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10

Strategies for Conserving Clinal, Ecotypic, and Disjunct Population Diversity in Widespread Species

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Why is a chapter on widespread species appearing in a volume on rare species? One answer to that is another question: Why focus on species in the first place? Granted, species do have a unique status. They are more or less closed units genetically, and species extinction signals the end of an evolutionary lineage that may have begun millions of years ago. By contrast, individuals and populations within species are lost and replaced all the time.

If a goal of conservation is to maintain species in the long term, then attention should focus on levels of biological organization within the species. Stripped of their taxonomic identities, organisms cluster naturally into hierarchical units of genetic similarity, with individuals at the lowest level and genetically related groups at the highest level. The species level is part of a continuum of genetic distinctness: diversity and rarity occur at every level, and much of this variation is adaptive. The evolutionary potential and resilience of species depend on the amount and structure of genetic variation at intraspecific levels. Widespread species, by virtue of their size, have complex genetic structures, and cause attention to be focused on these levels. During the early missionary phase of genetic conservation, it was appropriate and effective for the species level to receive the most attention. But now that conservation biology has advanced in its understanding of species and population biology, we recognize that we cannot ignore the unique, rare, and unusual variation that exists at intraspecific levels of most species.

This chapter reviews the genetic architectures of both rare and widespread species, and presents strategies for conservation of diversity within widespread species. Because widespread species by definition comprise many individuals and many populations, conservation strategies differ from those for rare species in that they aim not so much to preserve the entire species, but to sample genetically diverse individuals from some meaningful hierarchy of stands, populations, and races. This chapter draws attention to the inherent value and priority for conserving widespread species and emphasizes that intraspecific variation is important in determining conservation strategies for rare species as well. As forest geneticists, we have chosen as our examples tem-

perate-zone trees. We hope that the stories they provide will be useful for conservation of other taxa.

GENETIC ARCHITECTURE OF RARE VERSUS WIDESPREAD SPECIES

All species, whether rare or common, have a unique genetic profile that defines their hierarchical structure of variation, or genetic architecture of traits (Libby and Critchfield 1987). The existence of intraspecific levels of variation has profound implications for conservation of species. At each level, variation can affect fitness and viability. At the level of individuals, loss of variation (increasing homozygosity) can lead to inbreeding depression, which is often expressed as a severe decline in health (e.g., Libby et al. 1981; Sorensen and Miles 1982; Strauss 1987). At the population level, where variation is often related to adaptation to local environments, reductions in variation or other disruptions of the local gene pool may decrease the chance that a population can adapt to changing conditions. At the species level, loss of diverse populations reduces the potential of the species to respond to environmental changes at regional and global scales. Thus species existence depends to a large part on the genetic health of all the intraspecific levels. Similarly, the appropriate conservation strategy for a species also varies in response to the particular genetic architecture of each species.

Patterns of genetic variation can be grouped into general classes for specific taxonomic groups. Northern Hemisphere conifers have been widely studied, and we summarize some of the patterns of genetic variation that have been described for species in that group.

No or Little Genetic Variation Within Species

This situation is unexpected in most taxonomic groups, but especially in conifers, which typically are highly variable. Red pine (*Pinus resinosa*) is the best known example of this small class. Despite its wide distribution throughout the north central and northeastern United States and adjacent regions in Canada, and a reproductive system that favors the evolution and maintenance of variation, red pine is nearly invariant genetically (Fowler and Morris 1977). This situation is attributed to Pleistocene glaciation, which appears to have reduced red pine to a small area and eliminated variation. Red pine has subsequently recolonized vast areas and, within these areas, appears healthy and aggressive despite loss (presumably) of genetic variation (Barrett and Kohn, Chapter 1; Templeton, Chapter 12).

Species may be invariant in one part of the range and variable in another part. Western redcedar (*Thuja plicata*) grows as a scattered component of mixed conifer forests throughout the Pacific Northwest and British Columbia. In these regions, it appears to have little genetic variation (Copes 1981), perhaps another victim of Pleistocene climate changes. In its southernmost populations of northern California, western redcedar has some genetic variation. These areas may have been refugia for the species during the glacial periods, where variation persisted in moderate-size populations. Unusual environmental heterogeneity in northern California may also contribute to the evolution and retention of variation there.

No Variation Within Populations, Variation Among Populations

Torrey pine (*Pinus torreyana*) exemplifies this small class for conifers. Torrey pine is a rare California species known from only two small populations, one on the mainland near San Diego, and the other on Santa Rosa Island off the California coast, 280 km northwest of San Diego. Genetic studies show this species to have no variation within populations at any of 59 allozyme loci (Ledig and Conkle 1983). There are, however, differences between the populations at two allozyme loci and some variation in monoterpene composition (Zavarin et al. 1967). Thus minor differences between populations, presumably caused by founder events, are the only known genetic variation in the species.

Little Variation Within Populations, Little Variation Among Populations

This class of forest trees is slightly more common than the previous two. Santa Lucia fir (*Abies bracteata*), another rare California conifer that grows scattered in the rugged wilderness of the Santa Lucia Mountains, is an example. There is a modest amount of variation in this species, both within and among populations (Ledig 1987). The differences among populations follow no geographical pattern and are uncorrelated with obvious environmental factors.

These three classes describe patterns of variation that are considered exceptional for the outbreeding, long-lived conifers, but that may be more common for taxonomic groups with different reproductive systems. Geneticists concerned about long-term viability of conifer populations have usually advocated management that avoided inbreeding and that maintained large populations made up of unrelated individuals. These recommendations were aimed to minimize situations of low genetic variation within populations, such as occur naturally in the three classes described above. More commonly, conifers do not tolerate those genetic conditions. That there are so few examples in these classes may testify to this. The species that have survived well despite high homozygosity may be the few winners in an evolutionary game that extirpated many others. Managers have developed techniques to carry populations of normally outbreeding species through an inbreeding bottleneck to a point where high homozygosity is tolerated in the population (Barker and Libby 1974; Libby 1976; Templeton and Read 1983). This may mimic what occurs in the few successful natural instances.

High Variation Within Populations, Little Variation Among Populations

Several widespread conifers exemplify this pattern, either over their entire range or in certain portions of it. Incense-cedar (*Libocedrus decurrens*) is a widespread species common in mid-elevation mixed-conifer forests of California and western Oregon. Variation within populations in the Sierra Nevada portion of the range is relatively high, but only minor genetic differences exist among widely separated populations (Harry 1984, 1987). Low population differentiation but high within-population diversity in western white pine (*Pinus monticola*) is a common pattern throughout the ex-

tensive portions of its range in Oregon, Washington, and Idaho. Populations that extend into California, however, are distinct from one another and from the Pacific Northwest populations (Steinhoff et al. 1983). This pattern appears to reflect the relative degree of habitat heterogeneity over western white pine's range—low in the north, high in the south.

High Variation Within Populations, High Variation Among Populations

Conifers in this common situation can be divided into discontinuous and continuous geographical patterns. The difference between these patterns is often only a matter of scale.

Discontinuous

Abrupt genetic differences among populations—that is, large differences at many loci in repeated groupings—occur as a result of natural selection and/or genetic drift. When selection appears to be the primary factor leading to differentiation, as suggested by major differences in environment predictably being associated with particular combinations of genetic variation, the resulting pattern is called *ecotypic*. This may be recognized on a large scale, as in ponderosa pine (*Pinus ponderosa*), where at least four major ecotypes have been described: the Pacific, North Plateau, Rocky Mountain, and Southwestern races (Wells 1964; Smith et al. 1969; Read 1980) (Figure 10.1). The transitions among these races occur in more or less continuous pine populations over hundreds of kilometers. By contrast, on a small scale, bishop pine (*Pinus muricata*) is genetically differentiated into ecotypes that grow on and off pygmy-forest soils in northern California. The two ecotypes meet at abrupt ecotones only 0.5 km wide (Millar 1989).

Discontinuous variation also can occur primarily because of isolation of disjunct populations and genetic drift. In these cases, the pattern of differentiation may parallel the population disjunctions, and similar environments are not predictably associated with particular combinations of genetic variation. Certainly, selection is not suspended, but its role is less obvious, and the term "ecotype" is not used. In Monterey pine (*Pinus radiata*), for example, each of the five disjunct populations has genetically distinct traits (Bannister et al. 1962; Forde 1964; Millar et al. 1988; Morán et al. 1988). Although selective factors differ among the populations, isolation and genetic drift were probably more important in producing current patterns of population variation.

Continuous

Genetic changes often occur gradually over space rather than abruptly, in which case they are called *clines*. Clinal variation is usually ascribed to gradual changes in natural selection, but migration and genetic drift can also give rise to situations where genetic distance is correlated with geographical distance. At the level of the species range, differences among ecotypes may appear abrupt, whereas at the local level, the transition between races may be clinal (Sorensen 1979; see also Figure 10.1, ponderosa pine). This is common when populations are spatially continuous.

Where important factors of the species' selective environments vary gradually,

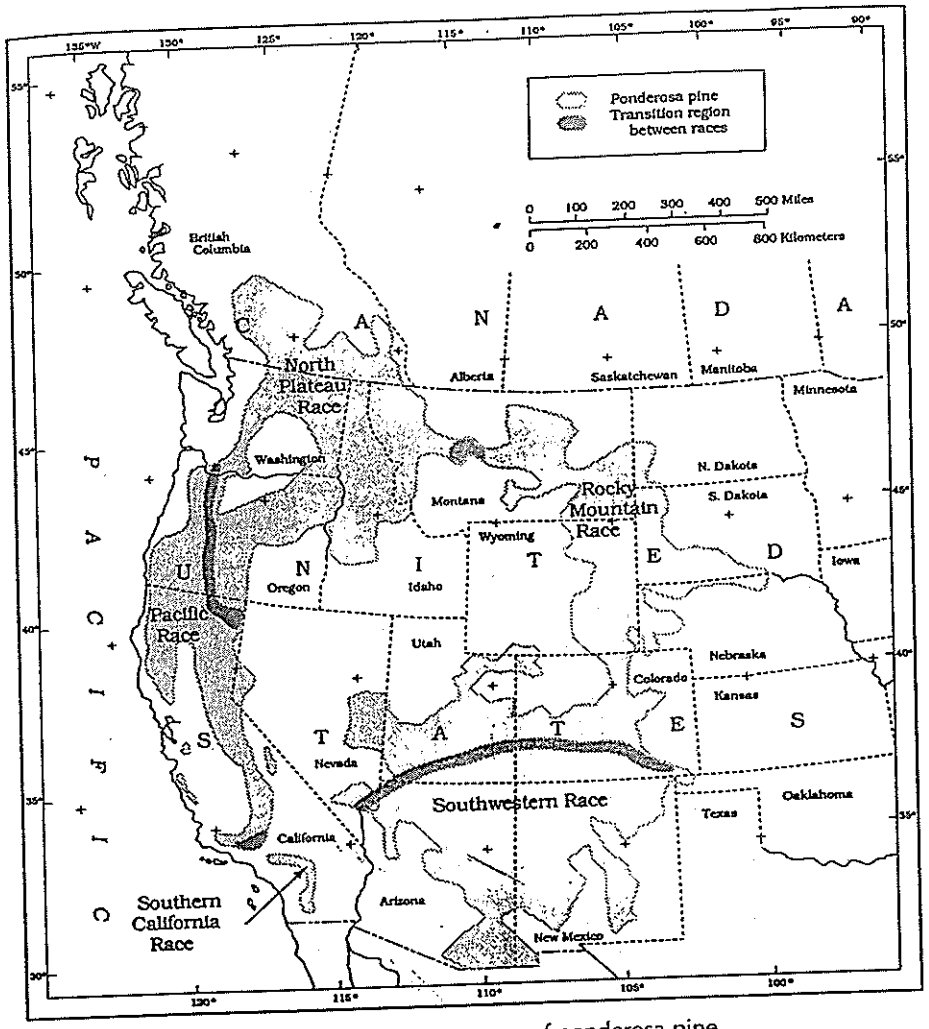

Figure 10.1. Major ecotypes of ponderosa pine.

such as climate, and when populations are large and more or less continuous, clines are likely to evolve. Many important physiological traits vary clinally in conifers. Most adaptive traits of ponderosa pine and white fir (*Abies concolor*) vary clinally from south to north and from low to high elevations in the Sierra Nevada of California (Callaham and Liddicoet 1961; Conkle 1973; Hamrick 1976; Libby et al. 1980). Average values for second-year height from a common-garden test in white fir vary clinally throughout the species range (Figure 10.2) (Hamrick and Libby 1972). Changes in allozyme frequency in knobcone pine (*Pinus attenuata*) vary clinally along the species' U-shaped distribution (Millar et al. 1988). Many other examples exist.

Clines occur not just in frequencies of genes, but also in levels of diversity within stands. In the southwestern United States, many conifers are most diverse in their more southern populations. Diversity within populations decreases clinally northward (Fins

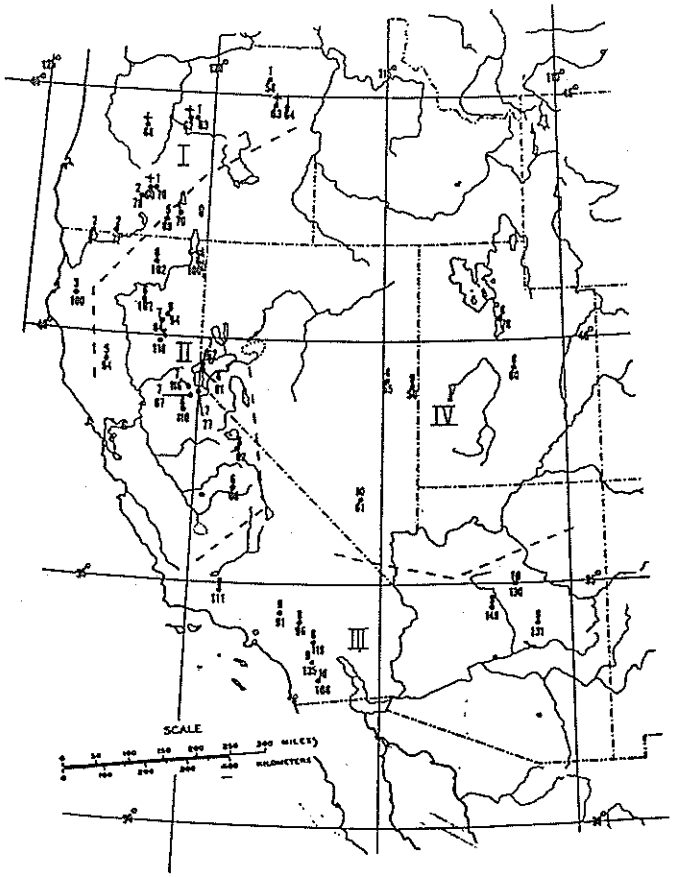

Figure 10.2. Clinical variation in average second-year height (values below black dots) of white fir in common-garden plantations; ecotypic variation in adaxial stomata rows (values above black dots) of white fir. Roman numerals indicate four regional groups indicated by multivariate analyses of nine traits. (From Hamrick and Libby 1972)

and Libby 1982; Steinhoff et al. 1983; Critchfield 1984; Furnier and Adams 1986; Ledig 1987; Millar et al. 1988).

In concluding this section on patterns of variation, we note that different patterns of variation may occur, depending on the traits studied. This discordance has been discussed for adaptive (e.g., survival and growth) traits versus presumably more neutral (e.g., biochemical) traits (Endler 1986; Libby and Critchfield 1987). In many conifers (e.g., lodgepole pine, *Pinus contorta;* Douglas fir, *Pseudotsuga menziesii;* and ponderosa pine), isozyme variation among populations is less than 10%, whereas a much larger proportion of total variation occurs among populations in obviously adaptive traits. For example, in lodgepole pine, 6% of the total isozyme variation is among populations, whereas 38% of total morphometric variation is among populations (Wheeler and Guries 1982). The discordance can occur even among traits within either of those categories. In bishop pine, large differences in allozymes yield one pattern,

survival and growth traits another, cone morphology a third, resin canal anatomy a fourth, monoterpene composition a fifth, and reproductive crossing relationships a sixth pattern (reviewed in Millar 1986). Obviously, this is a nightmare for taxonomists and conservationists, but is still entirely within the rules of evolution.

We have enumerated these patterns because on the whole they may occur in rare as well as in widespread species, differing only in geographical scale. Some generalizations are developing about the relative magnitudes of variation in rare versus widespread species. Despite the limitations of using one type of trait to infer a general pattern, enzyme polymorphisms—allozymes—are useful traits for comparing diverse taxa. Hamrick and his colleagues (Chapter 5 and references therein) have surveyed the relationships between allozyme variation and life-history traits for many taxonomic groups. For 28 conifers, endemic and narrowly distributed species had a lower index of variation than widespread species, although this difference was not statistically significant (Hamrick et al. 1981). On a broader taxonomic basis, the influence of geographical range (as well as other life-history traits) on genetic architecture has been compared by using allozyme data from 122 studies involving 91 plant species (Hamrick et al. 1979; Hamrick 1983). For the 91 plant species and four categories of geographical range (endemic, narrow, regional, and widespread), the highest levels of variation occurred in widespread species (Table 10.1). Overall diversity within the species and within individual populations on average was 10% larger in widespread species than in endemic and narrowly distributed species. Species with narrow to regional-size ranges had on average 10% more variability among populations than did endemic species. Of the few comparisons that involved pairs of closely related species with different distribution sizes, no generalizations were possible.

THREATS TO GENETIC DIVERSITY

Aside from strictly biological considerations, widespread species differ from rare species in ways that affect conservation. In forestry and horticulture, many widespread species are economically important, and census numbers of individuals usually increase, not decrease, under management. Intensive use and management of species lead to special conservation problems. The greatest risks are not to the species' existence but to the integrity of the native gene pools (Ledig 1986a, 1988; Millar 1987; Millar and Libby 1989). As such, the effects can extend throughout the species' range, wherever management occurs.

Table 10.1. Relationship of Geographical Range to Allozyme Variability in 91 Species of Seed Plants

Geographical range	No. of studies	H_t	H_s	G_{st}	A_p	P_a
Endemic	10	.275	.208	.200	3.26	.639
Narrow	31	.261	.177	.275	2.96	.609
Regional	38	.238	.154	.312	3.23	.573
Widespread	43	.380	.293	.253	3.70	.702

Key: H_t = total allelic diversity; H_s = mean diversity within populations; G_{st} = proportion of among-population to total diversity; A_p = mean number of alleles per polymorphic locus; P_a = the proportion of the total number of alleles found within each population.

Source: Hamrick (1983).

Some management activities have direct negative (*dysgenic*) impacts on gene pools. Foremost among these is high-grading, whereby the most valuable trees are removed each harvest cycle, and inferior trees are left to reproduce. For example, centuries of such dysgenic selection reduced the gene pools of once vigorous timber-producing forests of *Pinus densiflorus* and *P. thunbergiana* in Japan and Korea (S. K. Hyun pers. commun. 1960), *P. brutia* in Turkey (K. Jsik pers. commun. 1977), and *P. roxburghii* in the Himalayas (D. Khurana pers. commun. 1988) to stunted and malformed trees.

Although the dysgenic effects of high-grading are well understood, whenever some trees are left after harvest to regenerate the stand, diversity of their offspring may be affected. Pollen from the remaining trees contributes to the genotypes of future seeds not only in the harvest unit, but in the surrounding forests as well. If only a few trees are left to serve as seed parents (for natural regeneration), then inbreeding and its depression of viability are likely to build up in future generations.

Other actions tend to stir up the gene pool, disrupting rangewide genetic structure by changing allele and genotype frequencies in future generations. The actual effects on diversity and long-term adaptation of forests are usually unknown. *Artificial reforestation*—that is, planting nursery-grown trees back into native habitats—is a common practice of both operational forestry and intensive tree-breeding programs. If not carefully controlled, planting poses a significant threat to gene pools when nonlocal and novel genes are introduced into populations. The potential to disrupt natural genetic architecture increases as more lands are planted. Increasing concern for reforestation and restoration in many temperate forests has prompted enactment of laws requiring that planting follow disturbance of many kinds. If planting is not done with a serious regard for intraspecific variation, the species can be irrevocably affected.

Horticultural use of trees can threaten native gene pools in similar if more localized ways. The origin of trees used for landscaping is usually unknown and nonlocal. Thus when popular species are planted within or near their native ranges, it is unlikely, except for genetically homogeneous or extremely rare species, that they come from the same gene pool as the native population. Interbreeding of planted and native trees occurs, and foreign genes are introduced into native populations, often creating a permanent change. This situation occurs in two of the five populations of Monterey pine native to California, where Monterey pine is widely planted as an ornamental (Libby 1990). The planting stock came from New Zealand landraces, which derived mostly from a third native population several generations ago.

Similarly, it appears that the genetically distinct columnar form of Italian cypress (*Cupressus sempervirens*), which is a widely planted ornamental, has been invading native cypress forests in the Mediterranean region for millennia. Many trees of intermediate crown architecture are the results of interbreeding of the columnar cultivars with the normal broad-crowned forest trees (M. Vidakovic pers. commun. 1980).

In addition to forces that perturb the genetic structure of species, population extirpation is a serious threat to widespread species. The culprits are the same societal forces that threaten rare species—increasing urbanization and conversion to agriculture. In trees, for example, entire populations of MacNab cypress (*Cupressus macnabiana*)—a widespread but patchily distributed Californian species—have been lost because of land conversion. The entire type locality for the species at Whiskeytown, California, was drowned by construction of a reservoir, and a population at Grass

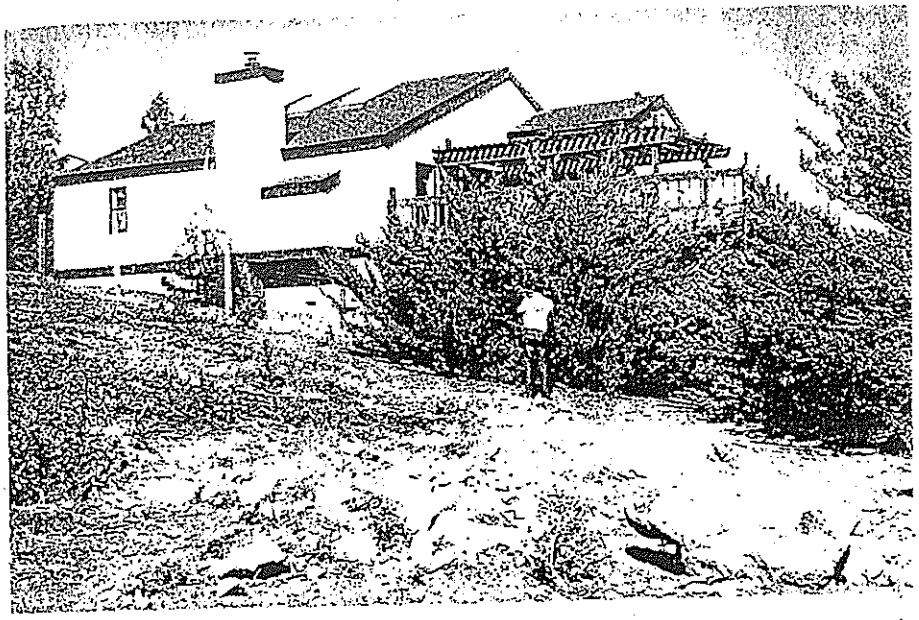

Figure 10.3. Remaining populations of MacNab cypress in Grass Valley, California, after construction of condominiums. (Photo by authors)

Valley, California, has been nearly obliterated by development of a condominium (Figure 10.3). In another example, the gracious valley oak (*Quercus lobata*), once a common symbol of California's Central Valley, has been forced by agricultural expansion into minor riparian refugia plus a few urban parks and estates. These stories are being repeated in most forested regions in the temperate zone.

ETHICAL CONSIDERATIONS

Conservation biology is continually faced with the dilemma of choice: Which taxa are chosen for protection from among the many in need? By limiting the field to the species level and by defining the rarest of rare species and the most endangered among these, there has been a hope that the job could be tackled. But we argue that the species level is only part of a continuum of genetic variation, and that rarity and endangerment occur importantly at levels within species. Species preservation per se may depend on conservation of intraspecific structure. This increases the number of candidates for protection far beyond our hope of even cataloging them.

Obviously, other criteria in addition to rarity and endangerment are needed to guide decisions. Those that have been discussed elsewhere include ecological importance and economic value (Oldfield 1984; Wilson 1988). A genetic criterion should also be added. Taxa would be ranked according to the quality (individual heterozygosity, stand and population diversity, number of hierarchical levels) and quantity of genetic variation they contain as well as to the uniqueness of the taxa, as measured by the genetic distance to the most closely related taxa.

Assigning ranks and assessing genetic value should not, however, follow a straight formula for maximizing diversity. For example, a monophyletic species such as *Ginkgo biloba* would rank higher than a "minor" species in a large section of a large genus such as *Pinus* or *Quercus*. Within species, however, it may not be the genetically distinct outlier populations that rank highest but the core populations from the center of a species range. In this case, within-stand diversity may be qualitatively more important than uniqueness of a population, although we hasten to add that certain types of distinct populations should rank high also (more on this later).

Another criterion is the cause of rarity. Some taxa are naturally rare, whereas others are artificially rare as a result of human actions. Does this situation confer different responsibilities for protection? The answer, we believe, is yes, for two reasons: ethically, we should fix what humans break, and biologically, the taxa that have not evolved under situations of rarity may be less stable than those that are naturally rare. The actual tolerance of naturally widespread species to reductions in population size and concomitant genetic changes depends on many factors, including paleohistory, life history, response to inbreeding, longevity, and phenotypic and developmental plasticity. That many widespread tree species do not tolerate reductions in population size well is indicated empirically by the severe inbreeding depression that occurs when N_e (effective population size) is lowered (e.g., Sorensen 1969; Libby et al. 1981; Sorensen and Miles 1982; Wilcox 1983), and theoretically by such models as genetic transilience (Templeton 1980).

Conservation of diversity within widespread species usually has an objective different from that for rare species. Because species existence is not threatened, it is the evolutionary fitness of the species that is being safeguarded. For rare species, by contrast, the final race against extinction is being fought. In the former case, conservation is the application of preventive medicine, whereas in the latter, it is crisis intervention (Templeton, Chapter 12). And, as in medicine, the benefits reaped by early and thoughtful maintenance are often greater and longer acting than those afforded by treating a battered system suffering a terminal illness. This is not meant to imply that endangered taxa should be given up and all conservation focused on healthy species. On the contrary, it is meant to draw attention to how much can be done and needs to be done before a taxon reaches the critical point. Perhaps this is the greatest challenge to conservation biologists in the long-run: that through our knowledge and influence we not only can rescue species from the brink of extinction, but also can prevent currently healthy taxa from ever becoming endangered.

CONSERVATION STRATEGIES FOR WIDESPREAD SPECIES

In the case of extremely rare plants limited to no more than a few small populations, the goal of conserving the entire species may be possible through habitat protection and *ex situ* preservation. For many other rare but not extremely rare species and for all widespread species, conservation will always involve protecting appropriate samples of the whole species. The goal in this case is to choose the samples such that the important and/or interesting genetic variation in the species is conserved. Such sampling decisions are based on information from genetic architecture studies.

The two main types of sampling are *in situ* habitat protection and *ex situ* germplasm

preservation. The pros and cons of each have been widely discussed (Frankel and Hawkes 1975). We take the approach of Falk (1987b) in advocating an integrated approach that involves both methods. Under normal conditions of climatic stability, we would emphasize the values of protecting genetically valuable populations through various levels of *in situ* management. The *ex situ* component would act both as a backup system to safeguard a wider range of variation than could be included in native habitat areas, and as a source of germplasm for research, monitoring, and restoration.

The specter of global climate change, however, threatens the value and security of *in situ* protection areas. If average or extreme temperatures change as fast as predicted, floristic changes will be enormous. On a local scale, there will be widespread population extirpation and vegetation shuffling. *In situ* areas that were originally chosen as prime habitat for specific communities may end up as completely different vegetation types. Although those areas may play new roles in conservation, their intended value as protection areas for specific types may be nil. In this scenario, *ex situ* collecting should play the primary role in sampling and preserving germplasm before it is lost.

We take an uncommitted stand regarding climate change. In the discussions that follow, we assume that both *ex situ* and *in situ* conservation will have value whether climate changes drastically or not. The two components together should be viewed as complementary, and when new information on climate change or other environmental threats becomes available, one or the other component can be favored and expanded.

Geographical Scale

The scale of concern is an important issue in the practical steps that follow. For many widespread species, it is unlikely that one centralized program of genetic conservation will be implemented. Rather, smaller political areas will act on a local level, coordinating, where possible, with other regions. In the following discussions, we assume the scale to be on the order of a single national forest of the U.S. Department of Agriculture Forest Service system. It is important to keep this scale in mind, since the numbers we recommend for conservation are intended to apply to small portions of the species range and not to the whole species.

Index Species

Although we are basically proposing a single-species approach, we consider that some species under consideration may effectively serve as index species for entire ecological communities. Choices among alternative strategies for the individual species will be weighted by their merit in conserving other, nontargeted species. In this discussion, the index species are commercially important tree species and their relatives. This is a realistic choice because trees command a large share of political and fiscal support, because they play central ecological roles in the communities they support, and because in general much more ecological and genetic information is available about them than about many of their associated plants, animals, or microorganisms. Trees are large organisms, with many aspects of their natural (genetic and ecological) history readily accessible. Other index species, such as the spotted owl, peregrine falcon, and black bear (few plant species have been used yet), are chosen for similar criteria.

The Land Component: *In Situ* Management

Genetic Management Systems

Conservation within native habitats protects and perpetuates the integrity of gene pools and of coevolutionary relationships. Individuals, populations, and species interact with one another and with changing environments and thus maintain their evolutionary fitness in ways that are nearly impossible elsewhere. There are, however, many forms of *in situ* management, with conservation objectives having different priorities among them (Salwasser 1987). Some conservationists consider strict reserves, established for the sole purpose of preservation, to be the only form of *in situ* protection. But because only small amounts of land will ever be available for strict reserves, and correspondingly small percents of widespread species will be included in these reserves, we are forced to consider ways in which conservation goals can be incorporated into the management of multiple-use lands.

We propose a composite category of lands called a *genetic management system*, distinct from other management categories because the objectives of genetic conservation would have higher priority over other uses. This category would group together many types of preexisting management classes. One extreme in the category would be strict nature reserves, such as wilderness areas, wild rivers, research natural areas, and other designated nature preserves. Less restricted areas would also be included if their management met certain genetic requirements. Examples include botanical areas and other special interest areas, streamside and riparian buffers, and such areas as spotted owl habitat areas and gene resource management units (GRMUs) (Riggs 1982; Krugman 1984).

For a particular national forest and for a species of interest, the boundaries of preexisting conservation areas would be overlaid on a map of the species distribution. A genetic gap analysis would then be made. This would entail systematically comparing the coverage of preexisting units in the genetic management system with the profile of genetic variation for the species of concern. Where gaps are located (criteria discussed in next section), candidate GRMUs would be sought.

The concept of GRMUs has been proposed and discussed at length both generally (Krugman 1984; Ledig 1988) and for specific cases (Riggs 1982). Authors have used the concept to imply somewhat different types of land management. We define a GRMU as *any designated forest area that meets minimum genetic management objectives*. GRMUs are not exclusive of many other forms of land use. For example, timber harvest could be allowed, although certain restrictions would apply. The main objective is to control the genetic composition of trees in the GRMU so as to maintain, restore, or mimic the natural genetic condition. Natural reforestation is preferred when timber harvest is excluded, but only if the seed and pollen parents are native and if the gene pool of the population has not been compromised (i.e., by high-grading, genetically positive selection, or extreme restriction of population size). If these restrictions do not apply or if planting is required for some other reason, strictly controlled planting is allowed if seeds from a certified native, local, and random sample of trees can be obtained. Species mixes must be maintained in the approximate proportions originally found on the site. To our knowledge, no GRMU has been expressly designated that fully exercises the multiple-use flexibility allowed under this concept.

For many species, it will be best to exclude timber harvest from the GRMU. Many forested areas are considered unharvestable because the lands are too steep, the soil is too erosive, the site is not capable of being replanted, or the growth rates are too poor. If these areas fit into the genetic management system, they can be maintained as de facto reserves because no timber management was planned for them anyway. The serious problem with these areas is that if fire is also suppressed on the GRMU or on surrounding areas, the association of species in the GRMU may change to a different successional type.

In many cases, however, the genetically most appropriate, interesting, and important stands are in designated commercial forests where timber harvest has been a top priority. If timber harvest is permitted on a GRMU, the type of harvest depends on the species and on the management of the surrounding lands. No type of harvest is automatically excluded, as long as a genetic prescription for the regenerating stand is written for the unit. When prescribed, artificial regeneration should be done with seeds obtained from a minimum of 20 randomly selected trees per species growing in the unit and chosen before harvest.

Placement and Design of Units in the Genetic Management System

The two major sampling considerations for conservation of widespread species are geographical placement of units and size of individual units. Many biological and nonbiological factors determine the final numbers. We will consider primarily the genetic ones, while acknowledging that practical concerns limit rather than increase the number and size of areas.

There has been much debate in the conservation community over the relative merits of a few large versus many small *in situ* conservation areas (Jarvinen 1982; Simberloff and Abele 1982; Janzen 1983). Most of the arguments have stemmed from ecological considerations of island biogeographical theory (e.g., Margules et al. 1982). However, the genetic system of the target species also affects the proper allocation and size of reserves. For highly inbreeding species where geographical differentiation is great and within-stand diversity is low (e.g., species of *Avena, Bromus, Elymus,* and *Hordeum* [Hamrick et al. 1979]), many reserves would be better than a few. The reserves could be small, with the major effort aimed at capturing geographical variation with many reserves. For temperate-forest trees, however, which are typically highly outcrossing and where within-stand diversity is high, large reserves would better maintain the important component of within-stand variability. If a few of these were strategically placed on the basis of patterns of genetic variation, much of the variability among populations could also be captured.

The first step in determining the sampling design is—if possible—to complete a study of the genetic architecture of the target species. Such a study can take many forms, from biochemical assays such as restriction-fragment-length polymorphism (RFLP), isozyme, or terpene analyses, to physiological or morphological studies in a common garden. The advantages of the biochemical assays are their speed and ability to yield precise genetic information. Common-garden tests are slow to yield information, and usually do not allow actual genotypic data to be estimated. They do, however, provide information about traits that more directly relate to adaptation than do biochemical analyses.

To determine optimal geographical placement of GRMUs, the following data are useful: magnitude and pattern of genetic variation in the species, amount of variation due to within- and among-stand factors, and geographical factors contributing to variation. These data usually can be obtained from the genetic analyses mentioned above. In addition, the following more specialized data are useful for each sampled region or stand: observed and expected heterozygosities, number of alleles at specific loci, and number of alleles in various frequency classes, including unique alleles.

We suggest that the highest priority for *in situ* conservation systems is to capture the core(s) of variability in the species. By this we mean areas that contain the common or shared genetic variability for a region. For economically important tree species, this area often defines the populations containing the most valued or useful trees as well, and it is important to conserve the base populations from which valued domesticated lines are desired. The number of cores depends on the regional patterns in the species. If a species has racial or regional-level variation (e.g., ponderosa pine, Douglas fir) or smaller-scale ecotypic variation (e.g., ponderosa pine on and off serpentine soils), then each ecotype would have its own core of variation. The percentage of variation that makes up each core depends on the amount of within- and among-stand variability for each species. For a wide-ranging conifer where much genetic variation is found within stands, each core might comprise over 95% of the allozyme variation and perhaps 75% of the adaptive variation in the race or ecotype. Areas that contain this core variation are often in the central portions of species ranges within regions on optimum sites for the species.

Each core of variability should be conserved by a minimum of two independent sites. There are two reasons for this: first, two sites reduce the risk of catastrophic loss (by fire, insects, climate change); and second, two sites capture some of the among-stand variability. To satisfy the second requirement, therefore, the two sites should not be as nearly alike as possible, but should sample some of the site differences within the central core of variation. Such core sites should be large (see below); if two independent sites seriously compromise the size of either one individually, then the single larger core site might be preferable.

As many more units as practical should be added to the genetic management system, but the core areas should be considered essential. For example, in doing a gap analysis, if the preexisting areas sample only the genetic margins or less variable portions of a species range (as is often the case with wilderness areas and other strict reserves [Harris 1984; Ledig 1988]), then the region would be considered insufficiently covered. In adding sites beyond the core, unusual genetic patterns and large discontinuities become important and should be added as possible. Among these, sites with high within-stand variability should be considered first, unless genetically impoverished sites are striking in their complement of unique alleles. The trade-offs between increasing the number of sites at the expense of losing size of individual units is discussed later, but the initial stage of selection should include more candidates than anticipated established areas. In the case of clinal variation or in other cases where no obvious core of variability exists, it would be best to locate two or three areas at significant genetic distance from one another along the main gradient of the cline. Secondary reserves would fill in gaps in the cline as well as any major discontinuities not contained in the primary areas.

After candidates for the genetic management system have been identified for the target species, the value of these sites for other native species (plants, animals, and microorganisms) should be evaluated. On the basis of available information on species occurrence and genetic variation in associated species, the candidate areas that maximize protection of associated native species should be chosen.

Other criteria may also influence selection of final candidate sites. For example, concerns about global climate change suggest that areas with potential escape routes for migration (e.g., whole drainages or at least large elevational spreads) or areas that are ecologically stable despite climate change (e.g., serpentine soils) should be preferred. Management of surrounding lands should also be considered. Whenever an area is situated such that adjacent lands are managed in a way that will compromise the genetic integrity of the GRMU, other areas should be considered. By corollary, any candidates that are adjacent to or in other ways complement other units of the genetic management system should be seriously considered. These areas not only have protected boundaries, but also add value to preexisting reserves by increasing their size and improving their integrity.

The other major sampling question for designing new units in the genetic management system is, How big should individual areas be? The answer depends on whether the goal is to maintain a population size that retains the current levels of diversity or to allow the population to decrease to some acceptable minimum number of individuals that hopefully will still ensure long-term survival of the population. Either way, the genetic integrity of populations depends both on the genetic structure of individuals within the GRMU and on management of adjacent lands.

The genetic factors influencing within-stand dynamics include inbreeding, genetic drift, immigration, and selection and are influenced by the reproductive system of the species. For many outbreeding species, where within-stand variation is normally high, inbreeding caused by reduced population size can cause a severe decline in health and vigor. These species will need relatively large populations to resist the long-term decline of heterozygosity as related individuals mate. The opposite is true for species that tolerate inbreeding. Genetic drift causes a decline in heterozygosity that is negatively correlated to effective population size. The rate at which heterozygosity declines depends not only on population size within the GRMU, but also on immigration from outside the unit. If immigration is reduced either unintentionally due to loss of the species outside the unit or intentionally due to buffer management (see below), drift has a larger effect than if a small amount of immigration is allowed (Crow and Kimura 1970). The drift effect of increasing homozygosity on the fitness of the organisms depends on initial heterozygosity and tolerance to homozygosity.

The uncertainties in these factors suggest that a conservative action would be to establish populations large enough to maintain current levels of heterozygosity. Of particular interest is variability at loci with adaptive effects. Unfortunately, this has proved recalcitrant to estimate. Formulae for calculating effective population sizes required to maintain heterozygosity have been developed for neutral alleles (Crow and Kimura 1970), and these have been applied to allozyme data. Calculation of this size depends on the relation of effective population size to actual census number, mutation rate, and current levels of heterozygosity. Based on allozyme data for ponderosa pine and Douglas fir, the effective population sizes range from 2230 to 910, 640, depending

on original heterozygosity and mutation rate (Table 10.2). For alleles with nonzero selective values, the N_e may be larger or smaller, depending on the type of selection on the locus, the intensity of selection, and the mutation–selection relationship.

Genetic variability within GRMUs is affected by conditions outside the GRMU primarily through gene contamination and secondarily by altered selection pressures due to human-caused disturbances. Some geneticists have argued that gene contamination via pollen from ill-adapted stock is not a problem, since maladapted individuals will be selected against in the GRMU. We take the conservative position that any foreign genes should be minimized in the GRMU, since deleterious foreign genes may not be eliminated quickly by selection, and they would cause a gradual decline in fitness. A swamping of deleterious genes, because of massive planting of areas adjacent to nonlocal populations, could cause a catastrophic loss of fitness in the GRMU population. Similarly, deleterious genes that act in late maturity or are deleterious only in rare episodic environmental conditions (droughts, freezes, etc.) will accumulate (Millar and Libby 1989). Either GRMUs must be large enough to effectively buffer populations from external effects (i.e., include their own buffer) or surrounding lands must be managed as a buffer. Either way, we recommend an active program of buffer management.

The size of the buffer depends on the extent of gene flow, which varies by species. In wind-pollinated tree species, viable pollen can disperse tens and even hundreds of kilometers (Lanner 1966; Koski 1970; Millar 1983), although most probably falls in the first hundred meters (Sarvas 1967; Wang et al. 1969). The potential impact from gene contamination depends on the degree of difference in allele frequencies and in local adaptation between the trees in a GRMU and those in the outside forests. In the unlikely event that there is no difference, then gene contamination has no effect, and a buffer against pollen contamination is unnecessary. At the other extreme, if trees near the GRMU have very different allele frequencies, as in plantations with nonlocal stock or highly domesticated trees, then an effective buffer is essential.

Table 10.2. Estimates of Effective Population Sizes (N_e) Needed to Maintain Current Levels of Allozyme Heterozygosities in Populations of Ponderosa Pine and Douglas Fir

Species	Region	H_e	N_e at $\mu = 10^{-5}$	N_e at $\mu = 10^{-7}$
Ponderosa pine	S. California	.082	2230	223,300
Ponderosa pine	N.W. California	.108	3030	302,700
Ponderosa pine	Sierra Nevada	.123	3510	350,600
Ponderosa pine	N.E. California	.157	4660	465,600
Ponderosa pine	N.W. California	.183	5600	560,000
Douglas fir	Sierra Nevada	.190	5860	586,400
Douglas fir	N.W. California	.209	6610	660,600
Douglas fir	N.W. California	.217	6930	692,850
Douglas fir	N.W. California	.229	7430	742,500
Douglas fir	N.W. California	.267	9110	910,600

Note: Estimates are calculated from Crow and Kimura's (1970) equilibrium heterozygosity formula for neutral alleles. Two mutations rates (μ) are given as a range.

Source: Millar (unpubl. data).

In the same way, the impact from altered selection pressures depends on the management of the external lands. If adjacent lands are only slightly modified, as with selection cuts, then a modest buffer suffices. If, however, these lands are completely altered, by clear-cut or conversion to nonforest use, then a wider buffer is necessary to ameliorate the selective effect on the genetic composition of the population in the GRMU.

The management requirements for the buffer resemble those for the GRMU: practice conservative forestry. Allow natural regeneration, or use local seeds. In many regions, there are not enough local seeds to meet all the planting demands (e.g., Riggs 1982). We suggest giving highest priority to the GRMU and the buffer zone when deploying the available certified local seeds. Harvest is allowed in the buffers, but selection cutting is preferred because the trees in the buffers should provide a physical barrier against both an altered microclimate and foreign pollen in the external zone. A loss of some of the resident species because of a shift toward later successional types is preferable to inappropriate populations of those species being planted in the buffer.

If natural regeneration is practiced in the GRMU and the buffer, harvest in the buffer can be timed to occur in the five years or so after harvest in the external zone. This is a good time to cut in the buffer, because then the regenerating trees in the GRMU are not in need of buffer against pollen contamination. Regeneration in the buffer is likely to come from the GRMU and not from outside because the external trees will still be immature. Also, the buffer should grow at the same rate as the outside area, and would guard against contamination as the stands mature.

If natural regeneration is not possible for the buffer and truly local seeds are unavailable, an alternative is to plant the buffer with a well-known, tested, and adapted exotic species (Ledig 1986a). Only those species known to be noninvasive should be chosen. For genetic reasons, a buffer of nonnative species is even better than one of native species because it acts not only as a physical but also as a genetic buffer of edge environment. A nonnative buffer will not become contaminated with genes from the native species, and thus will protect the GRMU longer than would a buffer of native species. Exotic species are easily identified and can be removed if necessary. Exotic species are far more desirable in the buffer than native species of nonlocal origin or even native improved stock. Obviously, the ecological effects of the exotic species on other organisms must be determined.

Once the genetic management system is established, the individual units should be defended and monitored actively. The boundaries of each unit should be clearly marked on the ground and on administrative maps used by forest officers. Opportunities that increase the area of units in the system, or establish new GRMUs, should be sought. If not already available, an ecological survey of the GRMUs and as many of the other units in the system as possible should be conducted. This survey documents baseline information about the areas, and should include a species inventory (plants and animals), soils information, and ecological data about the community types present. Scientific study in the GRMUs should be encouraged, especially pertaining to genetic aspects of both index and associated species. Comparisons between genetic data in strict reserve units and in those GRMUs where timber harvest or replanting is practiced would be useful to guide later management. If possible, reports from research studies should be accessible from a database maintained in a central location for each region.

Gene Banking: *Ex Situ* Management

Ex situ collections serve the essential role of filling the gaps and extending the range of genetic variation protected, and of providing germplasm to damaged habitats. In addition to their protective role if climate changes, *ex situ* collections act as insurance against other, more familiar disasters in the natural environment. Fire, insect epidemic, atmospheric pollution, subminimal viable population size, and changes in regulatory or management policies all threaten populations in natural reserves.

Ex situ collections can also provide important monitoring functions. If collections are made regularly, samples can be genetically analyzed, and changes in genetic composition of the protected stands determined. This would be especially useful if the native populations were thought to be at risk—for example, from gene contamination or inbreeding.

Finally, *ex situ* collections serve as a source of material for scientific research. The information that accumulates from research provides insight into the best conservation use of the collections.

Sampling Design

Many of the same concerns about designing an on-site genetic management system apply to *ex situ* collecting. Genetic information or inferences about genetic structure remain a necessary ingredient for planning.

As a first step, and to serve as backup, *ex situ* collection samples should be drawn from all *in situ* areas. The genetic gap analysis will provide information useful for locating additional collecting areas. These additional sites should be chosen to systematically sample a broader range of genetic variation than was included at *in situ* sites. Special emphasis should be given to genetically distinct populations (especially if they occur in unusual environments or in atypical ecological situations), to small or isolated populations, and to endangered populations. In all cases, efforts should be made to find the least disturbed populations to serve as collection stands. In all cases, detailed records on the collection should be kept, including as much of the following as possible: notes on access, location, and the physical, ecological, and management attributes of the site; number of collection (donor) plants; type and number of propagules; and mapped location of donor plants.

Although the total number of sites chosen for *ex situ* collecting will depend on the genetic structure of the species, in all but the most genetically uniform species there should be many more *ex situ* than *in situ* sites. The more genetically diverse a species is over its range, the more samples should be included. Transition areas between races or ecotypes, and steep gradients along clines should be emphasized. The optimal *ex situ* collections will include replicate samples from genetically similar areas, as well as extensive geographical coverage.

An example of partitioning a species range in forest trees is the practice of establishing seed zones and seed-transfer and collecting guidelines (e.g., Kitzmiller 1976) (Figure 10.4). Forested regions are divided into zones, which in theory include similarly adapted populations. Subzones are delineated to follow clinal or ecotypic contours within each zone. Seeds are collected from multiple stands within each subzone, and guidelines for the number of trees per stand are given.

The number of plants to sample at an area depends on the objectives for the col-

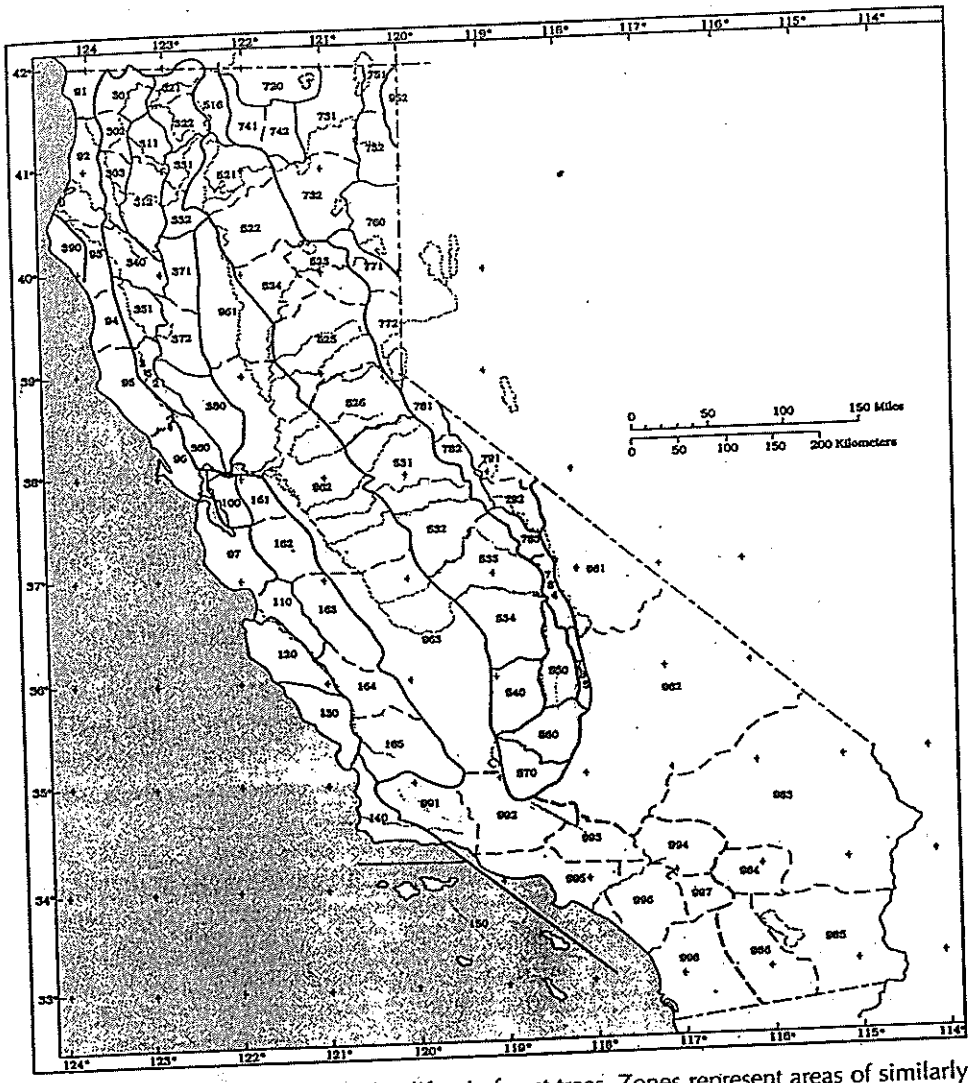

Figure 10.4. Seed-zone map of California forest trees. Zones represent areas of similarly adapted populations. Seeds for replanting after harvest are drawn from 155-m elevation bands within the same zone as the harvest unit.

lection. Collections may be done to duplicate actual allele frequencies of the native stands or to capture the largest amount of genetic variation without regard for frequencies (Marshall and Brown 1975). Much has been written about determining actual numbers for both situations (e.g., Brown 1975, 1978, 1988; Marshall and Brown 1975; Brown and Moran 1981; Chapman 1984; Yonezawa 1985; Namkoong 1988; Ryder 1988; Crossa 1989). Most calculations require genetic information, especially allele frequencies, within-stand diversities, and numbers of rare and unique alleles per population. For genetically variable species, the numbers of seed parents are usually smaller than those required for *in situ* conservation. This is especially true when the species is outbreeding because the genes in the embryos donated by the pollen parents

capture a wide range of local variation. Whenever possible, samples should be kept separate and labeled by parent tree. To reduce relatedness and increase genetic diversity, collection trees should be separated by at least 100 m. This distance depends on pollen flow distances and would be considerably less for species with pollen that does not disperse far.

Storage Considerations

Many kinds of *ex situ* germplasm storage are possible, including living plants in gardens, seeds in cold storage, tissue cultures, and DNA in genomic libraries. The advantages and disadvantages of these have been discussed elsewhere (e.g., Belcher 1980; Ledig 1986a; Bonner 1988, 1989; Millar 1991). We discuss here some special considerations of botanical gardens in maintaining *ex situ* collections.

In all that is done with storage of *ex situ* germplasm, one of the most important is recordkeeping. Proper identification is the first job of recordkeeping. A propagule without a name is useless to genetic conservation. (Although there are biochemical fingerprinting techniques that can help to identify orphan plants, they are expensive and time consuming.) The recordkeeping system should start in the field and track the location and storage conditions of the germplasm, following the germplasm wherever it goes.

The second job of recordkeeping is to maintain information about the performance of the propagules. If plants are grown in gardens or if samples are given to researchers who grow them, as much information as possible on morphology, pathology, growth, and other factors should be kept for that sample. The more information that is available for accessions in a collection, the more valuable the collection becomes. Even casual observations may prove important. All gardens should encourage scientific use of their germplasm, as this fosters an interactive flow of use and information.

Space is usually a problem at botanical gardens. Although many seeds can be stored in small freezers, adequate numbers of all but the smallest whole plants require large planting areas. Furthermore, if genetic information is to be collected from plantations, replication and regular spacing of the plants are required, which also increases the planting space. One option is for gardens to form consortia and to divide the samples of a widespread species among several gardens. Each garden would be responsible for one or several populations, although each population should be replicated in at least two sites. This has the advantages that the populations can be matched to gardens with the best growing conditions for the population and that replication guards against accidental loss at one garden. Furthermore, replication at different gardens can serve as a test of plant performance under various climates. This information may prove useful in the event of major climate change and the need to establish populations in new habitats. If pollination is to be allowed and seeds are to be collected from garden-grown plants, wind pollination can be allowed if only one population has been grown at the garden (but consider the problem noted below). A major disadvantage when the entire collection is not housed together is that relative performance of the populations cannot be compared, since plants from different populations are grown in different environments. Furthermore, dividing the collection makes maintenance, curation, and use more difficult. The benefits, however, may outweigh these problems.

A special problem of gardens is that they may be within gene-flow range of other members of the same species. This is a problem only when seeds are to be collected—

for example, to enhance or replenish stored seed collections—and not if clonal replication of the plants is planned. If the garden is outside the native range of the species, gene contamination can occur owing to planted ornamentals in the vicinity of the garden. The problem is potentially worse if the garden lies within the range of the species, where the native pollen cloud may outcompete the pollen from the planted individuals. This problem may be reduced by bagging female flowers, collecting pollen, and artificially pollinating the plants. This process, however, is time consuming and expensive.

CONCLUSIONS

We propose a five-step plan for conserving widespread species:

1. Study the genetic variation of the index species, or in some other way infer its patterns of genetic variation.
2. Plan and implement *in situ* genetic management systems for local regions (e.g., for trees, areas the size of national forests in the U.S. Forest Service system). Units in the system include strict reserves (e.g., wilderness areas, botanical areas) and multiple-use areas called gene resource management units (GRMUs) where genetic management is the main objective, but some manipulative practices may be allowed. The *in situ* areas should sample the genetic cores of the species for the local region as well as key areas representing important ranges of diversity. The final choice from among candidate units should be decided by the quality of protection that is afforded important associated species.
3. Plan and implement an *ex situ* germplasm conservation program to complement and extend the *in situ* program. *Ex situ* collection sites should be much more extensive and sample more genetically extreme and unusual sites than *in situ* units. Keep the collection viable by replenishing periodically.
4. Monitor genetic diversity over time in both *in situ* units and *ex situ* collections. Encourage research use and maintain files of all information on performance of the plants.
5. Actively promote the conservation use of both *in situ* areas and *ex situ* collections. *In situ* areas can be used as habitat for reintroduction or enhancement of endangered species (animals as well as plants). *Ex situ* collections should be offered when restoration of native habitat is needed (after fires, reclamation, etc.).

Conservation biologists have correctly pointed out (Salwasser 1987; Ledig 1988) that since only small percentages of native habitat will ever be fully protected in strict reserves, the crux of the problem lies in wisely managing the remaining lands. Although we also promote conservation-minded management on all wildlands to the extent that is practical, we know there are many practices that by their nature counter the goals of genetic conservation even when they are highly constrained. We do not think that the only conservation strategy is to abolish or severely restrict these practices. In many cases, this would only delay a diversity problem. Rather, an extensive and replicated conservation program such as we propose could release some wildlands for intensive management. This can be envisioned as a trade-off situation: the better the

conservation program, the more land that could be made available for other uses. By this, we are not condoning irresponsible or exploitive use of these areas. We are suggesting instead that intensive management of resources is not counter to conservation goals when it is part of a comprehensive program that also includes intensive conservation. With the possibility of relief from excessive environmentalist attack and government regulation, this approach may motivate some previously anticonservation groups to support large conservation programs financially.

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