ISOZYMES FOR TREE IMPROVEMENT

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INTRODUCTION

Multiple molecular forms of enzymes (isozymes) may be separated from one another using several techniques, the most common being electrophoresis. Isozymes are proteins composed principally of amino acids. As proteins, they may be negatively charged in a basic environment and thus when subjected to a polarized field they migrate. Relative migration rate of isozymes is a function of isozyme molecular weight, configuration, and amino acid and coordination complex composition, i.e., structure. Since protein structure, thus isozyme structure is the direct expression of genetic information contained in mRNA, then isozymes represent a relatively direct biochemical link to DNA and structural genes encoded therein. It is for this reason that study of isozymes and isozyme variability has been especially rewarding to plant and animal geneticists and breeders (Feret and Bergmann, 1976).

In tree improvement, isozyme investigations are just now beginning to provide answers to long-existing questions, particularly those pertaining to problems in population genetics of natural populations (e.g., Guries and Ledig, 1978). In addition, spin-off applications to understanding genetic behavior of domesticated populations are now being made (e.g., Adams and Coutinho, 1977).

Although isozymes may be used to "fingerprint" individuals (i.e., each individual genotype has its own unique set of isozymes) the real power of isozyme analysis lies in its ability to detect genetic markers. By studying patterns of isozyme variability in full-sib hybrids, or in megagametophytes of conifers, it is possible to identify individual loci, and to determine if an individual is homozygous or heterozygous at a particular locus. By surveying numbers of individuals in populations it is possible to identify polymorphic loci (loci possessing several alleles in a population). Thus, by studying allozyme (allelic isozymes) variability it is possible

to quantify genetic variation directly. Implications of this for the study of tree genetics and tree improvement are profound.

The purpose of this presentation is to briefly show the relevancy of isozyme analysis to tree improvement and tree improvement-related activities.

ISOZYME GENETICS

The DNA of a tree may be partitioned into several categories:

1. "Worthless" DNA - DNA having no apparent function; evolutionary relic, "mistake" or "noise".

2. Non-transcribed DNA – DNA serving a structural role.

3. Transcribed DNA with the final product functional (i.e., rRNA, tRNA).

4. Transcribed, translated DNA producing structural proteins and amino acids "sinks".

5. Transcribed, translated DNA producing a functional protein (i.e., enzymes).

6. DNA containing information dealing with gene regulatory processes (i.e., polymerase punctuation, etc.).

7. Non-nuclear DNA (i.e., mitochondrial).

By extrapolating Powell's conjectures (Powell, 1975) to forest trees, we may assume that category 5 DNA comprises only about 0.5% of the total DNA in the plant. Thus, although the figure may vary by a magnitude, it is safe to assume that isozyme analysis is capable of characterizing only a small part of the total plant DNA.

Isozyme analysis involves analytical techniques, most commonly electrophoresis. The electrophoretic technique separates isozymes (and allelic isozymes, called allozymes) on the basis of electric charge and molecular weight. Since, on an evolutionary scale, isozymes are generally thought to arise by mutation from a single progenitor (ignoring non-genetic pathways of isozyme formation), let's examine the efficiency of the electrophoretic technique in detecting mutations. Powell

(1975) by extrapolating data of Holmquist et al. (1972), determined the following frequencies of occurrence to be associated with mutations: (1) mutations caused by base changes with no change in amino acid sequences coded for – frequency: 32%; (2) mutations causing changes in amino acids, but with no change in the net electric charge of the protein – frequency: 46%; and (3) mutations causing changes in amino acids resulting in changed protein electric charge – frequency: 22%.

Since electrophoretic analysis of isozymes is the most commonly used tool for their identification, it is apparent that isozyme analysis usually is capapbe of detecting only about one-fifth of the mutationally altered enzymes. Thus, most research on isozymes to date has apparently been done investigating only about 0.1% of the genome (i.e., 0.22 x 0.005). Even though only 0.1% of the DNA may be all that is being sampled by isozyme analysis, it is apparent that a much larger sample of markers are available now for study of tree genetics than ever before, and their numbers appear to be increasing at an exponential rate because of the availability of isozymes.

There remain arguments among students of population genetics supporting both sides of the argument as to whether the array of isozyme variability exhibited in animals and plants is "random evolutionary noise", or reflects the outcome of "natural selection", or both. It is not within the scope of this presentation to review the theoretical literature and the following is in no way to be considered such. However, because the application of isozyme analysis to tree improvement is dependent on whether isozyme polymorphisms are significant in improving fitness, or just random variability, a brief discussion here is necessary.

Briefly, the "classical" school (Dobzhansky, 1955) held the view that most mutations were deleterious, that most members of a population were relatively homozygous, and that natural selection removed individuals carrying deleterious alleles. With the recent accumulation of isozyme data, the classical theory has been modified to what is often called the "Neutralist" ("non-Darwinian" or neo-classical) theory which states that the polymorphisms are selectively neutral and of no physiological consequence. The work of Kumura and Ohta (1971a, b) and that of Yamazaki and Maruyama (1975) will provide the reader with a springborad for the literature on "Neutralist" thought.

The "balance" school suggests that variability of potential adaptive value is always in populations. Under this theory selection and heterosis would be the primary forces maintaining isozyme variability in populations (Powell, 1975; Lewontin, 1973). Certainly recent evidence strongly suggests the balance school of thought is defendable (e.g., Milkman, 1975; Clegg and Allard, 1972; Vigue and Johnson, 1973; among others).

ISOZYMES FOR TREE IMPROVEMENT

Application of isozyme analysis (beyond merely describing isozyme variability and inheritance) to tree improvement is at least in part dependent upon whether isozyme variability in trees is the result of essentially random influences of genetic drift, or the results of balanced selection. If the latter is the primary mechanism responsible for the variability observed, then isozyme analysis will tell us much about the adaptive strategies used by woody plants. This knowledge can then be applied to the design of tree improvement strategies. Conversely, if the variability is of essentially "neutral" adaptive value, then isozyme analysis can be used only in the traditional sense of "gene markers" (of primary importance in domesticated populations).

The number of potential loci available for study now, using isozymes, is magnitudes greater than the number of Mendelian traits available in 1960. This is particularly true in higher plants and more specifically, in trees where simple Mendelian inheritance of easily measured traits is a rarely observed occurrence in natural populations. Albino seedlings (i.e., Zasada and Winton, 1970), aurea mutations (Walles, 1967) and similar rare events are potentially useful for studies of selfing and breeding structure of populations (Franklin, 1977); but their use for studies of natural stands in <u>situ</u> is limited by the usual negative selective value of such mutants and their consequent general absence from natural populations. Isozymes, however, <u>if environmentally stable,</u> may be studied in natural stands, in <u>situ</u> (i.e., Tigerstedt, 1973; Rasmuson and Rudin, 1971). In addition, isozymes make available for study of trees a large number of loci, permit study of multiple allelism and permit analysis of "codominant" genes (Rudin, 1976).

Evidence of the large amount of data generated on forest tree isozymes is provided by Table 1. Listed in Table 1 are most of the genetic marker systems now elucidated by macrogametophyte analysis in conifers. The

total number of systems encompass about 11 enzyme systems, approximately 240 alleles at 80 loci in 10 species. In addition to the systems listed in Table 1, more are elucidated but: (1) are yet unpublished, (2) pertain to angiospermous trees, or (3) are based on analysis of sporophyte tissues. By contrast, 16 years ago, Wright (1962) was able to list only five Mendelian characters.

To understand isozymes and their relevance to tree improvement, four points must be recognized: (1) artifacts may be created during extraction and purification procedures; (2) isozyme zymograms usually represent several loci (allozyme systems); (3) isozymes are often organ, tissue or cell specific; within the cell, isozymes vary with subcellular component; and (4) isozyme phenotypes vary with stage of differentiation, growth, and senescence; their presence may be influenced by both biotic and abiotic factors.

Because of these four considerations, it is important that, wherever possible, isozyme systems of known genetic control be used. By so doing, it is possible to: (1) be relatively sure artifacts are not being studied; (2) more easily identify changes in gene expression and avoid misinterpretation of isozyme complements; and (3) remove sources of variability not associated with experimental conditions (i.e., variation of unknown genetic or environmental origin) (Scandalios, 1974).

Tree improvement research may be broadly considered as research on adaptation (Bunting, 1976). In an effort to provide tree planters with seedlings most adapted to a set of cultural and economic conditions, research examining isozyme variation and its relation to adaptation may be of two types. Research may be designed to more completely understand the role of isozymes in adaptation of trees to specific environments, or research may be designed to find isozyme phenotypes correlated with some or several aspects of growth and yield. Usually these two objectives are closely correlated.

The ways in which isozyme analysis contributes to tree improvement research are many. These have been broadly reviewed by Feret and Bergmann (1976). However, there are a few uses which I would like to emphasize here.

Genetic improvement requires that variation be identified and manipulated. Identification of variation

by isozyme analysis seems to be a distinct possibility. Fowler and Morris (1977) have demonstrated that electrophoretic analysis of red pine isozymes reflects variation at the molecular level that is consistent with the traditional quantitative measures of variation for that species. Thus, perhaps isozyme analysis can be used to locate variable provenances (or other units of selection) for breeding trees for improved growth or adaptability.

Although it is generally recognized that most tree improvement is made by capitalizing on additive genetic variance, isozymes may be important, particularly in hardwood improvement, for understanding heterosis (e.g., Stairs, 1968). Evidence is present in the literature which suggests that heterosis and isozyme heterogeneity are correlated (Gupta and Singh, 1977; Schwartz and Laughner, 1969) and that "hybrid molecules" may be somewhat "better" than "non-hybrid" enzymes (Scandalios, 1974; Scandalios, Liu and Campeau, 1972). If improvement in enzymatic activity, thermostability or photosensitivity of isozymes can improve metabolic efficiency, and if heteromultimers are somehow "better" than homomultimeric isozymes, then analysis of multimeric isozymes may be a direct measure of genotypic potential for tree improvement. Similarly, if homozygosity or heterozygosity per se is valuable to tree improvement strategy (i.e., Stairs, 1968), then isozyme analysis can obviously aid in identifying genotypes of value.

The use of isozymes for the study of tree adaptation to stressed environments may also be of potential value especially to tree improvement workers developing populations for urban uses. In investigations on Avena, Clegg and Allard (1972) and Hamrick and Allard (1972) demonstrated a relationship between isozymes and moisture availability in natural environments. Peroxidase and esterases were used by Thom and Maretzki (1970) to study sugar cane adaptations to stress. Tigerstedt (1973) has compared Picea isozymes in marginal (stressed?) populations with centrally located populations. Recently Yang, Ching and Ching (1977) suggested heterozygosity differences among Douglas-fir provenances were caused by selection pressures due to moisture stress and soil type. These suggestions coupled with the implications of Drosophila research on temperature sensitive-isozymes (i.e., Koehn, 1969; Cochrane, 1976) suggest adaptation to stress may be studied using isozyme analysis.

There may be potential value in isozyme analysis for studying the role of gene duplication and ploidy in forest tree adaptation. This is particularly true when tree improvement efforts involve species with small and difficult to analyze chromosomes. Isozymes have been used to study trisomics in barley (McDaniel and Ramage, 1970), genetic redundancy in fish (Lim and Bailey, 1977), and chromosome complements in wheat and triticale (Tang and Hart, 1975). Genome identification in polyploid series of forest trees could be made using isozyme analyses. Lester (personal communication, 1978) has made preliminary investigations of some isozyme systems in Ulmus americana to determine if they can be used for distinguishing polyploid series.

There is little reason to expect that isozyme analysis cannot be used for taxonomic purposes, a use closely allied to those discussed in the previous paragraph and of key importance in programs utilizing interspecific hybrids for tree improvement. Adams and Coutinho (1977) and Tobolski and Conkle (1977) have effectively used isozyme analysis for identification of intersepcific pine hybrids. Cultivars of potato (Desborough and Peloquin, 1968), species of Nicotiana (Sheen, 1970), and clones of Cryptomeria (Miyazaki and Sakai, 1969) serve as additional examples of taxonomic application. Given the large amounts of variation within and among individuals and populations of trees (e.g., Conkle and Adams, 1977) it should be easy to use isozyme analysis as a relatively fool-proof method of identifying seed orchard clones, cultivars, etc. once standards of isozyme analysis are adopted. Plant registration and patent procedures could eventually make use of isozyme analysis for identification purposes (Santamour, personal communication, 1978).

Finally, survival, yield and growth improvement strategies are dependent upon an understanding of natural systems. Isozyme analysis, in addition to being an important tool for studying mating systems, selection intensities and multilocus organization (Rudin, 1976) is a valuable tool for studying the molecular basis of genotype environment interaction. The development of isozyme systems for study of trees in artificial environments and their correlative behavior in situ in plantations should provide predictive models for growth and yield. Juvenile-mature correlations may also be a fertile area for applications of isozyme analyses.

SUMMARY AND CONCLUSION

In summary, isozyme analysis of trees is making significant contributions to tree improvement. Applications are becoming increasingly frequent and in the future will probably be even more frequent. The full realization of the potential of isozyme analysis will be realized when cooperative research undertaken by silviculturists, physiologists and geneticists is integrated and applied to the basic problems of improving survival, growth and yield.

Table 1. A summary of segregating isozymes from the analysis of the haploid gametophyte tissue of conifers. Note: Abbreviated table headings and abbreviations in table body listed at end of table. Adapted and slightly expanded from Witter (1977).

ENZ	LOC	ALL	NUL	POP	IND	NP	DP	FQ	SP	AUT
EST	EST 1	6		1	146	+		.40	P.t.	C & A
	EST 1	4		1	146	+		.64	P.t.	C & A
	EST 1	2		1	201	+		.75	P.a.	Ba
	EST A	3		6	15-20	+		.93-1.00	P.a.	B-2 (B-1)
	EST B	3		6	15-20	+		.65-.83	P.a.	B-2 (B-1)
	EST C	2							P.a.	B-1
	EST	2		2	∿50	+		.73-.74	P.a.	T-1
	EST U	3		10	18-107	+		.43-.75	P.a.	T-2
	EST L	4		10	18-107	+		.30-.74	P.a.	T-2
	E6	2	+	3	15-20	+		.67-.85	P.p	F
	EST B	6		3	39-141	+		.62-.86	P.s.	R-1
	EST A	3+	+	1	15		+		P.a.	L-3
	EST B	3+	+	1	15		+		P.a.	L-3
	EST C	2		1	15		+		P.a.	L-3
	EST D	3+	+	1	15		+		P.a., P.r.	L-3 G & L
GOT	GOT 1	3		1	146	+		.77	P.t.	C & A
	GOT 2	3		1	146	+		.51	P.t.	C & A
	GOT 3	2		1	146	+		.79	P.t.	C & A
	GOT A	3			8				P.s.	R-2
	GOT B	2			8				P.s.	R-2
	GOT	2			7				P.r., P.t.	A & C

ENZ	LOC	ALL	NUL	POP	IND	NP	DP	FQ	SP	AUT
GOT	GOT B	6		3	39-141	+		.36-.59	P.s.	R-1
	GOT C	(2)							P.a.	L-2
	GOT A	5		4	7-28	+	+	.34-.50	P.v.	F & W
	GOT B	3		4	7-28	+	+	.91-1.0	P.v.	F & W
	GOT 1	3		Many	Many	+		N.G.	P.r.	G & L
	GOT 2	2		Many	Many	+		N.G.	P.r.	G & L
	GOT 1	2		5	25-111	+		192	A.b.	N
	GOT 2	2		5	25-111	+		.66-.99	A.b.	N
	GOT 3	2		5	25-111	+			A.b.	N
APH	PHOS	3	+	2	∿50	+		.76-.78	P.a.	T-1
	PHOS	3	+	10	18-107	+		.78-.93	P.a.	T-2
	A	3			5				P.a.	L-3
	SAP A	2							P.a.	B-4
	SAP B	4							P.a.	
	SAP B	5	+	18	200 seeds	+		.05-.78, .15-.96	P.a.	B-5
	SAP	4	+	6	200 seeds	+		.55-.93	P.m.	B-5
	APH	4		2	13, 7	+		.55	L.d.	M & B
	ACPH	7		1	146	+	+	.82	P.t.	C & A
LAP	LAP A	1			6				P.a.	L-1, L-2
	LAP B	4			6				P.a.	L-1, L-2
	LAP U	3	+	2	50	+		.53-.61	P.a.	T-1
	LAP L	2		2	50	+		.47-.61	P.a.	T-1
	LAP U	3	+	10	18-107	+		.36-.61, .34-.64	P.a.	T-2
	LAP L	3	+	10	18-107	+		.24-.74, .25-.75	P.a.	T-2

ENZ	LOC	ALL	NUL	POP	IND	NP	DP	FQ	SP	AUT
LAP	LAP A	4	+						P.a.	B-4
	LAP B	3							P.a.	B-4
	LAP B	3		6	15-20	+		.58-.69	P.a.	B-2
	LAP A	6	+	1	200 seeds	+		.38	P.m.	B-3
	LAP B	2			200 seeds	+		.77	P.m.	B-3
	LAP A	2	+						P.n.	N & B
	LAP B	3	+						P.n.	N & B
	LAP 1	4		1	146	+		.87	P.t.	C & A
	LAP 2	4		1	146	+		.53	P.t.	C & A
	LAP B	4		3	38-141	+		.94-.95	P.s.	R-1
	LAP	2			7				P.t., P.r.	A & C
	LAP 1	2	+	Many	Many	+		N.G.	P.r.	G & L
	LAP 2	2		Many	Many	+		N.G.	P.r.	G & L
	LAP 1	4	+	5	25-111	+		.47	A.b.	N
	LAP 2	2	+	5	25-111	+		.84	A.b.	N
PGM	1	2			7				P.t., P.r.	A & C
	PGM	3		1	146				P.t.	C & A
	PGM 1	2		Many	Many	+		N.G.	P.r.	G & L
	PGM 2	2		Many	Many	+		N.G.	P.r.	G & L
	PGM 1	1		5	25-111	+		N.G.	A.b.	N
	PGM 2	2		5	25-111	+		.88	A.b.	N
PGI	1	2			7				P.t.	A & C
	PGI 1	3		5	25-111			.96	A.b.	N
	PGI 2	1		5	25-111			N.G.	A.b.	N

ENZ	LOC	ALL	NUL	POP	IND	NP	DP	FQ	SP	AUT
6-PGD	1	2			7				P.t., P.r.	A & C
	6-PGD-1	5		Many	Many	+		N.G.	P.r.	G & L
	6-PGD-2	3		Many	Many	+		N.G.	P.r.	G & L
	6-PGD-1	2		5	25-111	+		.75	A.b.	N
GDH	1	2			7				P.t., P.r.	A & C
ADH	ADH	2		1	146	+		.99	P.t.	C & A
BANA	BANA	3		1	146	+			P.t.	C & A
MDH	MDH 1	3		Many	Many	+		N.G.	P.r.	G & L
	MDH 2	4		Many	Many	+		N.G.	P.r.	G & L
ACO	ACO	3+		Many	Many	+		N.G.	P.r.	G & L
GPI	GPI 1	2		Many	Many	+		N.G.	P.r.	G & L
	GPI 2	2		Many	Many	+		N.G.	P.r.	G & L
IDH	IDH	2		Many	Many	+		N.G.	P.r.	G & L
	IDH 1	2		5	25-111	+		.99	A.b.	N

ABBREVIATIONS

Column headings:

ENZ = enzyme, LOC = number of loci and designation, ALL = number of alleles

per locus, NUL = presence or absence of null alleles, POP = number of populations, IND = number of individuals per population, NP = natural populations, DP = domestic populations, FQ = frequency of most common allele, SP = species, and AUT = author.

Enzyme systems:

EST = esterase, GOT = glutamate oxalo-acetate transaminase, APH = acid phosphatase, LAP = leucine aminopeptidase isozymes, PGM = phosphoglucomutase, PGI = phosphoglucose isomerase, 6-PGD = 6-phosphogluconate dehydrogenase, GDH = glutamate dehydrogenase, ADH = alcohol dehydrogenase, BANA = endopeptidase, MDH = malate dehydrogenase, ACO = aconitase, GPI = glucose phosphate isomerase, and IDH = isocitrate dehydrogenase.

Authors:

A & C = Adams and Coutinho (1977), Ba = Bartels (1971), B-1 = Bergmann (1973a), B-2 = Bergmann (1973b), B-3 = Bergmann (1973c), B-4 = Bergmann (1974), B-5 = Bergmann (1975), C & A = Conkle and Adams (1977), F = Feret (1974), F & W = Feret and Witter, G & L = Guries and Ledig (1978), L-1 = Lundkvist (1974a), L-2 = Lundkvist (1974b), L-3 = Lundkvist (1975), L-4 = Lundkvist (1977), M & B = Mejnartowicz and Bergmann (1975), N = Neale (1978), N & B = Nikolic and Bergmann (1974), R-1 = Rudin et al. (1974), P-2 = Rudin (1975), T-1 = Tigerstedt (1973), T-2 = Tigerstedt (1974), and C & A = Conkle and Adams (1977).

Species:

P.a. = *Picea abies,* P.p = *Pinus pungens,* P.s. = *Pinus sylvestris,* P.r. = *Pinus rigida,* P.t. = *Pinus taeda,* P.n. = *Pinus nigra,* P.v. = *Pinus virginiana,* P.m. = *Pseudotsuga menziesii,* L.d. = *Larix decidua,* and A.b. = *Abies balsamea.*

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