Electrophoretic Evidence for Mosaic 'Diploids' in Megagametophytes of Knobcone Pine (Pinus attenuata Lemm.)

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Abstract

Abnormal allozyme phenotypes were identified in megagametophytes from trees of *Pinus attenuata* growing in southeastern Oregon. The expression of two allozymes where only one was expected suggests that some megagametophytes are mosaic 'diploids', having been formed by two or more megaspores during seed development. The occurrence of such allozyme phenotypes emphasizes the need to consider developmental processes and molecular structure in interpreting allozyme variation.

Key words: allozyme, megagametophyte, mosaicism, *Pinus attenuata.*

Zusammenfassung

Abnorme allozymatische Phänotypen wurden in Megagametophyten von Bäumen von *Pinus attenuata* aus Südost-Oregon identifiziert. Die Expression von 2 Allozymen, wo nur eins erwartet wurde, legt nahe, daß einige Megagametophyten „Mosaikdiploide" sind, die durch 2 oder mehr Megasporen während der Entwicklung geformt werden. Das Erscheinen von solchen allozymatischen Phänotypen unterstreicht die Notwendigkeit, Entwicklungsprozesse und die molekulare Struktur in die Interpretation der Allozymvariation einzubeziehen.

Introduction

The inheritance of allozyme variants as inferred from segregation analysis using conifer megagametophytes has been the focus of numerous electrophoretic studies and is now well-documented (Adams and Joly, 1980a; Bartels, 1971; El-Kassaby *et al.*, 1981; Guries and Ledig, 1978; Lundkvist, 1975; Rudin, 1975). Such direct analyses of the products of meiosis eliminate the need for controlled breeding to determine inheritance patterns, and also offer possibilities for linkage analyses (Adams and Joly, 1980b; Conkle, 1981; Guries *et al.*, 1978; O'Malley *et al.*, 1986) and the study of conifer mating systems (Cheliak *et al.*, 1983; Epperson and Allard, 1984). While genetic interpretations are usually straightforward, deviations from random segregation are frequent (e. g., Adams and Joly, 1980a; Cheliak *et al.*, 1984; O'Malley *et al.*, 1979; Rudin, 1975; Strauss and Conkle, 1986). Such departures from random segregation may provide important information on the genetic biology of trees with respect to linkage between allozyme markers and embryonic lethals (Cheliak *et al.*, 1984).

Seed development is a highly conserved process in the gymnosperms. Typically, four haploid megaspores are produced by the meiotic division of one megasporocyte (Foster and Gifford, 1974). Among the gymnosperms, only *Gnetum* and *Welwitschia* deviate from this pattern by yielding megagametophytes derived from all four megaspores (Willson and Burley, 1983). In *Pinus*, the megaspores are usually aligned in a linear tetrad and all but the one farthest from the micropyle degenerates. The remaining megaspore develops into the megagametophyte. Rarely, it appears that the megagametophyte may be derived from more than one megaspore (O'Malley and Kelly, 1988). In these instances, the tissue is a mosaic of cells descended from different megaspores which differ in their allozyme composition. A graphic example of such a mosaic was reported by O'Malley and Kelly (1988) involving *Gingko biloba.* In this note, we report an example of unusual allozyme variation in knobcone pine (*Pinus attenuata* Lemm.) megagametophytes which we interpret to be the result of an abnormal pattern of megagametophyte development.

Materials and Methods

As part of a survey of electrophoretic variation in conifers, we obtained seed from fifty knobcone pine growing near O'Brien, Oregon. The trees are located on a decomposed serpentine soil at an elevation of 140 m above sea level. The stand contained a number of dwarfed trees, possibly due to the unusual edaphic conditions of the site.

Seed handling, germination, and electrophoretic methodology, followed our standard procedures and are given elsewhere (O'Malley *et al.*, 1980). A total of thirty-one enzyme systems encoding forty-six allozyme loci were surveyed.

Results and Discussion

A total of 15 knobcone pine trees from the O'Brien population produced abnormal allozyme phenotypes in which some, many, or all megagametophytes expressed two allozymes where only one was expected given the haploid nature of the tissue (*Fig. 1*). Two-band patterns were recognized for seven allozyme loci: ACP-2 (acid phosphatase), ADH-3 (alcohol dehydrogenase), DIA-3 (diaphorase), GPD (glyceraldehyde-3-phosphate dehydrogenase), 6PG-1 (6-phosphogluconic dehydrogenase), PGI-2 (phosphoglucose isomerase), and PGM-1 (phosphoglucomutase). The occurrence of single and double band patterns in the megagametophytes from a single tree (assuming a single megasporocyte) can be explained by variation in the number of megaspores contributing to the megagametophyte. If the megagametophyte is derived from one megaspore (monosporic), every allozyme locus will be represented by a single band (fast or slow) on gels. If the megagametophyte is derived from three or four megaspores (tetrasporic), every heterozygous allozyme locus will be represented by a two-band pattern. When the megagametophyte is derived from two megaspores and no crossing over is allowed, all heterozygous allozyme loci would be represented on a gel by one band, or all by two bands, depending upon which two megaspores contribute to the megagametophyte. In many instances a megagametophyte which showed a two-band pattern for one allozyme also appeared to be two-banded for all other allozymes segregating in megagametophytes from that parent tree (*Table 1*). However, the truly diagnostic situations for the two-megaspore case involve cross-overs be-

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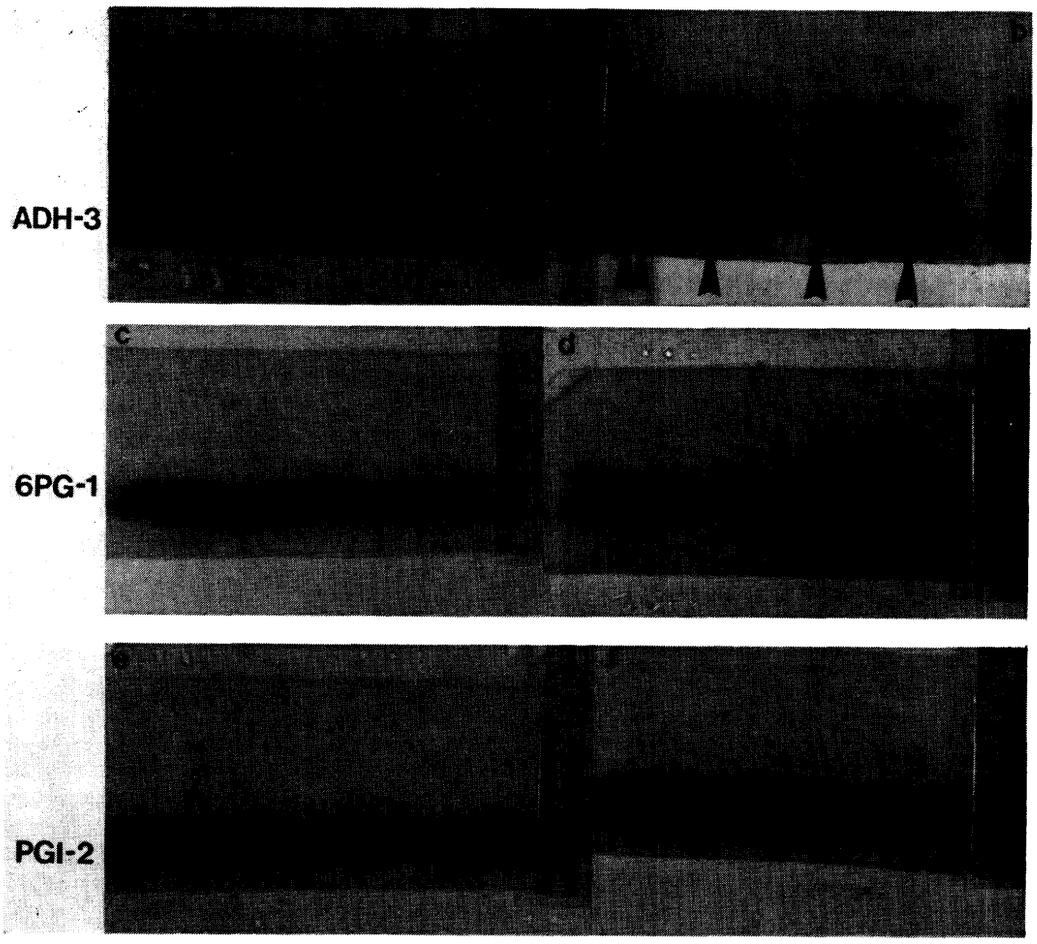

Figure 1a—f. — Allozyme patterns from normally segregating trees, and from trees exhibiting abnormal megametophyte development. a. normal ADH-3 with 1:1 segregation. b. abnormal ADH-3 with most sample slots having both fast (f) and slow (s) allozymes; arrows indicate a few normal samples. c. normal 6-PG-1 with 1:1 segregation; lowermost zone is IDH. d. abnormal 6-PG-1 with most sample slots having both fast (f) and slow (s) allozymes. e. normal PGI, with 1:1 segregation at PGI-2 locus. f. abnormal PGI-2 with all slots having both fast (f) and slow (s) allozymes.

	BOTH LOCI 1ST DIV SEGR.				A LOCUS 1ST DIV SEGR.				B LOCUS 1ST DIV SEGR.				A AND B LOCUS 2ND DIV SEGR.			
TETRAD	A B	A b	a B	a b	A B	A B	a B	a B	A B	A B	A b	A b	A B	A b	A B	A B
	A B	A b	a B	a b	A b	A b	a b	a b	a B	a B	a b	a b	a b	a B	a b	a b
	a b	a B	A b	A B	a B	a b	A B	A b	A b	a b	A B	a B	A B	A b	a B	a b
	a b	a B	A b	A B	a b	a B	A b	A B	a b	A b	a B	A B	a b	a B	A b	A B
ALLOZYME LOCUS A F			●	●			●	●	●	●	●	●	●	●	●	●
ALLOZYME LOCUS A S	●	●			●	●			●	●	●	●	●	●	●	●
ALLOZYME LOCUS B F		●		●	●	●	●	●			●	●	●	●	●	●
ALLOZYME LOCUS B S	●		●		●	●	●	●	●	●			●	●	●	●

Figure 2. — Allozyme patterns expected for megagametophytes derived from 2 megaspores for an individual heterozygous for two genes, A and B (assuming a single megasporocyte and survival of two lowermost megaspores.

Table 1. — Segregation data from tree PIAT 17 showing variation in the numbers of single (s is slow and f is fast) and double (D is both fast and slow) allozyme banding patterns and putative number of megaspores (1 or 2, only 2, and 2 or more) from which the megagametophyte was derived.

Locus	Megagametophyte Samples													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
ACP-2	f	D	s	f	D	s	D	D	D	f	f	f	D	D
ADH-3	D	D	f	f	D	s	s	f	D	s	D	f	D	D
GPD	f	D	s	s	D	f	s	D	D	s	D	f	s	D
6PG-1	f	s	f	s	D	f	s	D	D	f	D	f	s	D
Number of Megaspores:	2	2	1-2	1-2	2+	1-2	2	2	2+	1-2	2	1-2	2	2+

tween a locus and its centromere (second division segregation in tetrad analysis) leading to megagametophytes in which some heterozygous loci are represented by single bands and some by double bands (*Fig. 2*). Putative megagametophytes corresponding to expectations for one, two, and three or four megaspores can be identified from segregation data (*Table 1*). It is obvious that accession PIAT 17 (*Table 1*) yielded megagametophytes derived usually from 2 megaspores.

Diplcids which are heterozygous for dimeric allozymes have a characteristic three-band pattern (Gottlieb, 1981). The enzyme molecules consist of two subunits, each subunit being encoded by one of two alleles. In this situation, subunits combine to form 3 different molecules: 2 molecules comprised of subunits encoded by one or the other allele, and a third hybrid molecule comprised of one subunit encoded by each of the allelic forms of the gene. Examples of 'diploid' patterns in megagametophytes have been reported previously. The dimeric enzyme, malic dehydrogenase (MDH), is encoded by 4 genes in several species of conifers (O'Malley *et al.*, 1979; El-Kassaby, 1981). Two of the genes (not linked) apparently are expressed in the same compartment within the cell and produce a three-band pattern in which the intermediate band is an interlocus heterodimer. The result is a pattern similar in appearance to diploids heterozygous for dimeric allozymes. O'Malley and Guries (1983) identified a three-band allozyme variant of 6PG-1 (6-phosphogluconic dehydrogenase) which segregated 1:1 in the megagametophytes of 3 individuals of jack pine (*Pinus banksiana* Lamb). Segregation analysis of one tree strongly suggested that the three-band variant resulted from heterodimer formation between subunits encoded by a translocated 6PG-1 locus and subunits encoded by the standard 6PG-1 locus.

In contrast, the knobcone pine 'diploid' megagametophytes did not produce a heterodimer band for dimeric enzymes such as alcohol dehydrogenase (ADH), 6-phosphogluconic dehydrogenase (6PG-1), and phosphoglucose isomerase (PGI). This provides evidence that the two allozymes encoded by an enzyme gene are not expressed in the same cell. Weeden and Gottlieb (1979) analyzed complex plant isozyme patterns from electrophoresis of both normal diploid tissues and of crushed pollen. The two allozymes encoded by a heterozygote for a PGI gene appeared on gels loaded with pollen samples, but these haploid cells lacked the intralocus heterodimer bands seen in diploid cells because only one allozyme for each locus is encoded and expressed in each pollen grain.

The frequency of megagametophytes possessing two-band allozyme patterns varies from tree to tree, suggesting that megagametophytes are derived from different numbers of megaspores in different trees. However, if the linear tetrad is maintained and the megagametophyte is regularly derived from the two lower megaspores in some individuals, it may be possible to recover two of the 4 chromatids involved in meiosis and thus map the positions of segregating genes relative to their centromeres via half-tetrad analysis. A meiotic abnormality controlled by the elongate gene in maize (Rhoades and Dempsey, 1966) and 2N gametes in potatoes (Mendiburu and Peloquin, 1979) have been exploited for this purpose in plants.

Although the inheritance of allozyme variation in megagametophytes yields Mendelian ratios for the vast majority of cases, it is important to be aware of possible exceptions. The exceptions may provide useful genetic information but may also result in incorrect genetic interpretation if not understood. In conifer allozyme studies, extensive genetic analysis is not always feasible, but simple segregation analysis of unusual phenotypes is easily accomplished. However, it is important to take into account developmental processes and molecular structure as well as segregation analysis in interpreting allozyme variation. The allozyme patterns observed in this population of knobcone pine demonstrate that even fundamental assumptions concerning the inheritance of conifer enzyme variation should not be left unquestioned.

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Putative hybridization between P. caribaea Morelet and P. oocarpa Schiede: a canonical approach[1])

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Summary

Principal component and canonical correlation analyses were applied to three sets of traits (needle, cone and chemical compounds) measured on a sample of pine trees. A transect in a location at Honduras was defined were *P. caribaea* Morelet and *P. oocarpa* Schiede live sympatrically. Results suggest that natural hybridization is occurring between these species. Intermediate and outlying trees were statistically found. Intermediate trees have characteristics between the two parent populations and outliers are those sharing characteristics of both parental populations but for different sets of traits.

Key words: Hybridization, *P. caribaea*, *P. oocarpa*, principal components, canonical correlations.

Zusammenfassung

Die Hauptkomponenten-Analyse und die kanonische Korrelationsanalyse wurden auf drei Merkmalsgruppen (Kiefernnadeln, Kiefernzapfen und chemische Zusammensetzung des Holzmaterials) angewandt, die anhand einer Stichprobe von Kiefern gemessen wurden. In Honduras wurde ein Transect festgelegt, wo *P. caribaea* Morelet und *Pinus oocarpa* Schiede nebeneinander vorkommen. Die Ergebnisse lassen den Schluß zu, daß eine natürliche Hybridisierung zwischen beiden Arten vorkommt. Intermediäre und Ausreißerformen konnten statistisch abgesichert unterschieden werden. Die intermediären Formen weisen Merkmale auf, die in ihrer Ausprägung zwischen denen der beiden Elternpopulationen liegen, während die Ausreißerformen sich Merkmale beider Elternpopulationen teilen, jedoch für verschiedene Merkmalsgruppen.

1. Introduction

These two pine species are industrially important because they are fast growing trees and in short periods of about 15 to 20 years they yield economic quantities of wood, resin and seed. In some countries these species can be used in fuelwood plantations for energy (Burley, 1980). Total natural areas approximate to 2.5 million ha. and the plantation area exceeds 1 million ha. in 57 countries.

It has been suggested that *P. caribaea* Morelet and *P. oocarpa* Schiede may cross producing fertile offspring (Williams, 1955; Mirov, 1967). This motivated research on the possible hybridization of the two species developed at the Commonwealth Forestry Institute (CFI), Oxford, (UK) and the Tropical Products Institute (TPI), London.

A natural stand of *P. caribaea* var. *hondurensis* and *P. oocarpa* in Pinalejo is being studied. This paper is a follow up of the results published by Styles (1976); Fernandez de la Reguera *et al.* (1984) and Styles *et al.* (1982), where descriptions of the sampling procedures, the individual trees and their environment are presented. Statistical multivariate methods used in these references are mainly, principal component and canonical correlation analyses applied to selections of needle and cone measurements. This paper introduces the chemical compounds evaluated per tree and a different selection of variables based on a statistical screening process.

2. Material and Methods

The data were obtained from trees selected along a transect in Pinalejo, Honduras (15°23'N, 88°24'W). Altitude along the transect ranged from 300 to 900 m (approx.) above sea level. Ten altitude ranges, which were considered as sites, were defined, their limits not exactly fixed because of the natural spread of the trees and their availability at specific altitude. Ten trees were randomly selected per site, from those that were apparently healthy, mature and dominant, and aged 20 years or more.

The actual data is an extension of that used in Styles' papers. It includes the two bottom sites which were not considered by Styles, because he thought they were stands of pure *P. caribaea* trees, and a determination of the chemical compounds in the sampled trees.

A large number of variables were evaluated but a statistical screening of them reduced their number to 22 namely needle width (NWD), number of needles per fascicle (NNDL), length of sheath (SHEZ), number of stomatal rows on the dorsal side (STD), ventral side (STV) and in 5 mm section of the needle (ST5MM), total number of resin canals (RES); cone length (CLNG), cone diameter (DIAM), width at mid point of cone (WMD), distance to widest point of cone (DSWP), width of the umbo (UMBO), shape of cone (SHAPE); α-pinene (APAIN), myrcene (MYRC), limonene (LIMO), β-phellandrene (BFEL), allo-occinene (ALLO), camphor (CAM), longifolene (LFOL) and longicyclene (LCYC). These variables are those showing a linear trend

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