

Review paper

Allozyme markers in forest genetic conservation

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Application. Allozyme markers are useful in three main aspects of forest genetic conservation: assessing the amount and distribution of genetic variation; determining sampling plans for in-situ and ex-situ conservation; and monitoring changes in genetic diversity. Since allozymes represent only one type of polymorphism, they are best used in conjunction with other traits when information about adaptive variation is needed.

Abstract. Genetic diversity is important in tree-breeding, in managing rare and endangered tree species, and in maintaining healthy populations of widespread native tree species. Allozymes are useful in determining genetic relationships among species, where they can be used to assess affiliations of rare taxa and predict relative endangerment among species. Because allozymes sometimes yield different information about genetic variation within species than revealed by other traits, when estimates of total or adaptive genetic variation are important, allozymes are best used in conjunction with other traits. Allozymes are useful for measuring direct allelic diversity when designing ex-situ and in-situ conservation strategies. We demonstrate an application of canonical trend-surface analysis for determining locations of in-situ genetic conservation areas. Allozymes also serve as useful markers in monitoring the effects of forest management and other environmental changes on genetic diversity.

Introduction

Forest genetic conservation has its origins in the crop gene-resources movement (Frankel 1977). Initial concern focused primarily on economi-

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cally important tree species, and on the importance of conserving germ-plasm for future benefits to tree breeding. Because commercial tree species, unlike agronomic crops, had long rotation times and were planted in wildland settings, diversity was important for adaptability as well as increased productivity. Within such a context, the goals and techniques of genetic conservation became interwoven with long-term tree-improvement strategies (e.g., FAO 1975; Kitzmiller 1976, 1990; Zobel 1977; Namkoong 1984).

Increasing concern for environmental degradation has broadened the scope of genetic conservation to include non-commercial species, especially rare or endangered taxa, for their intrinsic values (Namkoong 1986; Ledig 1986a, 1988a, b). Forest geneticists view as serious losses the species extinctions and systematic impoverishment of populations that are occurring not just in tropical ecosystems, but in temperate forests as well. Trees are also increasingly valued for their role in ecosystem functioning. As structurally large organisms, trees create the physical and biological habitats on which many other plants and animals depend for their survival.

Allozymes in the service of genetic conservation

For many of the same reasons that allozyme markers have been useful in other aspects of forest genetics, they find applications in a wide range of genetic conservation situations. Research and application in forest genetic conservation can be divided into three steps: Assessing, conserving, and monitoring genetic diversity. Genetic conservation borrows heavily from the cumulative research of forest genetics, using information about genetic architecture, mating systems, gene flow, etc. as input for many conservation analyses. It is because so much basic knowledge exists about population genetic parameters in trees that we have been able to progress in conservation analyses beyond what has been possible in lesser known plants.

Assessing genetic diversity

Knowledge of baseline genetic diversity among and within species is needed for nearly all conservation analyses and applications. This information, whether measured or predicted, is essential for deciding what and how to conserve, for assessing whether genetic changes are occurring, and for determining whether genetic parameters are even relevant in a conservation plan. Assessing baseline diversity goes beyond the inventory or

description of taxa and genetic variation. In most cases it includes an analysis of pattern in the data and attempts to interpret the pattern in light of evolutionary history and ecological context.

Systematic studies

Knowledge of species boundaries and genetic relationships among species are critical to conservation. Both by legal mandates and general consensus among conservation biologists, genetic resources of an individual species are usually given higher priority for protection against extinction than populations within a species. Although in the temperate zone species extinction in historical times has been infrequent in tree species, 233 woody plant species have been identified by the Center for Plant Conservation as being of "significant conservation concern" in the United States (Falk 1990; Ledig 1991). For example, American chestnut (*Castanea dentata*) has been all but eliminated by an introduced blight early in this century (Burnham 1988). American elm (*Ulmus americana*) and white pines (*Pinus* sect. *Strobus*) (Kinloch 1972) also have been devastated by introduced pathogens. The ranges of Port-Orford-cedar (*Chamaecyparis lawsoniana*) (Zobel et al. 1985) and Florida torreya (*Torreya taxifolia*) (Godfrey and Kurz 1962) are being fragmented by diseases. Ozone and other pollutants are narrowing the gene pools of pines around urban areas, such as the Los Angeles basin (Miller 1973). Genetic diversity in Fraser fir (*Abies fraseri*) and red spruce (*Picea rubens*) in the Appalachians is being lost as a result of combined onslaughts by insects, climate change, heavy metal pollution and acid deposition (Hamburg and Cogbill 1988). In tropical forests, the rate of species extinction is high (Wilson 1988).

Allozymes are useful for evaluating species boundaries because they provide more-or-less standard yardsticks that make comparisons among taxa meaningful (Strauss 1992, this issue, pp. 125–158). They are also less subject to problems of homoplasy (that is, similar traits occurring in distinct taxa that are not due to common ancestry) than traits traditionally used to evaluate taxonomy. Because endangered taxa at the species level are eligible for protection under endangered species legislation, taxonomic determinations carry legal implications. Although listing under plant protection legislation involves an evaluation of many factors affecting endangerment, one important consideration is the degree of genetic distinction of the taxon. Allozymes can provide a quantitative estimate of distinction, thereby helping to determine conservation priorities (Millar and Libby 1991). In some cases, allozymes may indicate that a taxon traditionally accepted as a rare species (and maybe already listed) is actually well within the range of variation of a more widespread species. In other cases,

allozymes may draw attention to distinct taxa that hadn't been recognized on other grounds. Because of inconsistent correlations of allozyme with other traits (Hamrick and Godt 1989), conservation priorities probably should not be determined legalistically on allozymes alone, although they are important when combined with other data.

The cypresses (*Cupressus*) of southwest United States and Baja California provide an example. As many as 14 species (Wolf 1948) and as few as 7 (Little 1970) have been recognized, all of which occur as mosaics of disjunct populations associated with sterile soils. Santa Cruz cypress (*C. abramsiana*) is federally and state-listed as endangered. We analyzed allozyme variation in 62 populations at 25 loci of all taxa in Baja California and the United States, and found patterns of relationships that differed in some cases from those suggested by the published taxonomies.¹ For example, there was no evidence that Santa Cruz cypress is a distinct taxon. Its five populations were well within the range of variation of the Sargent cypress (*C. sargentii*) complex. The Santa Cruz cypress populations had lower genetic distance to populations of other taxa than to each other. The five populations of Tecate cypress (*C. forbesii*) in California, which were petitioned for state listing, were also within the range of another taxon, the Arizona cypress complex (*C. arizonica*, *C. glabra*). A sixth population of Tecate cypress, however, on Guadalupe Island, Mexico, was distinct from this group. Cuyamaca cypress (*C. stephensonii*), known from one small and declining population in the United States, but not currently being considered for listing, was a distinct taxon allozymically. In almost all taxa, the populations with the smallest sizes were more divergent.

In another example, Prober et al. (1990a) analyzed allozyme variation of the 12 "green ash" species of *Eucalyptus*, which include several rare species of conservation interest, and identified several relationships that had been either poorly resolved or intractable to morphological analysis. A major difference was the strong allozyme evidence for monophyly of members of the "dendromorpha" group, a relationship not indicated by morphology. Based primarily on analysis of allozyme distance and genetic diversity, the rarity in *E. paliformis* and *E. rupicola* was shown to be old, whereas rarity in *E. burgessiana* was mostly likely due to recent divergence. The allozyme analyses, together with morphological data, provided important information for setting conservation priorities and managing these rare species.

In some cases, distinct populations given species status are identified by allozyme markers as hybrid populations. For instance, in the Black Hills of South Dakota and Wyoming, distinct oak populations were originally classified as *Quercus mandanensis*. Allozyme analysis suggested instead that these populations resulted from hybridization between closely related

Q. macrocarpa and *Q. gambelii* with extensive backcrossing to *Q. macrocarpa* (Schnabel and Hamrick 1990). Thus, by clarifying the close relationship of these populations to non-endangered species, conservation efforts might be redirected to distinct endangered species.

Diversity within species

The literature on genetic architecture in natural populations of forest trees in rich (Conkle; Dancik; Müller-Starck; Moran; Loveless; Smouse; Hanover, this issue), and the envy of conservation biologists who work with lesser known taxa. Although most of these studies were not carried out intentionally for conservation purposes (and will not be reviewed here), like studies on systematics, they provide critical baseline data for conservation. For a species that has been identified for conservation efforts, information about genetic diversity within species provides the raw data for determining conservation guidelines.

Genetic conservation has been the major motivation for assessing diversity within rare or threatened tree species. Torrey pine (*Pinus torreyana*), endemic to two small and widely disjunct populations in California, had declined to about 500 trees by 1921 (Dusek 1985). No allozyme variation was found within either of the populations at any of 59 isozyme loci, although there were fixed differences at two loci between the populations (Ledig and Conkle 1983). The lack of genetic variability may have contributed to Torrey pine's decline; several growth and reproductive traits characteristic of the species seem to be maladaptive (e.g., seeds are retained in cones even when open; seeds germinate in cones; seed maturity takes six months longer than usual for related pines, Ledig 1986a). Active protection of both populations has increased the census to about 10,000 trees (Ledig and Conkle 1983). Should the numbers decline again, for example due to pest or pathogen epidemic or to climate changes, a small amount of genetic diversity could be reintroduced by crossing trees from the two populations.

Not all rare tree species are depauperate of genetic diversity. Monterey cypress (*Cupressus macrocarpa*) exists in two small California coastal populations with a total population about 10,000 (Conkle 1987). Monterey cypress resembles Torrey pine in its narrow endemism, habitat and ecology, and evolutionary history. Unlike Torrey pine, however, Monterey cypress maintains high heterozygosity within populations (0.18) with geographically structured variation both within and between the two nearby populations (5 km apart) (Conkle 1987). Although parts of the native populations are protected, expanding urban development on the Monterey peninsula creates a need for mitigation, and artificial regenera-

tion is commonly undertaken. The genetic data indicate that propagules should come from very local samples and should include many trees in each sample.

The general criticism that allozymes are neutral markers and do not adequately estimate adaptive (e.g., growth and form) variation (e.g., Hamrick and Godt 1989; El-Kassaby 1990) is especially relevant to conservation. Measures based on different loci and different traits often yield discordant results about geographic patterns (Smouse, this issue, pp. 179–196; Hanover, this issue, pp. 159–178; McDonald 1983). The lessons learned in forestry about seed transfer and the importance of local adaptation make it important that adaptive variation not be underestimated in conservation. Multivariate analyses of allozyme data seem to correlate with patterns based on growth and form better than single-locus methods (Conkle and Westfall 1984; Guries 1984).

Where datasets are available on different kinds of traits, one possibility is to use all genetic information together to assess patterns of variation important for conservation, as has been done in numerical taxonomy (Sneath and Sokal 1973). If different traits give discordant patterns of variation, the species is divided into the smallest reasonable units suggested by cumulative analysis of individual patterns. An example using bishop pine (*Pinus muricata*) is given in Fig. 1. This approach weights traits equally (as has been suggested in crop germplasm sampling, Marshall 1989), accepting the conservative position that we cannot know what genes are or will be adaptive in future environments. This approach, of course, depends on the number of traits available, which, if large, could result in over-partitioning the taxon. An alternative approach would be to use the trait or markers that give the most sensitive and random sample of the genome.

Correlates of genetic variation

Given the importance of baseline genetic diversity for conservation and the difficulty of obtaining such data, there has been an urgency in the conservation field to find correlates of genetic variation. The underlying hope is that some readily observable traits or attributes of species might allow indirect estimation of genetic architecture, obviating the need for direct genetic studies.

The most comprehensive efforts have been made over the past 15 years by Hamrick and colleagues (Hamrick and Godt 1989; Hamrick, this issue, pp. 95–124), who have analyzed correlations of life-history traits of plants with allozyme statistics from hundreds of studies. As the number of species included in the analyses increased (449 species in Hamrick and

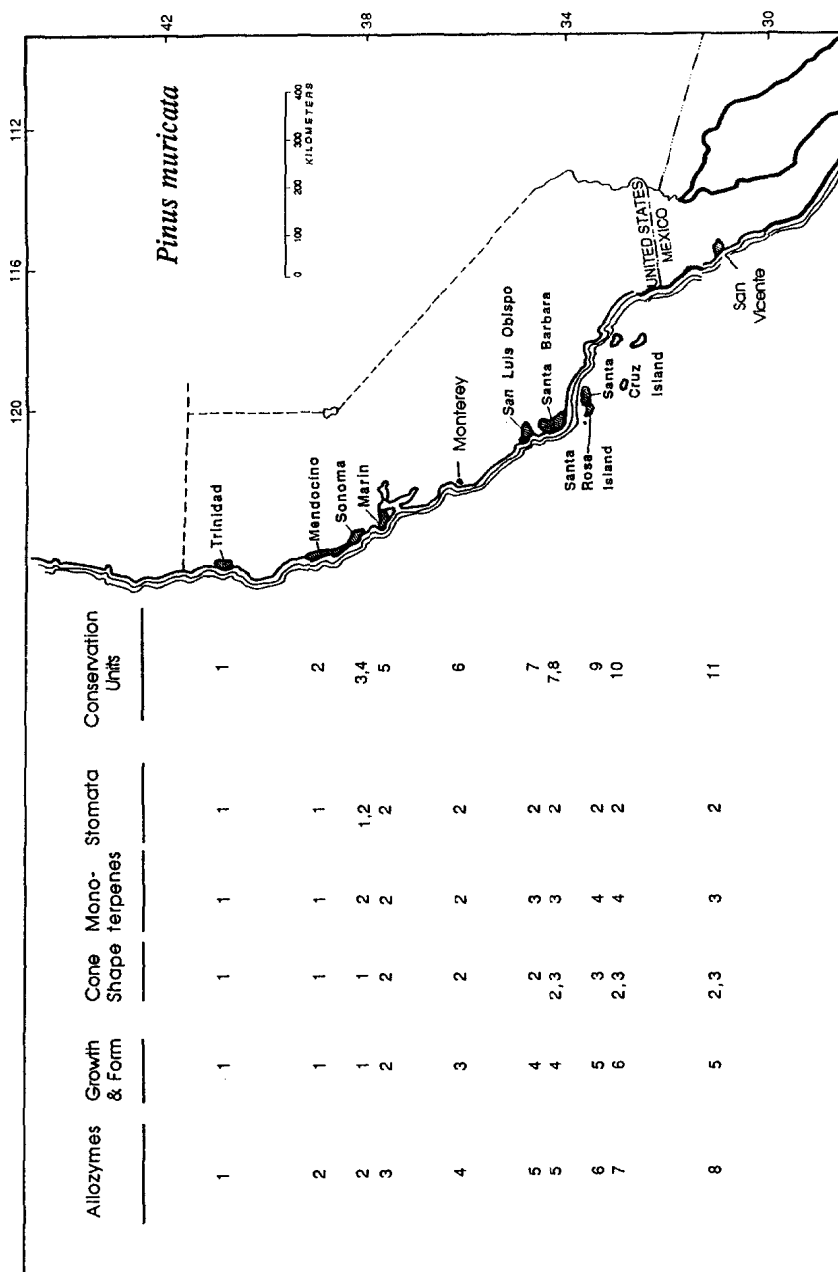


Fig. 1. Patterns of genetic variation in bishop pine (*Pinus muricata*) based on various traits, and conservation units derived from this information. Similarities among populations in each trait are indicated by identical numbers among populations; genetically dissimilar stands within a population are indicated by two numbers together. Despite quantitative variation in some traits, the groupings represent distinct clusters in all traits. Conservation units with different numbers should be treated as discrete groups in planning in-situ or ex-situ conservation. Data from Millar and Critchfield 1986; Millar et al. 1988; Millar 1989; unpub. data.

Godt 1989), certain traits appeared increasingly correlated with genetic diversity. Of special significance to conservation is the observation that geographic range accounts for the largest proportion of variability in genetic diversity at the species level. Widespread species contain significantly more allozyme diversity than narrowly distributed species. Narrowly distributed species, however, partition variation within species in the same way as more widespread species do. Partitioning of variation within species is correlated most highly with plant breeding system. Although these models provide generalizations, only about 30–50% of the variation among species is accounted for by the model (Hamrick and Godt 1989). Thus, direct genetic analyses of individual species are still necessary when genetic structure must be reliably ascertained.

Other correlations may exist at regional levels. For example, expected allozyme heterozygosities in conifers of western North America increase clinally with decreasing latitude (Ledig 1987, 1988b). This may result primarily from effects of the Pleistocene on conifer distributions. Northern populations were extirpated or severely challenged during glacial periods, whereas more southerly populations persisted, retaining high diversity. Following the return to warmer interglacial periods, northern regions were recolonized, with concomitant drift effects eroding genetic variability in the newly founded populations. For conservation, the most diverse conifer populations in western North America are southern ones; northern populations, although lower in total diversity, may contain unique alleles.

Conserving genetic diversity

Assessment of genetic diversity is only the prelude to genetic conservation. Whether conservation acts reactively to correct critical situations involving extremely endangered taxa or proactively to maintain diversity of currently stable species, decisions must be made about how and what germplasm to conserve. Traditionally germplasm has been conserved either in-situ, where organisms interact in natural or restored populations in wild habitat and condition, or ex-situ, where germplasm is stored in isolation from the natural habitat. Comprehensive programs that integrate both methods are most effective practically (Falk 1990; Millar and Libby 1991). In all cases, sampling decisions must be made about what genes to sample and from what locations.

Population viability analysis — Sampling in rare and endangered species

Soule (1987) has argued that the question “how much is enough” is the

quintessential issue in conservation biology. For rare and endangered species, population viability analysis (PVA) attempts to define minimum conditions for a population or species to maintain itself over an agreed-on time with an agreed-on degree of certitude (reviewed in Soule 1987). PVA is an analysis of risk because it acknowledges the impossibility of guaranteeing absolute survival of a group indefinitely and the need for society to decide on an acceptable risk. The time scale usually used is on the order of centuries and the probability usually over 95%. Many factors influence viability of populations, and these are categorized as genetic, demographic, environmental, and catastrophic (Shaffer 1981). Theoretical constructs have been developed that independently model genetic and demographic effects on viability, and significant advances in considering both aspects have been made. Nevertheless, we are still far from being able to model or measure all the main factors and their complex interactions.

Attempts at empirical PVA have been limited almost exclusively to animals, especially birds and large vertebrates, although application of theoretical PVA to plants (Menges 1990a) and empirical assessment of plants (Menges 1991) is beginning. In forest trees, although comprehensive analyses have not been made, some of the genetic components of population viability have been evaluated. In general, inbreeding has been viewed as the principal genetic threat to short-term population survival and genetic drift as the principal threat to long-term survival (Frankel and Soule 1981). General estimation of these parameters for trees is reviewed in Epperson, Mitton, Ellstrand, Smouse, and Hanover in this issue. Specific aspects relevant to conservation are considered here.

The relationship between allozyme heterozygosity and fitness is especially important to conservation. In trees, allozyme heterozygosity has been shown to be positively correlated with fitness (usually measured as growth) in many but not all cases (Mitton and Grant 1984; Ledig 1986b). Much discussion has centered on the source of fitness, whether due to heterosis or release from inbreeding depression. Regardless of the nature of the effect, outbreeding trees have been shown repeatedly to carry high genetic loads and suffer inbreeding depression (reviewed in Ledig 1986b). Species that are low in allozyme diversity, especially those that are naturally rare or those that have undergone bottlenecks in their geologic past generally suffer lower inbreeding depression than species that are high in diversity and have maintained large population sizes for long times. In species that survive bottlenecks and/or persist as rare populations, most seriously deleterious alleles seem to have been purged, although mildly maladaptive genes may be fixed (Ledig 1986b). By contrast, species whose distributions have been reduced by human actions may be at greatest risk of accelerated decline in population viability and numbers

due to rapid exposure of recessive deleterious alleles in homozygous condition. This effect has been demonstrated repeatedly in animal breeding programs when formerly large and outbred populations are reduced to small effective population sizes (Lande and Barrowclough 1987).

In an effort to assess relative viability and endangerment of two rare *Eucalyptus* species, *E. paliformis* and *E. parvifolia*, Prober et al. (1990b) measured several ecological as well as genetic parameters. In addition to allozyme structure, they estimated numbers of individuals (population size), number of breeding individuals (trees bearing fruit, an estimate of effective population size), stand densities and age structure, fire history, and gene flow among the populations. Considering these factors together, they were able to show that *E. paliformis*, although lower in genetic diversity than *E. parvifolia*, had high and stable effective population sizes, indicative of an old rare species. By contrast, *E. parvifolia*, which had high genetic diversity indicative of a formerly widespread species, had only recently undergone reduction in size. They estimated that it had low effective population sizes, was under threat of swamping through hybridization from several widespread *Eucalyptus* species due to its being relegated to marginal sites for the species, and therefore was at greater risk of extinction.

When populations of rare species cannot be protected in-situ, their germplasm may be removed and stored ex-situ. Brown and Briggs (1990) use the neutral allele model to determine the minimum number of individuals to sample for a rare species. They argue that rare alleles are probably insignificant to species survival and that the effort involved in obtaining them jeopardizes protection of other species. They show that allelic content of a sample is proportional to the logarithm of population size and sample size, a relationship independent of mating structure and population structure. This relationship has been shown to hold for the proportion of alleles with frequency greater than 5% surviving a bottleneck (Sirkkomaa 1983). From this "law of diminishing returns", Brown and Briggs argue that the first ten individuals randomly sampled from a population are as important genetically as the next ninety. Although small samples may preserve alleles, these guidelines seem to ignore the effect of small samples on genotypic compositions, the degree of inbreeding depression in the species, and subsequent survival probabilities.

Sampling in widespread species

For widespread species where imminent extinction is unlikely, the objective for conservation extends beyond sustaining minimum conditions for survival to promoting healthy populations with sufficient genetic diversity

to adapt to environmental stresses. Thus, sampling strategies depend on how much diversity is necessary to maintain forest health. This issue is nearly as intractable as that of minimum viable population size, although theoretical aspects have been considered (reviewed in Brown and Moran 1981).

1. *Sampling for ex-situ conservation*

Since the amount of time and money available constrains sampling, the topic of sampling efficiency has received considerable attention in the crop literature. Sampling to capture unique or locally rare alleles or to replicate natural allele frequencies in a sample may not be worth the effort necessary to get them (Marshall 1989). Efficient sampling captures the maximal amount of useful variation within practical limits (Allard 1970). The number of alleles per locus, usually measured by allozyme statistics, has been considered the best measure of genetic diversity (Marshall and Brown 1975; Asins and Carbonell 1987). Efficient sampling would occur when at least one copy of each important allele in a population was preserved. Marshall and Brown (1981) define priority of alleles in a population by subdividing alleles into four classes, based on frequency within the population ($> 5\%$ and $< 5\%$) and distribution among populations ($> 10\%$ and $< 10\%$). Highest priority in sampling should be given to the class of common local alleles. These alleles are apparently maintained in frequencies higher than the mutation rate and may be important elements of geographic diversity. Common widespread alleles are likely to be contained in all sampling strategies and rare local alleles are difficult to obtain. Modifications to this approach and applications to more sophisticated approaches when more information is available have been developed (in Marshall 1989).

Several allozyme datasets of trees have been divided into these classes. For six conifers, percent of alleles in the locally common class ranged from 0–17% (Adams 1981; Brown and Moran 1971). In 20 California conifers, this range was 0–32% (Table 1). The size of this class was unrelated to geographic distribution of the species (rare versus widespread; disjunct versus continuous populations), taxonomic affiliation, or ecological status. With a few exceptions, there was a common pattern for the classes of common widespread and rare local alleles to dominate, together accounting for over 80% on average, whereas proportions in the two other classes varied. The distribution patterns of alleles in fifteen *Eucalyptus* species (Prober et al. 1990b) were similar to the conifers, although in the eucalypts, the general pattern had fewer common widespread alleles and more common local alleles. This may be due to insect pollination in the eucalypts versus wind pollination in conifers. Efficient sampling strategies

Table 1. Percents of alleles with four types of distributions among populations of 20 California conifers

Species	No. pops. sampled	No. alleles	Common		Rare		Source
			Wide- spread	Local	Wide- spread	Local	
<i>Pseudotsuga menziesii</i>	62	217	44	32	5	19	(1)
<i>Pinus ponderosa</i>	60	199	54	28	1	18	(1)
<i>Cupressus macnabiana</i>	10	93	48	16	15	20	(1)
<i>Pinus muricata</i>	7	86	57	14	7	22	(2)
<i>Cupressus sargentii</i>	9	83	53	10	8	29	(1)
<i>C. goveniana</i>	2	48	65	10	2	23	(1)
<i>C. forbesii</i>	5	77	53	10	6	30	(1)
<i>C. bakeri</i>	6	67	64	9	1	25	(1)
<i>C. abramsiana</i>	4	55	65	7	5	22	(1)
<i>Chamaecyparis lawsoniana</i>	9	105	58	6	15	22	(3)
<i>Cupressus nevadensis</i>	4	64	59	5	14	22	(1)
<i>C. macrocarpa</i>	2	44	80	5	9	7	(4)
<i>C. pygmaea</i>	5	53	67	4	11	17	(1)
<i>Pinus radiata</i>	5	91	52	4	21	12	(2)
<i>P. torreyana</i>	2	60	97	3	0	0	(5)
<i>P. attenuata</i>	4	63	54	3	16	27	(2)
<i>P. jeffreyi</i>	14	52	78	0	19	4	(6)
<i>P. washoensis</i>	3	50	52	0	20	28	(7)
<i>Calocedrus decurrens</i>	3	122	33	0	43	24	(8)
<i>Sequoiadendron giganteum</i>	34	15	100	0	0	0	(9)

Common alleles are defined as having frequencies over 0.05 and widespread alleles are defined as being distributed in more than 10% of the populations. (1) Millar, unpubl. data; (2) Millar et al. 1988; (3) Millar and Marshall 1991; (4) Conkle 1987; (5) Ledig and Conkle 1983; (6) Furnier 1984; (7) Niebling and Conkle 1990; (8) Harry 1984; (9) Fins and Libby 1982.

for species having larger classes of locally common alleles would be to concentrate efforts on increasing geographic coverage and reducing the number of samples at a location.

The approach of Marshall and Brown (1975) has been criticized for its apparent emphasis on allozyme loci. It has been argued that sampling strategies for conservation should not be designed to capture allozyme variants per se since they are not directly useful to breeders, nor do they necessarily correlate with the quantitative variation that is useful (in Marshall 1989). These objections are met by using datasets from as many kinds of traits as are available, weighting each equally, considering the classes of alleles separately for each dataset, and sampling according to the most conservative set of guidelines.

Sampling for maintenance of a germplasm collection involves many of the same considerations as the initial sampling from natural populations. This issue is of increasing concern in crop gene-bank management, as the size of collections has increased to the point where many are impractically large and impossible to maintain (Frankel and Soule 1981; Marshall and Brown 1981; Marshall 1989). Ways of identifying unnecessary redundancy for the purpose of purging gene banks have been discussed (Roos 1988; Peeters and Martinelli 1989). Crossa (1989) reviews sampling design for regenerating collections with alleles of known frequencies, including effects of sampling neutral alleles.

2. *Sampling for in-situ conservation*

The concept of conserving genetic resources of trees in special genetic reserves or genetic resource management units (GRMUs) is not new (Hagman 1973; Krugman 1984; Ledig 1986a, 1988a, b; Millar and Libby 1991), but the idea has been little developed and even less applied. Although most natural areas (National Parks, Wilderness Areas, etc.) serve as *de facto* gene reserves, in practice their size and location are determined almost exclusively on non-genetic, primarily esthetic and ecological, grounds. If these natural areas are allowed to serve as the primary gene-pool reserves for forest trees, then we must accept that in-situ genetic coverage will be a hodge-podge of protection, and the job of systematically conserving genetic resources must be done in some other way, most likely through ex-situ preservation.

Far preferable is a systematic approach to in-situ genetic conservation, in which genetic architecture is first analyzed and locations of GRMUs are then determined from the genetic analyses. Established natural areas could serve as GRMUs when they coincide with the locations and specifications determined by genetic analyses. In other cases, GRMUs would be newly designated following the genetic analysis. GRMUs, unlike other natural areas, are not exclusive of many land uses, including timber harvest. A forested area designated as a GRMU must only be managed to meet minimum genetic specifications (Krugman 1984; Ledig 1988a; Millar and Libby 1991). If a GRMU cannot be established where recommended, then it represents a known gap that can be compensated in other ways.

One approach to estimating genetically adequate sizes of GRMUs for widespread species calls for conserving populations that are large enough to maintain current levels of genetic diversity. Since there are many measures of diversity (Brown 1978; Brown and Weir 1983; Asins and Carbonell 1987), which often yield different relative patterns, deciding on the appropriate one is important. One method for quantitative analysis

derives from the equilibrium equation for neutral mutations (Crow and Kimura 1970; Brown and Moran 1981). Effective population sizes required to maintain expected allozyme heterozygosities in equilibrium for most forest trees are large. For instance, in ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*) populations, heterozygosities ranged from 0.082 to 0.267 (Millar and Libby 1991). When conservative mutation rates are used (10^{-7}) in the equilibrium formula, the effective population sizes to maintain these heterozygosities range from 223,300 to 910,600. At appropriate stand densities, these numbers would correspond to areas far larger than are currently protected or potentially can be protected.

If the analysis is calculated to conserve heterozygosity over a finite rather than equilibrium time, then the required population sizes are smaller. Measures other than expected heterozygosity, such as number of alleles or effective number of alleles (Marshall and Brown 1975), may be more valuable indicators of diversity, and may give different results. For example, for a 90% probability of conserving all alleles of frequency equal to or greater than 0.01 for ten generations, the effective population size would have to be 916 (from Gregorius 1980).

Several criticisms of this approach have been raised. Neutral mutations may not be the variants of concern to conservation. The rates of advantageous mutations are not known, but are probably much lower. Once exposed to selection, however, these alleles will be kept in populations of modest size. Furthermore, Lande and Barrowclough (1987) have shown that estimates of variability based on single-locus statistics such as allozymes will be much lower than estimates based on traits with quantitative inheritance even if neutral mutation rates prevail. This is because variance in quantitative traits is obtained by mutation in any of the genes controlling the traits, thus yielding a higher effective mutation rate for the trait than occurs for single allozyme loci. Another criticism questions the value of maintaining current levels of heterozygosity. Most populations are in non-equilibrium states (Namkoong 1986) and current levels of population heterozygosity may have little to do with population stability or robustness.

Guidelines for deciding how many and where GRMUs should be for trees have been general. In the absence of direct genetic data, seed zones representing regions of physiographic similarity could be adopted as the zones for genetic conservation, with GRMUs placed in each zone. To the extent that allozymes have improved the delimitation of seed zones, they also contribute to genetic conservation (Westfall and Conkle 1992, this issue, pp. 279–309).

The Washington State Department of Natural Resources established a

network of gene pool reserves to conserve genetic resources of Douglas-fir (Wilson 1990). Reserves are designated in each 152 m elevation band within every seed zone in which the Department owns over 405 ha. Over 100 reserves have been established with average size of 10 ha. These areas are managed as strict reserves, with no timber harvest allowed.

In general, even if harvest were allowed on GRMUs, practical constraints will probably limit the ability to maintain as many or as large GRMUs for all commercial and widespread species as may be indicated by genetic analysis. We are developing an approach to designing systematic GRMU networks that incorporates pre-existing reserved areas where appropriate.² The process first identifies genetic targets, then analyzes gaps in protection. The gap analysis highlights areas that require greatest conservation attention.

We are studying the Eldorado National Forest (ENF) on the west slope of the central Sierra Nevada in California as an application of this approach.³ Mixed conifer forests blanket the low and middle elevations, where most of the production forests occur (USFS 1989). Reserved areas of varying sizes and types are scattered throughout the Forest. Excellent genetic data on allozymes and quantitative traits are available for several of the commercial conifers that sample stands throughout the Forest. We focus on white fir allozymes here (from Conkel and Westfall 1988 unpublished report), but intend to incorporate datasets for different traits in a more comprehensive analysis of tree species on the ENF.

The first step was to describe the geographic patterns of allozyme variation in white fir throughout the ENF. A canonical trend-surface analysis (CTSA; Lee 1969) of white fir allozymes over the range of the species in the western Sierra Nevada (Conkle and Westfall 1988) provided the source of data for the EMF portion of white fir's range. CTSA provides scores for the individual trees in the analysis, with the scores measured in multi-locus standard deviation units. Trees were grouped according to their scores into one of four classes (A, B, C, D), with the classes defined as above (+) or below (−) the mean in each of three primary vectors (A was − for first vector, − for second vector, − for third vector; B was −, −, +; C was −, +, −; D was −, +, +, respectively). The complex variation of white fir on the ENF (Fig. 2) is better understood when compared to regional trends in the species throughout the western Sierra Nevada (Conkle and Westfall 1988). The bulk of the middle-elevation populations on the ENF are part of a broad class (denoted by As in Fig. 2) that extends north of the ENF. A higher elevation class, above 1675 m elevation, is limited mostly to the ENF (Bs in Fig. 2). The extreme southern part of the ENF contains complex white fir stands that are transitional to a broad class that extends south of the ENF. These

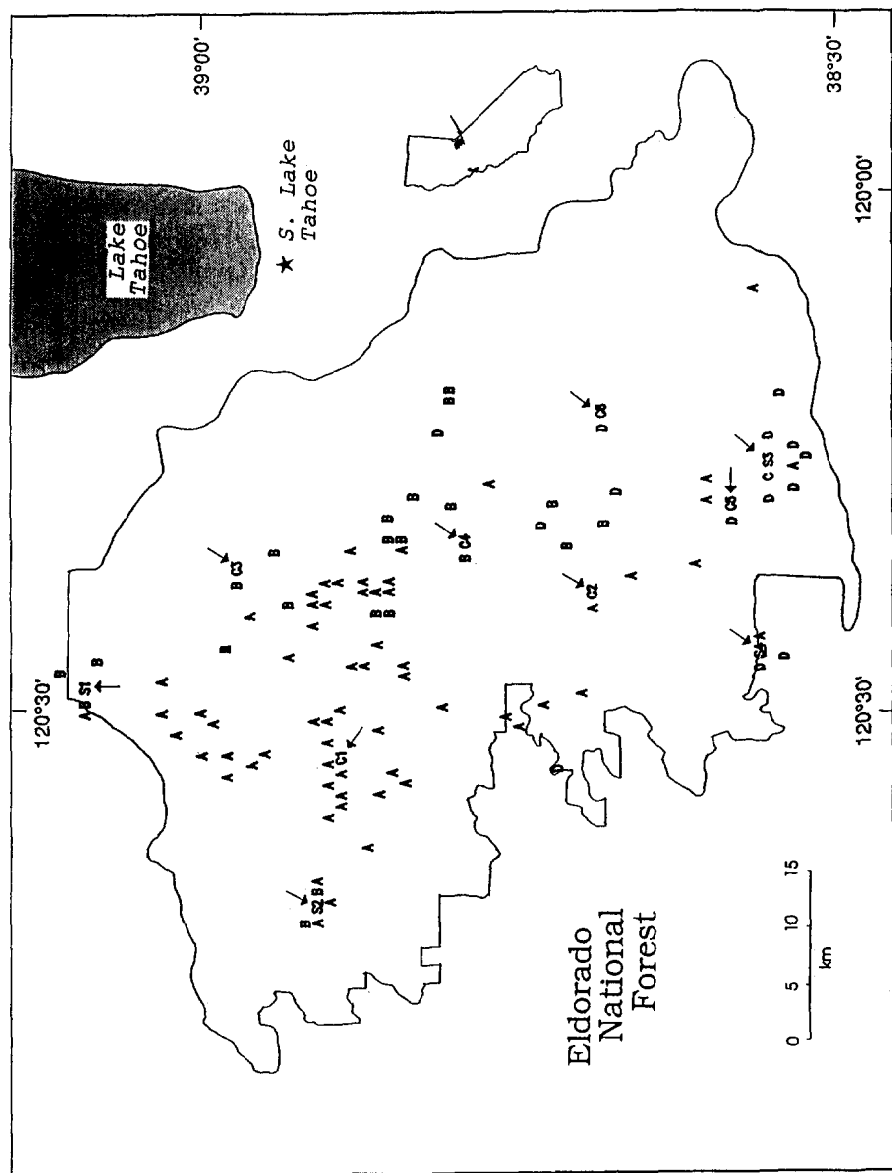


Fig. 2. Patterns of multi-locus allozyme variation in white fir on the Eldorado National Forest, California, as indicated by canonical-trend surface analysis. Letters indicate location of samples, and genetically similar samples are indicated with the same letter. Three conservation zones for white fir correspond to the A, B, and C/D pattern. Six core Genetic Resource Management Units (GRMUs) (C1–C6) and four supplemental GRMUs (S1–S4) are identified from the genetic data (arrows).

are the C and D classes (Fig. 2), which are subdivided by elevation. The transition between the A and D classes in the southern ENF represents the sharpest genetic shift in white fir on the ENF.

The data are further analyzed for genetic conservation purposes by progressive application of transfer-risk analysis (Campbell 1986; Westfall 1991). Based on the genetic data, three major zones are defined for white fir on the ENF that correspond to the A, B, and C/D classes described above. Zones here are defined by the risk that would be incurred if trees from one area were moved to another area (Westfall and Conkle, this issue, pp. 279–309). Risk measures the proportion of multi-locus genotypes that differs among locations, and is reflected by the changes in frequencies over space. Average transfer risk within zones is 5%; average risk among the extreme zones (i.e., A–D) is 22%. We consider two core GRMUs per zone, chosen to represent the average diversity within the zone, as the minimal goal (Millar and Libby 1991).

As a validation of the allozyme analysis, we computed transfer risks using growth, morphological, and phenological data from common-garden nursery (Hamrick 1976) and plantation (J. Jenkinson, U.S. Forest Service, unpubl.) trials of white fir. These tests contained central Sierra Nevada sources that spanned the elevation range of the allozyme dataset. Average transfer risk per 300 m elevation was 0.18 for the allozyme and 0.19 for the common-garden data. This close correspondence between independent traits confirms the values from the transfer risk analysis of allozyme data for white fir in the Sierra Nevada.

Locations of core GRMUs within zones were chosen such that the average transfer risk between them was greater than 6% (to capture some among-site diversity) and less than 12% (to avoid extremes of diversity within zones) (Table 2; Fig. 2). In addition to core GRMUs, supplemental GRMUs were nominated in areas that represent extremes in diversity, that is, where transfer risk within zones averaged greater than 10% to the core GRMUs (Fig. 2). In this way, six core GRMUs and four supplemental GRMUs were identified for the ENF.

The set of candidate GRMUs was then compared to a map of pre-existing areas such as Wilderness Areas, Spotted Owl Habitat Areas, etc., where management is compatible with GRMU status and where natural stands of white fir should exist. Pre-existing areas that coincide with candidate GRMUs were chosen first for GRMU status. Where candidate GRMUs are not included within pre-existing protected areas but protected areas exist nearby, the transfer risk of using nearby areas as GRMUs is assessed. On a genetic basis, these areas could acceptably serve GRMU status if their transfer risk is less than about 5–6%. Locations where risks between a candidate GRMU and its potential replacement site are greater

Table 2. Transfer risks among candidate Genetic Resource Management Units (GRMUs) identified for white fir on the Eldorado National Forest, California

GRMUs	Core						Supplemental		
	C1	C2	C3	C4	C5	C6	S1	S2	S3
Core									
C2	0.06								
C3	0.12	0.12							
C4	0.15	0.11	0.09						
C5	0.21	0.15	0.19	0.11					
C6	0.30	0.25	0.23	0.16	0.12				
Supplemental									
S1	0.11	0.14	0.09	0.13	0.22	0.26			
S2	0.11	0.07	0.09	0.06	0.12	0.19	0.11		
S3	0.16	0.10	0.17	0.09	0.05	0.17	0.18	0.09	
S4	0.19	0.13	0.20	0.15	0.11	0.21	0.22	0.12	0.10

Core GRMUs C1 and C2 are in the A zone; C3 and C4 in the B zone; and C5 and C6 in the D zone.

than 6% are identified as gaps in protection. These locations, especially any core sites, should receive attention for genetic conservation. Once replacement sites were identified as acceptable from the genetic model, they would be ground-checked for suitable species' habitat, and their long-term management status determined.

This process can be extended to multiple species or datasets based on different traits. Pre-existing areas that can act as acceptable GRMUs (i.e., within acceptable risk) for more than one species or dataset are given highest priority for GRMU status. Higher risks may be acceptable when attempting to designate GRMUs that represent many species.

Monitoring genetic diversity

Effective conservation requires an understanding not just of baseline genetic diversity but of changes in diversity. Conservationists are especially concerned with mitigating the effects of human actions on genetic diversity. Some actions have dispersed effects, such as introduction of exotic pathogens or insects, pollution of the atmosphere, and global climate change. Other actions are more localized, such as artificial reforestation and silvicultural practices.

Significant and consistent effects of atmospheric pollution on allozyme structure of trees have been reported (reviewed in Scholz et al. 1989). The

effects include altered allele frequencies, decreases in expected heterozygosities, and decreases in multi-locus allozyme diversities. These effects were observed not just in allozyme structure of affected stands, but in comparisons of tolerant and sensitive clones subjected to specific polluting chemicals such as sulphur dioxide and ozone. A possible explanation for the relationship in these data between genetic diversity and air pollution is that certain genotypes (especially inbreds) are more susceptible to multiple stresses (such as air pollution, insect and disease, competition), and are most likely to succumb to cumulative effects (Ledig 1991).

In contrast to the relatively large and consistent effects on allozyme diversity related to atmospheric pollution, studies that monitored effects of forest management on allozyme diversity have not shown consistent results (Muona, this issue). Allozyme diversity was 33% higher on average in natural populations of Scots pine (*Pinus sylvestris*) and Monterey pine (*P. radiata*) (in Adams 1981), whereas in two studies of Douglas-fir, diversity was similar in natural populations and seed orchards (Adams 1981). In another study of Douglas-fir, allozyme diversity in seedlots from seed trees and commercial seedzone collections was about the same as diversity in seed orchards (Adams 1981).

Small or insignificant effects on allozyme diversity have been reported due to silvicultural manipulations in commercial conifers. With minor exceptions, there were no significant differences in allele diversity among samples from four life-cycle stages in Douglas-fir stands in Oregon managed under a shelterwood system (Neale 1985). This was true despite large reductions in the number of parent trees in the shelterwood. Excess homozygotes generated in seeds from natural regeneration in a Scots pine (*Pinus sylvestria*) shelterwood were eliminated by natural selection after 10–20 years in the field (Muona, this issue, pp. 329–345).

Allozymes are especially useful in detective aspects of conservation, where their application is similar to identifying clones and evaluating contamination in seed orchards (e.g., Friedman and Adams 1985; Rajora 1988). Catalina mahogany (*Cercocarpus traskiae*) is an extremely endangered tree species of Santa Catalina Island, California, with only seven remaining individuals. The species has declined in recent years due to habitat degradation and overgrazing from introduced ungulates, leading the Center for Plant Conservation to begin a rescue effort for the species (Reiseberg 1988). Electrophoretic analyses indicated that two of the Catalina mahoganies were hybrids with the common mahogany (*C. betuloides*) on the island. This knowledge enabled conservationists to vegetatively propagate the five pure Catalina mahoganies with the intent of re-introducing them into fenced habitat on the island.

We used allozymes to evaluate genetic contamination in the northern-

most and smallest grove of giant sequoia (*Sequoiadendron giganteum*), where planting by local service clubs has resulted in establishment of young trees of unknown origin. One restoration plan for the grove recommended removing all trees of non-local origin. The possibility existed, however, that some of the young trees now in the grove might be natural regenerants. We genotyped mature and young trees and, by analyzing loci present in the young trees that were not present in the old-growth trees, determined that all but one young tree had to have been planted.⁴

Conclusions

Application of allozyme markers to forest genetic conservation is a natural extension of the uses to which allozymes have been applied in many areas of forest genetics. Allozymes are routinely used to estimate the amount and distribution of genetic diversity in natural populations and provide important baseline data to conservation. Although this application should continue, there is increasing need to focus on questions of special relevance to conservation, especially relating to how much variation is enough to ensure viability of rare taxa and to ensure healthy and robust forests of widespread species. Increased emphasis on monitoring changes in diversity due to human actions is also needed.

In many of these applications, the isozyme method is valuable because it provides direct information on allelic diversity that can be compared across taxa. For estimating relationships among species and microevolutionary parameters within species, such as gene flow and mating systems, and in conservation detective work, allozymes are excellent markers. In cases where allozymes are used to represent other kinds of genes (e.g., adaptive), or where conservation decisions are made on the basis of allozyme diversity per se, they may be misleading. Certain statistical methods may allow allozyme diversity to be partitioned such that the portion of allozyme diversity that correlates with adaptive variation is revealed. Conversely, variation may be assessed in multiple traits, and the conservative pattern indicated by all data be accepted.

Finally, many of the applications needed in genetic conservation may be served better by combining isozyme methods with new techniques emerging from molecular biology. The large number of DNA markers that can be analyzed, their broad representation throughout the genome, and the different scales of polymorphism of these markers may provide critical information for conservation. While nuclear markers may provide the large numbers of DNA sequences useful for comparing extant populations, organelle markers may be especially useful for tracing pedigrees and

phylogenies of threatened and endangered organisms. These techniques offer a great potential for extending the biochemical approaches that isozyme analysis affords to many new conservation situations.

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Notes

1. Millar, C. I. and Delany, D. L. (in prep.) Biosystematics of cypresses in Southwestern United States and Baja California. Systematic Botany.
2. Millar, C. I. and Westfall, R. D. (in prep.) A sampling strategy for in-situ genetic conservation. Conservation Biology.
3. This analysis is undertaken as an exercise of the approach, and nothing indicated here binds on ENF management and planning.
4. Millar, c. I. and Delany, D. L. (in prep.) Genetic contamination and natural regeneration in the Placer Grove, giant sequoia. Madrono.

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