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3 Conservation of Germplasm in Forest Trees

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1 Introduction

Forest trees meet human needs and contribute to natural ecosystems in unique and diverse ways. As a source of fuel and fiber, they are important commodities. As dominant members of many natural ecosystems, they play keystone ecological roles in plant and animal communities. As habitat for other organisms, forests are a reservoir of great natural diversity. In themselves, trees contribute taxonomic diversity through a wealth of species, from rare to widespread and through great genetic diversity within species. Forests purify water and cleanse the atmosphere. And, to increasingly crowded urban denizens, forests provide valued opportunities for solitude and recreation.

Understandably, there are as many kinds of demands for conservation of diversity in forests as there are diverse values of forest resources. Although some forest-conservation issues seem to apply specifically to the species, community, or ecosystem levels, genetic concerns in fact pervade all levels. The actual approach to forest-tree conservation and the methods used jointly depend on available genetic-conservation techniques and on the ultimate goal for perpetuating the germplasm.

1.1 Threats to Forest-Tree Germplasm

Conservation usually is motivated by a threat to natural resources, either an immediate crisis or a perceived future challenge. Because of the diverse roles that trees play in human and wild environments, trees face more kinds of threats than most classes of organisms. Over-exploitation, land conversion, and deforestation have extirpated populations and brought about species extinction, especially in tropical regions (references in Wilson 1988). Global climate change threatens both rare and widespread tree species with mass population extirpation and possible species extinction, especially at temperate latitudes (Hansen et al. 1987; Peters 1990; Davis 1991).

Forest trees also are threatened in a way that is not problematic for most other wild species. Because trees are managed in or near their native forests, the potential for gene-pool disruption due to production forestry and horticulture is

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high. From dysgenic to progenic harvest, from operational reforestation to tree improvement, from landscaping to restoration, trees are cut, grown, and replanted in ways that can contaminate, impoverish, or alter the natural genetic architecture of forests (Ledig 1986, 1988; Millar 1987; Millar and Libby 1989a, 1991).

The use of clones poses special threats to genetic diversity in restoration, tree improvement, and general forestry. With the ability to replicate exact genotypes comes the potential to reduce diversity in restored forests and plantations by planting many copies of one or a few genotypes. The risks associated with genetic monocultures have been widely recognized and discussed in agricultural (NAS 1972; Frankel and Hawkes 1975) and forestry contexts (FAO/UNEP 1975; Ledig 1986). Most modern forestry programs take active measures to guard against low diversity, although from a strict production standpoint, as few as seven clones per plantation have been recommended as safe (Libby 1981).

The level of genetic awareness is not yet as high in most programs for restoration or conservation of rare species as it is in production forestry (Millar and Libby 1991). The former activities to date have been based primarily on ecological and botanical but not genetic principles. Clonal propagation is commonly used, especially for noncommercial species. The incentive to plant many copies of a few clones may be high, either for ease in production, or because the number of remaining wild genotypes available is low. Increasingly, however, attention is being given to the genetic risks associated with clonal replication of a few genotypes in restoration and in programs for rare plant recovery (Ambrose 1987b; Holsinger and Falk 1991).

Despite these threats, the genetic wealth of many natural forests is still great. Forest trees are among the most genetically diverse organisms known; their evolutionary persistence may depend on this. Trees have evolved many life history traits that promote and maintain genetic variability at all levels under natural conditions. This variation is the raw material both for natural selection of adaptations to changing environments and for artificial selection of traits desired by humans. Unlike many agricultural crops, trees have only begun to be domesticated (Libby et al. 1969), so that the gene pools are rich and have not been exploited. It is this natural variation that is sought and protected by breeders, conservation biologists, poets, and backpackers, and by the many forest organisms that depend on trees.

1.2 Approaches to Conservation

An important challenge to conservation is to conserve genetic diversity in ways that protect the germplasm in its desired form. Conservation strategies range from preservation of germplasm in cloned DNA sequences and genomic libraries, through storing genetic samples as tissue cultures, seeds, seedling and clonal plantations, to conservation of dynamic and evolving gene pools in natural forests. In tree improvement, where a common goal is to perpetuate a supply of high-quality wood fiber, genetic diversity is important in providing raw material for selection, in avoiding deleterious effects of inbreeding, and in guarding against threats of destruction by pests and pathogens. The choice of

material for conservation is usually biased toward select phenotypes with desired commercial traits (Zobel 1977), or trees with broad adaptability (Ledig 1986). Germplasm for tree improvement is most often conserved in plantations and other kinds of gene banks. In the broader sense, when the goal is to perpetuate species per se, a combined approach of natural forest protection and gene banking is chosen (FAO 1975; Krugman 1984; Ledig 1986, 1988; Millar and Libby 1991). Selection of material in this case is intended to be random with respect to phenotype and genotype, and evolutionary resilience is sought. When the goal is to perpetuate forest trees as habitat for other organisms, the approach is to protect the entire natural community (Soule and Wilcox 1980; Harris 1984; OTA 1987). Trees are selected for their contribution to the desired ecosystem, and genetic aspects may be only one of many conservation criteria (e.g., demographic structure, population sizes, species mixes, etc.). It is important to realize that there are many methods of conservation, and they are not necessarily mutually exclusive. In practice, combined or integrated conservation methods will not only improve the success of individual programs, but will allow coordination among different types of conservation programs.

Efficient conservation activities for any of these goals benefit from the knowledge of genetic variation. This information guides the details of sampling and handling germplasm throughout the conservation process, including original collections and delimitations of land area, evaluation and monitoring, and distribution and use of the germplasm (Hawkes 1980). Fortunately for forest trees, a substantial literature is developing on basic genetic variability in species, and increasingly on the implications for conservation. Conservation of forest germplasm also stands to benefit from the lessons learned in agriculture, including the consequences of depleting native gene pools, and reducing variation within domesticated lines.

2 Use of Clonal Techniques for Germplasm Conservation

Whereas conservation strategies depend on knowledge of genetic variation for their conception, they depend on available technology for their implementation. New methods of clonal technology based on molecular and biochemical techniques are being developed, and their mastery broadens the range of conservation possibilities. Although clones are just starting to be used systematically, they have wide application in many conservation situations.

2.1 Advantages of Clones for Conservation

The advantages of using clones for genetic conservation relate to the ability to preserve and replicate exact genotypes without sexual recombination. The general consequence of this is to give control over genetic diversity, which is a critical tool for conservation (Libby 1983). Thus, although clonal forestry potentially threatens genetic diversity because monocultures of single genotypes

can be propagated, the same opportunity to control diversity can be used advantageously to deploy appropriate levels of known diversity (Libby 1987; Leakey 1987).

Clonal propagation offers the only or the most important method of conservation in situations where seeds cannot be stored or where seeds physically are unavailable. The most obvious example is for species with recalcitrant seeds (seeds that have maximum storage life less than a few years; Bonner 1985, 1990). Many tropical tree species fall into this category, and also several important temperate genera, for example, *Quercus*, *Acer*, and *Aesculus* (Bonner 1990, and unpublished 1985).

In other cases, seeds may be produced under normal conditions, but may be unavailable at the time of collection. This may occur due to stressed conditions of the plants, including insect damage and drought, to demographic structure of the population (all plants juvenile), or to seed abortion following inbreeding. The clonal method allows these nonreproductive individuals to be propagated. Even when a stand is sexually mature and healthy, variation in seed production among trees within a stand and among years may create conditions such that collections limited to seeds would be disadvantageously small and/or nonrandom.

Clonal propagation is especially valued when the genetic integrity of seeds is suspect. Many opportunities for pollen contamination of natural stands, plantations, or gardens and arboreta exist – with possible sources of contaminating pollen being landscaping trees, forestry plantations containing trees of nonlocal origin, and genetically improved trees (Wilson 1990; Millar and Libby 1989a). In addition to genetic contamination, seeds may be genetically poor quality because of inbreeding depression. In these cases, clonal propagules avoid problems with seed, and, if the identity of standing trees is certified, assure that native germplasm is collected.

In addition to capturing otherwise unavailable genotypes, clonal propagation affords a security measure for conservation. In any germplasm collection or restoration program, replication is an important insurance against loss. In gene banking, replicates within a site and across sites are used whenever possible. Clonal propagation allows replications of exact genotypes, which is the best insurance.

An important phase of the conservation process is to evaluate the germplasm being protected – natural forest, plantations, tissue culture, or DNA. Evaluating the performance of accessions makes the germplasm collections valuable and useful. With clonal replication, genotypes can be characterized with greater statistical efficiency than through seedling progeny tests. Clones can be tested in many environments and their performance characterized by site (Libby 1983).

Clonal propagation is valuable for distribution and use of conserved germplasm. As information about performance accumulates on individual clones, they become more valuable, and distribution of exact genotypes is likely to be preferred (Libby 1983). For restoration, and for production forestry, certain tested clones can be prescribed from among the appropriate pool of native and local genotypes, to meet specific site conditions.

Obviously there are many ways in which clonal methods and methods based on sexual recombination can be used together for conservation. At some phase

of a program, the safest route may be to use clones; at another phase, seeds may be cheaper and more reliable, and/or genetically more defensible. In other cases, the two methods may be used simultaneously.

3 Methods of Clonal Conservation

The methods of forest conservation are traditionally divided between in-situ and ex-situ approaches. I classify conservation methods by the packaging of the germplasm (e.g., tissue culture vs. whole tree; natural vs. artificial regeneration) and by the context of the germplasm collection (e.g., native forest, plantation, laboratory, or clonal archive). The diverse objectives for conservation, from preserving commercially valuable germplasm to conserving evolutionarily resilient populations, are met through combinations of these methods. Clones have application at every level, in circumventing biological barriers, and in providing alternatives and complements to methods with sexual reproduction.

The methods discussed below begin with tree germplasm conserved as wild trees in natural communities, and proceed through methods that conserve more reduced elements of germplasm, ending with DNA. In the former, the whole species plus associated forest ecosystems are conserved; in the latter, only fragments of germplasm are preserved in a highly artificial environment. Each method has advantages for certain goals, and strong programs are built by the integrated use of several methods.

3.1 Native and Near-Native Forests

Much germplasm-conservation effort calls for designation of in-situ forest reserves, where native trees are allowed to grow and evolve under natural conditions. The degree of management can vary from noninterventive (laissez-faire) to intensive. In strict reserves such as Wilderness Areas or Natural Areas, management is usually limited to suppressing catastrophic fires and prescribing manageable fires to reduce fuels and maintain target species. The opportunities for clonal manipulation are few, except in clonally reproducing species such as coast redwood (*Sequoia sempervirens*) or quaking aspen (*Populus tremuloides*), which are stimulated to sprout and coppice following fire. In these cases, a cleansing fire that stimulates clonal reproduction of existing trees will regenerate native germplasm on the site. This is preferred over seed regeneration if the pollen source is suspect, as in cases where trees of unknown or nonlocal source are planted nearby.

Gymnocladus dioica (Leguminosae) is a rare and endangered tree of north-central North America whose survival in natural stands has depended on its ability to reproduce vegetatively (Ambrose and Carey 1987). Although sexual reproduction occurs in populations in the United States, where within-stand levels of variation are high, most populations at the northern limits of its distribution in Canada are entirely clonal and genetically monomorphic within stands. Given the dioecious nature of the species, and the intensive land-clearing

and agricultural development in its northern habitat, it is doubtful whether *G. dioica* would have survived without its ability to expand clonally. Despite the low levels of regional genetic variability, many of the Canadian populations are continuing to expand clonally, and appear vigorous (Ambrose and Carey 1987).

In some cases, reserves may be more intensively used and managed. Gene Resource Management Units (GRMUs, also called Genetic Conservation Areas) are a much-discussed but as yet little implemented form of land conservation (NCGR 1982; Krugman 1984) that allow manipulative use of the forest. The goal of GRMUs is to retain native tree species in populations with natural gene frequencies (Ledig 1988; Millar and Libby 1991). As long as these goals are likely to be achieved, activities such as timber harvest and artificial regeneration are allowed. Since these uses must not interfere with retention of native germplasm on the site, all planting must be done with the objective of approximating the natural genetic architecture. Clones allow the propagation of old-growth and other valued genotypes that would be lost following sexual recombination. Clones are especially important when the GRMU is surrounded by managed land with native species of non-native or unknown origin. Seed from native trees in the GRMU may be contaminated with pollen genes of this non-native origin. Clones allow regeneration of known native genotypes. However, if clones are used exclusively for long periods of time, the natural evolution of these populations will have been frozen.

In other situations, such as in state parks or national monuments, the objective may be to favor vegetation of a particular historic period. Clones afford a means for preserving old genotypes. In such cases, the freezing of evolution may be exactly what is desired.

3.2 Restored and Mitigated Forests

Of all fields in conservation biology, restoration science may emerge as the most important in the next half-century (Bradshaw and Chadwick 1980; Cairns 1980). The urgency to restore native ecosystems follows in the wake of rampant land development and conversion, deforestation, and pollution of the natural environment. Many natural resource agencies are restoring degraded sites, removing exotics, and reintroducing native species. Federal, state, and local mitigation laws require land developers to restore or transplant disturbed native communities to protected locations outside the development. Endangered species laws require that populations of listed species be recovered and that species be restored to self-sufficiency.

The need for restoration will be most urgent, however, if even a fraction of the projected consequences of global climate change occur (Peters and Lovejoy 1991; Peters 1990). Following such a change, vegetation patterns will be completely altered, and our knowledge of plant adaptation must by then be great if we are to reconstruct new and useful forest communities (Peters 1985).

The new field of restoration biology has grown primarily from ecology disciplines (Jordan et al. 1988). Restoration at the community and species level has been the main focus, with only minor attention to the genetic level (Millar and Libby 1989a). This situation is changing rapidly with the recognition that

genetic source and genetic variability are important factors in plant survival and success on a site. With this awareness, agencies are incorporating genetic guidelines into their restoration policies (e.g., NPS 1988).

Clones have special value to restoration. They may be invaluable in rescuing endangered species, or any taxon for which census number is low. Since demographic stochasticity is a critical factor in the final stages of extinction, the most urgent goal in these circumstances is to increase plant numbers. Often this will be possible only with clonal expansion, since seed production may be absent or unreliable.

Clones are proving their effectiveness in the rescue efforts for several endangered species. For example, the Center for Plant Conservation, a national consortium of United States botanical gardens and arboreta, has used vegetative cuttings and tissue-culture methods to propagate wild material of the extremely endangered Catalina mahogany (*Cercocarpus traskiae*) (Reiseberg 1988). This species, endemic to Catalina Island in California, had declined in recent years to seven individuals, primarily because of habitat degradation and overgrazing from introduced goats, sheep, and feral pigs. Recent morphological and electrophoretic observations indicated that two of the seven remaining trees were hybrids with the common mahogany on the island, *Cercocarpus betuloides* ssp. *blanchae*. Cuttings from the pure Catalina mahogany are being propagated along with seedlings derived from wind-pollinated seeds from the island trees at Rancho Santa Ana Botanic Garden. Only the clones, however, are known uncontaminated genotypes. Both vegetative- and seedling-origin plants will be reintroduced into fenced habitat on the island.

Another species that may be aided by clonal propagation is giant sequoia (*Sequoiadendron giganteum*) (Fig. 1). The species per se is in no danger of extinction, as it is widely planted and protected by many in-situ and ex-situ collections. However, the northernmost native stand, the Placer Grove, is at risk. Disjunct from the rest of the distribution by 80 km, this tiny population of only six mature trees is genetically distinct and has the lowest within-stand genetic variation of all the groves (Fins and Libby 1982). Complicating conservation efforts is the presence of many young giant sequoias of uncertain origin that were planted in the grove by a local service group in 1951 (J. LaBoa, Tahoe National Forest, pers. commun. 1989). If pollen from any of these trees has contributed to the seeds of the mature trees, then the genetic integrity of naturally regenerated seedlings is compromised. Several conservation scenarios are being considered for the grove (W.J. Libby, pers. commun. 1989). The most prudent involves removing all planted sequoias and replanting with rooted cuttings of known native origin from existing clonal hedge banks. The hedge banks consist of plants from wind-pollinated families from five of the six native trees. Seeds that produced these hedges were collected before the contaminating sequoias were producing pollen.

Even widespread species may be threatened by extinction and require restoration. The American chestnut (*Castanea dentata*) has benefited from both natural cloning and ex-situ rooting of cuttings. Chestnut blight, caused by the foreign pathogen *Endothia parasitica*, all but eliminated the billions of chestnut trees that grew throughout the eastern United States. The species has survived only because of its ability to sprout from the base of killed stems. These sprouts grow for a few years, but typically become infected and die before they become

Fig. 1. Placer Grove of giant sequoia (*Sequoiadendron giganteum*), showing planted trees of uncertain origin in understory and mature native trees. This grove is the northernmost native population of giant sequoia, has only six surviving native trees, and differs from all other groves in several traits

sexually mature. Now, thanks to the chestnut's ability to sprout, there is hope for the species. A survey in 1984 (McKeen and Ambrose 1988) revealed individual chestnut trees that have regrown and remain blight-free. These clones are being tested for resistance, possibly maturation related, to *E. parasitica*, (J.D. Ambrose, University of Guelph Arboretum, pers. commun. 1988). If found to be resistant, such clones could be vegetatively propagated and reintroduced into chestnut habitat.

As in any clonal program, the danger of eroding genetic diversity is great in restoration, especially where disease resistance is involved. Often only a few individual survivors of a major epidemic remain in a species or population, or only a few resistant genotypes may be isolated. If these are clonally expanded and provide the basis for major restoration, then future problems may result from low diversity. Where possible, a phase of sexual reproduction should follow the initial clonal expansion of remnant or select wild genotypes. In some cases where the remnant individuals are related, inbreeding depression may reduce the vigor of sexually produced progeny. An innovative program for avoiding problems of inbreeding in captive propagation has been followed in the endangered Spekes gazelle (Templeton and Read 1983), where the captive

population was forced through an early inbreeding bottleneck. This approach may also be useful in restoration of severely reduced tree populations or species.

The use of clonal propagation in mitigation allows the exact replication of genotypes in a new environment. If the site to be developed is well studied, the plants are mapped and cloned before development, and a new site is chosen that closely resembles the original, then it may be possible to reconstruct the original population successfully, notwithstanding nongenetic problems. Even in the case of many rare and endangered species or populations, where the original or mitigation site is atypical of native habitat, clones afford the opportunity to prescribe adapted genotypes for unusual sites (Libby 1983).

Another type of mitigation that may be done in non-native habitat would resemble a tree foster home. Species endangered in one part of the world would be established in similar environmental conditions elsewhere where protection was possible. The intent would be to maintain an option to reintroduce the genotypes back into the native habitat of the species once the threatening force was ameliorated. Clones could serve as the vehicle for moving genotypes and for regenerating trees in the foster forest. Clones would more accurately and easily maintain identities in the new site than would seeds unless controlled pollination were possible.

Forest decline in Europe provides a potential example. The nature of the threatening agent, air pollution, is difficult to escape in situ. Clones from the threatened species could be brought to North America and established in sites of similar physical conditions. Vegetative propagules from these trees could later be used for re-establishment in native European sites when air quality is improved.

Some tropical forests also are candidates for this type of rescue. Many areas are in imminent danger of exploitive deforestation. For many tropical species whose seeds do not store well, some form of clonal propagation may be the only means of propagating the species (Mathias 1988). Propagules from many of the species on a site would allow the structural re-creation of a tropical forest community valued for research and education as well as conservation. However, because many of the native elements would be missing from these foster forests, notably the pollinators, continued clonal regeneration might be necessary.

Clones will have at least two significant roles in restoration related to global climate change. The first should begin immediately and involves using clones to test genotypes for their breadth of climatic adaptability. Extensive evaluations will allow more effective restoration after climate change. Although there is general consensus that average temperatures will increase and precipitation decrease (NRC 1983), the direction of local climate change remains unpredictable (Hansen et al. 1988). This means that testing should evaluate adaptive niches in several climatic dimensions, although tolerance for warmier, drier conditions should be emphasized.

Adaptation can be evaluated in many ways, from routine progeny and provenance testing in tree-improvement programs to replicated plantations specifically for this purpose in gardens and arboreta. Both the Center for Plant Conservation in the United States (Thibodeau and Falk 1987; Falk 1990) and the Canadian Plant Conservation Program (Ambrose 1987a) are incorporating this element of off-site evaluation into their conservation programs.

Clones will also serve a primary function in actual restoration of areas where native vegetation could not tolerate climate change. Previously native species may be restored to the sites using different ecotypes adapted to the new conditions. Where clonal evaluation has indicated only marginal tolerance to the new climate, mixtures of clones from diverse but potentially adapted populations may be used to provide a pool of variation for evolution to develop adapted populations in the new sites.

3.3 Plantations

The goal in the examples described above is to maintain germplasm in natural or near-natural conditions. This includes native species, typical age-class mixes, and natural spacings and regeneration, with the result that the conserved forest serves as valuable habitat for plants and animals other than the target tree species. Furthermore, the species of this ecosystem continue to co-evolve in natural or semi-natural conditions. Plantations, however, have a much simpler structure with more controlled design and no opportunity to evolve. They consist of one or two species (common in temperate zone) to several species (common where agroforestry is practised), usually of the same age, and have regular spacing among trees. Competing species are undesired. Whenever germplasm collections are planted within the native range of the species, there is a risk of gene contamination from surrounding wild trees in the seeds of the planted trees. Using clonal replicates of the collection trees ensures that no contamination of the original germplasm occurs. The only way to ensure this with seedlings would be to make controlled crosses.

Plantations may be established specifically for germplasm conservation or they may primarily serve production forestry and only opportunistically or secondarily serve conservation. Plantations designed specifically for germplasm conservation of commercially valuable tree species usually complement ongoing domestication programs (Wood and Burley 1980). The degree of a program's allegiance to domestication can be measured by the nature of the conservation collections. Clonal archives that closely complement tree-breeding programs include clones with traits of immediate value to breeding. The diversity conserved in such collections may not represent allele frequencies in natural stands.

An example of clonal archives maintained to support economically valuable species is the National Clonal Germplasm Repository, part of the National Plant Germplasm System. It was established for the express purpose of maintaining valuable germplasm of agricultural crops (Council for Agricultural Science and Technology 1985; Parfitt 1988). The five existing repositories clonally propagate plants, and grow them in the field and in greenhouses. All accessions are stored on the USDA Genetic Resources Information Network (GRIN). Germplasm is obtained from existing plant-breeding programs and also from sponsored collection trips. For some species, a virus-testing program has been developed, and isozyme electrophoresis is used as an adjunct to morphological descriptors to characterize the accessions. Germplasm maintained in this program is distributed as scionwood.

Examples of clonal plantations specifically for germplasm conservation of forest trees include Monterey pine (*Pinus radiata*), giant sequoia, and coast redwood programs (Libby 1990; Fig. 2). In each of these cases, efforts were made to collect random samples of the population that represent the natural genetic structure of the species. The giant sequoia clones originated as wind-pollinated seeds from native stands whose seedlings were clonally expanded with rooted cuttings; the redwood clones were collected primarily as stem cuttings from seedlings in the wild. In the redwood collection, some wild sprouts were rooted and subsequently propagated through tissue culture to achieve some rejuvenation (or at least some reinvigoration – see Bonga and von Aderkas, Chap. 12, Vol. 1) and then as rooted cuttings. All species are maintained as plantations of hedged plants, from which cuttings may be taken. The redwood program includes 180 clones from 90 populations with clonal gene-conservation plantations at two sites in California and a third planned (J. Kuser, Rutgers University, pers. commun. 1988).

Species of *Populus* also have been the focus of specific conservation efforts. The Ontario Ministry of Natural Resources in Canada maintains clonal hedge archives of four commercially important native species (D. Rogers, OMNR, pers. commun. 1988). Sampling of the species was done in a grid pattern in the late 1970s. Some of the clones from the archive have been used in the breeding program.

Fig. 2. Genetic-conservation hedge orchard of giant sequoia, University of California Russell Reservation at Lafayette, California. Samples of nearly half of the 84 native populations are maintained here and at a US Forest Service clonally replicated hedge orchard at Chico, California

The Poplar Research Center of Belgium has an active program of preserving the native *P. nigra* gene pool. With known native populations rapidly diminishing because of land conversion, a collection was made in 1959 of remaining native trees in Belgium, and these trees are periodically intercrossed. Resulting seedlings are screened and evaluated, and select clones are exported to the operational program (Strobl 1987).

Many clonal tree-improvement and general forestry plantations serve opportunistically as germplasm repositories. The basic dilemma when serving both tree-improvement and genetic-conservation objectives is that the goal of increasing gain through breeding requires a narrowing of the genetic base, which counters conservation objectives (Barnes and Burley 1987). Furthermore, selections for tree improvement are nonrandom whereas collections for long-term genetic conservation are best made randomly. Many tree-improvement programs, however, spend considerable time and effort in expanding their genetic collections beyond what would be necessary for current breeding, often at the expense of immediate gain (e.g., Kitzmiller 1976, 1990). Plantations associated with tree-improvement programs, such as provenance or progeny tests, maintain broad genetic diversity. For agricultural species, the IBPGR specifies these as field gene-banks, and systematically inventories them for useful germplasm (Withers and Williams 1986). Identities and pedigrees of single trees are maintained in these plantations and performance is evaluated, both of which increase the value of the germplasm to conservation. The relatively short duration of some of these plantations decreases their value, but since many of the clones in a clonal improvement program will be retained and planted in other sites, they are not necessarily lost.

There are many examples of such improvement and production programs that have used clonal opportunities to provide back-up material for conservation. In Germany, the Institute of Forest Genetics and Tree Breeding is making a concerted effort to add a genetic conservation element to already existing clonal plantations of Norway spruce (*Picea abies*) (H. Muhs, Institute of Forest Genetics and Tree Breeding, pers. commun. 1988). The basis for the collections is an international provenance experiment begun in 1964, which contained 1100 sample provenances. The clones were replicated at three sites and most have been evaluated for growth and isozyme traits. These provenance plantations have been intentionally maintained for conservation by thinning to favor random diversity rather than favoring trees with commercially valued traits.

In Yugoslavia, the Poplar Research Institute maintains a clonal archive of poplars that has a more restricted genetic base (Herpka 1987). Selection for disease resistance and growth is initially made on progeny of *Populus nigra* crosses. The best clones are planted yearly in genetic-conservation stool beds, which are felled and allowed to resprout every second year. These archives provide material for further selection.

Other examples of programs that use clones in conservation include the work on *Pinus caribaea* by the Oxford Forestry Institute (OFI). In 1963, the OFI began extensive seed collections of *P. caribaea* for provenance trials (Barnes and Burley (1990). The seeds were widely distributed and the plantations were periodically evaluated. By the late 1970s, the program had progressed to a stage where a second round of seed collections was planned. In the intervening years,

however, many of the original native stands either had suffered serious genetic depletion or had become inaccessible for security reasons. This catapulted the status of the original provenance plantations into being extremely valuable and in some cases irreplaceable germplasm collections. Subsequently, many of the trees in the provenance plantations were vegetatively propagated and distributed to regional centers throughout the tropics. Ex-situ conservation of this germplasm in subsequent breeding programs has been practised by applying theoretical concepts of multiple-population breeding (Namkoong et al. 1980) to practical plantation management (Barnes 1986).

In the past, botanical gardens and arboreta focused on display, education, and recreation as primary goals, and conservation was either ignored or accomplished as a consequence of these primary activities. More recently, many botanical gardens and arboreta are shifting emphasis such that conservation has become a primary goal (Simmons et al. 1976; Frankel and Soule 1981). In the past, garden plantings have included only a few individuals from one or two populations of each species. There is increasing recognition of the importance of appropriate genetic sampling for conservation collections in botanical gardens. The Center for Plant Conservation (CPC), with headquarters at the Arnold Arboretum near Boston, Massachusetts, is a consortium of 19 gardens and arboreta dedicated to genetic conservation of rare plants (Thibodeau and Falk 1987; Falk 1990). Gardens affiliated with the CPC are chosen for their ability to maintain and propagate conservation collections. Each garden is charged with protecting specific endangered taxa. Although the CPC gardens maintain ex-situ collections, mostly in the form of planted individuals, the focus of the program is on integrated strategies that transcend the dichotomy of in-situ versus ex-situ approaches (Falk 1987). Clonal propagation is commonly used for transferring germplasm from native sites to gardens, for expanding clones to be used in evaluation and dissemination, and for returning germplasm to the wild as restoration or mitigation plantings.

The CPC works with extremely endangered taxa, including many woody plants in addition to *Cercocarpus* discussed above. For example, the CPC is focusing on the Florida torreya (*Torreya taxifolia*), which has experienced a serious decline in recent years. These large trees once covered thousands of hectares in Florida and Georgia, but the species now stands on the brink of extinction. An interaction of environmental changes and a suite of fungal diseases threatens the species. The only remaining living plants arise as sprouts from the roots of top-killed adults but none lives to sexual maturity. Because disease is rampant in wild stands, habitat protection alone will not save the species. Together, the US Fish and Wildlife Service, The Nature Conservancy, and the CPC are mounting a multiphased project to save the species (McMahan 1989). A major aspect of this is collecting and propagating cuttings of wild plants from systematic population samples. These will serve as donors for subsequent clonal propagation and distribution to select botanical gardens and arboreta for research and conservation.

The major focus given by the CPC to the importance of genetic aspects in conservation has been instrumental in educating garden and arboreta staff about genetic conservation. In 1989, the CPC sponsored a conference on the Genetics and Population Biology of Rare Plants, which had the goal of

summarizing current knowledge on the importance of genetic variation for ex-situ conservation (Mlot 1989; Holsinger and Falk 1991).

Other garden consortia and individual arboreta have similar goals. The Canadian Plant Conservation Program, based at the University of Guelph Arboretum, Ontario, also focuses on the importance of genetic variation in ex-situ collections and plantations (Ambrose 1987a). The Canadian program emphasizes regionally rare plants of Canada's Carolinian zone, where species of the eastern North America deciduous forest reach their northern distribution limit. Ex-situ conservation stands that have been established through vegetative propagation include such woody taxa as *Castanea dentata*, *Gymnocladus dioica*, and *Rosa setigera* (J.D. Ambrose, University of Guelph Arboretum, pers. commun. 1988).

Even gardens that have not identified conservation as a major focus often house valuable germplasm. Gardens have traditionally collected unusual variants and cultivars of species, many of which are propagated clonally. Together these can present a large opportunistic store of variation in a species. In Port-Orford cedar (*Chamaecyparis lawsoniana*), for instance, over 200 distinct cultivars have been described and are clonally propagated (Dallimore and Jackson 1966). Many derive from wild origins. Because they are widely planted and recognized for ornamental purposes, their performance in a wide range of environments could be assessed, and a profile of the adaptive breadths for each cultivar described.

Such a pool of germplasm, although not originally intended for conservation, may be what will save Port-Orford cedar in the wild. Port-Orford cedar is endangered throughout its range by a fatal root disease caused by the fungus *Phytophthora lateralis* (Zobel et al. 1985). Several programs for control and recovery are underway, including a genetic resistance study, and a silvicultural program. Although to date resistance has not been found in the wild species, the ornamental cultivars may offer a useful pool of genetic variation. Not only is it a highly variable pool, but propagules have been repeatedly subjected to nursery conditions where various species of *Phytophthora* occur in abundance. Thus, if there is resistance, some selection may already have occurred in cultivation.

3.4 Regenerable Tissues

The tissue most commonly used for regeneration is the embryo contained in the seed. Tissues that are clonally propagated include cells, organs, and tissue masses, rootable stem and leaf cuttings, root sections that produce shoots, grafts, apomictic seeds, and embryoids encapsulated in matrix. Although these tissues are the vehicles for multiplying plants in any clonal conservation program, they may also serve as primary units for germplasm conservation.

The role of tissue culture in germplasm conservation is best illustrated by the comprehensive program of the International Board for Plant Genetic Resources (IBPGR, Withers and Williams 1986) for agricultural species. The IBPGR program cycles newly acquired germplasm through phases of multiplication, distribution, storage, characterization, evaluation, and disease indexing. To coordinate research and conservation efforts on agricultural crops, IBPGR

maintains a computerized international database related to all aspects of in-vitro conservation (Wheelans and Withers 1984).

Clonal techniques in the IBPGR program start in the field, by collecting tissue directly into culture media (Yidana et al. 1986). Germplasm is transported to the laboratory, multiplied in vitro, then distributed to participating institutions for storage. Tissues are stored either in traditional culture under slow-growth conditions, or by cryopreservation. Tissues for slow growth are usually cultured on solid media, to which protectant chemicals are added, and stored at low temperatures for several years (Henshaw 1987, and references therein).

Tissues cultured in slow growth are used for active research; the tissues are cloned and distributed to researchers and breeders. Long-term base collections not intended for frequent use are kept in cryopreservation at the temperature of liquid nitrogen (− 196 °C; Henshaw 1987). Before storage, clones are screened for specific disease-causing organisms, especially viruses (disease indexing), and infected cultures are cleaned up through serial propagation and thermotherapy (Walkey 1978, 1985). Since cell and tissue cultures are not easily identifiable by morphological descriptors, they are characterized by biochemical methods, most commonly electrophoresis. Some testing for disease resistance or nutrient tolerance can be done in culture. More extensive evaluations are made when the plants are grown out.

Concern for genetic instability (Scowcroft 1984) in cultures has motivated considerable research in the IBPGR program. The risk of instability resulting from the release of somaclonal variation in in-vitro systems ranges from minor to major, depending on the species and on the culture system used. There is evidence for some natural somatic instability in all living tissues (D'Amato 1978; Larkin and Scowcroft 1981;Roberts and Ellis 1984; Walbot and Callis 1985; Ahuja 1987a). In addition, in-vitro conditions themselves may be mutagenic to plant cells (D'Amato 1978).

In-vitro conservation programs for trees are not as extensive or as comprehensive as those for agricultural crops. Although there have been many advances in developing in-vitro techniques for clonally propagating tree tissues (Bonga and Durzan 1987a,b; Ahuja 1987b; Hasnain and Cheliak 1986; Hanover and Keathley 1988, and Chaps. 7–15, Vol. 1), there have been fewer examples of applications to long-term storage and conservation (Wilkins and Dodds 1983).

Experiments with traditional cold storage on *Populus tremula* clones indicate that branches with dormant buds can be stored and later cultured (Ahuja 1989). After a year in cold storage at 0 °C, − 5, − 8, − 18, and − 80 °C, only the buds stored at − 5, − 8, and − 80 °C showed good growth and differentiation in culture. Slow growth of cultures by storing cultures at low temperatures has successfully extended the storage of cultures from a few weeks to 1 year in several forest tree species (Table 1; IBPGR 1989). Research has also advanced on many fruit and nut species, where subculturing at 1-month intervals has prolonged storage indefinitely (IBPGR 1989).

Storing woody germplasm in liquid nitrogen is an exciting yet little researched or applied method (Sakai 1985; Ahuja 1989). For many species, it may provide safe storage far beyond typical durations under normal conditions (as long as two centuries, Bonner, unpubl. 1985). Cryopreservation is relatively simple and inexpensive, as long as a steady supply of liquid nitrogen is available

Table 1. Tree species that have been successfully stored and regrown under slow-growth tissue culture conditions. (IBPGR database 1989)

Species	Conditions (temp. reduction range, °C)	Storage length	Recovery rate (%)	Database reference
Betula pendula	Solid medium 25 – 4	16 weeks	100	Meier-Dinkel
B. pubescens	Solid medium 25 – 4	24 weeks	100	Meier-Dinkel
Larix decidua	Solid medium 22 – 4	2–3 months	Low	Bonga
Picea abies	Solid medium 21 – 4	24 weeks	30	Simola; Santanen
Pinus radiata	Solid medium 24 – 5	4.5 years	95	Aitken-Christie
Populus deltoides	Solid medium + activated charcoal 22 – 4	1 year	80	Overton; Sebastian; Vermeir; Evers
Populus tremula	Solid medium 22 – 5	1 year	[a]	Douglas
Pseudotsuga menziesii	Solid medium 25 – 6	4 weeks	[a]	Vermeir; Evers

[a]Not available.

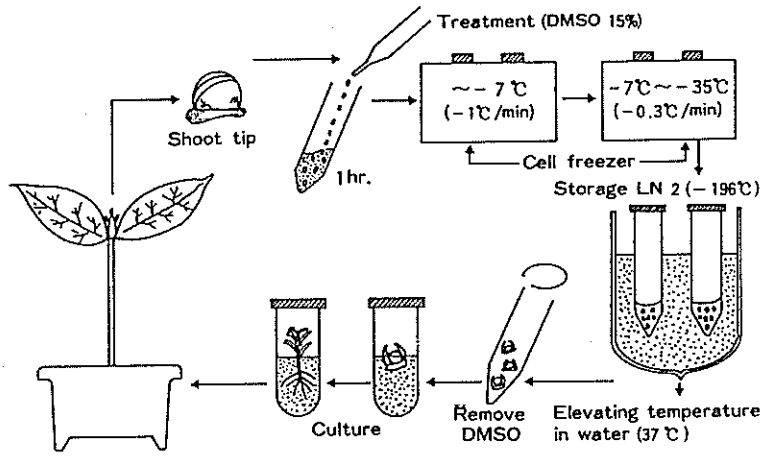

Fig. 3. Steps in cryopreservation of plant tissue, including preparation of tissues, slow freezing, storage in liquid nitrogen, thawing, and plantlet regrowth. (Reprinted with permission from the Institute of Forest Genetics, Republic of Korea)

(Fig. 3). Using appropriate cryoprotective compounds and rates of cooling and warming is critical to the survival of frozen cells (Finkle et al. 1985). Little is known about maximum possible lengths of storage, or the genetic damage that may accumulate in DNA during storage. Encapsulating excised embryos with

artificial coatings may protect naked tissue against damage during freezing (Bonner, unpubl. 1985).

Considerable progress has been made on cryopreservation of agricultural plant tissues (Sakai 1985; Kartha 1985). IBPGR (1989) lists successful cryopreservation (i.e., living tissue recovered from exposure to ultra-low temperatures) for 60 agricultural species. Tree species included are angiosperms such as *Malus*, *Morus*, and *Prunus*, *Phoenix dactylifera*, *Ulmus*, *Populus tremula*, *Fraxinus*, and *Platanus* (Table 2). Much less work has been done on cryopreserving conifer tissues (Sakai 1985; Aitken-Christie and Singh 1987; Table 2). A method using cryoprotectants, prescribed freezing and warming rates, and specific container materials (Finkle et al. 1985) was successfully employed for *Picea abies* and *Pinus taeda* (Gupta et al. 1987). Research on *Picea mariana* and *Pinus banksiana* is underway (D. Rogers, Ontario Ministry of Natural Resources, pers. commun. 1988). Seeds of *Larix decidua*, *Pinus sylvestris*, and *Abies alba* have been successfully recovered from ultra-low temperatures (Ahuja 1989). Seed germination of seeds stored in cryopreservation conditions was over 90% of the control

Table 2. Tree species that have been successfully stored by cryopreservation (temperature of liquid nitrogen, − 196 °C)

Species	Tissue	Storage length	Recovery rate (%)	Reference
1. Gymnosperms				
Abies alba	Seeds	6 days	100	Ahuja (1989)
Larix decidua	Seeds	6 days	100	Ahuja (1989)
Picea abies	Seeds	6 days	100	Ahuja (1989)
Picea abies	Embryogenic cells	10 min	50	Gupta et al. (1987)
Pinus sylvestris	Seeds	6 days	100	Ahuja (1989)
Pinus taeda	Embryogenic cells	10 min	50	Gupta et al. (1987)
2. Angiosperms				
Fraxinus	Callus	[a]	17–38	Shim (1987)
Malus spp.	Meristems	15 min	70	IBPGR (1989)
Malus spp.	Meristems	11 months	86	Katano (1986)
Morus spp.	Winter buds	1 month	50	IBPGR (1989)
Platanus				Sugawara and Sakai (1974)
Populus hybrids	Seeds	6 days	100	Ahuja (1989)
Populus hybrids	Callus	[a]	17–38	Shim (1987)
Populus tremula	Twigs with buds	2 years	14	Ahuja (1989)
Populus tremula	Excised buds	2 years	100	Ahuja (1989)
Prunus spp.	Shoot tips	24 h	[a]	IBPGR (1989)
Ulmus americana	Callus	4 min	42	Ulrich et al. (1984)
Phoenix dactylifera	Embryogenic callus	4 min	100 (after subculture)	Ulrich et al. (1982)

[a]Not available.

group. In many cases, storage was tested for a short time (2 days to 1 month), but tissue that can withstand short periods of supercooling should theoretically be able to tolerate much longer times (B. Finkle, Agricultural Research Service, pers. commun. 1988). In most cases reported, recovery of tissues was at least 50% the rate of the recovery from unfrozen tissues in the control group.

3.5 DNA

As molecular technology increases, we may expect to increase the usefulness of cloning and storing germplasm as DNA, either as whole genomes or as particular known and valuable sequences (Peacock 1984; Riemenschneider et al. 1988). Whole genomes can be stored as genomic libraries, and particular sequences can then be cloned when the need arises. DNA sequences are the most elemental form of germplasm that can be successfully preserved and recovered. Their usefulness depends on the ability to be transferred into a system where the genes can be incorporated and expressed. Sequences could be stored in bacterial plasmids or bacteriophage genomes. The recombinant molecules can then be cloned to produce potentially limitless copies of the stored sequence. Despite technical obstacles, progress is being made on transfer systems in higher plants including forest trees.

A major advantage of cloning and storing DNA for conservation is that DNA sequences potentially are transferable between species, and even among species in different kingdoms. The technology thus extends the use of known and valuable genes far beyond the reach of normal breeding. Transformation has been achieved to date in several forest trees (Ahuja 1988), including *Populus* species (Kriebel 1987; Chun et al. 1988), *Abies nordmanniana* and *Picea abies* (Clapham and Ekberg 1988), *Larix* (Diner and Karnowsky 1986), *Pinus densiflora* and *Quercus acutissima* (Choi et al. 1988), and *Pinus taeda* (Sederoff et al. 1986, 1987), where *Agrobacterium tumefaciens* was used as a vector. The work in these systems has been experimental and has not yet been applied to conservation.

Another advantage of DNA technology for conservation is the inert nature of DNA. This affords the theoretical ability to recover DNA from dead or extinct organisms. Although this technique has been successfully applied only in a few cases, the persistence of DNA suggests that the technique is possible for wide application. The inert nature of DNA makes it easy to store for conservation. Cloned DNA may be stored for indefinite periods by freezing at $-20\,^{\circ}C$ in appropriate buffer conditions (Mantell et al. 1985). For long-term conservation, DNA will most likely remain stable if genomic libraries are stored in inert state. In practice, metabolism of host vectors is reduced by cryogenic storage or freeze-drying, thus achieving a near-inert condition of DNA.

Recombinant DNA can also be maintained by normal culture of the vector bacterium. Appropriate measures must be taken to prevent genetic modification or loss of the sequence from the bacterium due to rearrangement and deletion of DNA, or to mutation followed by selection or genetic drift in the bacterial population. Instability in DNA collections occurs most likely in conditions where the host organism is maintained in an active condition. Plasmids are

commonly lost spontaneously from bacteria. If this occurs in a portion of the population, part of the target DNA may be lost, in a manner equivalent to genetic drift. If there is selection against bacterial cells containing recombinant plasmids, these cells will be eliminated faster. Another opportunity for genetic change through drift may occur during amplification, when the host organism is cloned to high number to obtain abundant DNA. DNA rearrangements during bacterial cloning and deletion of target DNA may be a serious source of genetic erosion in genomic collections. This is especially problematic in plant genomes, which contain large portions of repeat DNA. Some alleviation of this problem has been achieved by using selection systems (Loenen and Blattner 1983).

An international program called DNA BANK-NET, with headquarters at Baylor University, Texas, was initiated in 1988 as an international network of DNA-conservation banks. DNA BANK-NET is founded on the premise that DNA transfer between species will be the most common method of crop improvement in the next century and that DNA diversity will be sought. Each acquisition is documented by an herbarium voucher, and information on the source, collector, and date of acquisition is recorded in a computerized database. DNA transcription by polymerase chain reaction allows disbursement of DNA to private and public groups. In its first decade, DNA BANK-NET will focus on acquiring rare and endangered tropical species. Although agricultural crops are the most important focus of the network, forest-tree germplasm is also included.

In extreme cases where a species is reduced to a few individuals or a species is threatened by devastating disease, drastic measures to induce genetic variation may be taken in an attempt to preserve the species at all. Molecular biology offers tools for this extreme situation. Genetic variation may be stimulated by increasing the frequency of transposition of genetic elements (Saedler and Nevers 1985). Such mobile pieces of DNA, common in bacteria, were found in higher plants first in maize (McClintock 1956), but have been found in other angiosperms (Mantell et al. 1985). DNA with sequences and behavior similar to transposable elements has been discovered in conifers (C. Kinlaw, U.S. Forest Service, pers. commun. 1989). There is evidence that transposition may occur in response to environmental stimuli, such as virus infection (McClintock 1984). Variants resulting from transposition appear to be more stably inherited than variants from other mutagenic techniques such as irradiation (Herpka 1987). As these elements become better understood, they may prove useful in inducing variation in species that are low in variation or dangerously threatened (e.g., chestnut). Obviously, induction of variation would be done only in extreme situations, under controlled conditions, and would always be followed by monitoring, selection, and testing.

4 Integrated Strategies for Conservation

Security is crucial to conservation, and diversification of methods is one way to increase security. Combined conservation of specific germplasm, for example, in DNA banks, in tissue culture, and in plantations or natural stands allows

germplasm to be stored and used efficiently. Germplasm may be partitioned into base collections where germplasm preservation is highest priority, shorter-term active collections that are accessed frequently, and living collections in natural habitats where evolution proceeds. The time horizon for conservation varies from short to very long. For base collections, conservation activities ideally should be planned for centuries. This is difficult to achieve in a social environment of diminishing grant periods, scarce budgets, and transient public and institutional priorities. Dedication of even the longest projects often extends only to decades. Conservation projects that have existed for many years may be completely lost by even a short hiatus in funding (Libby 1990). An integrated approach, with germplasm replicated in different types of collections, increases the likelihood that the species being conserved will survive the many kinds of assaults that arise over the years.

Cloning technology offers the hope for rescuing neglected germplasm collections once support is secured. If collections are reduced in number or health, if seed production has been compromised by gene contamination, or if plants are still juvenile, then cloning will help to expand and replicate the bottlenecked germplasm. Although this does not increase the diversity of a compromised collection, it allows whatever is remaining to be secured and prepared for later augmentation of diversity through crossing, transformation, or mutagenesis.

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