not recommended as the younger trees will probably be further behind and not contribute much to the final stand.

5.4.7 Fire management

Adequate protection from fire is crucial for successful *ex situ* conservation. During the evaluation of 135 *ex situ* stands of tropical pines established in the late 1970s and early 1980s (see Section 5.5.2), fire was identified as the major threat to survival of the stands. In total 20 stands had been lost, and in 14 of these fire was reported as the key factor.

Appropriate fire lines should be cleared around the stands. During dry season or when a fire risk exists the fire lines should be kept free from vegetation. For some species, and after a certain age, controlled burnings may be desirable. Great care is necessary, however, since this method requires highly skilled staff and the existence of solid local experience.

5.4.8 Protection

In the early stages stands should be carefully monitored for any attack by insects or diseases. If an attack which may threaten the survival of the stand is identified, protective measures should be initiated following the best locally proven method. On the other hand, the outbreak of substantial pest problems should be considered as valuable information with respect to the appropriateness of the species in question for conservation or use in those areas or environments.

Depending on the species and on the environment in which it is planted, protection from wildlife and domestic stock may be critical in the early stages. In general, fencing with subsequent regular maintenance of the fence and monitoring of the stand is the most effective measure against browsing animals. However, this substantially increases the potential establishment and maintenance costs and careful consideration must be given to the potential animal damage that could occur.

5.4.9 Management

Thinning

Timely thinning is especially important for *ex situ* conservation stands, since the reproductive potential of species may depend on it. The objective of thinning is to maintain healthy trees with good crown development to ensure sufficient fruiting and flowering, and at the same time to retain an adequate population size in order to keep a high level of genetic diversity in the stand.

For monocultures or species plantings, thinning should in general be based on dominant height; this means that thinning should be carried out earlier on good sites than on poorer sites. As an example, for tropical pines established at 3 x 3 m it is recommended that the height of dominant trees should be around 8 m before thinning is carried out. On drier tropical sites it is advisable to carry out the first thinning 2 years after canopy closure in order to ensure complete suppression of weeds and grasses. An overall and very important guideline is that stands should be regularly monitored (every year) to assess the status of the stand in terms of thinning and crown development and subsequently to ensure that thinning operations are initiated accordingly.

Thinning regime

Thinning can be either **systematic** or **selective**. In seed production stands and commercial plantations thinning will normally be selective in order to favour superior individuals, whereas systematic thinning aims at maintaining the gene frequencies of the original population throughout the life of the stand. Systematic thinning will go some way towards static conservation, but in a living stand some level of adaptation will always occur especially in connection with generation turnover.

Systematic thinning is particularly relevant in cases where the sampled material is based on a few mother trees and there is a risk of losing entire families at the time of generation turnover. Systematic thinning is also recommended if material is conserved at only one site. While true systematic thinning may be the most effective method for maintaining genetic diversity, it could lead to the loss of superior phenotypes of potential value for local breeding programmes. This may reduce the ability of the conservation manager to justify further investments in these stands, and potentially compromises the view that these stands should be left to a degree where they experience some local ‘natural selection’ (see Chap. 2). Some compromises will ultimately have to be made by the local conservation forester.

5.5 Experiences with *ex situ* stands

5.5.1 Hardwoods in Indonesia

In the early half of the twentieth century the Dutch established plantings of a number of hardwoods in Indonesia. The original idea was not to consider these plantings as conservation stands *per se*: at the time of establishment they were simply meant as silvicultural trials to evaluate species for plantations. Nevertheless, these stands—many of which are now 60 years old—may provide valuable experience relating to the establishment and management of *ex situ* conservation stands. Indeed, in the literature today the Indonesian stands are often referred to as *ex situ* conservation stands (Subiakto *et al*. 2001; Sidiyasa *et al*. 2001).

In 1937 the Dutch Forest Research Institute, then, after independence, the Forestry and Nature Conservation Research and Developmental Centre (FNCRDC), established eight demonstration forests in western Java, which harbour dipterocarp collections of 5 genera and 41 species from Sumatra, Bangka, Java, Kalimantan and Maluku. Various studies have been conducted from these demonstration forests, including growth, yield, pest and disease and flowering patterns. The dipterocarp stands at the demonstration forests have become important seed sources for planting programmes. Thus, even though they were actually designed for research purposes, some of the plots are now considered valuable field genebanks.

As there is an increasing trend of encroachment and illegal logging even in protected areas, it has been argued that the safest way to conserve dipterocarp species is by *ex situ* conservation (Subiakto *et al*. 2001). However, as the demonstration forests also show, maintenance of dipterocarps *ex situ* is by no means simple (see Table 5.1). A number of stands have been lost outright, and most of the remaining stands have been diminished to a very few trees. To serve any purpose for conservation these stands will have to be infused with new material in order to maintain a minimum of genetic variation. Furthermore, most of the stands have reached maturity, which raises the question about regeneration. For some stands this will be easy, as abundant natural regeneration is now found within the stand. But for most stands there are only a few seedlings, or none at all.

For some species regeneration may be ensured by simply collecting seed to establish a new plot, but others will prove more problematic to regenerate. This is amply illustrated by *Dryobalanops lanceolata.* Three *ex situ* stands of this species were established in three

Table 5.1 Selected dipterocarp collections at FNCRDC demonstration forest

Species	Planting site	Planting year	Origin	Number of trees left	Natural regeneration
Dipterocarpus gracilis	Darmaga	1957	Sumatra	16	Few saplings
Dipterocarpus haselthii	Carita	1957	Java	8	Few saplings
Dipterocarpus tempehes	Haurbantes	1940	Kalimantan	21	None
Dryobalanops lanceolata	Haurbantes	1954	Kalimantan	2	No fruit, flowering only
	Pasir Hantap	1973	Kalimantan	6	Do not flower yet
	Darmaga	1987	Kalimantan	0	None
Hopea bancana	Haurbantes	1954	Sumatra	57	Few saplings
Hopea mengarawan	Haurbantes	1954	Sumatra	4	Plenty
		1958	Sumatra	34	Few
		1974	Sumatra	0	None
Shorea acuminatissima	Haurbantes	1940	Kalimantan	2	No fruit, flowering only
Shorea javanica	Pasir Awi	1958	Java	0	None
Shorea laepifolia	Haurbantes	n.a.	n.a.	0	None
Shorea leprosula	Haurbantes	1940	Kalimantan	3	Plenty
Shorea macrophylla	Haurbantes	1940	Kalimantan	0	Regeneration abundant. Poles up to 20 cm dbh.

For full list, see Subiakto *et al*. (2001)

different sites (see Table 5.1). One stand was lost; another, established in 1973, is down to six trees with no flowering yet observed. This shows that it may be many years before some of these long-lived trees initiate flowering, or perhaps that the flowering is somehow disrupted in the new environment. In the third and oldest stand, dating from 1954 and established at a different site, flowering is observed but no fruiting. This could be the result of a range of problems, such as lack of pollen or pollinators or critical temperatures during seed set after pollination or fertilization.

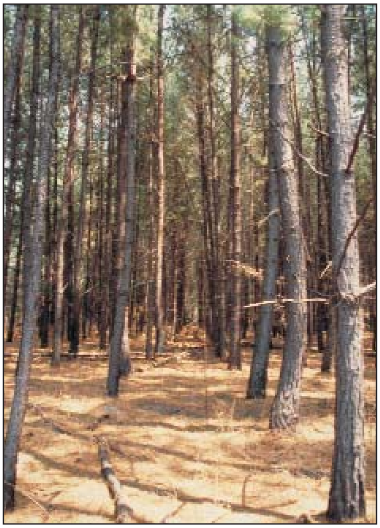
5.5.2 Tropical pines from Central America

Natural populations of *Pinus caribaea*, *P. oocarpa* and *P. tecunumanii* in Central America are under intense pressure from agriculture, grazing, overexploitation and fire. For this reason a network of *ex situ* conservation stands of the three pine species was established in the late 1970s (FAO 1985). DFSC and FAO recently assessed stands in 8 different countries to document their conservation status. The study included 135 *ex situ* conservation stands with a total area of 950 ha. The main conclusions are given below.

- Stands had been successfully established and survival was relatively good, but approximately 20% of the stands have been lost. Fire was the overriding cause of loss, along with substantial encroachment and illegal cutting that had damaged a number of stands to varying extents. Most stands were smaller than the 10 ha recommended at the time of establishment, but were

Assessing the success of ex situ *conservation of tropical pines. (Allan Breum Larsen/DFSC)*

nonetheless considered to have a sufficient number of individuals to secure an acceptable level of genetic variation.
- Only about one-half of the *ex situ* stands fulfilled the requirements for isolation from possible contaminating pollen sources. In most countries several stands were planted at one site, typically close to research or gene conservation stations. Grouping stands at one site provided advantages in terms of protection and management, but made it difficult to secure appropriate isolation.
- Flowering and seed setting were also generally very poor. The poor environmental match of many provenances may have reduced their reproductive potential. Moreover, stands were rarely thinned, which restricted crown development and thereby flowering.
- Poor isolation from contaminating pollen sources and limited cone and seed production restricted the use of the stands as seed sources. Therefore, regeneration of the stands seems doubtful. The costs involved and the poor prospects for recovering part of the expenses by seed sales will probably hamper the interest of institutions in regenerating the stands at the end of their rotation (Theilade *et al*. 2001).



Lack of thinning in an ex situ *conservation stand of* Pinus tecunumanii *restricts seed setting and compromises regeneration (Chati, Zambia, 1997). (Allan Breum Larsen/DFSC)*

5.5.3 Regeneration—the bottleneck for *ex situ* conservation stands

Comparing the experience from the Indonesian *ex situ* stands with that of *ex situ* conservation stands of tropical pines established some 30 years later, it is interesting to note the astonishing similarities in what went well and what failed.

For both the dipterocarps and the tropical pines it proved possible to establish stands over a wide range of conditions, and to some degree maintain the populations over several decades. In both programmes, however, it was difficult to ensure that the integrity of the stands was maintained. Most importantly, both programmes pointed to the uncertainty of regeneration, and this is likely to prove the weakest point of *ex situ* conservation stands. The question of regeneration of many *ex situ* conservation stands remains to be properly addressed (Cohen *et al*. 1991). Regeneration protocols have to be developed and practices implemented as older collections mature; otherwise there will be no security for these genetic resources.

5.6 Suggestions for developing *ex situ* gene conservation populations

5.6.1 General considerations on establishment of *ex situ* conservation stands

Tree species have an extraordinary diversity of reproductive systems, and these differences are major factors affecting the patterns of genetic variation within and among populations (Wilcox and Murphy 1985). In brief, self-pollinating and vegetatively reproducing species will vary more genetically between than within breeding populations (Allard 1960). For outbreeders, however, especially dioecious species, the reverse holds true though differences in gene frequency between reproductively isolated populations will tend to increase over time. In self-pollinating species, representative samples of a wide range of breeding populations should be sampled, and can be represented by comparatively few individuals. For outbreeders, individual populations should be well sampled, but fewer representatives of different populations will generally be necessary (Ashton 1988).

Burdon (1988, 1995) discusses issues involved in developing *ex situ* populations. The number of mother trees collected from and the final size of the *ex situ* stand will of course depend on the objective of the stand and funds available. As mentioned in Chap. 3, and suggested by Johnson *et al*. (2001), a minimum of 50 unrelated individuals per population should be used to establish a gene resource population. In order to ensure adequate conservation of the sampled material, each sampled mother tree needs to be represented by a reasonable number of progeny in the conservation stand. For example, if 50 unrelated trees were used to establish the conservation stand, using about 30 progeny per mother tree would provide a stand of 1 500 stems. Although this number does not generally meet the requirements we have proposed previously (for example a minimum size of 10 ha), it does show that a high level of genetic diversity can be conserved in a few thousand trees. The minimum of 10 ha is usually stipulated because it is the final number of stems at the time of rotation that is important rather than the number planted, and many natural losses as well as planned thinnings will occur. In addition to the genetic considerations it must be ensured that stand sizes are kept at a manageable level and that the burden of future management and regeneration is within the capacity of the institution in charge.

As mentioned earlier, multiple stands should be established to spread the risk of losing a population (see Table 5.1). If regeneration plans depend on wind pollination rather than controlled pollination, which is most often the case, larger stands should generally be considered in order to ensure that the pollen component in the next generation is from the appropriate population. (FAO (1992) has also provided additional general guidelines for establishment of *ex situ* conservation stands using common plantation practices.)

5.6.2 Pure or mixed conservation stands?

As mentioned in Section 5.3.1, conservation of forest trees *ex situ* has been mainly restricted to pioneer species, for which seeds are readily available, stored, and easy to propagate and grow. The design of *ex situ* stands has followed plantation practices, implying monocultures and even-aged stand structure. The use of single species and even-aged plantation design is feasible provided that resources are available for intensive management and regeneration. Using this design, the conservation officer will, after relatively few years, have to initiate thinning and start considering how to make the generation turnover, i.e. collect seed and establish a new stand. This is a costly procedure if it is not combined with utilization of the trees (Kjær *et al*. 2001). Furthermore, the plantation design may not be suitable for many tropical forest tree species of subsequent succession stages (see Box 5.1). Late-succession species usually occur in mixed and uneven-aged stands and are often shade tolerant. For conservation of these species to succeed *ex situ*, issues such as nurse crops, mixtures of trees, reproductive ecology, including maintenance of pollinators and the collection and handling of recalcitrant seed need to be addressed.

Thus, if conservation of trees *ex situ* is to play a role in the conservation of the numerous threatened tropical tree species, and not just for a few pioneer species, it may be necessary to think in terms of larger areas with a mixture of species. This will favour natural regeneration and long-term stability of established *ex situ* populations.

Box 5.1 Mixing species from several succession phases in *ex situ* plantings

The São Paolo Forest Institute has been conserving native Brazilian forest trees *ex situ* in its breeding programme since 1979. Initially, all species were established in pure stands. However, starting in 1990, with the discovery of the importance of respecting succession stages for better adaptation and growth of most native forest species, experimental mixed plantings were set up, combining several succession-phase species such as pioneers, initial secondary, late secondary and climax species. One such example is the experiment with *Guazuma ulmifolia* (pioneer) and *Genipa americana* (secondary), combined with *Peltophorum dubium* (initial secondary), *Myracrodruon urundeuva* (late secondary) and *Esenbeckia leiocarpa* (climax).

Conservation is in the form of plantations in experimental designs, which allows for the study of silvicultural behaviour, heritability of traits, population structure and monitoring of genetic variability. Another advantage is the possibility of transforming the experiments into seed orchards, which permits the recombination of material and perpetuation of populations by using their seeds for reforesting altered and degraded areas. The use of low-intensity selection within the *ex situ* field banks will allow the production of bred seeds with high genetic variability.

Source: Sebbenn *et al*. (2001)

5.6.3 International cooperation and donor concerns regarding *ex situ* conservation

Most threatened tree species are found in developing countries where funds for conservation programmes are limited. In some instances *ex situ* conservation of forest genetic resources may be the only option, but it is also a long-term activity with a large initial investment and continuing cost. Donor agencies have increasingly incorporated environmental considerations into their international development activities, but support is generally provided for protection of plants *in situ* because of the urgent need to protect ecosystems in the face of imminent change. Furthermore, *ex situ* conservation presents few immediately tangible benefits except for employment.

In order to partly ease the problems with funding, *ex situ* genetic conservation programmes may be successfully carried out by multiple organizations working cooperatively. Examples include the provenance studies carried out by IUFRO in the past and the current efforts of the Central America and Mexico Coniferous Resources Cooperative (CAMCORE). CAMCORE is a cooperative working to establish *ex situ* gene resource populations of tropical species of which many are threatened. At present, 24 organizations are members of the cooperative. Successful *ex situ* populations have been established for 22 conifer and 13 hardwood species (CAMCORE 2000).

Ex situ conservation is also the concern of international agencies such as IUFRO, IPGRI and FAO that have been instrumental in drawing global attention to the need for collection and conservation of forest genetic resources. *Ex situ* programmes coordinated through multilateral organizations usually have a reasonable time horizon for funding because of the commitment from member governments. However, secure long-term funding is rarely available because donors continuously reassess priorities and redirect limited funds.

Therefore, international centres cannot carry the conservation responsibility alone (Plucknett *et al*. 1987). In addition, many forest trees are outside the mandate of international organizations and are responsibilities of national programmes, many in the developing world.

5.7 Discussion and conclusions on the role of *ex situ* conservation stands

Ex situ conservation of tropical forest trees is hampered by the very large number of taxa that require protection, the large area needed for the cultivation of trees, and the lack of adequate methods for long-term storage of seeds of many species. Moreover, once in cultivation, continual propagation by seed would be limited in many species because pollinators may not be abundant or even present at the new site. On the positive side, the long life cycles of many tropical trees should ensure survival of the original material for many years (Bawa and Ashton 1991).

Ex situ conservation stands can have several important functions, including providing material for planting and breeding programmes. *Ex situ* stands with the proper design may be transformed into seed orchards, which achieve two objectives simultaneously: conservation of genetic resources and seed production. Examples of this shift include the *ex situ* stands of dipterocarps in Indonesia (see Table 5.1) and Brazilian species at the São Paolo Forest Institute (see Box 5.1). On the other hand, if interest in maintaining *ex situ* stands is so intimately tied up with seed sales this may endanger the maintenance and regeneration of the stands once demand is low, as was the case with the *ex situ* stands of tropical pines (Theilade *et al*. 2001). Often we assume that the traits of interest to tree breeders will remain constant. We do not foresee breeding programmes losing interest in improving rate of growth, but history shows that traits can come and go in terms of importance. Examples of traits added to breeding programmes include wood density, pulping characteristics, and disease and insect resistance. Therefore, an important function of gene resource populations, whether *in situ* or *ex situ*, is to maintain variation so that new traits can be incorporated into breeding populations in the future. Besides providing material for planting and breeding programmes, the accessibility of plants in cultivation presents research opportunities that are not possible with remote and dispersed wild populations, as well as opportunities for education and for increasing public awareness that would not otherwise exist. *Ex situ* stands generate knowledge on biology and silviculture. This role is vital if we are to have knowledge about plant populations on the edge of extinction that provides a sufficient basis for their management *in situ*.

So far, *ex situ* stands have served mainly to provide material for plantations and breeding programmes, but material conserved *ex situ* could be of great relevance to rehabilitation of *in situ* sites as well. The United Nations Environment Programme (UNEP) has requested support to establish centres for *ex situ* conservation, particularly to conserve samples for restoration of ecosystems (UNEP 1990). There is no doubt that the role of *ex situ* stands in providing material for rehabilitation of altered and impoverished forest stands, protected areas, and national parks will increase in the decades to come.

As stated throughout these three volumes, it is critical to conserve as many species as possible through *in situ* conservation, as there simply will not be enough resources globally to attempt *ex situ* conservation for many tree species. Where *in situ* and *ex situ* techniques for conservation come together most closely is in reintroductions and recreation of habitat for rare and endangered species (Prance 1997). As more native habitats are lost and some tree species are conserved only *ex situ*, it will be necessary to restore suitable habitats. Future *ex situ* conservation efforts on tropical trees should focus on creating habitats to move the species back into *in situ*-like situations. By mimicking *in situ* conditions, it may be possible to facilitate natural regeneration. Such a strategy will broaden the range of species that can be considered for *ex situ* conservation in living stands, and may very well be a regular component of large-scale reforestation and rehabilitation programmes already undertaken in many countries.

EX SITU CONSERVATION THROUGH STORAGE AND USE

by Ida Theilade, Leonardo Petri and Jan Engels

6.1 Introduction

In conservation of biological diversity, when the preservation of ecosystems falters, their fragments may have to be cared for piece by piece (Conway 1988). Due to the rate of deforestation in many parts of the world, and the number of reported threatened tree species, this may well be the case for many forest species in the decades to come.

In agriculture most crop species are conserved by *ex situ* means using seed banks, field banks and, in certain cases, tissue culture. In forestry, however, because of the long regeneration time required by trees, the preferred conservation approach is to incorporate *in situ* conservation principles into sustainable forest management, increasing the areas of protected forests and in some cases complementing these with *ex situ* conservation genebanks and conservation stands. Despite the urgent need, genetic conservation applied within the framework of forest resource management has been slow to develop and *ex situ* efforts are generally restricted to species and provenances of proven value (FAO 1993).

The potential role of *ex situ* conservation of forest genetic resources, as a complement to the widely used *in situ* strategies, should be further considered. This chapter deals with *ex situ* storage of seeds, pollen, *in vitro* cultures and DNA libraries of forest tree species. *Ex situ* conservation of forest trees in living stands—arboreta, seedling banks and *ex situ* conservation stands—is discussed in Chap. 5.

The aim of *ex situ* storage for conserving woody species is to maintain the initial genetic and physiological quality of the germplasm until it is used or regenerated. Achieving this requires consideration of genetic and environmental factors affecting the germplasm in storage. Since seed storage is the most commonly used short to medium-term *ex situ* conservation strategy for forest trees which produce orthodox seeds, seed biology and its implications for storage are discussed in more detail in the following paragraphs. Then *ex situ* conservation of forest trees by storage techniques of pollen, *in vitro* cultures, and DNA libraries is reviewed with special emphasis on the relative advantages and disadvantages of each technique.

Finally, the complementary role of *ex situ* conservation to other conservation strategies is discussed and the importance of well-defined conservation objectives highlighted. In any case, the purpose of *ex situ* conservation must be carefully defined before management planning. Unless *ex situ* material is regenerated and at some stage brought into use, its conservation has no meaning.

6.2 Practical application of knowledge of biology of forest seeds

Knowledge of seed biology is crucial for the proper handling of seeds, including storage. With respect to handling, the term ‘seed’ usually refers to the unit extracted from the fruit and handled as a unit during storage, pre-treatment and sowing. Seed handling encompasses a series of procedures beginning with selection of the best quality of seed source, through collection, processing, storage and pre-treatment to germination. Each link of this chain implies a potential risk of losing seed, and all links in the process are of equal importance (although they are not necessarily equally sensitive). If a seed dies as a result of careless handling during collection or processing, even the best storage conditions will not bring it back to life. However, a handling procedure becomes unacceptably expensive if a certain loss cannot be tolerated during the process. In some cases it is more economical to collect excess seed rather than to try and ensure all seed is viable after the storage period. This balance must be weighed in each situation (Schmidt 2000). The whole process of seed storage begins with collection of seeds of good quality, both physiologically and genetically. This section focuses on the implications of physiological aspects on *ex situ* storage.

6.2.1 Seed storage categories

Most agricultural crops have seeds that can be dried and stored at low temperatures for years without losing the ability to germinate. These have been termed **orthodox seeds**, because they are considered the most usual and widespread type. However, many tree species, particularly in the tropics, have seeds that do not follow the orthodox rules. They are difficult to store because they do not tolerate drying and have therefore been termed **recalcitrant seeds**. Other seeds do not seem to fit into either of these two categories and are called **intermediate seeds**. In reality, differences among species probably form a continuum from very orthodox to very recalcitrant seed. From a practical point of view, there are two factors that are critical for seed storage: seed moisture content and storage temperature. The current definitions of the three storage categories are as follows (Thomsen 2000):

- **Orthodox seeds** tolerate drying (desiccation) to low moisture content. They also tolerate storage at low temperatures, and can generally be stored for very long periods.
- **Recalcitrant seeds** are damaged by desiccation and those of tropical species may also be damaged by exposure to low temperatures. They are said to be desiccation- and chilling-sensitive.
- **Intermediate seeds** are seeds that do not fit into either of the above two categories. They can be desiccated, although not to such low levels as orthodox seeds, and they are often sensitive to chilling.

Fertilization of the ovule starts a chain of processes, which ultimately leads to the formation of a ripe or mature fruit or seed-bearing organ containing mature germinable seeds. For most species the maturity of fruit and seed coincides with dispersal. However, in some species, such as *Ilex opaca* and *Fraxinus* spp., seeds contain physiologically immature embryos (Nitsch 1971). The seeds here are not germinable at fruit maturity but require after-ripening. The phenomenon is often described as **dormancy**. On the other hand, some seeds may mature well in advance of fruit maturity. Germinable seeds can thus be obtained from still green pods of some *Leguminosae* and unopened cones of some conifers (Sedgley and Griffin 1989). However, seeds picked very early may often have reduced storability and vigour.

Fruit development differs according to fruit type. In fleshy, animal-dispersed fruits sugar substances develop in the pulp simultaneously with a drastic increase in moisture content (Sedgley and Griffin 1989). The fruit changes colour from green to a usually bright and conspicuous red, orange or yellow, and becomes soft. Simultaneously, the pulp usually loosens easily from the seed or stone.

Loss of water and concurrent desiccation occurs late in the development of dry fruits. Thick fruit walls of capsules, samaras and large pods become woody or papery thin walls. Dehydration of the dry fruit implies disintegration of chlorophyll; the fruit changes colour from green to typically yellow, brown or black.

The late events in seed maturation include formation of storage proteins and hormones, and (in orthodox seeds) dehydration. In dry fruits, dehydration of seeds is concurrent with the general dehydration of the fruits. In fleshy fruits, dehydration is the result of the increased osmotic pressure due to sugar formation in the fruit pulp (Schmidt 2000). The final moisture content of the seeds depends on the species and the external environment. It has important implications for the storage properties of the seeds. Orthodox seeds typically dry out to 5–10% moisture content during maturation. Recalcitrant seeds maintain relatively high moisture content, typically >40–50%. Intermediate seeds also maintain relatively high moisture content.

Although recalcitrance is predominant in some plant families such as *Dipterocarpaceae* (see Box 6.1), there seems to be no phytogenetic pattern and often two closely related species show very different storage behaviour (Thomsen 2000). Table 6.1 lists some characteristics that may help to give an idea of storage behaviour.

Table 6.1 Characteristics of orthodox versus recalcitrant/intermediate seed

Orthodox seed	Recalcitrant and intermediate seed
Low moisture content at shedding	Relatively high moisture content at shedding
Includes dormant and non-dormant species	Usually no dormancy
Perennial, woody or annual, herbaceous species	Mostly perennial, woody species
Found in all ecosystems	Often found in humid ecosystems
Usually small seeds	Often large, fleshy seeds

Source: After Thomsen (2000)

Freezing injuries occur if recalcitrant seeds, which maintain high moisture content, are chilled to subzero temperatures. In addition, some species have seeds that are sensitive to temperatures between 0 and 15 °C. If a species is never exposed to low temperatures in the area of natural distribution, temperature sensitivity should be expected. *Hopea odorata* is a typical recalcitrant species from the humid tropics. It is very sensitive to desiccation and does not tolerate temperatures below 15 °C. On the other hand *Quercus robur*, a typical temperate recalcitrant species, is sensitive to desiccation, but tolerates storage temperatures of –2 °C. Thus, a clue to the behaviour of the seed can probably be found in the natural environment of the species (Thomsen 2000).

The use and conservation of particular species is often limited by problems relating to seed collection and storage. Seed viability restricts the use of the species to a narrow range from the seed source. *Azadirachta indica* seeds, for instance, can have a limited viability, which makes immediate sowing necessary, even at times that are not optimal for seedling survival (Schmidt 2000). Improved seed handling techniques have recently been worked out for this species (Sacandé 2000). Such improved techniques are a key to the increased use and conservation of many species, particularly those with non-orthodox seed storage behaviour. The recent developments in storage technologies are reviewed in Section 6.3, and the possibilities and limitations of storage as a potential long-term conservation measure for tree species are discussed.

Box 6.1 Storage of dipterocarp seeds

Dipterocarpaceae, which makes up the major component of the South-east Asian tropical rain forests, is an example of a family where recalcitrant seeds are a major constraint in cultivation and also circumvent *ex situ* storage of seeds.

Early publications mentioning dipterocarp seed storage emphasize the extremely short-lived nature of these seeds. According to a review of the literature (Tompsett 1992), the longevity of seed from 79 tropical recalcitrant species ranged from 14 days for *Shorea dasyphylla* to 365 days for *Hopea hainanensis*. In addition, reduced germination following temperatures below 16 °C (chilling damage) occurs in all moist dipterocarp seeds so far examined. Some authors have divided dipterocarp species according to the susceptibility of their seeds to chilling damage: seeds of *Shorea* species in the 'red meranti' and 'balau' groups were said to be especially sensitive, while all other dipterocarps studied were tolerant to 4 °C.

Dipterocarps have recalcitrant seeds and are therefore difficult to establish and conserve ex situ. *(Ida Theilade/FLD)*

Seeds of *Hopea*, *Cotylelobium* and *Vatica* tend to have greater desiccation tolerance than *Shorea* seeds, but most *Dipterocarpus* seeds are relatively intolerant. Several reports indicate optimum storage of recalcitrant dipterocarp seed at temperatures in the range 15–21 °C. Other *Dipterocarpus* species, for example *D. turbinatus*, are not recalcitrant.

Associations between desiccation tolerance and seed size, habitat, seed desiccation rate, and longevity have been observed among the species of the *Dipterocarpaceae*. Thus, seed of three *Shorea* species from different habitats have different desiccation tolerance. The low-rainfall species *S. roxburghii* has seed which can be dried safely to 35% whereas the monsoon or rainforest species *S. almon* and *S. robusta* cannot be safely dried below 40% moisture content. Smaller seeds dry faster and tend to be more desiccation tolerant. The seed with the greatest desiccation tolerance (*S. roxburghii*) is also the seed with the greatest longevity.

In summary, recalcitrant seeds should be kept at moisture contents above their lowest safe values. Ventilation is needed to remove toxic gases and to prevent anoxia. The value of fungicides in improving storage life of dipterocarp seeds has yet to be established. More work is needed to assess the optimum moisture content required for storage of seeds of particular species.

Based on a review of the literature on storage of dipterocarp seeds by Tompsett (1992)

6.2.2 Storage of orthodox seed

Storage of orthodox seeds is the most widely practised method of *ex situ* conservation of plant genetic resources. About 90% of the 6.1 million accessions stored in genebanks are in fact maintained as seed (FAO 1996). The techniques involve drying seeds to low moisture content (3–7% fresh weight basis, depending on the species), and storing them in hermetically sealed containers at low temperature, preferably at – 18 °C or cooler (FAO/IPGRI 1994). Seeds of many orthodox species may be conserved in this way for several decades and possibly centuries. A series of practical documents are available in the literature covering the main aspects of seed conservation, including design of seed storage facilities for genetic conservation (Cromarty *et al*. 1982); principles of seed testing to monitor the viability of seed accessions maintained in genebanks (Ellis *et al*. 1985a); methods for removing dormancy and germinating seeds (Ellis *et al*. 1985b); and methods for processing and handling seeds in genebanks (Hanson 1985).

An attempt has been made to develop a 'low-input' alternative to the conventional cold storage of seed. The technique is called **ultra-dry storage** and allows preservation at room temperature. It is considered a useful low-cost option when no adequate refrigeration can be provided. On the other hand, it has been argued that drying seeds beyond a critical moisture content may provide no additional benefit to longevity and may even accelerate seed ageing rates (Ellis *et al.* 1988; Vertucci and Roos 1993; Walters and Engels 1998). Research on various aspects of ultra-dry seed storage, including drying techniques such as sun/shade drying (Hay and Probert 2000) or vacuum/freeze drying (Côme 1983) and their applicability to a broader number of species should therefore be continued.

Box 6.2 Case study: the Millennium Seed Bank Project

The Millennium Seed Bank Project aims to conserve seed collections of woody and other species, primarily from the world's tropical and subtropical drylands. The Project has evolved from work on wild species started by the Royal Botanic Gardens (RBG), Kew, UK in the 1960s and builds on years of collaboration with botanical, agricultural and forestry organizations across the globe. The impetus of the Convention on Biological Diversity (CBD), and the threat that some 48 000 plant species may be brought to the edge of extinction within 20 years, has influenced the ambitious aim of conserving seed samples from 24 000 plant species during the years 2000–2009. The Project is managed by RBG Kew's Seed Conservation Department (SCD) based at the Wellcome Trust Millennium Building, Wakehurst Place, West Sussex, UK. The Project receives substantial funding from the UK's Millennium Commission, the Wellcome Trust and Orange plc.

Before the main phase of the Project began, the collection numbered over 11 000 population samples of 5 500 plant species. All samples are stored long-term to internationally accepted standards. Included within the existing collection are base collections of land stabilization and agroforestry species held on behalf of FAO/IPGRI and ICRAF.

Samples are collected and, where permitted, distributed, under material transfer

continued

The Millennium Seed Bank Project. Scientists from Kenya discuss the processing of Combretum collinum *seed with a member of RBG Kew staff at the Wellcome Trust Millennium Building. (Trustees of the RBG, Kew)*

agreements (MTAs). Seed samples are provided in small research quantities only. The key purpose of the collection is to underwrite conservation. This contrasts with many forest seed collections where more immediate use is the aim. Sampling has been directed initially at the species level, with the aim of at least one population sample per species. Within each population, the sampling aims to capture a wide representation of the alleles present. These initial samples will be used to learn more about the biology of the species and thereby formulate further sampling strategy.

In order to minimize the need for regeneration, with all of the attendant problems, it is important that sample life is maximized. Therefore, a significant research programme, based on some 25 years of experience, underpins the Project. This work feeds into a major training initiative and a database that should facilitate seed conservation in many countries.

6.2.3 Storage of non-orthodox seed

Seeds which shed at relatively high moisture content (>40–50% fresh weight) are unable to withstand desiccation and are often sensitive to chilling. These seeds therefore cannot be maintained under conventional seed storage conditions and are described as recalcitrant (Chin 1988). Even when they are stored in an optimal manner their life span is limited to weeks, occasionally months. An IPGRI protocol to determine the precise seed storage behaviour of unknown species is available (Hong and Ellis 1996).

Of more than 7 000 species for which information on seed storage behaviour has been published, approximately 3% are recorded as recalcitrant and an additional 4% as possibly recalcitrant (Hong *et al.* 1996, http://www.ipgri.cgiar.org/themes/exsitu/seed_compendium.htm). However, the percentages of species producing intermediate and recalcitrant seed cited are likely to be largely underestimated. These figures are based on scientific and technical publications, which, by default, concern mainly temperate species. It can be expected that a large proportion of the species for which no information is available, which are predominantly of tropical or subtropical origin, exhibit recalcitrant seed storage behaviour. As an example, it has been estimated that a majority of the species in tropical forest ecosystems have recalcitrant seeds (Ouédraogo *et al.* 1996).

More recent investigations have identified species exhibiting intermediate storage behaviour. The term has been used to describe seeds which tolerate a greater degree of drying than recalcitrant seeds but are less desiccation-tolerant than orthodox seeds (Ellis *et al.* 1990, 1991). Furthermore, intermediate seeds are often sensitive to chilling. Even though a continuum in desiccation sensitivity is observed from highly desiccation-sensitive to relatively tolerant (Berjak and Pammenter 1994), it may be impossible to achieve the long-term conservation at –20 °C that is possible for orthodox seeds. Included in the intermediate category are some economically important species such as coffee, citrus, rubber, oil palm and many tropical forest tree species.

Before undertaking any technically demanding research on methods to improve storage of recalcitrant and intermediate species, it is appropriate to examine the development pattern of seeds and perform preliminary experiments to determine their desiccation sensitivity as well as to define storage conditions. In collaboration with DFSC and numerous institutions worldwide, IPGRI has developed a protocol for screening tropical forest tree seeds for their desiccation sensitivity and storage behaviour (see Box 6.3). Even if such experiments do not make it possible to define long-term storage conditions, increased storage periods may make all the difference for the use of a species if it allows storage of seeds until the next planting season, or transport from the seed source to a prepared planting site.

6.3 Other techniques for storage of non-orthodox species

Despite decades of research on storage methods for recalcitrant seeds, only limited progress has been made. For the storage of non-orthodox species, it may therefore be necessary to turn to the other techniques listed in this section.

6.3.1 Cryopreservation

This term refers to seed storage at ultra-low temperature, usually in liquid nitrogen (–196 °C). Coupled with *in vitro* culture, this technique often represents the only safe and cost-effective

Box 6.3 Recalcitrant tropical forest tree seed project

The use of many high-value, tropical forest tree species in tree planting and conservation programmes is hindered by problems associated with seed handling and storage. The seeds of a large proportion of tropical tree species lose viability very fast and die soon after they have been collected from the tree. They are said to be recalcitrant seeds because they are difficult to handle and are difficult to store over long periods of time.

Some of the species that have recalcitrant seeds are highly desirable, but difficult seed handling often prevents their use. In order to try and overcome the problem, IPGRI and DFSC, together with national institutes, initiated a project on handling and storage of such seeds. In the first phase of the project, a protocol for determining the minimum moisture content and optimal storage conditions was developed and tested on about 30 tropical forest species of economic interest.

In the second phase of the project (1999–2001), more than 20 national partners from Africa, Asia and the Americas worked together to screen the desiccation tolerance and storage behaviour of several additional tropical forest tree species. A total of 52 species was studied and they were all screened by two replicating institutions.

continued

Regional training workshops in three continents provided practical experience to technicians from national institutions in the application of the screening protocol developed in the first phase. The workshops also provided opportunities for collecting and replicating partners to meet and discuss practical problems on their target species.

Seeds of Neobalanocarpus heimii *germinate immediately after seed fall, hindering supplies for conservation and reforestation programmes. (Ida Theilade/FLD)*

Communication between project members was fostered through an informal research network. The network was maintained through a newsletter, which has proved to be an effective tool for publishing and disseminating the protocol and technical papers as well as other relevant information on trials initiated, progress achieved, practical problems encountered and suggestions to solve them. The results are available on the DFSC website (www.dfsc.dk). Furthermore, a compilation of the results from the project is available in a book currently in press (Sacandé *et al.* 2004).

The results showed that many of the species are less difficult than expected. Slight modifications of the procedures may lead to longer safe storage periods. For instance, a light drying of the seeds or storage at a certain temperature will in some cases make it possible to keep the seeds for a couple of months instead of a few weeks. These results may make all the difference for the use of the species. In some tropical countries, for example Vietnam, there are more than 1 000 indigenous species of which some hundreds would be desirable to grow. If proper seed handling methods could be established, some of these species could be included in the tree planting programmes contributing to the national economy and greatly improving the biodiversity of the country.

The image above shows seeds of *Neobalanocarpus heimii.* Locally known as Chengal, this species is highly sought after because of its durable and heavy timber properties. Endemic to peninsular Malaysia, it is listed on the IUCN Red List as vulnerable. Its highly recalcitrant seed germinates 4–7 days after seed fall, which hinders seed supplies for conservation and reforestation programmes.

option for storage of non-orthodox species. Seeds of mahogany (*Swietenia macrophylla*) or neem (*Azadirachta indica*) are relatively small and tolerant to desiccation, and can thus be cryopreserved directly after partial desiccation (Hu *et al*. 1994; Marzalina 1995; Berjak and Dumet 1996). In cases when seeds are not amenable to cryopreservation, excised embryos or embryonic axes should be used. In this case, selecting embryos at the right developmental stage is critical for the success of cryopreservation.

In addition to the desiccation technique preferentially employed for freezing, other methods, including pregrowth–desiccation, encapsulation–dehydration and vitrification should be experimented with (Engelmann 1997; Engelmann and Engels 2002).

With species for which attempts to freeze whole embryos or embryonic axes have proven unsuccessful, various authors have suggested using shoot apices sampled on the embryos, adventitious buds or somatic embryos induced from embryonic tissues (Pence 1995; Berjak *et al*. 1996). This might be the only solution for species that lack well-defined embryos. However, in this case, more sophisticated tissue culture procedures have to be developed and mastered. Moreover, using adventitious explants would reduce the range of genetic variability sampled (Pence 1995; Berjak *et al*. 1996), especially when using somatic embryogenesis, since response to inducing treatments is generally highly genotype-specific and somatic embryo cultures might be obtained from only a limited number of genotypes (see for example Percy *et al*. 2000). The disadvantage of cryopreservation is the overall difficulty of regeneration of whole plants.

6.3.2 Seedling conservation

This technique refers to the conservation of young seedlings arrested in their development by storage at low temperature and/or under low light intensity. This technique has been tried out with *Symphonia globulifera* and *Dryobalanops aromatica* (Corbineau and Côme 1986; Marzalina *et al.* 1992).

6.3.3 *In vitro* conservation

This conservation method involves the maintenance of explants in a sterile, pathogen-free environment and is widely used for the conservation of species which produce recalcitrant seeds or no seeds at all, or for material which is propagated vegetatively to maintain particular genotypes (Engelmann 1997). Although research on *in vitro* techniques only started a few decades ago, this technique has been applied for multiplication, storage and the collection of germplasm material for more than 1 000 species (Bigot 1987).

In vitro techniques can be effectively used for collection, multiplication and storage, particularly with problematic species (Engelmann 1997). Techniques have been developed to introduce recalcitrant seeds and vegetatively propagated material *in vitro*, under aseptic conditions, directly from the field (Withers 1995). This approach will allow germplasm collections to be made in remote areas (in the case of highly valuable recalcitrant seeds, for example), or when the transport of the collected fruits would become prohibitively expensive because of their size or weight. Also, in cases where the target species would not have seeds to be collected, or when budwood would quickly lose viability or is highly contaminated, the establishment of aseptic cultures in the field will facilitate collecting and improve its efficiency (Engelmann and Engels 2002). Plant material with high multiplication rates can be propagated in an aseptic environment through tissue culture systems such as micropropagation and somatic embryogenesis. The miniaturization of explants is an interesting option to reduce space requirements and consequently labour costs for the maintenance of germplasm collections (Ashmore 1997).

Different *in vitro* conservation methods can be employed, depending on the storage duration required (Engelmann 1997; Withers and Engelmann 1998). For short- and medium-term storage, various techniques have been devised which make it possible to reduce growth and to increase the intervals between subcultures. *In vitro* conservation techniques using slow-growth storage have been developed for a wide range of species, including temperate woody plants, fruit trees and horticultural species. Despite the availability of such techniques, the *Report on the State of the World's Plant Genetic Resources for Food and Agriculture* (FAO 1996) indicates that worldwide only around 38 000 accessions are conserved *in vitro*. Most conservation programmes are unable to meet requirements for relatively sophisticated equipment, reliable electricity supply and trained staff. In addition, it should be borne in mind that only a limited amount of genetic diversity can be maintained *in vitro*.

6.3.4 Pollen storage

This technique is comparable to seed storage, since most pollen can be dried to less than 5% moisture content on a dry weight basis and stored below 0 ºC. Some species, however, produce pollen with recalcitrant-type storage characteristics. There is limited experience on the survival and fertilizing capacity of cryopreserved pollen more than 5 years old (Towill 1985). However, pollen has a relatively short life compared to seeds (although this varies significantly among species), and viability testing may be time-consuming. For these reasons, despite being a useful technique for species which produce recalcitrant seed (IPGRI 1996), pollen storage has been used only to a limited extent in germplasm conservation (Hoekstra 1995). Other disadvantages are the small amount of pollen produced by many species; the lack of transmission of organelle genomes via pollen; the loss of sex-linked genes in dioecious species; and the lack of plant regeneration capacity, although there are indications that pollen can be regrown into whole plants (Hoekstra 1995). On the other hand, pollen transfer of pests and diseases is rare with the exception of some virus diseases, thus allowing the safe movement and exchange of germplasm.

6.3.5 DNA storage

This storage method is rapidly increasing in terms of importance. DNA from the nucleus, mitochondrion and chloroplasts is now routinely extracted and immobilized into nitrocellulose sheets where it can be probed with numerous cloned genes. With the development of the polymerase chain reaction (PCR), it is now routinely possible to amplify specific oligonucleotides or genes from the entire mixture of genomic DNA. These advances have led to the formation of an international network of DNA repositories for the storage of genomic DNA (Adams 1997). The advantage of this technique is that it is efficient, simple and takes up little space. The main disadvantages, besides demanding requirements for capacity and equipment, lie in problems with subsequent gene isolation, cloning and transfer. The obvious problem is that it does not allow the regeneration of entire plants (Maxted *et al.* 1997).

6.4 Some considerations on *ex situ* storage of trees

The design of any *ex situ* conservation programme, and the decision about which technologies to use, must start from considerations of the biological material in question: why do we want to conserve it, and how is it going to be used in the future? Purpose must be strictly defined as a prerequisite to management planning. In the following, some important limitations and options inherent in *ex situ* conservation with seed are discussed.

6.4.1 The genetic consequences of *ex situ* conservation

A serious problem with *ex situ* seed storage is that due to natural variability within samples, 'artificial' losses of alleles inevitably occur. In other words, if a new population is established from seed which has been stored for a period of time, the progeny have been subjected to an artificial selection the genetic consequences of which we generally do not know. Whether *ex situ* conservation takes the form of plants, seed or tissue culture, genetic rejuvenation through controlled cross-pollination of regenerated plants is periodically obligatory, and new 'selected' gene combinations are inevitably introduced each time. The extent of the problem, with respect to absence of natural selection in storage, depends on the generation time of the species and the population structure. On the other hand, in some circumstances, an absence of true natural selection could be an advantage in maintaining potentially useful alleles that might have been lost in the wild.

Ex situ storage of small samples can therefore be expected to lead inevitably to unpredictable genetic change. The proportion of fit genotypes in progeny intended for reintroduction into the wild can thereby be greatly reduced, especially if the original wild donor populations are already small and in decline, and hence already suffering an accumulation of deleterious gene combinations (Hedrick 2004). For all practical purposes, therefore, *ex situ* conservation of species leads inevitably, and perhaps irreversibly, to some form of domestication (Ashton 1988), whether useful or not.

6.4.2 Limitations of science and technology

When evaluating the effectiveness and limitations of presently available *ex situ* methods for genetic conservation of perennial woody species, it becomes clear that, with present knowledge, the storage of recalcitrant seed, the storage of pollen and *in vitro* culture are viable only as short-term measures. The only secure medium- to long-term *ex situ* conservation method presently available for large-scale use in forestry is the storage of orthodox seeds. Problems related to regeneration of seed stocks must always be kept in mind. Currently, all methods of *ex situ* preservation of live plant material require periodic regeneration and sexual reproduction of the stock. The latter presupposes some knowledge of the breeding system and pattern of genetic variability of the species concerned. This knowledge is often not available for tropical tree species. Thus, storage *per se* may not be the most difficult problem in long-term *ex situ* conservation (FAO 1993).

The task of *ex situ* conservation is not made easier by the many cases where there may be mutual interdependence on other species. For example:

- Oaks (*Quercus* spp.) cannot generally be regenerated without symbiotic fungal flora on their roots, on which they depend and which differs between species.
- Large and heavily protected fruits from a large class of valuable timber trees of *Meliaceae* and *Burseraceae* in South-east Asia appear to be dispersed exclusively by hornbills, as only the strong bills of these birds possess the capacity to open the thick husks and extract the large arilate seeds from within (Terborgh 1990).

In some cases, such trees and their symbionts will prove exceptionally difficult to conserve *ex situ*. However, intensive care and biotechnology can preserve some diversity that would otherwise be lost.

Unfortunately, technically demanding applications often come at a high cost, and whereas the capability and the money to apply advanced technologies to preservation is located mostly in wealthy northern countries, the largest problems of species' loss are in poor tropical countries. *Ex situ* interventions do not contribute to habitat preservation. For less developed countries, habitat preservation might be the only realistic conservation strategy.

6.4.3 What is the specific role for *ex situ* storage of trees?

True orthodox seeds (from species such as *Pinus, Picea or Acacia*) have been successfully stored for up to 50 years. With improved protocols for seed collection, handling, processing and storage, the longevity of orthodox seeds could be extended to 75–100 years (FAO 1993). On the other hand, despite two decades of research towards developing effective storage methods for conserving seeds of recalcitrant tree species, only short-term storage methods have been developed, which extend storability from a few days or a few weeks to 8–12 months. These results were achieved by cryopreserving excised embryos from *Hevea brasiliensis* and *Theobroma cacao*, followed by regeneration using *in vitro* techniques (FAO

1993). Such methods are too expensive for activities on an operational scale, especially in developing countries. However, the potential for cryopreservation has been demonstrated and some institutions like Fort Collins in the USA and the National Bureau of Plant Genetic Resources (NBPGR) in India are looking at the longer-term prospects. At present, *ex situ* technology is a treatment that can be used to buy time to preserve options for a few populations and species judged to be of special value.

In some cases *ex situ* storage may be the only option for conservation over a short period of time before the species is reintroduced in its natural environment. The loss of wild populations is not always the result of irreversible habitat change. It can come about for transient economic or cultural reasons, such as unsustainable harvest or temporarily intensified slash and burn, or for correctable environmental reasons, such as certain mining operations, ongoing construction or introduced environmental weeds. Where the threat and destruction may be temporary it makes sense to relocate or store a threatened population for later reintroduction (Ashton 1988). In such cases the genetic make-up of the species should as far as possible be maintained (see Box 6.4 on the toromiro tree, *Sophora toromiro,* from Easter Island).

Ex situ seed samples can effectively serve as means to conserve important characteristics of the populations, and therefore important attributes of the species. *Ex situ* conservation programmes for provenances of tropical pines represent just such an example (CAMCORE 2000). For most forest tree species, specific gene combinations can be conserved only by conserving the genotype, which typically involves some kind of clonal propagation. Thus, it is imperative for long-term conservation that managers of *ex situ* population samples define, as carefully as information allows, the characteristics they are intending to conserve: all alleles in a subsample or only some, and, if some, then which? Once a clear set of priorities has been defined, the role of what in effect will become a form of 'gene library' will be considerable (Ashton 1988).

Box 6.4 *Sophora toromiro (Leguminosae):* the lost tree of Easter Island

Easter Island (Rapa Nui) is one of the most remote and smallest inhabited islands. With an area of a little more than 150 km^2 it shares with many other small islands the fate of having suffered dramatic environmental degradation and a high level of species extinction. One of the extinct plants of Easter Island is the toromiro tree (*Sophora toromiro*). It has, however, survived in cultivation in several botanical gardens.

With the main stage of environmental destruction on Easter Island having passed, the opportunity now exists to restore degraded habitats and reintroduce lost components of the flora. The conservation and reintroduction of *S. toromiro* is being coordinated by the Toromiro Management Group, a collaborative consortium of botanic gardens, geneticists, foresters and archaeologists. The first scientific collection of *S. toromiro* was made in 1774 during the second of Cook's voyages. Notes at that time suggest that the species survived as scattered thickets on the island and that its valuable timber had many uses: for building materials, household articles, canoes and carvings. The causes of loss of original shrub and forest can be traced to the ecological change following colonization by Polynesians around 400 AD. Already the first European visitors commented on the island's treeless state, and the process of degradation was completed in 1866 with the introduction of rabbits, sheep, pigs, horses and cattle.

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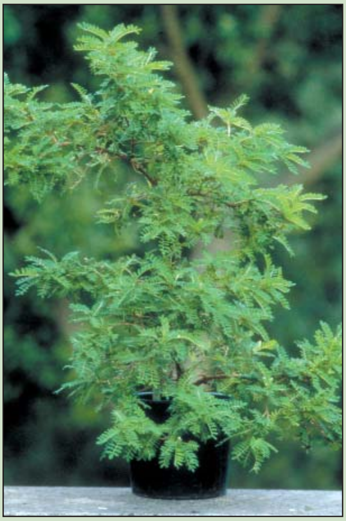
The last surviving wild *S. toromiro* specimen was growing on the inner slope of Rano Kau crater, protected from predation by livestock. This specimen survived until 1960 when it was chopped down for firewood. It was also the source of the seeds collected by Thor Heyerdahl during the 1955–56 archaeological expedition to Easter Island. The seeds were initially presented to the Naturhistoriska Riksmuseet in Stockholm but in the autumn of 1958 seeds were transferred to the Göteborg Botanical Garden and six or seven were sown. Stocks currently in cultivation descended from four seedlings germinated at the Göteborg Botanic Garden in 1959. This initial stock was derived from one tree and was likely to have arisen as a result of self-pollination. In addition, *S. toromiro* is grown at the National Botanic Garden in Chile, and there are a number of trees growing in private gardens and nurseries, but lack of documentation on their origin obscures their conservation value.



S. toromiro*—a descendant of the last surviving wild tree of Easter Island grown from seed at the Botanic Garden, Copenhagen. (Omar Ingerslev)*

The Toromiro Management Group has undertaken a global search and located all available toromiro trees in cultivation. A directory of trees is held at the Royal Botanic Gardens, Kew, UK, with associated data from genetic fingerprinting. It is evident that very little genetic diversity exists within the surviving population. The species apparently persisted at low population numbers over a number of decades; between 1917 and 1960 it probably persisted as one wild individual. It is likely that this bottleneck will have implications for the taxon's long-term survival, which will depend on efficient management of the surviving genotypes. Using genetic data, a propagation programme is being designed to establish plantations of trees in Europe, Chile and Easter Island. The Toromiro Management Group is working with the Corporación Nacional Forestal on the repatriation of toromiros to Easter Island. In 1995 an experimental introduction using 150 plants from Bonn and Göteborg botanic gardens was undertaken, but failed.

The future of the toromiro tree lies in promoting and maintaining collaboration between botanic gardens holding stock and the conservation authorities in both Chile and Easter Island. A single-species conservation project like this will only succeed if integrated within broader issues of protected area planning and habitat restoration. This in turn needs to be put into the context of the island's prevailing social and economic requirements. Easter Island has a population of 2 800 people, so any planned reintroduction programme must take into account the views and opinions of the islanders. Furthermore, the planned reintroduction and restoration programme will work within the constraint that the habitat degradation on Easter Island has been so profound that natural establishment of toromiro trees is no longer feasible. Efforts will focus initially on establishing cultivated field genebanks and assess the ability of restored island habitats to support reintroduced populations.

Toromiro is one of the few examples of *ex situ* storage and cultivation having prevented (or delayed) extinction of a tree species. But it is also an example of how difficult it is to reinstate a flora component once associations and habitat of the species have been altered. Hopefully the toromiro tree will soon again form part of the biological and cultural heritage of Easter Island.

Based on Maunder (1997) and Maunder *et al*. (1999)

The immediate role of *ex situ* conservation of rare and endangered trees lies more in research and education *per se* (see Section 5.7). This role is absolutely vital if we are to have knowledge about plant populations on the edge of extinction that provides a sufficient basis for their management. This point is brought home most poignantly by consideration of the humid tropics, where species diversity is at its greatest. There, long-lived perennials are in the majority, making cultivation easier, but few plants possess seed dormancy and many are of formidable size. Knowledge of the cultural requirements of the minority is non-existent. It is obvious that *ex situ* conservation cannot be an end in itself under such circumstances (Ashton 1988).

As we have said throughout these guidebooks, *ex situ* methods are complementary to *in situ* conservation, not an alternative. Of course, the fact remains that tree species, populations and alleles are becoming extinct in the wild, and some can and should be conserved *ex situ*. When extinction in the wild becomes a reality, the means to control artificial selection is greatly reduced, and the possibility to reintroduce alleles lost in culture is no longer available. Nonetheless, *ex situ* conservation must often be deemed preferable to loss (Raven 1976). In these cases a conservation strategy for a particular tree species, population or character requires a holistic approach, combining the different *ex situ* and *in situ* conservation techniques available. Selection of appropriate methods should be based on a range of criteria, including the biology of the species in question (Engels and Wood 1999), practicality and feasibility of the particular method chosen, as well as the cost-effectiveness and security afforded by its application (Maxted *et al*. 1997).

CHAPTER 7

CONCLUSIONS

by Alvin Yanchuk

Ex situ and *in situ* conservation—managing for a continuum of genetic diversity

Ex situ conservation will continue to be an important part of the maintenance and management of forest genetic resources. As with any of the approaches in gene conservation, not all aspects can be covered by one approach—each has to be evaluated with respect to the objectives of the conservation effort, the biological limitations of the species and the limitations of the organization.

Genetic variation is ultimately affected by genes that vary in their frequencies within and among populations. However, this variation is usually gradual across the distribution of a species, so genetic diversity is usually a continuum of variation and the terminology of *ex situ* or *in situ* conservation should only serve to guide us with respect to some of the general conservation and management objectives of each approach. For instance, we have seen that *ex situ* ('out of place') conservation can be both static and dynamic (or 'evolutionary'), large or small numbers of genotypes can be collected, populations can be managed *in situ* by various approaches, seed can be stored, and so on, but all of this should lead towards some conservation goal that can realistically be attained.

The future of gene conservation in forest trees

It is well known that forestry will continue to be pushed to more marginal lands, as the Earth's population grows and higher-quality land is used for agricultural crops or converted to urban uses. Different species and populations of trees will have to be found that are well adapted to these marginal sites. Species and provenance testing, pre-breeding or improvement programmes will need to produce different populations of trees that are adapted to many different climatic and soil conditions. Projected climate change scenarios for many parts of the globe over the next 100 years, if realized, will increase the speed of these changes. We will need to develop a new dimension to our planning and management of forest genetic resources. The only way to achieve this is to have access to a wide range of genetic diversity, with relevant information on species distributions, patterns of genetic variation and the critically important understanding of the biology of the species (for example reproductive methods, life history characteristics, adaptive and productivity potential in various climates). In this sense research will continue to be an important part of our ability to manage forest genetic resources in the future. For species for which we can initiate some types of studies, whether it is with molecular genetic experiments, or inter- and intraspecific common garden trials that develop some pre-breeding or breeding opportunities, we may have to focus on maintaining yields or products from trees rather than the conventional aim of improving or optimizing yields. For instance, marginal improvements in some traits, such as survival or pest resistance, may be more important than improvements in growth. Even simple selection strategies, such as phenotypic selection, albeit less efficient, may be more effective as they are simpler to implement and local values are selected. After all, this was the basis of crop breeding until the last 100 years or so. Likewise, for *in situ* populations, their relevance in a scenario of rapid climate change may be important somewhere else; therefore, it may be important to build programmes that can test such potential future problems.

Throughout these three volumes, we have also emphasized the need for involving local users of forest resources, as an important means of assisting with the conservation of forest genetic resources. Programmes that aim to distribute plant material to local people who will benefit from the species and its management have been shown to be a critical link in successful genetic conservation of forest trees. This approach not only ensures that people are given a chance to identify their own needs in the planting programmes, but can also expand the potential for among-population variation to build over time as there may be different selection pressures for environmental conditions or traits. Many important crop and animal genetic resources are being conserved by such local use and 'on the farm' conservation. Although this may not be directly applicable to forest trees in the short term, our knowledge of desirable trait values (such as growth and adaptability) relates to the present, not to the unknown future, so a base of variation needs to be maintained in case these trait values change.

Over the last decade there has been a tremendous development in interest in conserving forest genetic resources (e.g. ITTO 2000). We have only been able to point out a few of the examples of initiatives under way in these three guidebooks, but we hope they provide the reader with an overview of the needs, methods and approaches, and examples of how and where forest genetic resources can and are being conserved.

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ACRONYMS AND ABBREVIATIONS

ACF	American Chestnut Foundation
BCFS	British Columbia Forest Service
CAMCORE	Central America and Mexico Coniferous Resources Cooperative
CBD	Convention on Biological Diversity
CGIAR	Consultative Group on International Agricultural Research
DFSC	Danida Forest Tree Seed Centre
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization
FLD	Forest & Landscape Denmark
FNCRDC	Forestry and Nature Conservation Research and Developmental Centre
G x E	genotype x environment
GIS	geographic information system
GPS	global positioning system
ICB	incomplete block
INIBAP	International Network for the Improvement of Banana and Plantain
IPGRI	International Plant Genetic Resources Institute
ITTO	International Tropical Timber Organization
IUCN	World Conservation Union
IUFRO	International Union of Forest Research Organizations
MPBS	multiple population breeding system
MTA	material transfer agreement
NBPGR	National Bureau of Plant Genetic Resources
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
RBG	Royal Botanic Gardens
RCB	randomized complete block
SCD	Seed Conservation Department
STS	sequence tagged site
UNEP	United Nations Environment Programme
WCMC	World Conservation and Monitoring Centre

INDEX

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GLOSSARY of technical terms

ACCESSION A plant or seed sample, strain or population held in a genebank or breeding programme for conservation and use.

ADAPTIVE GENETIC VARIATION Difference in genotype due to the adjustment of a population to changes in environment over generations. The adjustment is associated (at least in part) with genetic changes resulting from selection imposed by the changed environment.

AGROFORESTRY A natural resources management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels.

ALLELE An alternative form of a gene. Alleles are located on corresponding loci of homologous chromosomes. They have different effects on the same trait or development processes and can mutate from one to another. They may affect the phenotype quantitatively and/or qualitatively.

BACKCROSS Crossing an individual with one of its parents or with the genetically equivalent organism. The offspring of such a cross are referred to as the backcross generation or backcross progeny.

BIOLOGICAL DIVERSITY The variety of life forms, the ecological roles they perform and the genetic diversity they contain (sometimes abbreviated to biodiversity).

BIOTECHNOLOGY (i) 'Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use' (Convention on Biological Diversity). (ii) 'Interpreted in a narrow sense, a range of different molecular technologies such as gene manipulation and gene transfer, DNA typing and cloning of plants and animals' (FAO's statement on biotechnology).

CLINAL Related to the variation in one or more phenotypic characters or allele frequencies across an environmental gradient.

CLONAL ARCHIVE A clonal archive (or clone bank) is a collection of genetic individuals which are retained for: (i) the commercial production of propagules, (ii) implementing a breeding strategy, (iii) genetic conservation. The individuals within the clone bank may be raised from seeds but more commonly are grafts whereby the stem, or scion, from the genotype selected in a genetic test has been grafted on to a juvenile rootstock in the nursery before planting out in the clone bank. It is common for there to be multiple copies (ramets) of each clone and these are usually planted adjacent to each other within the clone bank.

CLIMAX The period of equilibrium that is reached as a result of the gradual slowing down of the rate of continual development in a plant community. It is characterized by the fact that it maintains itself. The conditions it creates are stable only for the offspring of its own kind. It defines the terminal stage in ecological succession for a given environment.

CONSERVATION (of a resource) The actions and policies that assure its continued availability and existence.

CONSERVATION (of genetic resources) The management and human use of genetic resources so that they may yield the greatest sustainable benefit to present generations, while maintaining their potential to meet the needs and aspirations of future generations.

CRYOPRESERVATION The preservation or storage at very low temperatures (usually in liquid nitrogen). It is a form of conservation for some seeds and tissues.

DORMANCY A period in the life of an animal or plant during which growth slows or completely ceases.

ECOSYSTEM dynamic complex of plants, animal and micro-organisms, communities and their non-living environment interacting as a functional unit.

DIPLOID The status of having two complete sets of chromosomes.

ECOTYPIC Related to the adaptation of a population or a strain of an organism to a particular habitat.

EFFECTIVE POPULATION SIZE (Ne) The number of individuals in an ideal population which has the same level of genetic drift and inbreeding as the population from which it is drawn.

ENCAPSULATION The process of enclosing fragile organic material in a protective, nutritive casing, usually of a semi-solid nature. It is used for planting or moving somatic embryos, either individually or in rows.

***EX SITU* (CONSERVATION)** The conservation of components of biological diversity outside their natural habitats.

FINGERPRINTING Method of identification that compares fragments of DNA. It is sometimes called DNA typing.

FOREST MANAGEMENT OR WORKING PLAN A plan for regulating all forestry activities for a set period of time through the application of prescriptions that specify targets, action and control arrangements.

FRAGMENTATION The process of transforming large continuous forest patches into one or more smaller patches, creating areas of geographical discontinuity.

GENE In the genome of an organism, a sequence of nucleotides (DNA sequence) to which a specific function can be assigned.

GENEBANKS facility where germplasm is stored in the form of seeds, pollen or *in vitro* culture, or in the case of a field gene banks, as plants growing in the field.

GENE FLOW Exchange of genes between populations owing to the dispersal of gametes or zygotes.

GENE POOL The total sum of genetic material of an interbreeding population.

GENE(TIC) CONSERVATION All actions aimed at ensuring the continued existence, evolution and availability of genetic resources.

GENETIC DIVERSITY The sum total of genetic differences between and within species.

GENETIC DRIFT Change in allele frequency from one generation to another within a population, due to the sampling of finite numbers of genes that is inevitable in all finite-sized populations. The smaller the population, the greater the genetic drift, with the result that some alleles are lost, and genetic diversity is reduced.

GENETIC RESOURCES The economic, scientific or societal value of the heritable materials contained within and among species.

GENETIC VARIATION Variation due to the contribution of segregating genes and gene interactions.

GENOMICS The research strategy that uses molecular characterization and cloning of whole genomes to understand the structure, function and evolution of genes and to answer fundamental biological questions.

GENOTYPE The sum total of the genetic information contained in an organism or the genetic constitution of an organism with respect to one or a few gene loci under consideration.

GERMPLASM The total genetic variability available to a particular population of organisms, represented by the pool of germ cells (sex cells, the sperm or egg) or plant seeds. Also used to describe the plants, seeds, or other parts useful in plant breeding, research and conservation efforts, when they are maintained for the purpose of studying, managing or using the genetic information they posses (same as genetic resources).

HETEROZYGOSITY The proportion of heterozygous individuals at a locus or of heterozygous loci in an individual. He is expected heterozygosity; Ho, observed heterozygosity.

HERITABILITY The degree to which a given trait is controlled by inheritance, as opposed to being controlled by non-genetic factors.

INBREEDING Mating between individuals that have one or more ancestors in common, the extreme condition being self-fertilization, which occurs naturally in many plants.

***IN SITU* (CONSERVATION)** The conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties.

INDIGENOUS SPECIES Species existing in, and having originated naturally in, a particular region or environment.

INTERMEDIATE SEED Seed that can be desiccated, although not to such low levels as orthodox seed, and is often sensitive to chilling.

INTRASPECIFIC GENETIC VARIATION Genetic variation within a species.

IN VITRO Outside the organism, or in an artificial environment; literally 'in glass', i.e. in the test tube.

ISOZYME Multiple forms of a single enzyme.

LANDRACE An early, cultivated form of a crop species, evolved from a wild population, and generally composed of a heterogeneous mixture of genotypes.

LOCUS (PL. LOCI) A stretch of DNA at a particular place on a particular chromosome.

MOLECULAR MARKER A molecular selection technique of DNA signposts which allows the identification of differences in the nucleotide sequences of the DNA in different individuals.

ORTHODOX SEED Seed which is desiccation tolerant.

OUTCROSSING The crossing of genetically unrelated plants or animals; crossbreeding; outbreeding.

PARENT TREE A pollen donor and/or producer of ovules.

PHENOTYPE The observable characteristics of an individual, resulting from the interaction between the genotype and the environment in which development occurs.

PIONEER SPECIES The first species or community to colonize or re-colonize a barren or disturbed area, thereby initiating a new ecological succession (used synonymously with colonizing species).

POLLINATOR A living organism transferring pollen, e.g. insect, bird or bat.

POLYPLOID Organism, tissue or cells having more than two complete sets of chromosomes.

POPULATION A group of individuals of the same species occupying a defined area and genetically isolated to some degree from other similar groups.

PROGENY Synonym of offspring.

PROVENANCE The geographical and/or genetic origin of an individual.

RECALCITRANT SEED Seed which is desiccation-sensitive, with a short, hydrated lifespan in storage typically ranging from a few days to several months. Recalcitrant seed behaviour is most prevalent in tree species from tropical, humid zones with larger seeds (>3–5 g).

ROTATION AGE The age at which a forest stand is considered mature and ready for logging.

SELF(ING) To pollinate with pollen from the same flower or plant.

SPECIES SITE MATCHING TECHNIQUE A critical operation in plantation forestry, aimed at verifying the compatibility of a species (sometimes a provenance) with the site of plantation. Different approaches can be adopted: (i) field trials, (ii) gathering of data from natural stands in order to evaluate the similarity in environments between the seed source and any candidate site for planting, and (iii) gathering of data from existing plantations to produce predictive species-site compatibility models.

SEQUENCE TAGGED SITE Short, unique DNA sequence that can be amplified by the polymerase chain reaction and thus tagged to the site on the chromosome from which it was amplified.

SUCCESSION-PHASE SPECIES Vegetation structure and composition changes during succession phases from pioneer plant species, that first colonize a site, usually following a disturbance, to climax plant species, which occur when the rate of development of the vegetation slows down to reach more stable conditions. Succession is often directional and sometimes predictable and, although defined in terms of species composition, succession is always accompanied by changes in the environment (soil depth, soil nutrients, light characteristics, fuel characteristics, etc.). Succession-phase species are found during the transition period between the two extreme conditions (vegetation colonizing a newly exposed habitat devoid of life, or that have already supported life, and climax forest).

THINNING Gradual removal of trees crowding or shading the preferred species or individuals.

ULTRA-DRY STORAGE A ‘low-input’ alternative to the conventional cold storage of seed. Seeds are stored in airtight containers at room temperature after processing to adjust their moisture levels. This is not as effective as low-temperature methods for seeds that can withstand low temperatures. It is worth considering when maximum longevity is not important and refrigeration facilities are not readily available.

VEGETATIVE REPRODUCTION In plants, the formation of a new individual from a group of cells, without the production of an embryo or seed. Vegetative, somatic, non-sexual reproduction of a plant without fertilization.

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This guide is the third volume of a series of three that deal with the conservation of forest (trees and shrubs) genetic resources. This volume addresses technical requirements, and some applied approaches and experiences with the *ex situ* conservation and management of forest genetic resources. It outlines the role of *ex situ* conservation and reviews some of the strategies that may be employed, the managed development of the *ex situ* populations, as well as methods for storage, in the field and in seed banks, for *ex situ* genetic resources. With Volumes 1 and 2, and with other publications that are also addressing forest gene conservation issues at regional levels, we hope we have been able to provide the users of these manuals with an integrated view of the conservation approaches that can be used for the management of forest genetic resources around the world.

Forest & Landscape

Forest genetic resources conservation and management

IPGRI/FAO/DFSC