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Inheritance of Allozyme Variants in Bishop Pine (*Pinus muricuta* D. Don)

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Isozyme phenotypes are described for 45 structural loci and 1 modifier locus in bishop pine (Pinus muricata *D. Don,*) *and segregation data are presented for a subset of 31 polymorphic loci from 19 enzyme systems. All polymorphic loci had alleles that segregated within single-locus Mendelian expectations, although one pair of alleles at each of three loci showed significant segregation distortion. The consistency of resolution and segregation at many loci in bishop pine makes electrophoretic analyses feasible for many purposes in this species.*

KEY WORDS: allozymes; inheritance; segregation; *Pinus muricata.*

INTRODUCTION

Forest geneticists routinely apply electrophoretic techniques to analyze basic and applied problems in tree species. Although related species often resemble one another in general isozyme characteristics, species can differ in the numbers of loci for the same enzyme (Adams and Joly, 1980; El Kassaby, 1981), in the band phenotypes for similar loci (Adams and Joly, 1980; El Kassaby *et al.*, 1982), in the interaction of products at two loci (El Kassaby, 1981), and in the expression of modifier loci (Harry, 1983). To avoid misinterpretation, it is important to document band inheritance before beginning electrophoretic analyses in a species.

This paper reports an analysis of inheritance, based on megagametophyte segregation in bishop pine (*Pinus muricata* D. Don), of 31 polymorphic loci from 19 enzyme systems and 15 monomorphic loci from 3 additional

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enzyme systems. Bishop pine [subsection *Oocarpae* (Critchfield and Little, 1966)] is a maritime species that ranges discontinuously from northern California to Baja California. The detection of allozyme variation in one locus allowed Millar (1983) to describe patterns of gene flow and hybridization in bishop pine. Further applied and basic research on this highly variable species could benefit by using the many isozyme loci that can be detected.

MATERALS AND METHODS

Wind-pollinated seeds were collected from 600 trees in native stands of Sonoma and Mendocino Counties, California. Megagametophytes and embryos were dissected from germinated seeds and subjected to electrophoresis following the techniques and using the gel systems of Conkle *et al.* (1982). Briefly, I used horizontal 12.5% starch gels at three different *p*H levels (gel system A—Tris–citrate, *p*H 8.3; gel system B—Tris–citrate, *p*H 8.8; and gel

Table I. List of Enzymes, Enzyme Commission Reference Numbers, and Gel Buffer Systems Used for Bishop Pine Electrophoresis

Gel buffer system[a]	Enzyme	Abbreviation	EC number
A	Alcohol dehydrogenase	ADH	1.1.1.1
	Alanine aminopeptidase	ALAP	3.4.11.1
	Leucine aminopeptidase	LAP	3.4.11.1
	Menadione reductase	MNR	1.6.99.2
	Peroxidase	PER	1.11.1.7
	Phosphoglucose isomerase	PGI	5.3.1.9
B	Acid phosphatase	ACP	3.1.3.2
	Catalase	CAT	1.11.1.6
	Glucose-6-phosphate dehydrogenase	G-6PD	1.1.1.49
	Glutamate dehydrogenase	GDH	1.4.1.3
	Glutamate oxaloacetate transaminase	GOT	2.6.1.1
	Mannose phosphate isomerase	MPI	5.3.1.8
	Sorbitol dehydrogenase	SRDH	1.1.1.14
	Superoxide dismutase	SOD	1.15.1.1
C	Aconitase	ACO	4.2.1.3
	Aldolase	ALD	4.1.2.13
	Fructose diphosphatase	FDP	3.1.3.11
	Isocitric dehydrogenase	IDH	1.1.1.42
	Malate dehydrogenase	MDH	1.1.1.37
	6-Phosphogluconic dehydrogenase	6PGD	1.1.1.44
	Phosphoglucomutase	PGM	2.7.5.1
	Shikimate dehydrogenase	SKDH	1.1.1.25

[a]Gel systems of Conkle *et al.* (1982).

system D—morpholine citrate, *p*H 6.1). Twenty-two enzyme systems stained consistently enough to be included in this study (Table I). Some stain recipes and electrophoretic procedures were modified or added to the techniques of Conkle *et al.* (1982) (Table II).

I initially analyzed five megagametophytes and one embryo from each of the 600 trees and identified trees that were putative heterozygotes for each locus. Assuming 1:1 segregation at heterozygous loci, an expected 6% of the heterozygotes would be missed by sampling megagametophytes this way. If, however, severe segregation distortion existed at a locus, more heterozygotes would be undetected.

For each polymorphic locus, I pooled segregation data from all heterozygotes identified. When there were at least 10 heterozygous trees for each locus (at least 50 megagametophytes per locus), I calculated chi-square values testing the expected 1:1 ratios on the pooled data and the heterogeneity chi-square values among trees. For loci with fewer than 10 heterozygous trees (fewer than 50 megagametophytes), I analyzed additional seeds, where possible, from 1–5 heterozygous trees, using 10–42 additional megagametophytes per heterozygous tree per locus. These data were pooled and analyzed as above. One locus appeared only in embryos, and although I could not verify inheritance, I describe it because it stained clearly and was highly polymorphic.

The following nomenclature was used: an enzyme and its band phenotypes were designated by the enzyme's abbreviation in capital letters

Table II. Modifications to Electrophoretic Procedures of Conkle et al. (1982) Used for Bishop Pine Studies

(A) Modifications to stain recipes

Enzyme	Stain components that differ	
ACO	*cis*-Aconitic acid	200 mg
	Isocitrate dehydrogenase	60 U
ADH	95% ethyl alcohol	2 ml
ALAP	L-Alanine-naphthylamide	50 mg
	Fast black K salt	40 mg
ALD	Glyceraldehyde-3-phosphate dehydrogenase	780 U
GOT	Fast blue BB salt	50 mg
G6PD	D-Glucose-6-phosphate	250 mg
IDH	DL-Isocitric acid	200 mg
	1% $MgCl_2$	1 ml
LAP	Black K salt	40 mg
MNR	Menadione	25 mg
PGM	α-D-Glucose-1-phosphate	250 mg
	Glucose-6-phosphate dehydrogenase	80 U
SKDH	Shikimic acid	250 mg

Table II. Continued

(B) Additional stain recipes

Enzyme	Stain buffer	Stain components	
FDP	25 ml 0.2 M Tris–HCl, *p*H 8.0	Fructose-1,6-diphosphate	150 mg
		NADP	1 ml
		NBT	1 ml
		PMS	0.5 ml
		Glucose-6-phosphate dehydrogenase	100 U
		Phosphoglucose isomerase	100 U
		1% $MgCl_2$	1 ml
	Procedure: Add stain components to warm stain buffer and incubate gels in dark.		
MPI	25 ml 0.2 M Tris–HCl, *p*H 8.0	Mannose-6-phosphate	20 mg
		NADP	1 ml
		NBT	1 ml
		PMS	0.5 ml
		Glucose-6-phosphate dehydrogenase	100 U
		Phosphoglucose isomerase	100 U
	Procedure: Add stain components to warm stain buffer and incubate gels in dark.		
SRDH	60 ml 0.2 M Tris–HCl, *p*H 8.0	Sorbitol	5 g
		NAD	1 ml
		NBT	1 ml
		PMS	0.5 ml
	Procedure: Add stain components to warm stain buffer and incubate gels in dark.		

(C) Miscellaneous modifications

Wicks: Paper wicks were cut narrower than recommended (14 × 2 mm).
Extraction buffer: Add to buffer,
D-Glucose-6-phosphate 20 mg/100 ml buffer
Bovine albumin 40 mg/100 ml buffer

(Table I); each locus of an enzyme was designated by the enzyme's abbreviation, with the second and third letters in lowercase. When multiple loci occurred for an enzyme, the enzyme phenotypes and loci were numbered, with the most anodally migrating locus designated 1. Within each locus, the most frequent allele was assigned the value of 1.00. Other alleles of the locus were designated by a mobility value expressed relative to the most frequent allele (1.00). Negative mobility values were given to cathodally migrating alleles.

An allele lacking stain activity (null), observed at only one locus, was designated with a lowercase "n." Nomenclature for the modifier locus is described below.

RESULTS AND DISCUSSION

General Comments

The general phenotypes of bishop pine's isozymes resembled those found in related species, both in numbers of loci for given enzymes and in band phenotypes (numbers of bands and patterns of stain intensity). Fifteen monomorphic loci and 31 polymorphic loci resolved consistently in the 592 trees that had sufficient sound seeds and interpretable band patterns (Table III). Additional zones that appeared inconsistently in some systems were excluded. The number of alleles observed and verified at polymorphic loci varied from two to five, and megagametophyte band phenotypes at a locus varied from null to quadruple-banded. Seven enzymes had a single zone of activity (ACO, ACP, ADH, GDH, IDH, SOD, SRDH), while the rest had multiple zones (maximum, four zones in MDH). Coelectrophoresis of embryos and megagametophytes showed substantial variability between tissues in band expression for many enzymes.

Monomorphic Loci

Three enzyme systems, GDH, SRDH, and SOD, were invariant; each had a single band in megagametophytes and embryos. The phenotypes in these zones resembled those of corresponding polymorphic loci of other conifers, where single-locus Mendelian segregation has been shown (Table III). The other 11 monomorphic zones (ALAP2, CAT2, FDP2, FDP3, G6PD2, MDH4, MPI2, 6PGD2, PGI1, SKDH2, and SKDH3) occurred as invariant bands in enzyme systems that also had polymorphic loci banding in different zones. The monomorphic MDH, 6PGD, and PGI zones resembled polymorphic loci in other conifers where single-locus Mendelian segregation was reported (Table III). Inheritance of the remaining monomorphic zones has not been investigated in other conifers, and these zones should be considered putative loci in bishop pine.

Polymorphic Loci

The 31 polymorphic loci comprised 30 megagametophyte loci and 1 embryo locus. Megagametophyte band phenotypes at a single locus were either all single (most common), all double [either with two equally staining bands

Table III. Descriptions of Gametophyte and Embryo Phenotypes, Allele Designations, Quaternary Enzyme Structures of 22 Bishop Pine Enzymes, and References to Inheritance Studies in Other Conifers

Locus	Gametophyte phenotype	Alleles	Expression of gametophyte bands in embryo[a]	Gametophyte enzyme structure[b]	References reporting inheritance in other conifer species
Got1	Single	1.00, 1.07 0.98, 0.76	Same	Dimer	Rudin, 1975; Conkle and Adams, 1977; Yang *et al.*, 1977; Guries and Ledig, 1978; Rudin and Ekberg, 1978; Witter and Feret, 1978; O'Malley *et al.*, 1979; Yeh and Layton, 1979; Adams and Joly, 1980; Eckert *et al.*, 1981; Neale and Adams, 1981; El-Kassaby *et al.*, 1982; Fins and Libby, 1982; Wheeler and Guries, 1982; King and Dancik, 1983; Cheliak and Pitel, 1984; Harry, 1984; Neale *et al.*, 1984
Got2	No bands	1.00, 0.73	Embryo locus	Dimer	
Got3	Triple and double	1.00, 1.92, 1.92a	Same	—	
Idh	Double	1.00, 1.36, 0.96, 0.86	Same[c]	Dimer	Guries and Ledig, 1978; Yeh and Layton, 1979; O'Malley *et al.*, 1979; Adams and Joly, 1980; Eckert *et al.*, 1981; Neale and Adams, 1981; El-Kassaby *et al.*, 1982; Wheeler and Guries, 1982; King and Dancik, 1983; Loukas *et al.*, 1983; Cheliak and Pitel, 1984; Harry, 1984; Neale *et al.*, 1984
Lap1	Single and null	1.00, 1.06, n	Same	Monomer	Bergmann, 1973; Tigerstedt, 1973; Lundkvist, 1974; Conkle, 1971; Simonsen and Wellendorf, 1975; Lundkvist and Rudin, 1977; Rudin, 1977; Yang *et al.*, 1977; Guries and Ledig, 1978; Rudin and Ekberg, 1978; O'Malley *et al.*, 1979; Adams and Joly, 1980; Eckert *et al.*, 1981; Neale and Adams, 1981; Fins and Libby, 1982; King and Dancik, 1983; Loukas *et al.*, 1983; Cheliak and Pitel, 1984; Harry, 1984
Lap2	Single	1.00, 1.06	Same	Monomer	

Mdh1	Single	1.00, 1.09	Same	Dimer	Simonsen and Wellendorf, 1975; Guries and Ledig, 1978; Rudin and Ekberg, 1978; O'Malley *et al.*, 1979; Yeh and Layton, 1979; Adams and Joly, 1980; El-Kassaby, 1981; Neale and Adams, 1981; El-Kassaby *et al.*, 1982; Wheeler and Guries, 1982; Harry, 1983; King and Dancik, 1983; Harry, 1984; Neale *et al.*, 1984
Mdh2	Quadruple	1.00, 1.62	Same	—	
Mdh3	Single	1.00, 1.29, 0.91	No bands	—	
Mdh4	Single	1.00	No bands	—	
Mmd2	Modifier	f/s	Indeterminable	—	
Mnr1	Diffuse band	1.00, 0.71	Different	—	
Mnr2	Single	1.00, 1.13	Same	—	
Mnr3	Single	1.00, 1.42, 0.71, 0.17	Same	—	
Mpi1	Single	1.00, 0.97	Same	—	El-Kassaby *et al.*, 1982; Wheeler and Guries, 1982
Mpi2	Single	1.00	Different	—	
Per1	Single	−1.00, −0.88		—	Mitton *et al.*, 1977; Synder and Hamaker, 1978; Copes, 1979
Per2	Single	−1.00, −0.55		—	
Per3	Double	−1.00	Same	—	
6Pgd1	Single	1.00, 0.91	Same	Dimer	Guries and Ledig, 1978; O'Malley *et al.*, 1979; Yeh and Layton, 1979; Adams and Joly, 1980; Eckert *et al.*, 1981; Neale and Adams, 1981; El-Kassaby *et al.*, 1982; Wheeler and Guries, 1982; King and Dancik, 1983; Loukas *et al.*, 1983; Cheliak and Pitel, 1984; Harry, 1984; Neale *et al.*, 1984
6Pgd2	Single	1.00	Same	—	
Pgi1	Single	1.00	Different	—	Simonsen and Wellendorf, 1975; O'Malley *et al.*, 1979; Yeh and Layton, 1979; Adams and Joly, 1980; El-Kassaby *et al.*, 1982; King and Dancik, 1983; Harry, 1984; Neale *et al.*, 1984
Pgi2	Double	1.00, 1.38, 0.81	Same	Dimer	

Table III. Continued

Locus	Gametophyte phenotype	Alleles	Expression of gametophyte bands in embryo[a]	Gametophyte enzyme structure[b]	References reporting inheritance in other conifer species
Pgm1	Single and double	1.00, 0.95	Same	Monomer	Simonsen and Wellendorf, 1975; Guries and Ledig, 1978; Mitton *et al.*, 1979; O'Malley *et al.*, 1979; Yeh and Layton, 1979; Adams and Joly, 1980; Eckert *et al.*, 1981; Neale and Adams, 1981; El-Kassaby *et al.*, 1982; Wheeler and Guries, 1982; King and Dancik, 1983; Loukas *et al.*, 1983; Harry, 1984; Neale *et al.*, 1984
Pgm2	Single	1.00, 0.94	Same	Monomer	
Pgm3	Single	1.00, 1.11, 0.86	Same	Monomer	
Skdh1	Single	1.00, 1.05, 0.85	Same	Monomer	Harry, 1984; Neale *et al.*, 1984
Skdh2	Single	1.00	Same	—	
Skdh3	Single	1.00	Same	—	
Sod	Single	1.00	Same	—	El–Kassaby *et al.*, 1982; Fins and Libby, 1982; King and Dancik, 1983; Harry, 1984
Srdh	Single	1.00	Same	—	O'Malley *et al.*, 1979; Wheeler and Guries, 1982

[a]Comparison within present study of bands from megagametophytes to bands from their derived embryos.
[b]Structure inferred from band phenotypes of heterozygous embryos—see text. Dash (—) indicates structure was indeterminable because (1) a different locus was expressed in the embryo, (2) a heterozygous embryo was not seen, (3) no bands appeared for the embryo, or (4) the embryo bands stained poorly.
[c]Embryo had the same main band as the gametophyte but sometimes lacked the weaker trailer band(s).

(FDP1) or with one strongly staining band plus a weakly staining "trailer" band (ACO, IDH, and PGI2)], single and double (ACP and PGM), double and triple (GOT3), triple (ALD2), or quadruple (CAT1, with bands of equal staining intensity; and MDH2, with two strongly staining bands in the middle, plus fast and slow weakly staining bands) (Table III). The single locus (Got2) that stained clearly and consistently only in embryos had a single-banded homozygous phenotype and a triple-banded heterozygous phenotype.

A single nonstaining locus modified the mobility of the MDH2 structural locus. The MDH2 phenotype had four bands that moved in unison. Most of the observed MDH2 variation was between the two slowest-migrating morphs (MDH2-1.00 and MDH2-0.95). Phenotype MDH2-0.95 was present at low–moderate frequencies in many stands and was associated with two of the three populations sampled. The cosegregation of the two morphs and a third morph (MDH-1.62) in one tree suggested that these bands were affected by a modifier locus: the structural locus expressed a fast and a slow morph, resulting in a major mobility difference between phenotypes, while the modifier locus caused a minor mobility shift in the slow morph, from MDH2-1.00 to MDH2-0.95. Although four phenotypes are expected given this interpretation, I observed only three.

If the structural alleles are designated Mdh2-1.00 and Mdh2-1.62, and the modifier alleles, Mmd2-f (fast) and Mmd2-s (slow) (after Harry, 1983), then the genotypes of the three observed megagametophyte phenotypes are as follows: MDH2-1.00, Mdh2-1.00/Mmd2-f; MDH2-0.95, Mdh2-1.00/Mmd2-s; and MDH2-1.62, Mdh2-1.62/Mmd2-f. The last genotype was inferred from several heterozygous combinations where I found only MDH2-1.00 and MDH2-1.62 segregating. Since these trees were homozygous for Mmd2-f (i.e., MDH2-0.95 was absent), the genotype of MDH2-1.62 must be Mdh2-1.62/Mmd2-f. Of the five trees that had Mdh2-1.62, four were Mdh2-1.00/1.62, Mmd2-f/f, and only one appeared to be a double heterozygote, although I observed only three bands segregating. I had few viable seeds from this tree and only eight megagametophytes stained clearly. If segregation was 1:1 at Mdh2 and Mmd2, there was a 10% probability of missing one of the four expected phenotypes. I expect that the fourth phenotype would migrate slightly slower than MDH2-1.62.

Analogous patterns of inheritance involving a modifier locus have been described for MDH in another conifer and in angiosperms (Harry, 1983, and references cited). Harry investigated the complex inheritance of this MDH zone in megagametophytes and embryos of incense cedar (*Calocedrus decurrens*), concluding that the observed band patterns and frequencies were consistent with those expected if an unlinked, polymorphic, nonstructural locus modifies a polymorphic, structural locus. In incense cedar, although the alleles of the structural locus are codominant, the alleles of the modifier locus

show dominance. The megagametophyte phenotypes and mobility shifts of MDH2 in bishop pine are similar to those described for incense cedar.

Embryo Phenotypes

I grouped the embryo band patterns resulting from coelectrophoresis of megagametophytes and embryos into four classes (Table III): in most cases, the embryo bands stained at the same mobility as one and/or the other megagametophyte bands or at both megagametophyte mobilities plus an intermediate band suggestive of the heterodimeric form of a dimeric enzyme. In these cases, I assumed that the same genes were expressed in both tissues. A second pattern showed positive staining in the embryos but at a different mobility than the megagametophytes and not clearly related to the megagametophyte bands. In these cases, I assumed that the genes were differentially expressed or modified in the two tissues. Sometimes the most common embryo and megagametophyte bands were so similar in mobility that differences between the tissues became apparent only when rare variant megagametophyte bands stained at a different mobility than the embryos. A third pattern resulted when no bands stained in the embryo at the megagametophyte zone. I interpreted this as inactivity of the megagametophyte locus in embryo tissue (although possibly the locus is expressed in embryos, but electrophoresis did not detect it). Finally, I did not interpret embryo activity in zones where embryo staining was inconsistent.

When the megagametophyte locus was clearly expressed in the embryo and when heterozygous embryos were found, the quaternary structures of the enzymes were inferred (Table III). If the phenotype of the megagametophyte was single-banded and the heterozygous embryo was double-banded, the enzyme structure was inferred to be monomeric; if the embryo phenotype was triple-banded, the structure was inferred to be dimeric. In ACO, where the megagametophyte phenotype was double-banded, I interpreted the quadruple-banded heterozygous embryo phenotype to indicate a monomeric enzyme, as no intermediate bands appeared in embryos. In IDH and PGI2, where the megagametophyte phenotype had a strongly staining and a weakly staining band, the heterozygous embryo phenotype was triple-banded. It lacked the weakly staining bands of the megagametophyte but expressed an intermediate band between the two strongly staining megagametophyte bands, suggesting dimeric structure.

Additional Observations on Specific Loci

GOT3. Although heterozygous embryos were rare and difficult to interpret, the clearest gels had six bands. Four of these were identical in mobility to the fastest bands and the slowest bands of the two megagametophyte alleles, with

the remaining two bands staining halfway between the two fastest and the two slowest bands. A rare third allele of Got3 (1.92a) had two bands identical in mobility to Got3-1.92 but did not have the third intermediate band. Rare GOT3 phenotypes in incense cedar lacked either the fastest or the slowest bands but had the intermediate band (Harry, 1984).

LAP1. When the genotype of a female parent tree was inferred from megagametophytes as Lap1-n/Lap1-n (homozygous null), a paternal contribution of Lap1-1.00 or Lap1-1.06 could be detected and resulted in an embryo phenotype of a single band. If, however, the maternal genotype was not homozygous Lap1-n and the maternal contribution to the embryo was Lap1-1.00 or Lap1-1.06, the paternal genotype of the embryo could not be clearly determined, since a 1.00/1.00 phenotype was indistinguishable from a 1.00/n phenotype.

MNR1. The megagametophyte phenotype is a darkly staining, diffuse band.

PER. Major differences in staining intensity among megagametophytes on the same gel occurred and related generally to stages of seed germination. Designation of Per3 in bishop pine as a single locus with a double-banded phenotype is tentative since no variation occurred in these zones. Although the bands at this zone could be the expression of two independent loci, the single-locus interpretation agrees with results from other conifers.

Segregation Analyses

Deviations from 1:1 expectations for 22 polymorphic loci involving 31 combinations of alleles were nonsignificant for all but 3 combinations. Heterogeneity among parents was never significant, and *P* values were large.

The Mmd2 modifier locus and Mdh2 structural locus were among the loci that had nonsignificant deviations from 1:1. To test Mmd2, I observed MDH2-1.00 (= Mmd2-f) and MDH2-0.95 (= Mmd2-s) phenotypes segregating from a single tree. Similarly, to test Mdh2, I observed MDH2-1.00 and MDH2-1.62 phenotypes segregating from a heterozygous tree, which was homozygous for Mmd2-f.

Alleles of ACP and ALD2 deviated significantly in certain combinations (Acp-1.00/1.02 and Ald2-1.00/1.52) from 1:1 segregation, favoring the common allele (1.00). In other heterozygote combinations involving the common allele at these loci, deviations from 1:1 were nonsignificant. Segregation ratios in Aco 1.21/0.89 heterozygotes also differed significantly from 1:1, favoring the 1.21 allele. Both alleles, however, in combination with allele 1.00, conformed to Mendelian expectations. The observed deviations in these three

allelic pairs may not indicate actual segregation distortion at these loci, since in 31 trials, 2 (1%) are expected to differ at the 5% probability level.

Deviations from 1:1 expectations have been observed in other conifers sporadically when recorded either for single trees or for pooled data [ACP—*Pinus sylvestris* (Rudin and Ekberg, 1978) and *P. strobus* (Eckert *et al.*, 1981); GOT—*P. sylvestris* (Rudin, 1975; Rudin and Ekberg, 1978) and *P. virginiana* (Witter and Feret, 1978); LAP—*P. taeda* (Adams and Joly, 1980), *P. sylvestris* (Rudin and Ekberg, 1978), *P. strobus* (Eckert *et al.*, 1981), and *Picea abies* (Lundkvist, 1974); MDH—*P. sitchensis* (Simonsen and Wellendorf, 1975), *Pinus sylvestris* (Rudin and Ekberg, 1978), and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982); PER—*Pinus taeda* and *P. palustris* (Snyder and Hamaker, 1978); G6PD—*P. taeda* (Adams and Joly, 1980); PGI—*P. taeda* (Adams and Joly, 1980) and *Picea glauca* (Cheliak and Pitel, 1984)].

CONCLUSIONS

The 46 isozyme loci described here for bishop pine comprise 30 polymorphic structural loci, 1 polymorphic modifier locus, and 15 monomorphic loci. Bands at these loci stained clearly and consistently. Many loci were expressed in both embryos and megagametophytes. Sufficient allozymic variability exists to encourage isozyme analysis for further genetic studies with bishop pine.

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