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Isozymes in Studies of Aquatic Plants[1]

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ABSTRACT

Electrophoresis of proteins can be a useful technique for assessing genetic relationships among populations or species of aquatic plants and other organisms. The use of this technique, its limitations, advantages, and other practical considerations are reviewed.

Key words: electrophoresis, enzymes, hydrilla, waterhyacinth, *Cabomba* sp. *Zostera, Typha.*

INTRODUCTION

Isozyme analysis by electrophoresis in studies of aquatic plants is becoming increasingly widespread. Scientists in the areas of biological control, chemical control, and systematics realize that knowledge of the extent and pattern of genetic variation can be of great importance in the detection of ecotypes or races of a species which may differ considerably in their susceptibility to predators, parasites, or herbicides (Winder and Harley 1976). Electrophoretically separated enzymes are also commonly used as genetic markers for the purpose of taxon identification, confirmation of ancestry (Torres and Bergh 1978, Torres and Tisserat 1980), and systematic comparisons (Gottlieb, 1977).

Gel electrophoresis is a relatively easy and inexpensive technique for genetically screening large numbers of individuals. In general, tissues are homogenized, and a crude or purified protein extract is inserted into a gel matrix, often starch or acrylamide, through which is passed an electric current. Enzymes and other proteins migrate through the gel a characteristic distance based primarily upon net amino acid charge. Differences in net charge of

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polypeptides indicate underlying differences in amino acid sequence which in turn indicate differences in DNA base sequence. Other factors, such as molecular size and conformation, may also affect the rate of enzyme migration. Following electrophoresis, specific enzymes are visualized as discrete colored bands by soaking the gels in staining solutions containing the proper substrates, cofactors, and dyes. (See Brewer 1970; Shaw and Prasad 1970, for detailed descriptions of electrophoretic techniques and enzyme staining procedures).

In assessing the genetic composition of natural populations, it is ultimately desirable to examine the exact nucleotide base sequence of the DNA of individuals comprising the populations. Although now technologically possible, DNA, RNA, and amino acid sequencing may be time consuming, detailed processes. Electrophoresis provides a good approximation of differences in DNA base sequence, quantifiable as allele frequencies. Differentially migrating bands on the gel possess different net amino acid charges and therefore different amino acid sequences. Such alternate molecular forms of a gene are referred to as alleles. The frequency of occurrence of all alleles at several gene loci may be compared between different populations and a mathematical estimate of genetic relatedness obtained (Hedrick, 1971; Nei, 1972; Rogers, 1972; Nei, 1978). Precise nonsubjective analysis is not easily accomplished with morphological studies. In addition, electrophoretic phenotypes (banding patterns) are usually not influenced by the environment, as morphological characters often are, eliminating a large source of variation. Also of importance, most enzymes studied by electrophoresis exhibit co-dominant inheritance patterns. Consequently, hybrids will possess bands of both parents.

Electrophoresis does have limitations. First, it can not distinguish amino acid substitutions that do not differ in charge, such alleles would migrate the same distance on the gel even though their amino acid sequences differ. The amount of electrophoretically detectable variation is an underestimate of the amount actually present in nature. Secondly, the exact number of amino acid differences which exist between electrophoretically distinct enzymes can not easily be determined. Thirdly, only a limited number of enzyme systems can be conveniently analyzed, and this sample may not be representative of the genome as a whole.

Electrophoresis, however, can be a very powerful tool for analyzing the genetic composition of plant populations. When combined with other types of biochemical or morphological data, this technique can be valuable in making taxonomic assessments. Aquatic plants lend themselves well to analysis by electrophoresis: populations are often well delineated entities, the same population can be reanalyzed from season to season or year to year, and organs may be electrophoresed without killing the organism so individuals may be reanalyzed.

EXAMPLES OF ELECTROPHORESIS

This section will review several examples of how electrophoresis has been used to explore problems in aquatic botany. It is hoped that these examples will provide a better insight into the potential benefits of isozyme analysis.

Seagrasses are one of the most extensively sampled group of aquatic plants. Banding patterns have been obtained in more than 30 species representing 12 seagrass genera from throughout the world. In most species there exists no intraspecific variation in banding patterns, even across large geographic distances. Electrophoretic banding patterns, however, are distinct among species and this has been useful both in identification of seagrasses and in illuminating phylogenetic relationships within genera and subfamilies. Though no formal inheritance studies have been performed for the seagrasses, it is assumed that levels of heterozygosity are extremely low (McMillan, 1982). Even populations that exhibit physiological differentiation, such as in chill tolerance (McMillan, 1979), show no corresponding isozyme differentiation (McMillan, 1980).

Gagnon and co-workers (1980) used electrophoresis to genetically compare an annual and perennial form of the eelgrass, *Zostera marina,* one of the few seagrass species exhibiting protein polymorphism. The two forms differ morphologically and it was speculated that barriers to gene flow may also exist due to differences in flowering time. Gagnon and co-workers (1980) compared annual and perennial forms from two study sites on the Bagaduce River estuary in Maine. They discovered that at 17 gene loci the two forms were allelically identical. In addition, a Chi-square test indicated completely random mating among the forms. Their conclusion from these results is that morphological and phenological differences should be attributed to environmental differences and not underlying genetic differences.

A study of a similar nature was conducted by Wain and Martin (1980) on the waterhyacinth, *Eichhornia crassipes.* Three growth forms: small (ca. 15 cm tall), medium (45-60 cm) and "super" (90-120 cm) commonly exist in Florida. These growth forms also differ in susceptibility to the waterhyacinth weevil and in relative abundance of certain metals (Cooley et al., 1979). Horizontal starch gel electrophoresis of 14 zones of activity indicated that the three growth forms are genetically indistinguishable. As with *Zostera marina,* the differences among waterhyacinth forms appears to be phenotypically based. Specifically, the "super" forms have been observed in shallow water environments, commonly in regions of low dissolved oxygen content and relatively high iron levels; thus the largest form may well represent the response of the plant to the presence of iron(II) species.

Sharitz and co-workers (1980) examined ten enzyme systems in three North American species of *Typha* sampled throughout the United States. No intraspecific allelic variation was observed within *T. angustifolia* or *T. domingensis,* and very little variation was detected in *T. latifolia.* The species were found to be electrophoretically distinct (species diagnostic) for three of the ten enzyme systems. Electrophoresis provided an unambiguous means of identifying these species of *Typha* despite the fact that floral and vegetative morphology may be quite variable and sometimes intermediate in character. There was no electrophoretic evidence of hybridization occurring among these species. The presence of individuals possessing intermediate morphological characters does not necessarily indicate hybridization; mor-

phological similarities may instead represent responses to a similar environment.

Reproduction in *Typha* is primarily vegetative and as a consequence very dense stands may be formed. Krattinger (1983) estimated the actual number of distinct genotypes comprising such stands of *Typha latifolia* L. in Switzerland. The technique used was isolectric focusing (IEF) of pollen grain proteins. IEF is a method of electrophoresis in which a pH gradient is established in the gel. Proteins migrate through the gel until they reach the point of electric neutrality. The isoelectric point of a protein is the one pH at which the protein carries no net charge, owing to the presence of an equal number of individual positive and negative groupings (Bohinski, 1976). Krattinger (1983) discovered that populations of *Typha latifolia* are composed of several different genotypes and not just a single genotype. The number of distinct genotypes within a population decreases with age, apparently due to intraspecific competition.

Fanworts (*Cabomba* sp.) are submersed aquatic plants widely distributed in the southeastern United States but whose geographic range extends west into Texas and north to Illinois, Michigan and New England. Three taxa are commonly attributed to the United States; *C. caroliniana* var. *multipartita* (green fanwort), *C. caroliniana* var. *caroliniana* (fanwort), and *C. pulcherrima* (purple fanwort). *C.c. multipartita* is believed to be a cultivated form of *C.c. carolinia* arising from the aquarium industry. Genetic relationships between the three fanworts from eight sites in Florida were determined by gel electrophoresis (Wain, Haller, and Martin 1983). Very similar allele frequencies at 12 presumed gene loci in each taxon indicated that morphological differences are probably best ascribed to environmental factors.

The alligatorweed, *Alternanthera philoxeroides,* (Mart.) Griseb., is an aquatic mat-forming plant first introduced into the United States from South America around 1897 (Kay and Haller 1982). It was a major aquatic weed in the southern states prior to the release of biological control agents during the mid 1960's and early 1970's. The plant has an amphibious nature and has invaded cultivated fields, most notably soybean fields near infested waters in the lower Mississippi River valley. Alligatorweed exhibits considerable morphological variation, much of which is directly attributable to environmental conditions. For example, plants grown under highly saline conditions develop thickened leaves which revert to normal upon transfer to fresh water. Other traits, however, remain distinct when grown in a common environment. Kay and Haller (1982) provided evidence for the existence of two distinct alligatorweed biotypes, broad-stemmed (BSA) and narrow-stemmed (NSA). These biotypes exhibited statistically significant differences in stem width and length when maintained in a common environment.

Wain, Haller, and Martin (1984) compared isozyme patterns among two alligatorweed biotypes and the results validate the assumption of genetically distinct populations. The mean genetic identity among BSA and NSA was 0.886, a value comparable to that found among subspecies of other plants (Wain, 1983). In order to supplement isozyme data, leaf length-to-width ratios were compared between biotypes. A one way nested analysis of variance (leaves nested within plants) indicated a highly significant difference ($F = 36.63$; $p > F = 0.0001$), the broad-stemmed form having a greater ratio. No significant difference was found among leaves within plants ($F = 0.99$; $p > F = 0.5228$). All experimental plants had been maintained in a common environment for several months prior to analysis. Since alligatorweed has been in this country for such a short period of time, it is reasonable to believe that these biotypes represent separate introductions of previously differentiated populations.

Individuals of *Hydrilla verticillata* (L.f.) Royle from throughout the world were enzymatically analyzed by Verkleij and co-workers (1983). It was found that large genetic differences exist among geographically disjunct populations, possibly indicating different survival strategies (Verkleij et al., 1983). *Hydrilla verticillata* as a species exhibits considerable genetic variation in comparison to other aquatic plants studied to date. Fifteen of the eighteen enzymes analyzed were polymorphic. Since genetic variation was not measured within populations, however, it is difficult to compare these results with those obtained in studies of other plants. Although the breeding system of *Hydrilla* varies among different strains, reproduction is primarily vegetative and it is doubtful that much intrapopulational genetic variation exists. All plants analyzed from the United States, with the exception of an anomalous genotype collected in Washington, D.C., had identical banding patterns and would appear to be ramets of the same clone. Variations in isozyme banding pattern could not be correlated with either variations in chromosome number or morphology.

Grant and Proctor (1980) reported the results of a survey of isozyme variation in 12 species of the green alga *Chara*. The enzymes GOT, HEX, and GDH were monomorphic and single banded within each species but varied among species. A tremendous amount of allelic variation, however, was found at loci coding for PGI. In some species, 6 and perhaps as many as 8 loci are encoding as many as 16 different alleles. Such variation is much greater than would be expected for these predominantly self-fertilizing haplobiontic species. The electrophoretic evidence suggests that the genetic variation discovered at PGI is attributable to functional gene duplication arising through polyploidy. Gene duplication followed by differentiation via mutation may be an important evolutionary mechanism for maintaining biochemical versatility (MacIntyre, 1976; Gottlieb, 1982). This is especially true in haploid and predominantly self-fertilizing organisms. Gene duplication may be responsible for the large number of electrophoretic forms of MDH and GDH found in the haploid armored dinoflagelate *Peridinium* (Hayhome and Pfiester, 1983).

In summary, gel electrophoresis is a tool that can be effectively applied to the characterization of aquatic plants for purposes of identification, potential management, and illuminating evolutionary relationships[3]. Some plants that

[3]A computer program which calculates the statistics of genetic identity and genetic distance may be obtained by writing to R. P. Wain. This program was written for an Apple II plus in BASIC but can easily be modified to run on any microcomputer. We will mail you a listing of the program or if you include a blank five-and-a-quarter inch diskette we will transfer the program to your diskette.

exhibit environmentally induced differences (e.g. waterhyacinth and *Cabomba*) are actually genetically similar and populations within each species probably may be managed in a similar manner. Other plants, such as alligatorweed, exist as genetically distinctive biotypes which field biologists have noted differ in their responses to treatments of control.

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