WITHIN-STAND CLUSTERING OF EASTERN WHITE PINE GENOTYPES

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Abstract.--Canonical variate scores representing the age and location of eastern white pines in a three hectare unmanaged forest stand show a relationship to canonical scores representing genotypes of eastern white pines. Trees with similar genotypes and age grow in groups. The patterns of clustering may be related to establishment.

Additional keywords: Pinus strobus L., Smouse Scores, canonical correlation, genetic structure.

A large proportion of genetic variability in eastern white pine appears to be within stands, rather than among stands (Ryu and Eckert 1983), but the spatial distribution of the genetic variability within stands is not well understood. Electrophoretic analysis of tree enzymes can help in understanding genetic variability in forest stands. A better understanding of local genetic variability in eastern white pine could be applied to decisions affecting stand thinning and seed collection for breeding purposes. This paper describes the relationship of tree location to the allelic composition of eastern white pines growing in a three hectare unmanaged forest stand, and presents evidence suggesting that tree establishment may be in part responsible for clustering of trees with similar genotypes.

METHODS

The Study Site

The forest stand selected for this study is located in Durham, N.H. It is representative of the unmanaged eastern white pine and hemlock stands in New Hampshire. The stand is bordered by previously thinned stands on two sides, a wet area on the third, and a red pine stand on the fourth side. The location of all 500 eastern white pines in the stand was recorded on a x/y coordinate map, and the age of 160 randomly selected trees was estimated from incremental cores taken at breast height.

Isozyme analysis

Foliar tissue samples were collected from all 500 eastern white pines. sling shot was used to remove one year old foliage from sun-exposed portions of tree crowns. Samples were stored on ice while in the field and frozen (-20°C) in the laboratory until analyzed.

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Horizontal starch gel electrophoresis was used to determine allelic composition of foliar samples for the following seven enzymes: Leucine aminopeptidase (LAP E.C. 3.4.11.1), Phospho-glucose isomerase (PGI E.C. 5.3.1.9), Glutamate-oxaloacetate transaminase (GOT E.C. 2.6.1.1), Shikimate dehydrogenase (SDH E.C. 1.1.1.25), Fluorescent esterase (F-EST E.C. 3.1.1.2), Phosphoglucomutase (PGM E. C. 2.7.5.1), and Malate dehydrogenase (MDH E. C. 1.1.1.37). Each sample was frozen in liquid nitrogen and ground to powder with a mortar and pestle. An extraction buffer (Table 1) was added, the mixture was stirred until a paste formed, and then the extracts were absorbed into paper wicks through lens paper covering the paste (Gorman and Kiang, 1977). Electrophoresis was carried out at 3°- 5°C. Migration of most enzymes required approximately four hours under conditions described by Adams and Joly (1980), however malate dehydrogenase gels required twenty hours at 20 milli-amperes. Gel and electrode buffer recipes, and electrophoretic conditions are summarized in Table 2. Stain recipes are described in Table 3. The agar overlay method was used for SDH and MDH. F-EST gels were scored immediately, all other stains were developed at least one hour in darkness at 37°C.

Table 1.--Extraction buffer for fifty samples of white pine foliage.[a/]

COMPONENT	AMOUNT
Germanium dioxide[b/]	0.150 g
Sodium borate (decahydrated)	2.200 g
Sodium bisulfite	0.600 g
Soluble polyvinylpyrolidone (PVP-40T)	8.000 g
L-ascorbic acid	8.000 g
Diethyldithiocarbanic acid	0.550 g
Potassium phosphate buffer (0.16M KH_2PO_4, pH. 7.0)[c/]	16.000 ml
2-phenoxyethanol	1.000 ml

a/ Based on recipe of Mitton et al. 1977.
b/ Dissolve in 150 ml of boiling water. Cool solution to 2°-3°C.
c/ Mix with dry chemicals to make a paste, then add the germanium dioxide solution, and then add 2-phenoxyethanol.

Table 2.--Enzymes, buffers and electrophoretic conditions used for analysis of eastern white pine foliage.

ENZYMES	References for buffers and conditions for electrophoresis	Gel and Electrode buffers[a]
Leucine aminopeptidase (LAP E.C. 3.4.11.1)	Scandalios 1969	1
Phospho-glucose isomerase (PGI E.C. 5.3.1.9)	Scandalios 1969	1
Glutamate-oxaloacetate transaminase (GOT E.C. 2.6.1.1)	Hamrick 1980[b]	2
Shikimate dehydrogenase (SDH E.C. 1.1.1.25)	Hamrick 1980[b]	2
Fluorescent esterase (F-EST E.C. 3.1.1.2)	Mitton et al. 1977	3
Phosphoglucomutase (PGM E.C. 2.7.5.1)	Mitton et al. 1977	3
Malate dehydrogenase (MDH E.C. 1.1.1.37)	Neale 1980[c]	4

[a] Buffers and conditions: (1) stock a, 12.0 g. LiOH (monohydrate) - 118.9 g. H_3BO_3; pH 8.1; stock b, 62.0 g. Tris - 16.0 g. citric acid (monohydrate); pH 8.3; gel, 1 part a and 9 part b; electrode, a only; amperage, 15 - 40 mA; duration, about 4.5 hours; (2) gel, 121.1 g. Tris- 25.2 g. citric acid (monohydrate); pH 8.45; adjust pH with 0.2 M citric acid; electrode, 166.0 g. boric acid - 20.0 g. NaOH; pH 8.0; amperage, 45 - 55 mA; duration, about 4 hours; (3) gel, 14.5 g. Tris - 5.8 g. citric acid (monohydrate); pH 7.7; electrode, 115.875 g. boric acid - 4.65 g. NaOH; pH 7.5; amperage, 5 - 25 mA; duration, about 4 hours; (4) gel, 1 : 20 dilution of electrode buffer; electrode, 76.838 g. citric acid (anhydrous); pH 6.1; adjust pH with morpholine; amperage, 20 - 25 mA; duration, 20 hours.

NOTE: For each enzyme system, combine distilled water with-dry chemicals to total 10 liters of buffer. Use first amperage for 15 minutes, remove wicks, then use second amperage.

[b] Hamrick, 1980; personal communication by Ryu, 1982.

[c] Neale, 1980; personal communication by Ryu, 1982.

Table 3.--Stain recipes for foliar enzymes in eastern white pine.[a/]

ENZYME	COMPONENTS	AMOUNT
LAP	L-leucyl-napthamide	35.0 mg
	.2M Tris maleate buffer pH 3.8	89.0 ml
	.2M NaOH	45.0 ml
	Black K salt	90.0 mg
	H_2O	53.0 ml
PGI	1M Tris HC1 pH 8.0	4.6 ml
	MTT	30.0 mg
	PMS	7.5 mg
	G-6-PDH (10 u/ml)	6.5 ml
	Fructose-6-P	10.5 ml
	NADP	10.0 mg
	H_2O	170.0 ml
	$MgCl_2$ (10%)	8.8 ml
PGM	1M Tris HC1 pH 8.0	3.8 ml
	MTT	19.0 mg
	PMS	7.5 mg
	G-1, 6-DP (.05%)	2.5 ml
	G-6-PDH (10 u/ml)	13.0 ml
	NADP	8.0 mg
	H_2O	147.0 ml
	$MgC1_2$ (10%)	3.8 ml
	Na_2 G-1-P (1%)	12.5 ml
GOT	Pyridoxal-5'-phosphate	8.0 mg
	L-aspartic acid	320.0 mg
	ketoglutaric acid	160.0 mg
	1M Tris HC1 pH 8.8	14.0 ml
	Fast blue BB salt	240.0 mg
	H_2O	186.0 ml
F-EST	4-methylumbelliferyl acetate	35.0 mg
	Acetone	20.0 ml
	1M Na acetate pH 5.0	15.0 ml
	H_2O	57.0 ml
	Apply with paper towel, score immediately in UV light	
SDH	.05M Tris HC1 pH 8.0	13.5 ml
	MTT	7.5 mg
	PMS	9.0 mg
	(-) shikimic acid	12.0 mg
	NADP	12.0 mg
	Agar solution (1%)	18.0 ml
MDH	.25M Tris HC1 pH 8.6	12.0 ml
	L-malic acid	47.0 mg
	MTT	22.5 mg
	PMS	4.5 mg
	NAD	23.0 mg
	Agar solution (1%)	18.0 ml

[a/] Recipes are for three gel slices. Pour stain solutions over sliced gels and incubate at least one hour in darkness at 37°C, unless otherwise indicated.

Evidence for genetic control of enzyme variants

Preliminary evidence for genetic control of enzyme variants was obtained from segregation ratios in haploid tissue of heterozygous trees. Cones were collected in a clonal orchard where the genotypic identity of open pollinated parent trees is known from previous isozyme analyses. The clones are representative of trees growing in New Hampshire. Cones were not available in the natural stand. Cones were allowed to dry in sunlight, seeds were extracted, and stored at -15 °C until needed. Two days preceding analysis, seeds were removed from storage, soaked in 10% H_2O_2 for twelve hours, and retained in distilled water for the remaining 36 hours. The two day seed treatment was carried out in plastic petri dishes, illuminated by a 60 watt tungsten lamp. Megagametophyte tissue of each seed was dissected from the seeds up to 12 hours before analysis, and was stored individually in one drop of water at -15 °C. Immediately preceding electrophoretic analysis, the tissue was crushed with a glass rod, and an extract of the paste was absorbed into paper wicks. Electrophoresis and staining of the megagametophyte enzymes was identical to that described for foliar tissue enzymes.

Data analysis

The raw isozyme scores were converted into continuous "Smouse Scores" (Smouse et al. 1982), and analyzed by canonical correlation analysis (Gittins 1979). Canonical correlation analysis was used to evaluate the strength of relationship between the set of genotypic variables (represented by isozymes) and the within-stand location of the trees. The relation of age to the spatial distribution of the genotypes and to enzyme composition was also explored. Plots of predicted canonical variate scores for the enzymes were used to identify regions of the stand which contain trees with similar genotypes. Plots showing the distribution of trees with selected enzyme variants important in the formation of the canonical variate scores were used to explain the basis for some of the observed patterns. The relationship of tree age to allelic composition of individual trees, and the distribution of trees with similar age were used to suggest a possible reason for the patterns that were observed.

RESULTS AND DISCUSSION

Enzyme variants observed

The electrophoretic analysis showed the presence of enzyme variants for six of the seven enzymes surveyed. Foliar isozyme band patterns will be discussed separately for each enzyme system. Tests of significance for deviation from expected 1:1 ratios of enzyme variants in haploid megagamethophyte tissue of seeds from heterozygous mother trees are summarized in Table a. A graphic summary of patterns for each system at corresponding values is shown in Figure 1. Each zone of staining activity on a particular gel is encoded by a gene, with one or more allozymes encoded by allelic forms of the gene. The fastest migrating zone of each enzyme is designated locus one, the following locus two, etc. Alleles at each locus are identified as "a" for the fastest allele, "b" for a slower allele, etc.; the fast allele of the first Mdh locus is represented as Mdh-1a, for example. Heterozygote genotypes are represented as "a/b", and homozygotes as "a/a".

Table 4.--Observed ratios of allozymes from megagametophytes of heterozygous parent trees and chi-square analysis of ratios to expected 1:1 allozyme segregation.

Enzyme	Alleles a/b		Observed Number a	b	Deviation x^2(1:1)	Probability
SDH	a	b	150	145	.08	.78
MDH-1	a	b	131	140	.29	.61
	a	c	67	97	5.48	.02
	b	c	53	44	.84	.39
F-EST	a	b	52	49	.09	.77
PGI-2	a	b	64	81	1.99	.17

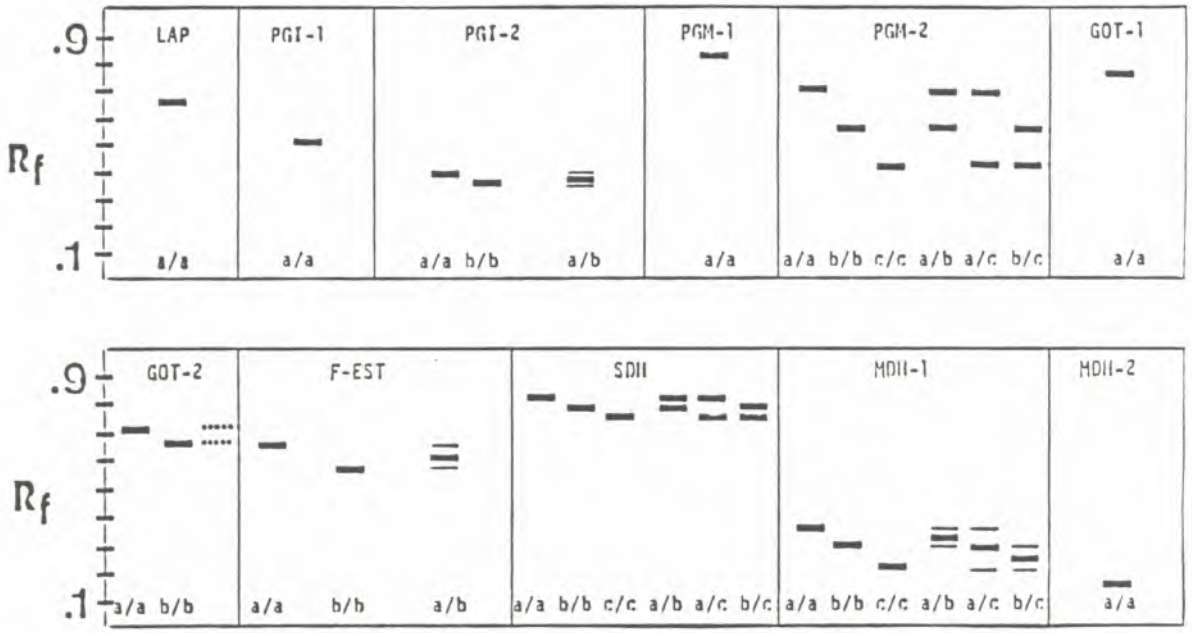

Figure 1.-- Band pattern and genotype designation for LAP, PGI, PGM, GOT, F-EST, and MDH in eastern white pine foliage. R_f is the migrational distance of enzymes relative to that of the buffer front (depression in gel), from the origin. Solid lines represent observed genotypes and dotted lines represent expected but not observed genotypes.

Leucine aminopeptidase (LAP) E.C. 3.4.11.1

A single enzyme band appeared consistently on gels with foliar samples stained for LAP. Two other isozymes, one slower and one faster, were occasionally observed, but not included in this study. The three zones of activity correspond to those seen by Ryu (1982). Eckert et al. (1981) observed similar bands in megagametophyte tissue of heterozygous white pines, but also detected additional loci. 1:1 segregation of allozymes in heterozygous white pine megagametophytes indicated the allozymes were under genetic control in megagametophyte tissue.

Phosphoglucose Isomerase (PGI) E.C 5.3.1.9

Two zones of activity were observed on gels with foliar samples stained for PGI. One band was observed for Pgi-1 and two variants were observed for Pgi-2. Pgi-2 bands of homozygotes appeared darker than those of heterozygotes. The heterozygotes displayed a dimeric pattern, with a darker band between the two lighter fast and slow bands. This type of pattern was reported for other conifers by Adams and Joly (1980), and by Guries and Ledig (1978). The first and second allozymes of Pgi-2 in heterozygous megagametophyte tissue segregated in a 1:1 ratio.

Phosphoglucomutase (PGM) E.C. 2.7.5.1

Two zones of activity were seen on gels with foliar samples stained for PGM. The fast migrating zone contained one dark band similar to that observed by Eckert et al. (1981) in megagametophyte tissue. Three different lighter bands were seen in the slow migrating zone, both in homozygous and heterozygous monomeric form. Heterozygote bands were lighter than homozygote bands. All six possible band combinations were observed at the slow locus. Ryu (1982) observed nearly identical numbers of enzyme bands at compatible Rf values in foliar tissue and in the megagametophyte. Eckert al, al. (1981) observed 1:1 variant segregation ratios in megagametophyte tissue of heterozygous eastern white pines, suggesting the allozymes are under genetic control.

Glutamate-oxaloacetate Transaminase (GOT) E.C. 2.6.1.1.

Two zones of activity were observed on gels stained for GOT, the fast zone had one isozyme band and the slow zone had two variants. Got-2 was present in only two of the 500 trees examined. No heterozygotes were detected. A third, even slower zone was occasionally visible, but was not intense enough for consistent scoring. Three GOT zones with compatible Rf values and nearly identical numbers of enzyme bands have been reported for both megagametophyte and foliar tissue, with two monomeric bands at the second locus (Ryu 1982). Eckert et al. (1981) used segregation of allozymes in heterozygous white pine megagametophytes to demonstrate genetic control of the GOT variants.

Fluorescent Esterase (F-EST) E.C. 1.1.1.2

One F-est locus containing two isozyme variants was scored for foliage. One slower locus was also visible, but its interpretation was unclear. First locus heterozygotes exhibited a dimeric pattern as reported for eastern white pine (Ryu, 1982) and for ponderosa pine foliage (Mitton al., 1979). The allozymes segregated in megagametophyte tissue as expected.

Shikimate Dehydrogenase (SDH) E.C. 1.1.1.25

One zone of three isozyme variants was observed for this enzyme in foliar samples. All six possible monomeric patterns were seen in the trees sampled for this study. Homozygote bands stained more intensely than did heterozygote bands. The first and second allozymes segregated as expected in megagametophyte tissue. No seeds were available to test segregation of the third allozyme.

Malate Dehvdrozenase (MDH) E.C. 1.1.1.17

Two zones of activity were seen for MDH in foliar samples, a fast zone with three enzyme variants and a slow zone fixed for one variant. All three homozygote and three dimeric heterozygote patterns of bands were observed at Mdh-1. Megagametophyte enzyme band pairs from heterozygous mother trees segregated in a 1:1 ratio as expected for a single locus under genetic control. Homozygote stains of Mdh-1 appeared darker than heterozygote stains, but comparison of the foliar band patterns to those of megagametophytes was hampered by differential migration of isozymes from the two tissues. Chemicals present in the foliar extracts, not found in the megagametophyte extracts may be responsible for this problem (Pitel and Cheliak, 1983).

Relationship of tree location to genotype

Canonical correlation analysis of enzyme scores and tree location variables showed two significant canonical correlations (Table 5). The first correlation accounted for 11.6 percent of variation in the data, the second correlation accounted for 6.8 percent of variation in the data. The correlations between the canonical variates were statistically significant and sufficiently strong for identifying spacial distribution patterns. Two levels of relationship were observed between the isozyme scores, and the distribution of the 500 trees. The first level is represented by the first pair of canonical variates V1 and W1 and the second level is represented by V2 and W2.

Table 5.--Canonical correlation analysis of isozyme variables and location of eastern white pines in a three hectare unmanaged forest stand -- dimensionality analysis.[a/]

K	r_k	r_k^2	F	Num df	Den df	PR>F
1	.341	.116	2.342	50	2251.79	0.0001
2	.261	.068	1.496	36	1852.98	0.0297
3	.145	.021	0.762	24	1436.25	0.7883

a/ K = number of dimensions
r = canonical correlation coefficient
r_k^2 = squared canonical correlation coefficient
F = approximate F based on likelihood ratios testing the null hypothesis (Ho) that the canonical correlation in the current row and all that follow are zero.
N = 508 observations
Isozyme variables (Smouse Scores): SDH1, SDH2, PGM21, PGM22, GOT21, PGI21, MDH11, MDH12, FEST11, FEST21
Location variables: X, X^2, Y, Y^2, XY

Remember that V represents a linear combination of the enzyme variables and W represents a linear combination of the location variables. In canonical correlation analysis the linear combination of each set of variables is calculated so that the correlation between V1 and W1 is maximized. The second set of canonical variables (V2 and W2) is then extracted, using a portion of the remaining variance not extracted by the first set of canonical variables. The canonical correlation coefficient (r) is a measure of the relationship between each pair of canonical variates. The relative importance of individual raw variables that contributed to the formation of the canonical variate scores may be determined from correlations between the canonical variates and raw variables (table 6). F-EST and SDH were most highly correlated with V1 and PGI was most highly correlated with V2. Y and Y^2 were most highly correlated with both W1 and W2. This indicates that the canonical variates represent the distribution of trees by F-EST and SDH (first canonical pair) and PGI (second canonical pair) isozyme scores in predominantly the Y or the SE to NW direction (see figure 2). The XY term is also important, and represents the bi-directional south to north pattern seen in figure 2b. This diagram shows that predicted canonical variate scores change in two directions (X and Y). A change in predicted scores represents a change in allelic composition or genotype of trees.

Table 6.--Canonical correlation analysis of isozyme variables and location of eastern white pine for a three hectare unmanaged forest stand -- correlations between the original variables and canonical variables.

Canonical Variate	V1	V2	W1	W2
Isozyme Variables (Smouse Scores)				
SDH1	0.4394	-0.0874	0.1499	-0.0228
SDH2	-0.5045	0.0992	-0.1721	0.0259
PGM21	-0.3226	-0.0820	-0.1101	-0.0214
PGM22	0.2551	0.0292	0.0870	0.0076
GOT21	0.3020	0.1259	0.1031	0.0329
PGI21	-0.1573	0.8590	-0.0537	0.2245
MDH11	0.1380	0.0374	0.0471	0.0098
MDH12	-0.1612	0.0862	-0.0550	0.0225
FEST11	0.3268	0.2062	0.1115	0.0539
FEST12	0.7558	0.2490	0.2579	0.0651
Tree Location Variable				
X	0.0022	-0.0701	0.0066	-0.2681
X^2	-0.0010	-0.0469	-0.0030	-0.1795
Y	0.2634	-0.1510	0.7721	-0.5777
Y^2	0.2750	-0.1463	0.8059	-0.5599
XY	0.1226	-0.1413	0.3593	-0.5406

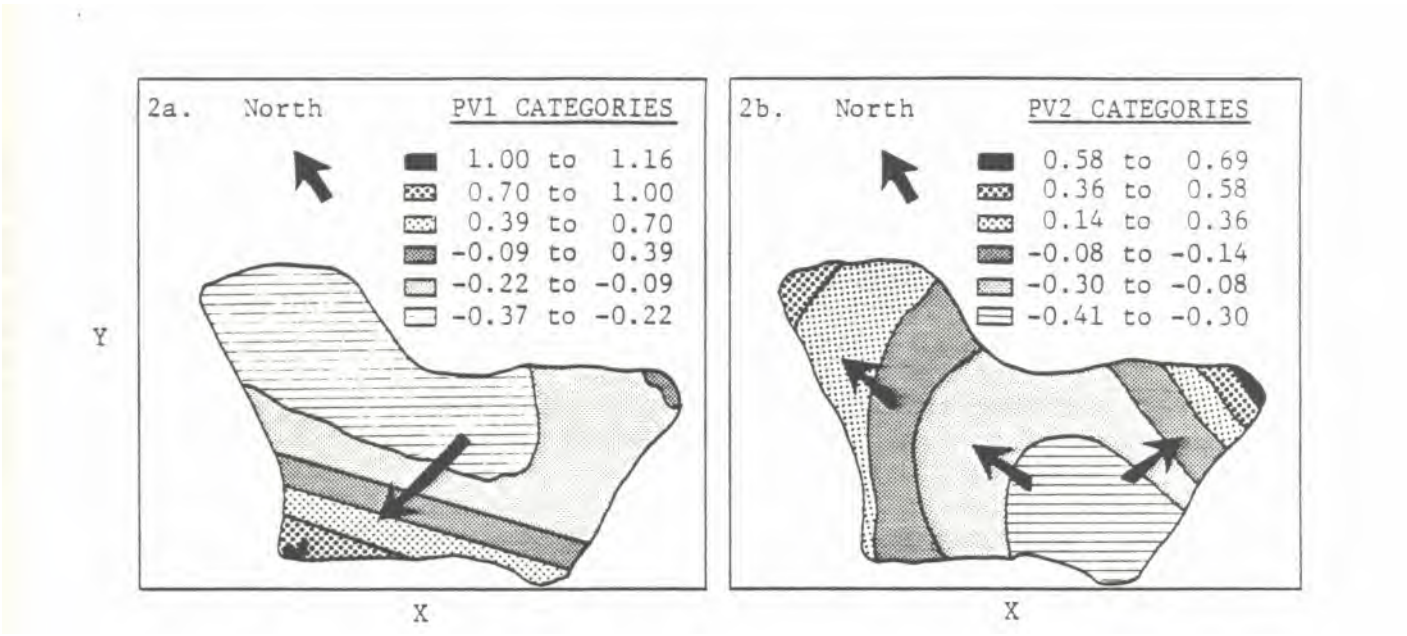

Figure 2.-- Regions of a three hectare forest stand containing eastern white pines with similar predicted canonical variate scores representing allelic composition. The scores were predicted from tree location. The arrows show the direction in which predicted scores increase in the stand. Differences in scores reflect allele frequency changes throughout the stand.

The change in tree genotype is directional and continuous in the stand when described by predicted canonical variate scores, and the change is related to the discrete variation in single enzymes. A simple plot showing the distribution of trees homozygous for the slow allele of PGI, one of the enzymes important in forming the second canonical variate, helps interpret the PV2 patterns. All but two trees with this genotype are found primarily in the southern section of the stand (figure 3). Trees in this section of the stand also have the lowest predicted PV2 score (figure 2b).

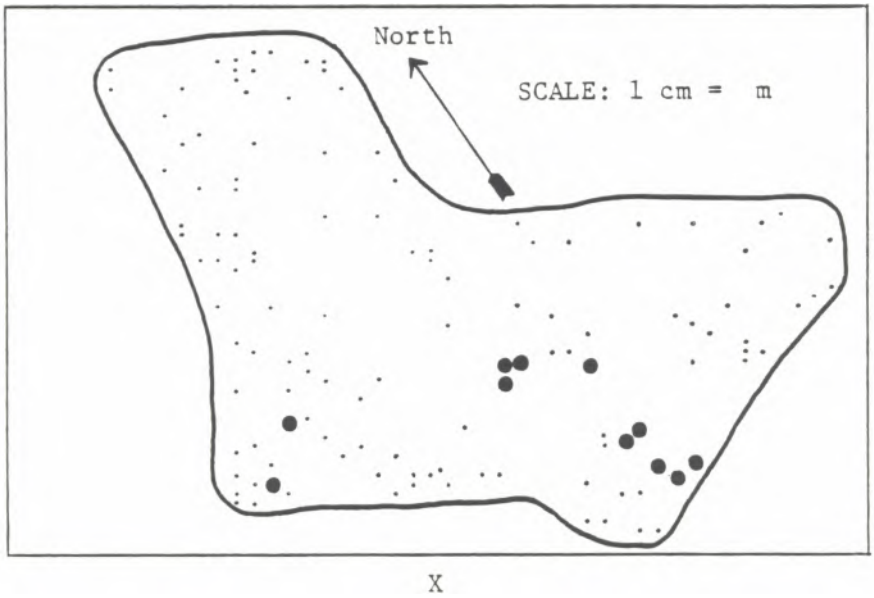

Figure 3.--Spatial distribution of eastern white pines homozygous for the slow allele of Phosphoglucose Isomerase.

Relation of age to distribution of genotypes

Additional canonical correlation analysis (not shown), using the reduced set of 130 trees for which age is known, and including location variables, produced predicted genotype distribution patterns similar to those discussed above. The contribution of raw variables to the formation of the canonical variates also remained similar. The first correlation coefficient was .48 and the second canonical correlation was not statistically significant. Age was more highly correlated with its canonical variate (W1) than was the Y coordinate, but it was less strongly correlated with W1 than was the X coordinate. Canonical correlation analysis for the same 130 trees excluding the variable age yielded almost identical results as did the analysis with age. This suggests that although age is related to enzyme composition of eastern white pines in this stand, the spatial distribution of trees explains most of the variation in the enzyme composition. The apparently unimportant role of age in explaining enzyme composition of the trees may be in part due to the fact that tree age measurements were estimates, and the number of trees with exactly the same age was also low. This hinders evaluation of the true association between age and genotype.

Plots of age categories (figure 4) show that trees of younger age occur in distinct clusters along the outer perimeter of the study area. These clusters could have formed following the death or fall of mature trees. If only one, or a small number of trees contributed seeds for the establishment of seedlings in these openings, clustering of trees by similar allelic component could be expected. No cones were seen on the white pines in the study area during the past four years and fewer than ten seedlings were found. It is not known how many mature trees contributed to the establishment of the white pines which play a role in the patterns seen in this study, but cone production, limited to a small number of mature trees could have resulted in the establishment of adjacent trees with similar genotypes.

Significance of the observed patterns

Interpretation of the relationship between the enzyme canonical variate scores and the location canonical variate scores is difficult, because the biological significance of X and Y is not clear. The coordinates would have more meaning over an extended latitudinal and longitudinal range. The value of the canonical correlation analysis therefore remains in its ability to summarize groups of variables, to point out patterns in the data, and to show which raw variables are important in forming those patterns. Those raw variables must then be used to help explain the patterns. The clustering of trees by age (figure 4) and by genotype (figure 3) support results from the canonical correlation analysis. Both canonical variate plots and raw variable plots suggest the distribution of trees with similar isozyme makeup is not random. Most of the isozymes related to the genotypic clustering observed this study occured in low frequencies. The significance of the patterns therefore depends in part on whether rare alleles are considered biologically important. Pare alleles may not be important for the current population of trees, but they may help white pine survive through environmental changes in the future. Additional studies are necessary to search for patterns of genotype distribution in other white pine forest stands. Until such studies are completed, this study will serve to remind foresters that local eastern white pine stands can be genetically structured. This information is

important to foresters whose objective is to conserve current genetic resources, and who wish to understand better how selective thinning of trees may affect the genotypic composition of future generations.

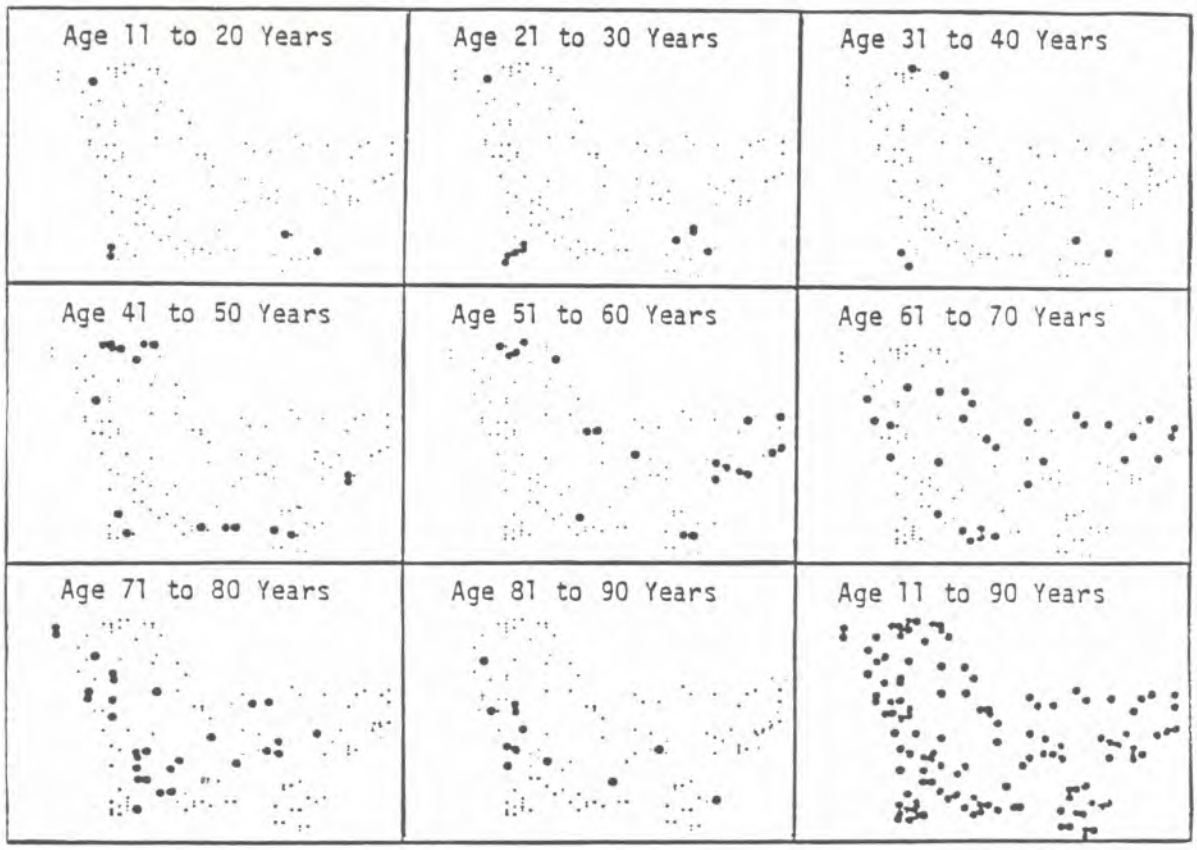

Figure 4.--Spatial distribution of eastern white pine in a three hectare forest stand, sorted into age categories.

CONCLUSIONS

The distribution of eastern white pines with similar allelic composition, growing in an unmanaged three hectare stand is not random and may be related in part to the establishment of young trees in natural openings, created following the death of mature trees. Plots of canonical data can be used to map genetic variation in forest stands.

LITERATURE CITED

Adams, W. T. and R. J. Joly. 1980. Genetics of allozyme variants in loblolly J. Heredity 71:33-40.

Eckert, R. T., R. J. Joly and D. B. Neale. 1981. Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can. J. For. Res. 11:573-579.

Gittins, R. 1979. Ecological Applications of Canonical Analysis. In: L. Orloci, C. R. Rao, and W. M. Stiteler (eds.), Multivariate Methods in Ecological Work, pp. 309-535. Int. Co-operative Publishing House, Fairland Maryland.

Gorman, M. B. and Y. T. Kiang. 1977. Variety-specific electrophoretic variants of four soybean enzymes. Crop. Sci. 17:963-965.

Guries, R. P. and F. T. Ledig. 1978. Inheritance of some polymorphic isoenzymes in pitch pine (Finus rigida mill.) Heredity 40 (1):27-32.

Mitton, J. B., P. Knowles, K. B. Sturgeon, Y. B. Linhart, and M. L. Davis. 1979. Association between heterozygosity and growth rate variables in three western forest trees. In: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 27-34.

Mitton, J. B., Y. B. Linhart, J. L. Hamrick and J. S. Beckman. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado front range. Theor. Appl. Genet. 51:5-13.

Pitel,J.A., and W. M. Cheliak. 1983. Extraction and characterization of isoenzymes from vegetative tissues of five conifers. Proceedings of the seventh North American Forest Biology Workshop. "Physiology and Genetics of Intensive Culture". July 26-28, 1982. University of Kentucky, Lexington, Kentucky. pp. 321-326.

Ryu, J. B. 1982. Genetic structure of Pinus strobus L. based on foliar isozymes from 27 provenances. Ph.D. Dissertation, The University of New Hampshire, Durham, N.H.

Ryu, J. B. and R. T. Eckert. 1983. Foliar isozyme variation in twenty seven provenances of Pinus strobus L.: Relationship of three foliar isozyme alleles with height growth and multivariate patterns of range-wide variation. Proceedings of the Seventh North American Forest Biology Workshop. "Physiology and Genetics of Intensive Culture." July 26-28, 1982. University of Kentucky, Lexington, Kentucky. pp. 179-187.

Scandalios, J. G. 1969. Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Genet. 3:37-79.

Smouse, P. E., R. S. Spielman, and M. H. Park. 1982. Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations. The American Naturalist 119:445-463.