ENZYME POLYMORPHISM IN FOREST TREES

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INTRODUCTION

In studies of genetic differences among trees, forest biologists have found that variations fall into two categories. In the first, characterized by metric traits, the phenotypic response results from the combined activity of many genes having minor effects. In the other, characterized by mutants and some resin and disease-resistance factors, the phenotypic response results from the activity of a few genes having major effects.

The genetic analysis of enzymes falls into both categories. Enzyme polymorphs are likely to have only slight effect on tree phenotypes. But they allow the specific classification of individuals that result from segregation of single genes.

Before I discuss how information from genetic analysis of enzymes can be applied to the study of forest trees, let me first review some basic concepts about enzymes and inheritance.

ENZYMES AND THE CONTINUUM FROM DNA TO TREE SPECIES

A hierarchy of activity leads from the basic genetic material of cells to phenotypic differences between families, races, and species. A simplification of this sequence is: DNA--RNA--Enzymes--Physiological Processes--Tree Phenotype--Family--Species. DNA has the reproducible specific array of base pairs that encodes genetic information. RNA transmits the code to sites where enzymes are produced. Enzymes are proteins with specific sequences of amino acids prescribed by the DNA. The amino acid sequence determines the enzyme structure, thereby imparting a specific function. Each enzyme catalyzes a specific substrate-to-product reaction. Such reactions underlie all physiological processes. These processes produce tissue, differentiate organs, and ultimately promote the tree phenotype.

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As primary gene products, enzymes are only one step removed from the genetic code. And different molecular forms from trees readily yield to genetic analysis (Bartels 1971, Conkle 1971a, Feret and Stairs 1971, Rudin and Rasmuson 1973). When family relationships between different seeds or trees are known, the inheritance of isozymes can be determined by gel electrophoresis. The enzyme bands result from specific substrate-to-product reactions. Therefore, they provide a link between inherited enzyme differences and specific physiological processes. The genetic analysis of enzymes may eventually provide a means for resolving differences in physiological response.

GENETIC ADAPTATION AND ENZYME DIFFERENCES

Past mutations in the chromosomal material provided the inherited variants that have led to the differentiation of taxa through the processes of evolution. Enzyme differences in forest trees are useful for detecting this level of differentiation (Feret 1972, Hamaker and Snyder 1973).

Genetic studies of enzymes must consider the current developmental stage of individuals. The stage of plant development defines the tissues and physiological processes that are active, and tree enzymes differ between growth stages (Conkle 1971b). The types of enzymes active in seed respiration are likely to represent a different sample of the genome than the enzymes active during growth, reproduction, and senescence. Enzymes of these three processes are likely to differ from stage to stage.

Though taxa and tissues can be characterized by enzyme differences, the adaptive importance of individual variants in selection processes has been questioned. Evidence strongly suggests that enzyme variants are selectively important and that natural variation in enzymes is maintained by active selection pressures (Johnson 1974). Numerous studies on a variety of organisms show that allelic gene frequencies change across geographic gradients; differentiation in assumed to be related to selection pressures exerted by environmental gradients. Similar evidence is emerging in forest trees (Bergmann 1971, 1973a, b, c, 1974; Muhs 1974; Rudin et al. 1974; Sakai et al. 1971; Sakai and Miyazaka 1972; Sakai and Park 1971; Tigerstedt 1973). Other evidence relates enzyme variability to substrate variability: enzymes with variable substrates are more polymorphic than enzymes for single substrates. Also, enzymes which limit the rates of reactions and by their actions regulate processes, show more polymorphism than do nonregulatory enzymes.

All of this evidence suggests two conclusions: First, certain enzyme polymorphisms are influenced by natural selection; second, some enzyme systems and certain gene loci are more polymorphic than others and are therefore more useful as monitors of inherited enzyme variation.

ANALYSIS OF CONIFER ENZYMES

The work of Lewontin and Hubby (1966) during the mid-1960's established the genetic importance of isozyme analyses. Their enzyme studies demonstrated that the technique could be used to assay allelic differences leading to estimates of gene frequencies and and average genetic heterozygosity.

Genetic analyses of conifer enzymes were first reported in the early 1970's. The technique separates enzyme molecules with different net static charges. For conifer gel electrophoresis, crude extracts from macerated tissues are absorbed on paper wicks (Conkle 1972.) The wicks are placed in a gel matrix to which a direct electric current is applied. The rate and direction of enzyme migration in the gel depends on the kind and size of net static charge on the molecules. Each gel can be sliced and stained for various enzyme systems. Enzyme activity is detected by immersing gel slices in a buffered solution of specific substrates and staining the reaction products with specific dyes.

The stained gels display a series of bands. When offspring of known families are examined, bands with different migration rates can be related to specific gene loci and to the segregation of allelic forms at individual loci. Segregation ratios of bands from various offspring conform to hypotheses of Mendelian inheritance. The pattern of banding can be considered a phenotype, from which the plant genotype is inferred. Single enzyme runs yield information on numerous individuals, with bands stained for various enzyme systems.

My studies have focused on the inheritance of isozyme band patterns in conifer seed and seedlings. The standard laboratory procedure is to germinate stratified seed. Embryos and gametophytes are separated and macerated when the radicle is 3 to 5 millimeters long. Clear bands are obtained for alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP) and catalase systems. Later, when the cotyledons are fully expanded and epicotyl growth is beginning, strong band patterns are discernable for esterase, phosphatase, and peroxidase systems. For these later growth stages, roots and seedling tops are assayed separately, as numerous band differences exist between the two tissues.

The inheritance of isozymes has been examined for Douglas-Fir (*Pseudotsuga menziesii* (Mirb.) Franco), and for ponderosa (*Pinus ponderosa* Laws.), Jeffrey (*P. jeffreyi* Grev. and Balf.), Coulter (*P. coulteri* D. Don), sugar (*P. lambertiana* Dougl.), and knobcone (*P. attenuata* Lemm.) pines. Varying numbers of families of each species were studied, but only knobcone pine seed collections represented a geographic sample of the species. Douglas-fir seed were families from southern Oregon stands. Ponderosa, Jeffrey, Coulter, and sugar pines were California seed collections; all but Coulter pine were from the central Sierra Nevada.

In general, the ADH and LAP systems stain for fewer bands than esterase, phosphatase, and peroxidase systems. Catalase bands are few in number but more difficult to work with owing to the transitory nature of the stain. The first inheritance studies were on the ADH and LAP systems; they are the easiest to subject to genetic analysis.

Genetic analyses were performed by using both diploid (embryo or seedling) and haploid (gametophyte) tissue. In one experiment, I worked with seedlings of a selfed knobcone pine family (Figure 1). Plant extracts were supplied by wicks spaced along the bottom of the gel, with enzyme migration toward the anode. The bands of the darkly staining zone are of three phenotypes. Seedlings have bands in: (1) the upper location only, (2) the lower location only, and (3) in both the upper and lower locations and a band with intermediate migration. On this gel, five seedlings had the upper band, 10 had three bands, and five had bands in the lower location. My interpretation is that seedlings with only the upper band are homozygous fast genotypes, seedlings with three bands are heterozygotes and those with only the lower band are homozygous slow genotypes. This family conforms to the 1:2:1 Mendelian segregation ratio expected from a selfed heterozygous parent.

Conifers and other gymnosperms are unique among seed plants in having sufficient haploid female gametophyte tissue for enzyme analysis. Figure 2 shows a gel with ADH bands from gametophyte and embryo tissues of a selfed family. From left to right are pairs of bands from eight seeds, with the gametophyte first and the embryo, which has a homozygous fast genotype. The third and fourth positions show a gametophyte with a slow allele and an accompanying embryo with the homozygous slow genotype. Fifth and sixth are a gametophyte with the fast allele and an embryo with a heterozygous genotype. Since an egg is part of the gametophyte, it has the same genotype as the rest of the gametophyte. Thus, this embryo received the fast allele from the egg (gametophyte phenotype) and--since it is heterozygous--had to receive the slow allele from the pollen. The haploid gametophytes approach the expected 1:1 ratio, while the embryos approach the 1:2:1 ratio for a heterozygous diploid. The combined analysis of gametophyte and embryo identifies the alleles contributed by the egg and pollen for each seed.

The analysis of isozyme inheritance in knobcone pine was undertaken with approximately 70 parent trees grown from seed collected throughout the species range. Trees were selfed or crossed to produce known families. Three loci could be identified for ADH and three loci for LAP (Figure 3a). For ADH, A and B zones are monomorphic, but zone C is polymorphic and band patterns result from the activity of three alleles from a single genetic locus. For LAP, band patterns are from three loci with two alleles at each locus (Figure 3a). Similar family analyses of three other western pines--ponderosa, Jeffrey, and Coulter--Douglas-fir, and sugar pine have

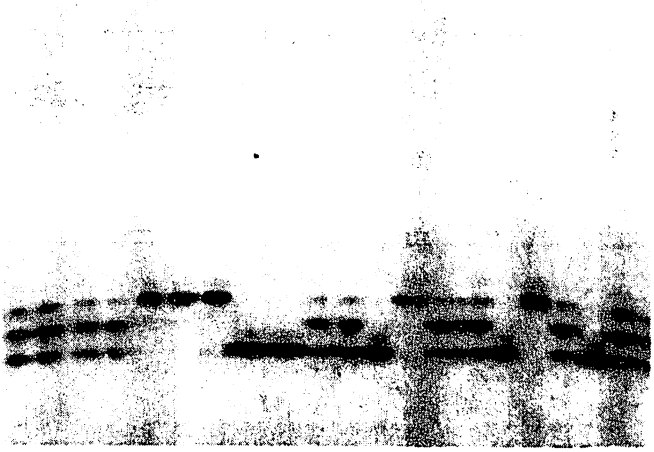

Figure 1. Alcohol dehydrogenase isozyme bands from 20 seedlings of a knobcone pine selfed family. Five seedlings have homozygous fast migrating bands, 10 are heterozygous, and five are homozygous for slow bands.

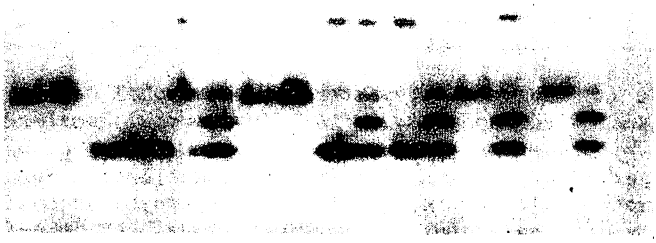

Figure 2. Alcohol dehydrogenase isozyme bands from gametophytes and embryos of eight seed from a selfed knobcone pine family. Pairs of bands are from the gametophyte and embryo of the same seed. The eight pairs of gametophytes and embryos show haploid and diploid segregation.

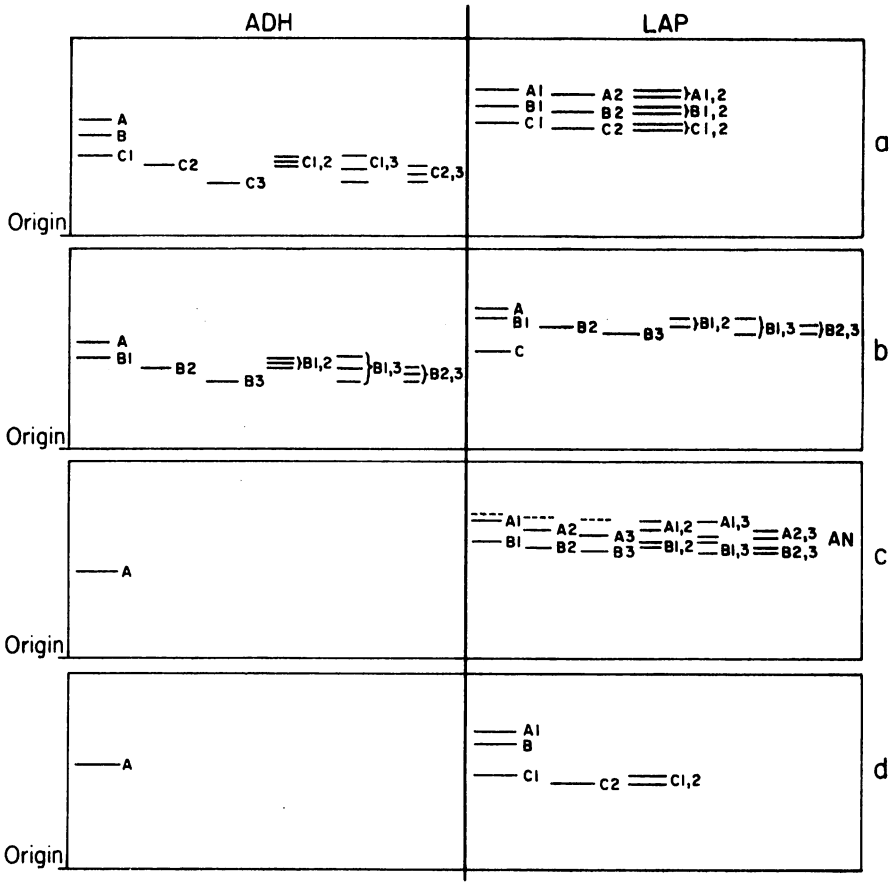

Figure 3. Isozyme band patterns from alcohol dehydrogenase (ADH) and leucine aminopeptidase (LAP) for several conifers: a, knobcone pine; b, ponderosa, Jeffrey, and Coulter pines; c, Douglas-fir; d, sugar pine. Capital letters represent loci. For polymorpic loci the number suffixes represent the gametophyte or homozygous diploid, and mixed numbers show heterozygous types. AN in c-(LAP) signifies a null (non-staining) form present within the A zone.

also identified allelic polymorphism (Figure 3). The relatively limited numbers of parents included in these studies probably do not represent a total sample of allelic variability in these taxa.

Even with a limited genetic base, it is common to find multiple alleles for various loci within taxa. For example, the A zone of Douglas-fir LAP shows four allelic types; one is a null allele that is easily identified from the combined analysis of gametophytes and embryos.

Plant tissues differ in the banding patterns of each enzyme system. Examples of tissue differences are the esterase and peroxidase systems of tops and roots (Figure 4a, b). Tops, including cotyledons and apical bud, were compared with roots of seedlings at the cotyledons expanded stage of development. In knobcone pine, both the esterase and peroxidase systems--virtually absent in the early phases of seed germination--yielded darkly-staining bands at this stage (Figure 4). Esterases from seedling tops stain strongly, while roots show weakly-staining bands. Peroxidase bands migrate both cathodically and anodically and some bands show presence-absence differences between tissues.

A preliminary investigation of Douglas-fir suggests that ADH is sensitive to environmental preconditioning. In the pines I have analyzed, germinating seed always possessed active ADH, but Douglas-fir seed yielded no bands. Since ADH is active in anaerobic respiration, the aerobic germination procedure may not provide the environment needed to activate Douglas-fir's ADH system. To test the hypothesis, I germinated Douglas-fir seeds to the stage of radicle emergence, submerged them in water for a week and then analyzed them for ADH activity. Both gametophytes and embryos of submerged seed showed strongly-staining bands for ADH (Figure 5). My tentative explanation is that ADH systems in Douglas-fir are activated by anaerobic pretreatment.

CONCLUSIONS

Until recently, forest biologists had only limited means of resolving differences at the level of individual gene loci. The above examples illustrate the genetic resolving power of the electrophoresis technique. As gene markers, isozymes are useful in describing genetic differentiation within and between taxa and for verifying hybridity. They should also prove useful for monitoring pollen migration, determining the degree of selfing, and testing panmixis. Isozymes offer a means of studying phenotypic response over environmental gradients where genetic material can be identified by specific allelic combinations.

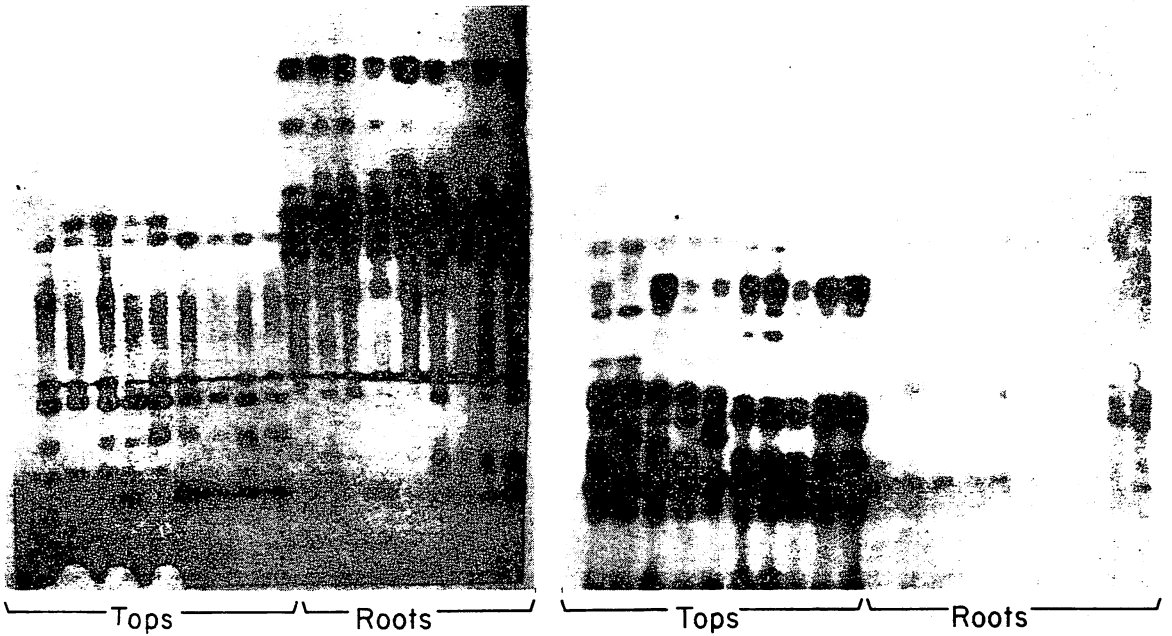

Figure 4. Isozyme band patterns for peroxidase, left, and esterase, right, enzyme systems in young seedlings of knobcone pine. The left half of each gel shows bands obtained from seedling tops. The right half of each gel has bands from seedling roots.

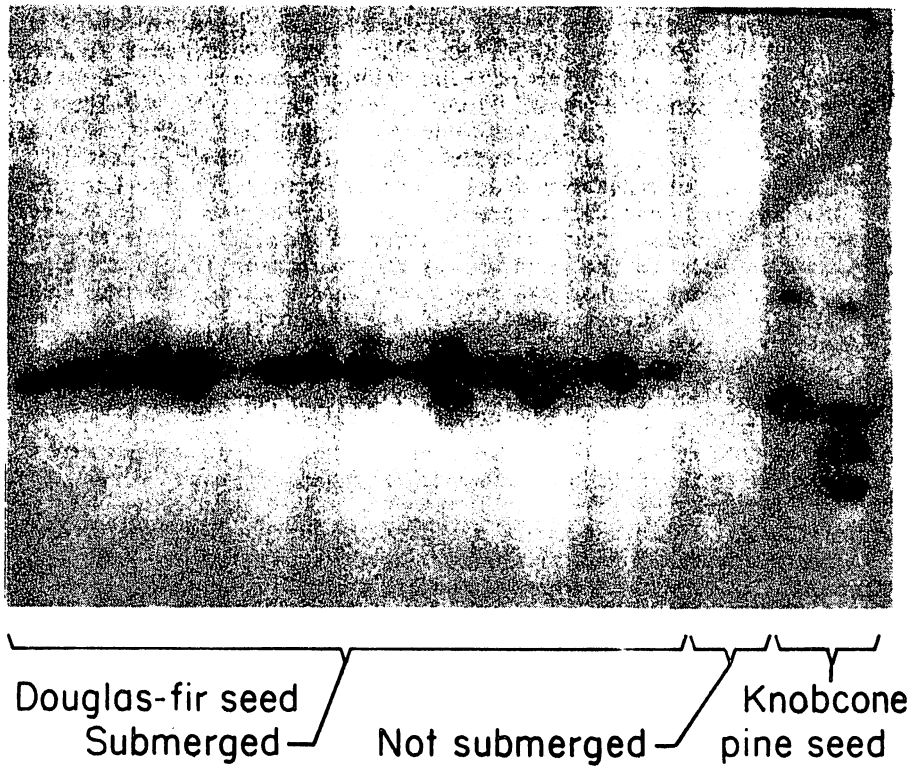

Figure 5. Alcohol dehydrogenase bands from gametophytes and embryos of Douglas-fir seed submerged before assay. The bulk of the gel displays dark staining bands from the submerged seed. On the right end of the gel is a zone with no activity from a Douglas-fir seed that was not submerged. On the far right are bands from the gametophyte and accompanying embryo of a knobcone pine seed.

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