HIDDEN ELECTROPHORETIC VARIATION AT THE ACONITASE LOCUS OF *PINUS BANKSIANA*[1/]

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Abstract.--The aconitase locus (ACO) of jack pine (*Pinus banksiana* Lamb.) is highly polymorphic. Screening ACO variation simultaneously on two electrophoresis buffers revealed more variation than detected using a single buffer and more hidden variants than detected at four other allozyme loci screened on four electrophoresis buffers. The characteristics of the jack pine ACO polymorphism suggest that intragenic recombination could play a role in maintaining genetic variation at this locus.

Additional keywords: cryptic variation, heterozygosity.

Interpreting electophoretic variation depends on an understanding of the biochemical basis of genetic variation in enzymes and proteins (Gottlieb 1981). One-pass gel electrophoresis commonly has been used to assess levels of variability for certain genes in natural populations. Additional variation that is hidden from one-pass methods may be detected by a sequential analysis of samples on a series of electrophoresis buffers differing in pH and/or gel concentration. Such a sequential analysis also may be done using temperature treatments or higher resolution methods such as isoelectrofocusing. Empirical studies on well-characterized proteins (eg. hemoglobin) have shown that simple concepts of electrophoresis involving (primarily) electrical charge variants are inadequate to explain levels of detectable variation (Ramshaw et al 1979). It is not clear what portion of the total genetic variation at a locus is detected by one-pass methods (small amounts, e.g. Ramshaw et al 1979, or large amounts, e.g. Ayala 1982). Other studies have shown that one-pass methods detect most of the electrophoretically detectable variation, but the number of new electromorphs found through more intensive screening may be roughly proportional to the original number of variants reported (ie. variable allozymes become more variable, Coyne and Felton 1977, Ayala 1982, Shumaker 1982, Buchanan and Johnson 1983).

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The significance and implications of hidden electrophoretic variation are not yet fully known. The existence of cryptic alleles suggests that heterozygosity has been somewhat underestimated in most studies (Ayala 1982, Shumaker et al. 1982) and cryptic variants can provide increased resolution for studies utilizing electrophoretic data (Coyne and Felton 1977, Aquandro and Avise 1982a,b). We encountered hidden variability during a study of outcrossing rates in jack pine (Pinus banksiansa Lamb.) while attempting to score variation at the aconitase locus (ACO) on gels made with different electrophoresis buffers. This paper reports the results of a study undertaken to quantify the extent and nature of hidden variation for five jack pine allozyme loci.

MATERIALS AND METHODS

Megagametophytes from 30 single tree jack pine seed collections were analyzed for electrophoretic variation; most of the seed was collected from two northern Wisconsin populations (13 trees from each). The trees were chosen to include representives of the allelic variation already recognized. Thus, the sample cannot be considered random and suitable for estimating gene frequencies, especially for rare alleles. Six megagametophytes per individual were examined on four different electrophoresis buffers and stained for four enzymes to provide a maternal tree's genotype (probability of misclassifying a heterozygote < 0.05) (Table 1). Mobility differences were attributed to allelic variation at allozyme loci.

Table 1. Electrophoresis buffers and enzymes used to screen for genetic variation. Sources of buffers and staining methods are described elsewhere (Guries and Ledig 1978, O'Malley et al 1980).

Buffer	pH	Composition	Allozymes resolved[1]
AC	6.2	citric acid, (aminopropyl) morpholine	ACO, MDH1, 6PG1, 6PG2, PGI2
HC	7.0	histidine, sodium citrate	ACO, MDH1, 6PG1, 6PG2, PGI2
TM	7.4	tris, maleic acid	MDH1, 6PG1, 6PG2, PGI2
TEB	8.5	tris, EDTA, borate	MDH1, 6PG1, 6PG2, PGI2

[1] Enzymes and abbreviations: aconitase (ACO), malic dehydrogenase (MDH), 6-phosphogluconic dehydrogenase (6PG), and phosphoglucose isomerase (PGI).

RESULTS

The four electrophoresis buffers revealed 13 alleles for the four allozyme loci (MDH1, 6PG1, 6PG2, and PGI2; Table 2). A single electrophoresis buffer distinguished an average of 85% of the total number of alleles; the best buffers distinguished 92% of the alleles. Substantially more variation (10 alleles) was detected for ACO than for the other four loci. ACO resolved well on only two buffers (AC and HC), but the two buffers combined distinguished 10 electrQmorphs (Figure 1). A single buffer detected an average of only 50% of the alleles for this locus while the best buffer separated 60% of the total number of alleles.

Table 2. Electromorphs detected for MDH1, $6P_1G1$, 6PG2, and PGI2 using four electrophoresis buffers. Numbers enclosed in brackets (e.g. (2 + 3)) indicate that the buffer failed to separate the two electromorphs.

		Buffer			
Locus	Allele	AC	HC	TM	TEB
MDH1	1	1	1	1	1
	2	(2 + 3)	(2 + 3)	(2 + 3)	2
	3	-	-	-	3
6PG1	1	1	1	1	(1 + 2)
	2	2	2	2	-
6PG2	1	1	1	1	(1 + 3)
	2	(2 + 4)	2	2	(2 + 4)
	3	3	3	3	-
	4	-	4	4	-
	5	5	5	5	5
PGI2	1	1	1	1	1
	2	2	2	2	(2 + 3)
	3	3	3	3	-
Alleles detected:	13	11	12	12	9

[1] See Table 1 for explanation of buffer and enzyme abbreviations.

DISCUSSION

ACO appears to have substantially more hidden variation than the other four allozyme loci studied. Allozyme loci fall into two general categories, weakly polymorphic or highly polymorphic (Coyne and Felton 1977, Gottlieb 1981, Buchanan and Johnson 1983); ACO may be an example of the latter group. More cryptic variation has been found for highly polymorphic loci than for weakly polymorphic loci. The HC and AC electrophoresis buffers distinguish different sets of amino acid substitutions within the aconitase molecule. The

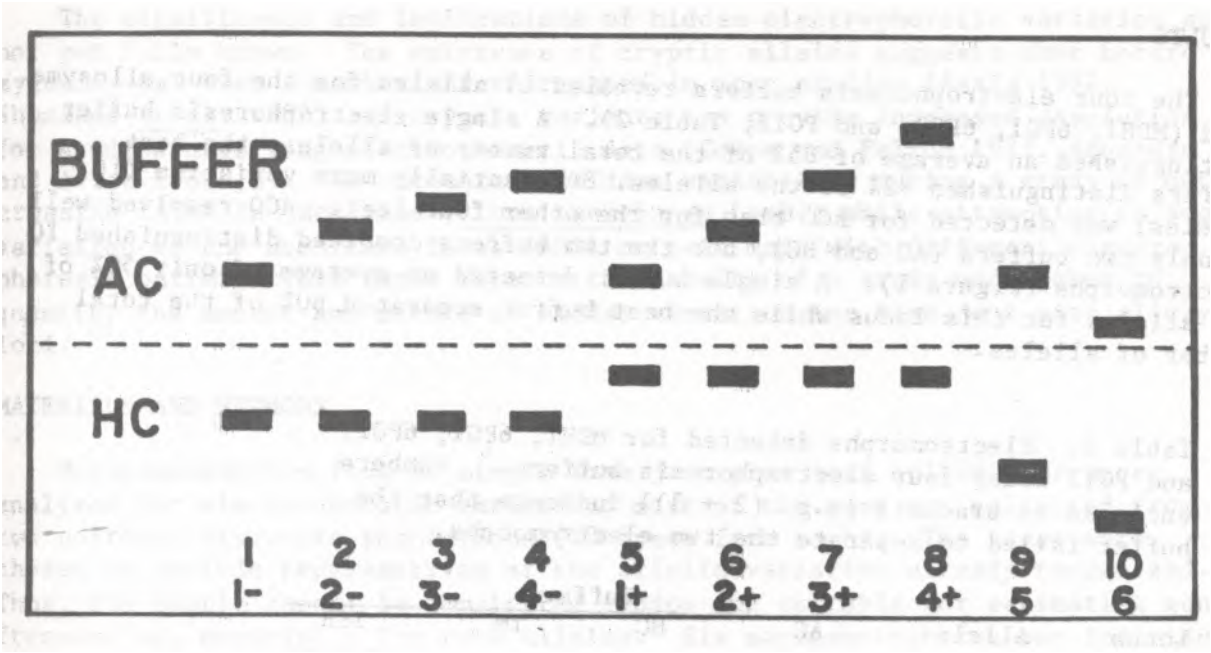

Figure 1. Diagrammatic representation of electromorphs detected at the aconitase locus; allele designations (e.g., 1- or 1+) represent putative amino acid substitutions detected on HC gels.

HC buffer separates two major electromorphs while the AC buffer separates four stepwise electromorphs within each of these two major electromorphs. Thus, the two mobility classes distinguished by the HC buffer may result from an amino acid substitution that is virtually "silent" on the AC gel and can be used to categorize the AC variants. In addition, two electromorphs are detectable on both types of gels (i.e., 9-5, 10-6).

The characteristics of the ACO polymorphism of jack pine strongly suggest the influence of intragenic recombination (Watt 1972). Intragenic recombination and mutation are both processes which result in the formation of new gene products, but mutation is more random, generating identical variants at very low frequencies. A limited number of different alleles (2^n) can be assembled from a small number (n) of preexisting nucleotide substitutions through intragenic recombination. In particular, if a substitution is found that can be used to categorize the alleles (electromorphs) already recognized at a locus, the number of potential alleles will double. Evidence has been found for generating electrophoretic variants through intragenic recombination for a highly variable esterase locus in Drosophila virilis (Tsuno 1982). The rate of mutation to electrophoretic variants is estimated to be 10^-6 (Voelker et al 1980) but mutation-like events caused by intragenic recombination can occur at a much higher rate, 10^-4 (Tsuno 1982). Highly variable genes may contain introns (intervening sequences not translated into protein) that facilitate crossing-over within the gene (Gilbert 1978).

Theoretical studies have suggested that intragenic recombination may play an important role in the origin and maintenance of genetic variation at some loci (Watt 1972, Strobeck and Morgan 1978, Morgan and Strobeck 1979). Intragenic recombination leads to an increase in the frequency of the common allele, an increase in the number of rare alleles, and an increase in homozygosity. Ancestry and genetic relationships are often inferred from similarity of electrophoretic phenotypes, but this would be misleading if some alleles have multiple origins due to recombination of existing nucleotide substitutions within a gene in different populations (Morgan and Strobeck 1979).

The evidence is limited for intragenic recombination occurring in natural populations. It provides one possible explanation for the increased frequency of rare alleles in hybrid zones (Barton et al 1983). Intragenic recombination has been invoked as an explanation for the observed variation at the phosphoglucomutase-1 locus (PGM1) in humans from the western Pacific area (Takahashi et al 1982). The number of alleles doubled from four to eight when isoelectrophocusing revealed that each of the original four electrophoretic variants could be subdivided into two classes (+ or -). This additional information allowed the construction of a phylogeny for the PGM1 alleles and the increased resolution provided biogeographical insight on the sequence of colonization in the western Pacific.

In most cases, simple one-pass gel electrophoresis detects most of the variation revealed by more exhaustive methods. For weakly polymorphic loci, this method is efficient in detecting variation when the best buffers are chosen. However, a substantial amount of undetected variation may exist for some allozyme loci. The additional variation could provide important information for studies where individuals are uniquely identified (e.g. clones, Adams, 1981) or population differences are analyzed (Coyne and Felton 1977, Aquandro and Avise 1982a and 1982b), thus justifying the increased effort. Outcrossing studies do not benefit from this additional resolution because computer programs generally cannot accomodate many alleles (e.g. Ritland and Jain 1981).

All loci are not equivalent with respect to degree of polymorphism (O'Brien et al 1980, Gottlieb 1981). One explanation for the differences among loci is the potential for intragenic recombination (Morgan and Strobeck 1979). Our study of hidden variation at the ACO locus of jack pine doubled the number of detected alleles. The HC buffer revealed a putative amino acid substitution 'hidden' on the AC buffer, thus most of the original alleles detected on the AC buffer could be classified with respect to the HC-detected substitution. Clearly, methods of analysis that treat all loci as equivalent (eg. heterozygosity, genetic distance) should be interpreted with caution. As shown in this study, the nature of the variation at the ACO locus is different from the less polymorphic loci examined.

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