Inheritance of esterase and acid phosphatase isozymes in Virginia Pine and the application of the isozyme technique to a seed orchard population[1])

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Summary

The inheritance of esterase and acid phosphatase isozymes in Virginia pine (*Pinus virginiana* Mill.) was determined by an analysis of the segregation patterns among the megagametophytes of seed collected from individual trees. Both esterase and acid phosphatase phenotypes consisted of multiple isozymes which segregated in a 1:1 ratio, consistent with the behavior of allelic variants at a single gene locus. The esterase phenotypes were unusual. The variants were usually not characterized by differences in isozyme mobility, but instead, segregated for differences in staining intensity. Acid phosphatase segregation patterns indicated control by a minimum of two independent loci. Although genetic control of the two enzyme systems was demonstrated, allelic designations were not assigned to each phenotype. The problems associated with an allelic interpretation when phenotypes consist of multiple isozymes are discussed.

A seed orchard population and a natural population of Virginia pine were analyzed in terms of the three enzyme systems (EST, APH, GOT) described for Virginia pine. The measurement of the effects of artificial selection on the genetic structure of forest tree populations using the isozyme technique is discussed.

Key words: Isozymes, Virginia pine, artificial selection, electrophoresis.

Zusammenfassung

Der Vererbungsmodus von Isoenzymen der Esterase und sauren Phosphatase bei *Pinus virginiana* Mill. wurde durch eine Segregationsanalyse mit Megagametophyten von Samen festgestellt, die von Einzelbäumen gesammelt wurden. Sowohl die Phänotypen der Esterase als auch der sauren Phosphatase bestehen aus multiplen Isoenzymen, die im Verhältnis 1:1 segregieren. Diese Segregation deutet auf eine Kontrolle durch einen einzigen Genlocus mit allelischen Varianten hin. Die Phänotypen der Esterasen waren etwas ungewöhnlich. Die Varianten waren in diesem Fall nicht wie gewöhnlich durch verschiedene Wanderungsgeschwindigkeiten charakterisiert, sondern anstelle dessen durch Unterschiede in der Färbungsintensität. Die Segregationszahlen bei den sauren Phosphatasen deuten auf eine Kontrolle durch mindestens zwei unabhängige Loci hin. Obwohl die genetische Kontrolle beider Enzymsysteme nachgewiesen wurde, konnte die allelische Konstitution nicht für jeden einzelnen Phänotyp festgestellt werden. Die Probleme, die mit der Interpretation des Vererbungsmodus verbunden sind, wenn der Phänotyp aus multiplen Isoenzymen besteht, werden diskutiert.

Eine Samenplantagenpopulation und eine natürliche Population von *Pinus virginiana* wurden analysiert in Bezug auf die 3 Enzymsysteme (EST, APH und GOT), die für *Pinus virginiana* beschrieben worden sind. Die Größe der Effekte, die die künstliche Selektion bei der Anwendung der Isoenzymtechnik auf die genetische Struktur der Forstbaumpopulation hat, wird diskutiert.

Introduction

Seed orchards are an increasingly important aspect of forest tree improvement programs. As greater land area is devoted to improved seed stock it is essential to understand the changes in the genetic structure of planted populations resulting from domestication. The genetic characteristics of seed orchard populations have been intensively studied using quantitative methods. Quantitative analysis, however, cannot measure several important parameters of population structure: it cannot measure the fraction of genetic variation present in selected populations relative to the total variation present in natural populations; nor can the genetic organization of individuals within each population be measured. To measure these parameters it is necessary to have genetic markers which can be interpreted in terms of allelic variants at specific gene loci.

Electrophoretic analysis of protein variation provides the most efficient method available to determine genetic variation at a large number of gene loci. Conifers are particularly amenable to population analysis because characteristics of their life cycle allow the genetic control of specific isozymes to be determined in a single generation without breeding experiments. The haploid megagametophyte tissue of each seed is the product of one of the four megaspores produced in meiosis. Since the degeneration of three of the four daughter cells is presumably random, different segregational genotypes of the megagametophyte should be randomly distributed among seed from a single parent tree. Thus, in a diploid tree heterozygous for a particular gene locus, one would predict a 1:1 segregation ratio of alleles among megagametophytes collected from a single tree.

Virginia pine (*Pinus virginiana* Mill.) is one of several forest tree species for which improved breeding programs have been established. In order to analyze a seed orchard and natural population of Virginia pine, the genetics and inheritance patterns of glutamate oxalo-acetate transaminase (GOT), esterase (EST), and acid phosphatase (APH) in the megagametophyte tissue of the seeds were studied. The formal genetic analysis of the GOT system has already been presented (Witter and Feret, 1978). The inheritance patterns of esterase and acid phosphatase and the changes in population structure associated with artificial selection are described and discussed in this paper.

Materials and Methods

Unopened cones were collected from individuals in four populations of Virginia pine. Tree samples were collected from two natural populations at Blacksburg, Virginia (n = 7), and the Reynolds Homestead Research Center at Critz, Virginia (n = 20). The third population sample was from a clearcut area at Critz, Virginia planted with nursery-run stock obtained from the Virginia Division of Forestry (n = 29). Individual trees from these populations were selected on the basis of assessability and cone production. Cones were also collected from each of twenty-six clones in a "first-generation" seed orchard population at Appomattox, Virginia (n = 26). All cones were oven-dried at 37° C. Seed was collected and stored at 4° C until used.

Each electrophoretic sample was a crude protein extract of the megagametophyte from a single seed. The gameto-

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phyte was excised and placed in a 1 ml centrifuge tube containing .075 ml, 0.1 N Tris (2 amino 2-hydroxymethyl 1, 3 propanediol)-HCl buffer, pH 8.0 (Firenzuloli *et al.*, 1968). The tissue was ground with a glass rod and then centrifuged at 3,500 × G for 10 minutes. Electrophoresis was performed using discontinuous, vertical polyacrylamide slab gel electrophoresis according to the method of Davis (1964). The procedure employed a 3.3 percent spacer gel and a 7.5 percent running gel. Samples were initially run at 50 V for 105 minutes followed by 175 V for 130 minutes. Esterase activity (α-napthyl acetate substrate) and acid phosphatase activity were localized in the gels according to the methods of Scandalios (1974).

A minimum of five seeds from each tree were analyzed. Assuming heterozygous individuals were segregating in a 1:1 ratio this would give a 1/16 chance of misidentifying heterozygous individuals as homozygous. When a single tree had two or more mutally exclusive isozyme patterns among its seeds, larger sample sizes were run. The occurrence of isozyme bands in a 1:1 or 1:1:1:1 ratio was tested using the Chi-square test.

Table 1. — Segregating phenotypes and Chi-square analysis of heterozygous esterase phenotypes in the female gametophyte tissue of *Pinus virginiana*.

Segregant Phenotype	Tree I.D.	n[1]	Segregation Ratio		χ^2	P
1:2	427	20	Observed:	11:9	.20	.5-.7
			Expected:	10:10		
1:3	225	15	Observed:	8:7	.07	.7-.8
			Expected:	7.5:7.5		
1:3	E	9	Observed:	5:4	.11	.7-.8
			Expected:	4.5:4.5		
1:7	240	14	Observed:	6:8	.29	.5-.7
			Expected:	7:7		
1:7	399	22	Observed:	10:12	.18	.5-.7
			Expected:	11:11		
9:10	398	21	Observed:	8:13	1.19	.2-.3
			Expected:	10.5:10.5		
1:11	410	20	Observed:	9:11	.20	.5-.7
			Expected:	10:10		
8:12	292	20	Observed:	7:13	1.8	.1-.2
			Expected:	10:10		
1:14	324	13	Observed:	7:6	.08	.7-.8
			Expected:	6.5:6.5		
13:12	G	16	Observed:	8:8	0	> .95
			Expected:	8:8		
3:4	J	19	Observed:	8:11	.47	.3-.5
			Expected:	9.5:9.5		
5:6	P	17	Observed:	6:11	1.47	.2-.3
			Expected:	8.5:8.5		
8:7	Y	17	Observed:	7:10	.53	.3-.5
			Expected:	8.5:8.5		

[1]n = sample size

Results and Discussion

Esterase

Nine to twelve isozyme bands were detected per megagametophyte. A maximum of two esterase phenotypes were found among seed from any individual tree and in all cases the two phenotypes occurred in a 1:1 ratio. The esterase phenotypes were complex, consisting of multiple isozymes of variable staining intensity. Phenotypes were described on the basis of the following system of nomenclature: six isozymes served as standards and were numbered EST 1—EST 6, where EST 1 was the isozyme of greatest relative mobility from the origin. Isozymes not having the same relative mobility as the standards were defined in terms of slower mobility. For example, EST 4^s had a mobility slightly slower than that of EST 4. Intensity differences were defined only in terms of strong (S) or weak (W), while no activity at a given isozyme position was designated null (N). A total of 14 esterase phenotypes were found in seed from the four sample populations. The esterase phenotypes are illustrated in *figure 1*. The experimental segregation ratios of these phenotypes among megagametophytes from individual trees and the Chisquare analysis testing conformance to a 1:1 segregation ratio are given in *table 1*.

The most significant aspect of the inheritance patterns of esterase isozymes in Virginia pine is that the majority of the segregants involved isozymes of differential staining intensity rather than isozymes of different relative mobility. Barley is the only other plant species in which this unusual pattern of inheritance has been described (Kahler and Allard, 1970).

In haploid tissue a 1:1 phenotypic segregation ratio is the ratio predicted by characteristics controlled by a single locus with two alleles per locus. According to classical genetic analysis each esterase phenotype observed may be considered an allelic variant. This interpretation would require that each esterase "allele" determines multiple isozyme forms. However, there is no adequate genetic model, consistent with all of the observed phenotypes, to describe the observed formation of multiple isozymes and isozymes of differential intensity in terms of the product of a single structural gene locus.

Esterases are a complex and heterogeneous class of enzymes. Considered as a class they have a generalized enzymatic function and show developmental (Conkle, 1970) and seasonal variation (Kelley and Adams, 1977) as well as tissue

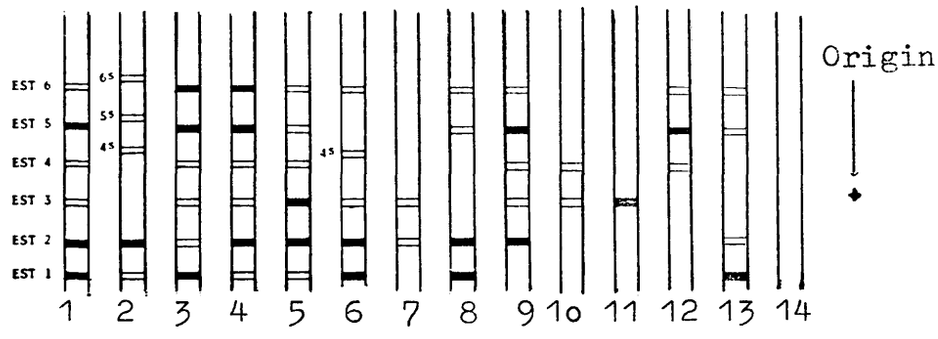

Figure 1. — Schematic representation of observed esterase isozyme phenotypes in Virginia pine macrogametophyte tissue.

and substrate specificity (MacDonald and Brewbaker, 1975). Given the diverse functions of the esterases, the complex inheritance patterns observed are hardly surprising. A brief review of the genetic basis and inheritance pattern of esterase isozymes in other conifer species, maize, and barley is presented here to illustrate common patterns of inheritance, mechanisms by which multiple isozymes can be generated, and the potential problems with interpretation and application of this particular enzyme system in Virginia pine.

In the conifers, multiple esterase isozymes are observed to behave as products of single alleles on the basis of segregation ratios in the macrogametophyte tissue. The various allelic phenotypes observed are: (1) a single isozyme segregating with a null allele *(Pinus pungens;* Feret 1974); (2) alleles of one, two, three or four isozyme bands at a single locus, some isozymes being common to the different allelic forms *(Picea abies;* Bergmann, 1973); (3) an allele of a single isozyme segregating with an allele consisting of two isozymes; one of the isozyme bands common to each of the two alleles *(Picea abies;* Tigerstedt, 1973).

While analysis of isozymes in megagametophyte tissue demonstrates the inheritance patterns of isozymes, it is not possible to analyze the behavior of isozymes in the diploid condition or to determine the mode of gene action in diploid tissues. The genetic control of esterase isozymes in diploid plant tissues has been most extensively studied in maize and barley. Seven barley loci (Kahler and Allard, 1970) have exhibited the following allelic control of isozyme phenotypes: (1) alleles controlling phenotypes exhibiting single isozymes of different relative mobilities and exhibiting a co-dominant pattern in heterozygotes (locus EA, EB); (2) alleles controlling single isozyme phenotypes segregating with null alleles rather than isozymes of different relative mobility (locus EE, EF, EG); (3) alleles controlling two isozymes in homozygotes exhibiting codominance and producing quadruple isozyme bands in heterozygotes (locus EC, ED). Aside from the evidence presented here *(Table 1),* the ED barley locus is the only other clearly documented case of esterase isozymes having allelic intensity variants. The ED locus has four alleles controlling isozyme phenotypes. Three alleles have two isozymes, and the fourth is a null. The isozymes of each allele show different patterns in staining intensity: either both isozymes are of equal, intense staining; both isozymes exhibiting equal, intermediate staining intensity; or the leading isozyme stains intensely while the trailing isozyme is much fainter. Controlled crosses between individuals homozygous for alleles of identical relative mobility but differing in their intensity patterns showed that the intensity variants are inherited as characteristics controlled by alleles at a single gene locus.

Isozymes differentiated in terms of staining intensity present special problems when assigning allelic designations to particular phenotypes, in spite of the fact that the phenotypes segregate as alleles. The ED intensity isozymes of barley showed variation among hybrids and from gel to gel. While it was possible to detect (within progeny from single crosses) the differential inheritance of intensity variants, the experimental variation among different hybrid combinations made it impossible to determine whether there was an equivalent genetic basis for intensity variants derived from different individuals. This same problem applies to intensity segregants in Virginia pine. Relative intensity differences could not be equated between seed samples from different individuals trees. Only alleles based on differences in relative mobility can be unambiguously equated between samples.

Intensity variants have a theoretical significance in studies concerned with assessing genetic variation. The intensity variants of esterase isozymes are an example of an additional source of cryptic, genetically based, protein variation. In this case, the source of additional genetic variation cannot be analyzed because variation in stain intensity cannot be quantified and standardized by known methods, to date.

Multiple esterase isozyme phenotypes generated by single gene loci appear to be common in plant species. The basis of multiple isozyme formation and the mode of gene action is variable (Schwartz 1964, 1967; Harris 1968; MacDonald and Brewbaker 1974, 1975). One important difference between the isozymes of conifers and those of maize and barley is that the number of isozymes controlled by alleles at a single locus in maize and barley is consistent, while single conifer loci may have alleles comprised of a variable number of isozymes.

In Virginia pine, we have demonstrated that there is a genetic basis for the esterase isozyme differences in the megagametophyte tissue. Heritable differences involve not only isozymes of different relative mobility but also differential stainability; the latter may be a function of either variation in isozyme concentration or activity. Two significant problems in assigning allelic designations to Virginia pine esterase phenotypes are: (1) the lack of evidence to distinguish whether the observed phenotypes are the product of a single locus or multiple linked loci and (2) an inability to equate intensity variants as genetically equivalent between individual trees.

While analysis of phenotypic segregation in haploid megagametophyte tissue is sufficient to demonstrate heritable differences in isozymes, it is not sufficient to distinguish isozyme phenotypes resulting from a single locus or multiple linked loci. In general, it is assumed that multiple isozymes are the product of a single gene locus in the absence of independent segregation among multiple isozymes or the appearance of recombinant phenotypes. If the observed isozyme patterns are truly the product of a single gene locus then it is necessary to determine how multiple isozymes are formed. The possible mechanisms by which multiple isozymes can be derived from a single structural locus include: (1) polymeric series, (2) conformational isozymes, (3) differential conjugation of polypeptide, (4) modification or cleavage of the polypeptide, (5) artifacts. In the case of the multiple isozymes described for Virginia pine several mechanisms can be omitted as likely explanations of the observed phenotypes. Artifactual esterase isozymes have been described in *Picea abies* (Bartels, 1971). The consistent segregation patterns and the reproducibility of within-tree phenotypes in multiple electrophoretic runs extending over a five month storage period argue against an artifactual basis of the isozymes of Virginia pine. The esterase phenotypes are not consistent with those phenotypes seen in isozymes generated by a polymeric series (Harris, 1968). While a polymeric series cannot be discounted as the basis of the observed phenotypes, it is unlikely.

To clarify the genetic basis of the esterase isozymes of Virginia pine the following lines of evidence might be investigated: (1) expression of esterase phenotypes in diploid tissues of parental trees, (2) mode of gene expression in diploid tissue of controlled hybrids, (3) substrate and inhibitor specificity of isozymes (e. g., MacDonald and Brew-

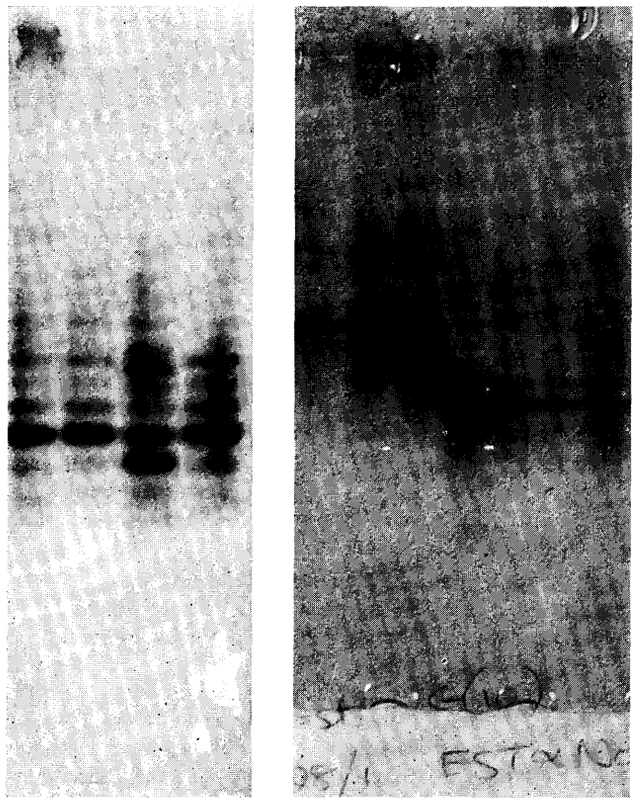

Figure 2. — Photographs of a polyacrylamide slab gel stained for esterase activity. 2a shows segregation of phenotypes 1 and 2 from Tree 427; 2b shows segregation of phenotypes 12 and 13 for Tree G.

BAKER, 1975, (4) tissue and developmental specificity. Information of this type would aid in determining whether the six major isozymes described here are a homogenous class and, in the absence of recombinant phenotypes, would help differentiate multiple isozymes as the product of a single locus or multiple linked loci.

Acid Phosphatase

From 2 to 5 acid phosphatase isozymes were observed in gels from each megagametophyte. Most trees which showed segregation among megagametophytes had two phenotypes segregating in a 1:1 ratio, although one tree had four phenotypes segregating in a 1:1:1:1 ratio.

Acid phosphatase phenotypes consist of multiple isozymes. To describe the phenotypes five standard acid phosphatase isozymes were designated APH 1—APH 5. Isozymes having relative mobilities different than those of the standards were designated fast (F) or slow (S) relative to isozymes APH 1—APH 5. The acid phosphatase phenotypes from the four sample populations are illustrated in *figure 3*. The experimental segregation ratios are given in *table 2*.

As with the esterases, acid phosphatase phenotypes are too complex to assign a specific allelic designation to particular isozyme phenotypes. Additional experiments such as those described above are necessary to determine the genetic basis of multiple isozymes. In order to be consistent with all the trees sampled, allelic designations would involve segregation at a minimum of three loci, each locus having alleles controlling null, single and double banded phenotypes. With this degree of variation, assigning allelic designations would be arbitrary and without a firm genetic basis for each phenotype. The segregation of four phenotypes does indicate that a minimum of two gene loci control the acid phosphatase isozymes in the region of APH 1—APH 5.

Table 2. — Segregating phenotypes and Chi-sqare analysis of heterozygous acid phosphatase phenotypes in the female gametophyte tissue of *Pinus virginiana*.

Segregant Phenotype	Tree I.D.	n[1]	Segregation Ratio	x^2	P
7:5	94	18	Observed: 7:11 Expected: 9:9	.89	.3-.5
7:5	242	18	Observed: 7:11 Expected: 9:9	.89	.3-.5
7:5	292	15	Observed: 8:7 Expected: 7.5:7.5	.07	.7-.8
6:5	277	21	Observed: 11:10 Expected: 10.5:10.5	.05	.8-.9
6:5	227	17	Observed: 7:10 Expected: 8.5:8.5	.53	.3-.5
6:5	56	9	Observed: 4:5 Expected: 4.5:4.5	.11	.7-.8
6:5	442	8	Observed: 3:5 Expected: 4:4	.50	.3-.5
1:2	27	16	Observed: 7:9 Expected: 8:8	.25	.5-.7
8:9	58	15	Observed: 8:7 Expected: 7.5:7.5	.07	.7-.8
3:2	U	19	Observed: 7:12 Expected: 9.5:9.5	1.32	.2-.3
10:6:7:12	307	27	Observed: 10:6:7:4 Expected: 6.75:6.75:6.75:6.75	2.78	.2-.3

[1]n = sample size

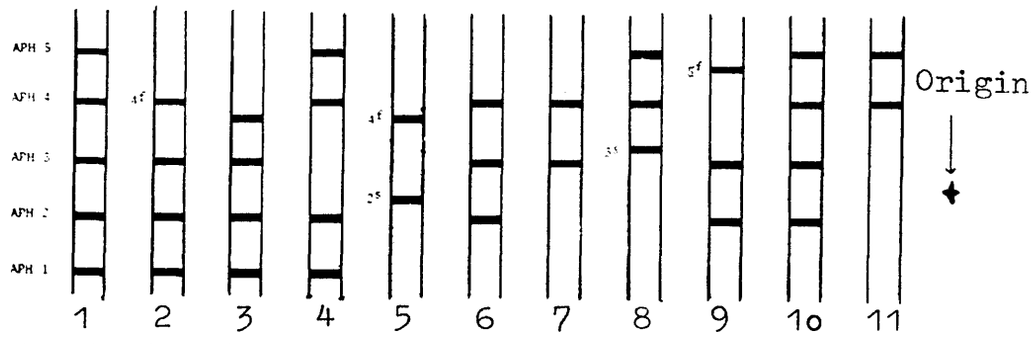

Figure 3. — Schematic representation of observed acid phosphatase isozyme phenotypes in Virginia pine macrogametophyte tissue.

Acid phosphatase is another of the most extensively studied enzyme systems in conifers. In *Picea abies* described alleles include phenotypes of the null, single, and double banded types (TIGERSTEDT, 1973; LUNDKVIST, 1975; BERGMANN, 1974 b). In *Picea abies* the enzyme was found to be a dimer of randomly associated subunits from a single locus(LUNDKVIST, 1975). Two other species, *Larix decidua* and *Pseudotsuga menziesii,* also have acid phosphatase alleles controlling null, single and double banded isozyme phenotypes (MEJNARTOWICZ and BERGMANN, 1975; BERGMANN, 1975).

The complexity of interpreting acid phosphatase isozymes coded by a single allele is best illustrated by the AP_1 locus in maize (EFRON, 1970). The AP_1 locus has three alleles: S, I, and F. Each allele has two possible isozyme forms. For all 3 alleles, one of the two isozymes forms was found to be specific to pollen, while the other form was specific to scutellar tissue. In leaf tissue both forms were found. EFRON (1970) proposed that differential isozyme formation from a single allelic form could be based on: (1) conformational isozymes with conformation dependent on a tissue specific cellular environment; (2) tissue specific enzymatic addition of charged groups; (3) the formation of allelic complexes with two different proteins to form different isozymes; (4) very closely linked loci. Complex patterns such as these underscore the necessity for a conservative approach in analysis of isozyme patterns.

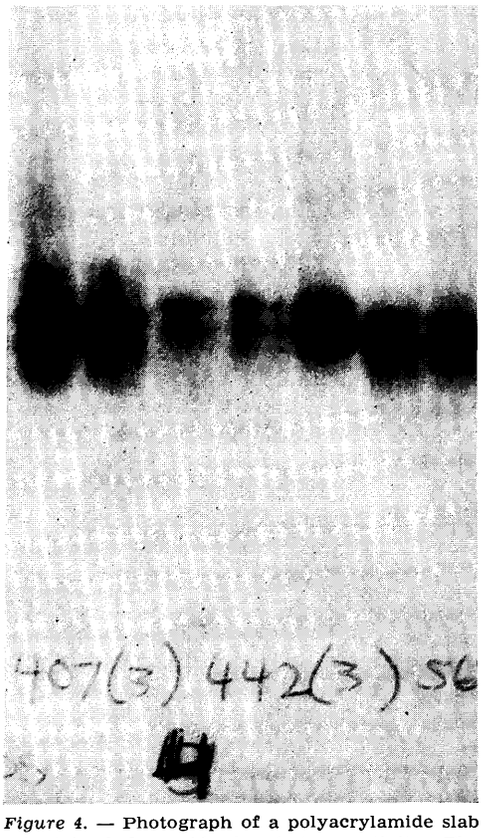

Figure 4. — Photograph of a polyacrylamide slab gel stained for acid phosphatase activity. The samples are from Trees 442 and 568 and show segregation of phenotypes 5 and 7.

Table 3. — Frequency of heterozygous individuals among four populations of *P. virginiana* with respect to 4 enzyme systems, GOT A, GOT B, EST, and APH.

	N[1]	GOT A	N	GOT B	N	EST	N	APH
Reynolds, natural stand	20	.65	20	.05	17	.41	18	.67
Blacksburg, natural stand	7	.72	7	.00	6	.33	6	.33
Reynolds, planted stand	28	.63	28	.04	25	.36	28	.39
VDF seed orchard	25	.40	25	.00	22	.36	25	.52

[1]N = sample size. N may be variable within a population for each enzyme system due to low activity of some seed sources in particular enzyme systems (n instead of N).

Population Analysis

Only one of the three enzyme systems, GOT, used to analyze the Virginia pine populations could be interpreted in an allelic sense (WITTER and FERET, 1978). No significant differences were found in allelic frequencies between the natural and seed orchard populations of Virginia pine. A significant difference was found in several genotypic frequencies. The seed orchard population had a greater number of individuals of the GOT A4/A4 genotype and fewer of the A2/A4 genotype (WITTER and FERET, 1978).

For the reasons presented here, it is best not to assign an allelic designation to the segregating esterase and acid phosphatase phenotypes although genetically they behave as single alleles. Phenotypic segregation among megagametophytes from individual trees does make it possible to identify idividuals as homozygous or heterozygous for each enzyme system. The frequency of heterozygous individuals in the four populations was calculated for each enzyme system *(Table 3).* By direct count the frequency of individuals in each population heterozygous for none, one, two and three Virginia pine enzyme systems was calculated *(Table 4; Figure 5).* Statistically, the Critz natural population had significantly fewer individuals homozygous for all three enzyme systems than did either the Critz planted population or the VDF seed orchard population (arc sin transformation t-test; SOKAL and ROHLF, 1969). Graphical analysis of the relative cumulative frequency distributions of the three populations showed the frequency distributions to be different for the VDF seed orchard population and the two Critz populations (ZAR, 1974). The individuals in both Critz populations were normally distributed relative to the percentage of heterozygous enzyme systems per individual. The VDF seed orchard population did not conform to a normal distribution but was skewed towards a greater than expected frequency of homozygous individuals.

Conclusions

Isozyme Genetics

The patterns of inheritance of acid phosphatase and esterase isozymes in Virginia pine are complex. Our results and a brief review of the results from other species indicate that the genetic control of these isozyme systems cannot always be explained in terms of a simple one allele:one polypeptide model. Some applications of the electrophoretic technique, such as clonal identification (STERNBERG, 1976), do not necessarily require a complete assessment of the genetic basis of isozyme variation. However, when applied to the analysis of the genetic structure of populations, isozyme variation does require a more rigorous interpretation. Genetic analysis of populations based on changes in allelic and

Table 4. — Frequency of individuals heterozygous for 0, 1, 2 and 3 enzyme systems (GOT A, EST, APH) among three populations of *P. virginiana*.

Population	n[1]	Frequency of heterozygous enzyme systems per individual .00	.33	.66	1.00
Critz, natural	16	.00*	.38	.44	.19
Critz, planted	25	.16*	.44	.28	.12
VDF seed orchard	22	.32*	.23	.27	.18

[1]n = sample size

* Significant difference in frequencies between Critz natural and planted stands; Critz natural stand and VDF seed orchard. All others are non-significant.

Blacksburg omitted due to small sample size (n = 5).

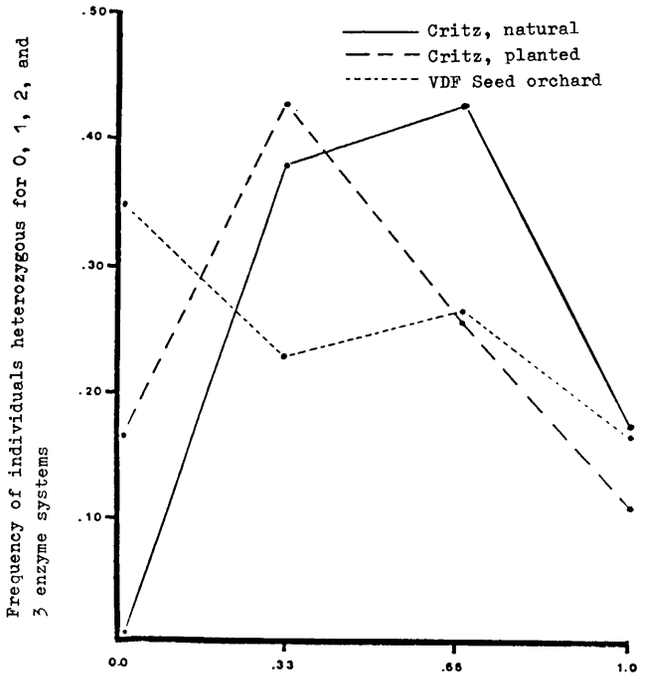

Figure 5. — Frequency of *P. virginiana* individuals heterozygous for 0, 1, 2 and 3 enzyme systems (GOT A, EST, APH) among three populations of *Pinus virginiana* (Critz, natural; Critz, planted; VDF seed orchard). Plotted from data in *table 4*.

genotypic frequencies make it essential that isozymes accurately reflect allelic variation.

There is evidence that enzyme systems such as the esterases should be applied to population analysis cautiously. Kahler and Allard (1970) demonstrated in barley that there were interactive effects between two of the barley esterase loci such that $EB^{2.7}$ was expressed in individuals heterozygous for the $EA^{2.0}$ allele but was not expressed in $EA^{2.6}$ homozygotes. Novel, non-predicted genotypes could be expected in an enzyme system which has interacting loci. Two studies of esterases in forest tree species have described unpredictable isozyme patterns. Feret (1971) analyzed esterase isozymes of *Picea glauca* full sib progeny and found that the progeny often lacked parental phenotype patterns or possessed novel patterns. Bergmann (1974 a) analyzed Finnish populations of *Picea abies* according to the genetic hypotheses of an earlier analysis. Ten to twenty-five percent of the individuals in each population had new esterase isozyme patterns which were not in accord with the earlier genetic model.

Population Comparisons

The effects of selection on the genetic structure of a population can be measured in terms of the characteristics of both the total population and as the sum of the individuals within a population. The significant characteristics of the population as a whole are: (1) the total allelic diversity of a population; (2) allelic and genotypic frequencies. Population of statistical equivalence in terms of these gross characteristics may be quite different in terms of their organization. Population organization depends on the distribution of genetic variation among indivduals. This is most commonly measured in terms of genotypic frequencies and the degree of individual heterozygosity.

GOT analysis of the seed orchard population and the Critz natural population showed that while statistically the two populations have the same information, it is not distributed identically among individuals (Witter and Feret, 1978). The frequency distribution of relative individual heterozygosity showed the seed orchard population to be relatively depauperate of individuals of intermediate heterozygosity. The limited number of enzyme systems, small sample sizes, and the non-allelic nature of two of the enzyme systems studied do not allow a definitive statement on the effects of domestication of Virginia pine. The data suggest a testable hypothesis, however. If individuals in natural populations of Virginia pine are normally distributed for the percent heterozygous loci/individual, selection of individuals for first generation seed orchard stock is non-random and primarily favors individuals with extremes of homozygosity, with extremes of heterozygosity favored secondarily. Selection for homozygosity is in keeping with the only demonstrated statistical difference in genotypic frequencies between populations.

There have been relatively few studies of the effects of artificial selection on the genetic structure of select populations. The effects of artificial selection have been studied in two ways, the design being dictated largely by the availability and the breeding history of the select populations. In the case of species which have been bred over many generations, changes in genetic structure are inferred from the genetic structure of existing cultivars or varieties. This only allows a comparison of existing variation between cultivated varieties and modern wild populations. With an experiment of this design, the nature of the original, select population and the genetic changes associated with the intervening generation of selection cannot be known. In cases where the parental population, the initial select population, and subsequent selected populations are available, the changes associated with each generation to produce the endproduct of selection can be documented. The major effect of artificial selection has been predicted to be the erosion of genetic variability and the divergence between selected populations at loci which are the targets of selection as well as the genome as a whole in cases where sampling (selection) causes "founder effects" (Williams and Brown, 1956; Levin, 1976).

The erosion of genetic variation in response to selection, both artificial and natural, does not seem to be as drastic

as previously predicted. In the species studied, there appear to be factors which act to maintain polymorphisms even in the face of extensive inbreeding. The degree to which domesticated populations maintain the variability present in wild populations depends on the following factors: (1) the population size of a founding population of primary or secondary selection. These populations act as genetic bottleneck and determine the absolute amount of genetic variation present in domesticated populations. The size of a genetic bottleneck is not an independent function of the selected population alone, but is related to the amount and distribution of genetic variation among wild populations; (2) the number of selected populations and the different selection goals associated with them; (3) the breeding system and the strength of the factors which favor individual heterozygosity (ALLARD *et al.*, 1972.)

The electrophoretic technique is applicable to many of the questions associated with the effects of selection at all stages of the domestication process in forest tree species. Characteristics of forest tree species which are usually regarded as a hinderance to an efficient breeding program make improved tree populations an ideal model for assessing the effects of intensive artificial selection. The persistence of populations and the long generation times of trees make experimental material from each selected generation available. The extensive quantitative analysis associated with improvement programs ensures accurate, detailed records of breeding histories. The selection pressures exerted to produce improved populations of tree species are extremely high because the land area and management required for seed orchards necessitate that populations be small. These are characteristics that make improved tree populations amenable to complete genetic analysis. Specific applications of the electrophoretic technique to each stage of domestication are detailed below.

The first stage in the domestication of a forest tree species involves the establishment of a seed orchard population of select individuals derived from one or more natural populations. The important domesticated populations are not represented by the first generation orchard, however. The populations of actual significance are outplanted progeny from the first generation orchard and the advanced generation orchards and their progeny. It is these populations which represent the real outcome of selection and domestication.

Two effects can be assessed by measuring the genetic structure of a first generation seed orchard relative to natural populations: the genetic qualities favored by artificial selection and the extent of the bottleneck effect associated with the establishment of the artificial population. These are the two parameters for which we have made a preliminary estimate by comparing a first generation seed orchard population and a single natural population. As already stated, in a first generation seed orchard of Virginia pine selection has produced a population of individuals in which individual heterozygosity levels have a non-normal distribution. Additional data are necessary to confirm whether artificial selection in Virginia pine is actually associated with selection for high levels of individual homozygosity. The GOT analysis of the two populations substantiated the probabilistic nature of the total genetic diversity present after selection: at naturally monomorphic loci (GOT B1 $>$ .95 in Critz natural and planted populations) rare allelic types will be largely eliminated in small, selected populations unless they affect selected characteristics. At polymorphic loci the effects of selection on total genetic variation will depend on the distribution of variation between populations and the extent of sampling within and between populations. The lack of baseline data for natural Virginia pine populations makes it impossible to determine the effect of selection on the polymorphic GOT A locus (WITTER and FERET, 1978).

The progeny of the first generation seed orchard represent the first improved population. Electrophoretic analysis of the first generation progeny can quantify the differences between natural populations and first generation progeny in terms of individual genotypes. The degree to which selection is effective will be a function of the difference between the genotypic array of natural populations and that of seed orchard progeny. Because individual genotypes can be described it is possible to determine the degree to which improvement is dependent on the characteristics of genetic organization such as individual levels of heterozygosity or on specific allelic arrays. The genetic basis of quantitatively analyzed characteristics such as GCA and SCA is not known: electrophoretic identification of specific genotypes can determine whether these characteristics are associated with either specific genotypes or specific types of genetic organization. The mating system of natural populations of *Eucalyptus* has been determined by electrophoretic analysis (BROWN *et al.*, 1975; PHILLIPS, 1977). It can also be used to determine whether mating is truly random in seed orchards and the degree to which inbreeding occurs in the production of progeny from the first generation seed orchard (FERET, 1978).

Advanced generation seed orchard populations represent additional levels of selection: measurement of these populations can determine whether there is continuity of the genetic characteristics of selected individuals and the effect of additional genetic bottlenecks. In some cases advanced generations are based on very few genotypes. In these generations there is a possibility of significantly reducing the variation present at naturally polymorphic loci. For this reason, it is important to determine the significance of polymorphisms in selected species and to determine whether efforts should be made to maintain them in artificial populations.

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Propagation of Hybrid larch by summer and winter cuttings

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Summary

Summer and winter cuttings from young Hybrid larch [*Larix* × *eurolepis* (HENRY)] were rooted at high levels (> 90%). The rate of rooting of summer cuttings was increased by indole butyric acid treatment though the final level was unaffected. Rooting decreased when the cuttings were collected later in the growing season. Cold storage of winter cuttings increased rooting whereas warm storage of more than three weeks resulted in extensive callus development but a large reduction in subsequent rooting. Extended warm storage followed by cold storage drastically reduced rooting.

Key words: Rooting, Hybrid larch, Summer cuttings, Winter cuttings.

Zusammenfassung

Sommer- und Wintersteckлinge von Lärchenhybriden [*Larix* × *eurolepis* (HENRY)] bewurzelten sich mit Erfolg (> 90%). Behandlung mit Indolbuttersäure erhöhte das Bewurzelungsvermögen der Sommersteckлinge, blieb jedoch ohne Einfluß auf den Gesamterfolg. Die später in der Vegetationsperiode geschnittenen Steckлinge wiesen geringere Bewurzelung auf. Kühlraumlagerung während der Wintermonate begünstigte das Bewurzelungsvermögen; dagegen trat nach Warmlagerung von mehr als drei Wochen beträchtliches Calluswachstum an der Schnittfläche der Steckлinge auf, jedoch auf Kosten des darauffolgenden Bewurzelungsvermögens. Längere Warmlagerung mit nachfolgender Kühlraumlagerung verminderte das Bewurzelungsvermögen ganz erheblich.

Introduction

Larix × *eurolepis* (HENRY) is frequently superior in the F_1 generation to the two parent species in terms of growth (HOLST, 1974), form (ELIASSON and CARLSON, 1963), drought resistance (KEIDING, 1962) and canker resistance (KIELLANDER, 1958). It has been planted extensively in the United Kingdom and demand has always exceeded the supply of seed (BIGGIN, 1977). Production of seed has been low due mainly to differences in the flowering times of the two parent species in seed orchards and the irregularity of good seed years. It is envisaged that, in the short term and until production from seed orchards can be increased, the demand for Hybrid larch seedlings might be met by vegetative multiplication from seedlings of superior genotypes.

The vegetative propagation of plants from stem cuttings has been used extensively in the horticultural industry for both herbaceous and woody species. The production of rooted stem cuttings of conifers has become important in recent years for producing clonal plants for experimental purposes (BURDEN and SHELBOURNE, 1974), for the testing of genetically superior stock produced by tree improvement programmes (RAUTER, 1974) and for commercial forestry (KLEINSCHMIT, 1974).

Larix spp. have been propagated vegetatively from cuttings although the levels of rooting achieved have tended to be low. ISIKAWA (1969) demonstrated that cuttings from young *Larix kaempferi* clones rooted best but rooting declined with increasing lignification of the cuttings and other workers found that the level of rooting also declined with the age of the donor clone (SAKAMOTO, 1972; OKADA, 1968). CHANDLER (1959) achieved 40% rooting with cuttings collected in August from *Larix* clones under four years old. Rooting at other periods of the summer, and from older ortets, was lower, although it could be increased by treatment of the cuttings with plant hormone solutions or commercially available rooting powders.

Hybrid larch is deciduous and two very different types of cutting are available at different times of the year, ie