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Using Isozyme Analysis to Aid in Selecting Genetically Superior Ponderosa Pine for Coal-Mine Spoil Reclamation

Abstract

Isozyme analysis was used to investigate the genetic population structure of ponderosa pine from the Colstrip area in eastern Montana. Results are discussed in terms of designing a progeny test to select trees which produce seedlings with a superior ability to survive on regraded coal-mine spoils. It is estimated that selection of 75 parent trees divided among 5 stands will efficiently sample the genetic diversity present. Due to the natural stand history, a large amount of gene flow between stands and the relatively small area being reforested, gene conservation is not of concern. As a result, the three or four parent trees whose progeny have the best survival rates can be used for future seed collection. Selection of three or four trees should allow a large genetic gain even though only 75 parents are being tested.

Introduction

Mine-spoil reclamation has become important in many parts of the world in recent years. Local laws often require that native species be established, and that the productivity of reclaimed land be equal to or greater than what it was before mining. Most research effort is put towards the engineering problems of regrading the spoils to create a medium suitable for the desired plant species, or towards the cultural techniques of seeding and planting. Fewer studies have investigated the potential for intraspecific selection of plants exhibiting genetic superiority in survival or growth on the reclaimed sites.

Most plant species show a great deal of variability that can be attributed to genetic differences among individuals. This variability has been used in agriculture, horticulture and forestry in selecting and breeding lines considered superior for the specific improvement objectives. In reclamation work, Plass (1969) found a great deal of variation among open-pollinated families of Virginia pine (*Pinus virginiana*) in their ability to survive on acid surface-mine spoils. Certain hybrid poplar clones have also shown superior survival ability on mine-spoils (Jones 1973, Davidson 1980). Geographic location of the seed source has been found to influence a plant's ability to survive on mine-spoils for alder (*Alnus glutinosa*) (Funk 1973), Austrian pine (*Pinus nigra*)

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(Neys and Cech 1977), and several species of birch (*Betula* spp.) (Davidson 1977). Scanlon (1979) reports differential survival and growth on mine-spoil sites among families for various shrub species. Taxonomic lines within a number of plant species have also been found to exhibit varying abilities to survive on soils with high heavy-metal concentrations (Antonovics *et al.* 1971).

Plants of high genetic quality, in terms of survival on mine-spoil areas, could provide economic benefits for reclamation efforts in two ways: 1. increased survival for a normal amount of site treatment and planting expense, or 2. satisfactory survival for less than normal site treatment and planting expense.

At Colstrip, in southeastern Montana, large tracts of land are being strip-mined for coal. The dry climate in this area hinders reclamation efforts, although reasonable success has been achieved with grass and shrub species. Indigenous ponderosa pine (*Pinus ponderosa* var. *scopulorum*) is generally limited to rocky upland sites (Richardson 1981), and preliminary efforts to establish this species on regraded mine-spoils have failed (MSU 1978).

To insure a high genetic quality in seed being used for reforestation efforts, a selection program is being carried out to identify open-pollinated ponderosa pine families that exhibit a superior ability to survive on mine-spoil areas. (Mine-spoils used in this study consist of soils that are reconstructed and graded as the first stage of reclamation.) This paper describes the use of isozyme analysis to estimate the amount and distribution of genetic variation in ponderosa pine from the Colstrip area, and its application to the selection of parent trees.

Isozyme Analysis

The use of electrophoresis to estimate certain genetic characteristics of populations has become widespread since the late 1960's. The technique uses an electric current to separate enzyme proteins in a gel medium. An enzyme is coded by the DNA found at certain chromosome locations (loci); the various forms of an enzyme coded at different loci are called isozymes. Isozyme variants result when alternate gene forms (alleles) are found at a chromosome locus. The isozyme variants will often migrate at different rates through the gel. Following electrophoresis, the gel is stained for the desired enzymes; isozyme variants can be detected by their location on the gel. (See Harris and Hopkinson (1976) for detailed procedures). Data from isozyme analysis allows accurate estimates of genetic variation and other genetic characteristics.

In conifers the amount and distribution of genetic diversity in quantitatively measured growth characteristics is reflected in estimates from isozyme analysis. Ponderosa pine from the northern Rocky Mountains exhibits a great deal of isozyme variation, with about 88 percent of the variation being due to genetic differences among individuals within a stand, and 12 percent due to differences among stands (O'Malley *et al.* 1979). This result is in general agreement with a study by Madsen and Blake (1977) which attributes 28.5 percent of the genetic variation in two year height growth to differences among stands. Most coniferous species exhibit a large amount of isozyme variability as well as a great deal of variability in quantitative characteristics. However, species such as red pine (*Pinus resinosa*) (Fowler and Morris 1977, Allendorf *et al.* 1982) and western red cedar (*Thuja plicata*) (Copes 1981) exhibit very little genetic variation in quantitative characteristics, and have also shown little isozyme variability.

Isozyme analysis offers a fast and inexpensive method of estimating the genetic structure of populations. This is basic but important knowledge which can improve the efficiency of a program aimed at selecting and breeding for the genetic improvement of a characteristic.

Methods

Ponderosa pine in the Colstrip area is generally found in small stands isolated by grasslands. To investigate the amount and distribution of genetic variation in these isolated ponderosa pine stands, open-pollinated seeds were collected from 50 trees throughout each of 6 stands. The stands were separated by varying distances ranging from 0.9 to 13.6 kilometers. Stand areas and densities are shown in Table 1.

TABLE 1. Size, area, density, and genetic heterozygosity (H_e) for six ponderosa pine stands sampled near Colstrip, Montana.

Stand	Number of trees*	Area (hectares)	Trees per hectare	H_e (percent)
1	260	4.4	59	12.8
2	320	3.1	74	13.2
3	920	8.4	110	12.7
4	400	3.4	118	11.5
5	2550	12.4	181	10.4
6	225	5.2	43	13.9
All stands combined				$H_e = 12.6$

*Number of trees of reproductive age estimated from air photos.

To estimate allele frequencies in the present stands, as well as genotype frequencies in the seed crops, one seed from each tree was screened for isozyme variants in megagametophyte and embryo tissue over 23 isozyme loci. Fresh ponderosa pine seeds from the Colstrip area do not require stratification (Woods and Blake 1981), therefore, seeds were soaked in water for 18 hours and germinated at room temperature on moist filter paper. Megagametophytes and embryos were dissected from the seeds, when the radicle had extended about one centimeter, and were crushed in 0.2 to 0.5 ml of distilled water in proportion to the amount of tissue present. Homogenate from the megagametophyte and embryo of each seed was absorbed into filter paper wicks and placed separately in a starch gel for electrophoresis.

Electrophoretic methods, enzymes, and allelic variants are described by Woods *et al.* (1983). Enzyme stains are described by Allendorf *et al.* (1977). For a general description of electrophoretic techniques in conifers see Conkle (1972).

Results

The amount of genetic variation, as estimated from isozyme analysis, is generally expressed as the average proportion of loci per individual (seed) exhibiting more than one electrophoretically detectable isozyme allele. This ratio is called the genetic heterozygosity (H_e). Values of H_e for individual stands varied from 10.3 to 13.7 percent, with an average over all stands of 12.6 percent (Table 1). No significant (prob. > 0.05) differences in H_e were found among stands.

The amount of genetic heterozygosity in ponderosa pine from the Colstrip area

(12.6 percent) is lower than for ponderosa pine from the northern Rocky Mountains in the U.S. (18.6 percent) (Allendorf *et al.* 1982). It is also lower than the average for 20 coniferous species (20.7 percent) compiled by Hamrick *et al.* (1981) (see Fig. 1). However, levels of *He* in Colstrip pine are high relative to many plant and animal species, and also to some conifer species, indicating that considerable genetic variation exists in these populations.

The genetic heterozygosity in populations can be partitioned into the amount of genetic diversity within subpopulations (stands) and the genetic diversity which is due to differences among subpopulations (Nei 1973). In the Colstrip pine stands, an average of 98.5 percent of the genetic heterozygosity is found within individual stands and 1.5 percent is due to differences among stands. Although the 1.5 percent is small, significant gene frequency differences exist among stands at some loci.

Discussion

In order for tree improvement to be successful, genetic variation must be present in the species and populations from which parent trees are chosen. Isozyme analysis indicates that ponderosa pine in the Colstrip area is genetically variable, and local populations will likely provide the variation needed for improvement. Without sufficient variation

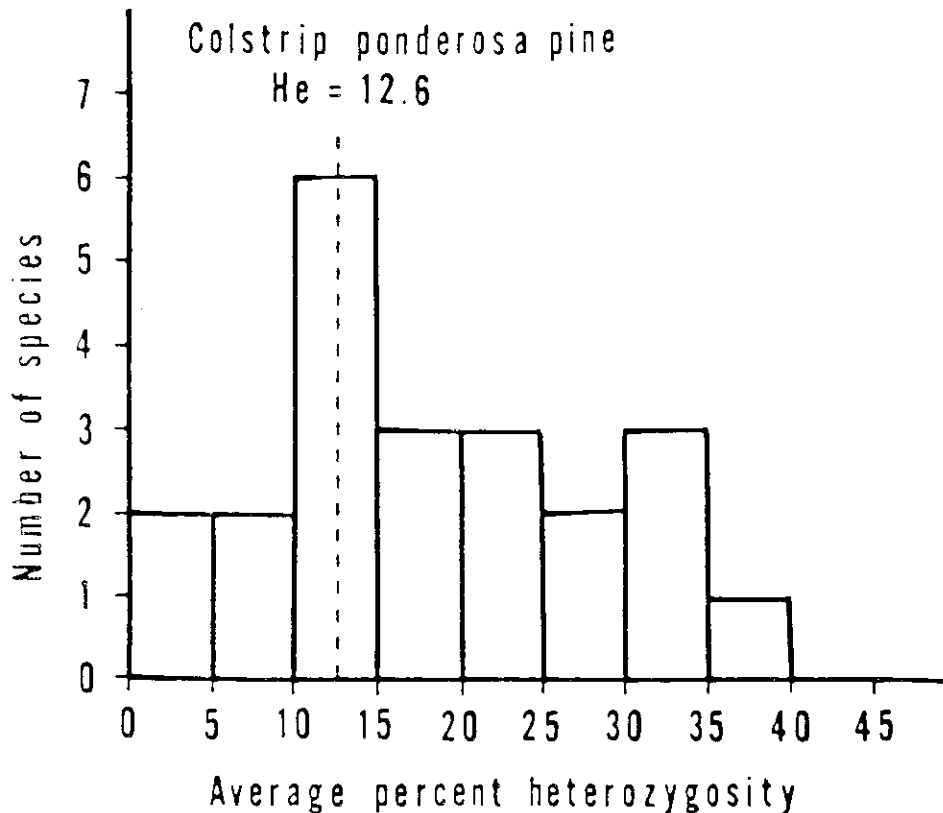


Figure 1. Distribution of the average heterozygosity (*He*) found in 22 conifer species (data from Hamrick *et al.* 1981).

in local stands, seed would have to be collected over a large geographic area to obtain the variable genotypes needed. Using local trees as progeny test parents, avoids the survival and growth problems often encountered when seed is moved from its natural origin. Also, the use of local trees involves less time and expense in seed collection and maintenance of selected parents.

Selection of Parents for Progeny Testing

In most tree improvement projects, parent trees with the desired phenotype (i.e., rapid volume growth, straight bole, etc.) are selected from natural stands. If progeny from these parents also exhibit these traits, then an initial genetic gain is made from the phenotypic selection. The trait of mine-spoil survival is not exhibited by a naturally occurring tree, therefore parents cannot be selected on this basis. To determine which parent trees produce progeny most able to survive on regraded mine-spoils, seedlings from various trees must be planted in an appropriate design, and survival rates determined (progeny test). On the basis of progeny survival, parent trees can then be selected for operational seed collection and, if desired, future breeding. Timber production is not the objective of this mine-spoil reforestation, therefore selection for traits such as rapid height growth is not of concern for this study.

Since parent trees cannot be chosen on the basis of their phenotype, it is desirable to choose parents which will represent a cross section of the genetic variation present in local stands. Isozyme analysis indicates about 98.5 percent of the genetic diversity exists within a stand. Therefore, by choosing parent trees from a single stand, genotypes will be drawn from a pool containing all but 1.5 percent of the genetic variation. Significant genetic differences also exist among stands, however, so by sampling parent trees from a number of stands a more diverse cross section of genotypes can be included (Brown 1978). Isozyme analysis results allow parent trees to be chosen in a design that efficiently samples the local genetic diversity.

Seed production varies among trees in ponderosa pine. Open-pollinated seed is used to produce seedlings for the progeny test, so good seed production is necessary to select parent trees. Parents producing progeny with superior survival ability will be used for future operational seed collection. If seed production is heritable, then trees from selected parents established on mine-spoils will also be good seed producers and stands are more likely to become self-regenerating.

Selection of Genetically Superior Trees

Following the progeny test, parent trees whose progeny had the best survival rate on the mine-spoils will be chosen for future seed production. The amount of genetic gain (G) achieved by selecting these parents is dependent upon selection differential (S) and the degree to which a trait is passed from parent to offspring (heritability = h): $G = Sh$. Selection differential is a measure of the difference between the average progeny survival rate over all parents and the average survival rate of progeny from the best selected parents. The fewer number of best parents chosen, the higher the selected average and the greater the selection differential and genetic gain.

Generally in tree improvement work, gene conservation is of concern (Adams 1981). Large scale forestry operations may significantly alter the genetic resource; measures are required to minimize the loss of genetic diversity. A trade-off is reached

between conserving a reasonably large superior population and genetic gain. In order to achieve a large selection differential, as well as to maintain a sufficient number of superior trees, many individuals (300-1000) are usually tested and the best 40 or 50 selected for a breeding population.

The conservation of genotypes, by selecting a large number of superior parents from progeny test results, is considered unnecessary in this project for the following reasons:

1. Strip-mine spoil reforestation involves small areas of land (relative to the areas reforested in large-scale forestry operations), and only small patches will be reforested, with most areas being reclaimed to grasslands.
2. Open-pollinated families will be used, therefore, even with a small number of female parents, a large number of male parents will be included.
3. Areas will only be mined and reforested once, and stands will regenerate naturally after they are established. Isozyme analysis indicates a great deal of pollen movement takes place between stands (Woods *et al.* 1983), causing a broadening of the genetic base in future generations.
4. The natural fire history of ponderosa pine in the Colstrip area indicates it is common for stands to originate from a small number of parents (Richardson 1981).

Selection of three or four parent trees with the best progeny survival should be sufficient to meet seed requirements. By selecting such a small number of genetically superior trees, a high selection differential and genetic gain should be obtained from a progeny test involving a small number of parents. For this project, a progeny test involving 75 parent trees, selected over 5 different stands (15 per stand), is considered sufficient to achieve a large genetic gain, while keeping the investment in seed collection and progeny testing small.

In the Colstrip area, obtaining a high survival rate of seedlings planted on mine-spoils is the main objective; wood volume production is of little concern. Seedling mortality is highest following planting, and the success or failure of a seedling will likely be determined within one or two years. This is a great advantage in terms of parent tree selection. The short period of time required to obtain results means that the investment in progeny testing need only be carried on for one or two years before superior seed can be used operationally.

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