Genetic Control of Isozyme Variation in Masson Pine, Pinus massoniana Lamb.

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Summary

Isozyme variations in 17 enzyme systems in masson pine (*Pinus massoniana* LAMB.) were studied by polyacrylamide vertical slab gel electrophoresis using haploid megagametophyte tissues. The evidence for a 1:1 segregation ratio of allozyme variants in heterozygous families indicated that the allozyme variants were encoded by a total of 98 alleles at 35 loci. No variations were observed at 7 loci, namely, *Adh-2, Sdh-1, G2d, Mdh-4, Got-1* and *Lap-2,* and the remaining 28 loci were polymorphic, each having more than 1 allele. Linkage relationships were studied for 187 2 locus combinations of the 26 polymorphic loci by conventional χ^2 analysis. Sixteen pairs of 18 loci from the 187 2-locus combinations showed significant joint segregation. Four linkage groups composed of the 18 loci were identified.

Key words: Isozymes, inheritance, linkage, masson pine, polyacrylamide gel electrophoresis, megagametophytes.

FDC: 165.3; 165.5; 174.7 *Pinus massoniana.*

Introduction

Isozymes have been widely used as genetic markers in studies of forest trees. Such studies have had an impact on various areas of forest genetics. For example, isoenzymes have been used (1) to certify the identity of clones, (2) to judge the localities from which seeds originate, (3) to determine the validity of controlled crosses, (4) to study the genetic efficiency of seeds orchards, (5) to determine the effectiveness of supplemental mass pollination, (6) to aid in the selection of economically important traits, (7) to estimate the genetic variability in natural populations, and (8) to characterize the relationships among congeneric species (ADAMS, 1983; HUANG, 1985). A knowledge of the inheritance of electrophoretically detectable polymorphisms is essential if they are to be used in such genetic studies. A knowledge of linkage relationships among isozyme loci is also important for the use of isozymes in marker-aided selection of important and economically valuable traits and for correct understanding of aspects of population genetics, such as genetic structure, mating system, and linkage desequilibrium. In addition, the information obtained from linkage maps of various species is useful for the interpretation of phylogenetic relationships (CHELIAK and PITEL, 1985).

The inheritance of isozymes and the linkage relationships between isozyme loci have been studied in many coniferous species, for example, in *Pinus densiflora* (NA'IEM *et al.*, 1989 and 1993; NA'IEM, 1991), *Pinus koraiensis* (TOMARU *et al.*, 1990), *Pinus rigida* (GURIES and LEDIG, 1978; O'MALLEY *et al.*, 1986), *Pinus strobus* (ECKERT *et al.*, 1981), *Pinus sylvestris* (RUDIN and RASMUSON, 1973; RUDIN, 1975 and 1977; RUDIN and EKBERG, 1978; NIEBLING *et al.*, 1987), *Pinus taeda* (ADAMS and JOLY, 1980a and b), *Pinus thunbergii* (SHIRAISHI, 1988a and b), *Abies mariesii* (SUYAMA *et al.*, 1992), *Pseudotsuga menziesii* (EL-KASSABY *et al.*, 1982a and b; ADAMS *et al.*, 1990 and *Cryptomeria japonica* (TSUMURA et al., 1989).

Masson pine (*Pinus massoniana* LAMB.) is widely distributed in China, ranging from Shantung, Kiangsu, and northern Taiwan west to Szechuan and south to northern Vietnam (CRITCHFIELD and LITTLE, 1966). It is frequently used for afforestation in China. However, little information is available about the genetic control of isozyme variations. In this paper, we report details of the inheritance and linkage relationships for 35 loci that encode 17 enzyme systems, as analyzed in megagametophyte tissues of masson pine.

Materials and Methods

Cones were collected from a total of 166 trees in 6 natural populations in the fall of 1988. The populations were distributed in Yuqin, Duyun, Jianhe and Suiyang. The cones were air-dried, and seeds were extracted and stored at —20 °C prior to the analysis of isozymes.

The seeds were soaked in running water at 4 °C for 48 hours, and then they were incubated on moistened filter paper in petri dishes at 4 °C for 3 weeks. Haploid megagametophytes were individually excised from stratified seeds for analysis by polyacrylamide gel electrophresis on vertical slab gels. The electrophoresis was performed by the procedures of DAVIS (1964) and ORNSTEIN (1964) with slight modification. Methods for the extraction of enzymes and staining were slightly modified versions of those described by CONKLE *et al.* (1982), MARTY *et al.* (1984) and SHIRAISHI (1988b). Seventeen enzyme systems were analyzed in this study (*Table 1*).

Table 1. — Enzymes examined in this study, with their abbreviations and Enzyme Commission (E.C.) reference numbers.

Enzyme	Abbreviation	E. C. no.
Alcohol dehydrogenase	ADH	1.1.1.1
Shikimate dehydrogenase	ShDH	1.1.1.25
Glycerate dehydrogenase	G2DH	1.1.1.29
Malate dehydrogenase	MDH	1.1.1.37
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49
Glutamate dehydrogenase	GDH	1.4.1.2
Diaphorase	DIA	1.6.4.3
Glutathione reductase	GR	1.6.4.2
Glutamate oxaloacetate transaminase	GOT	2.6.1.1
Phosphoglucomutase	PGM	2.7.5.1
Esterase	EST	3.1.1.
Amylase	AMY	3.2.1.
Leucine aminopeptidase	LAP	3.4.11.1
Alanine aminopeptidase	AAP	3.4.11.1
Fumarase	FM	4.2.1.2
Phosphoglucose isomerase	PGI	5.3.1.9

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In a heterozygous tree, allozymes are expected to segregate according to a 1:1 ratio in megagametophytes. The χ^2-test was used to examine the difference between the expected ratio of 1:1 and the segregation observed in heterozygous families. When several trees were studied that had the same allelic compositions (genotypes), after confirmation of homogenous segregation among the different families by a χ^2-test, the data were pooled over families for further statistical analysis of deviations from the expected ratio of 1:1.

Linkage analysis was performed as described by MATHER (1951) and BAILEY (1961). For a 2-locus (*A-B*) combination, the χ^2 values (χ^2A, χ^2B and χ^2H) were used for testing each of the deviations from the single-locus segregation ratio (1:1) and the heterogeneity of 2-locus segregations among families if there was more than 1 family. Then, the χ^2 value (χ^2L) was used to test the joint segregation (independence of loci). Recombination values (R) and their standard errors (SE_R) were estimated by BAILEY's (1961) procedure: the maximum likelihood estimator is given by $R = NR/n$ and the standard error is given by $SE_R = (R(1-R)/R)^{1/2}$, where NR is the number of recombinant types observed in n megagametophytes (BAILEY, 1961). A single-locus segregation distortion at only 1 locus does not disturb the linkage estimation. However, if single-locus segregations at both loci deviate from expectations, the linkage estimation is not valid. In such case in this study, another test for linkage analysis was made, as described by BAILEY (1961) and by RUDIN and EKBERG (1978).

Table 2. — Designation of loci and the alleles detected at each locus for 17 enzyme systems in masson pine.

Enzyme	Locus	No. of alleles	Allele and its Rf [1]					
			o	*a*	*b*	*c*	*d*	*e*
ADH	*Adh-1*	2		28	31			
	Adh-2	1		32				
	Adh-3	4	null [2]	33	34	36		
ShDH	*Shd-1*	1		35				
	Shd-2	4		36	37	38	40	
G2DH	*G2d*	1		14				
MDH	*Mdh-1*	2		18	20			
	Mdh-2	2		29	33			
	Mdh-3	1		32				
	Mdh-4	3		32	34	52		
6PGD	*6Pg-1*	2		18	22			
	6Pg-2	2		20	22			
G6PD	*G6p*	3		18	19	19H [3]		
GDH	*Gdh*	3	null	15	16			
GR	*Gr*	2		20	22			
DIA	*Dia-1*	3		19	20	21		
	Dia-2	2		26	28			
	Dia-3	4		29	35	38	44	
	Dia-4	4	null	58	59	60		
GOT	*Got-1*	1		18				
	Got-2	3		26	28	30		
	Got-3	2		32	35			
PGM	*Pgm*	4		51	54	55	57	
EST	*Est-1*	3	null	44	46			
	Est-2	6	null	61	62	63	66	70
	Est-3	6	null	60	70	79	82	85
AMY	*Amy*	5		46	49	52	53	56
LAP	*Lap-1*	1		42				
	Lap-2	1		60				
AAP	*Aap-1*	4		40	41	43	45	
	Aap-2	4		44	47	48	50	
	Aap-3	3		51	58	64		
FM	*Fm*	3		18	21	24		
PGI	*Pgi-1*	3		15	16	18		
	Pgi-2	3		24	26	28		
17	35	98						

[1]) Migrational distances of encoded proteins relative to that of bromophenol blue.
[2]) Non-active protein.
[3]) Intensely stained band.

Results and Discussion

Inheritance

We investigated the inheritance of isozymes in megagametophytes of 166 trees using 17 enzyme systems. Forty-three heterozygous trees in all were used for the genetic analysis. The results showed that the 17 enzymes were encoded by a total of 35 loci with 98 alleles (*Table 2*). No variations were observed at 7 loci, and 28 loci of the 35 loci were polymorphic. Multiple alleles numbering more than 4 were observed for *Est-2, Est-3* and *Amy*.

We present here a description of and a discussion of the genetic control of isozyme variants separately for each enzyme system in masson pine. The allelic variants found at each locus are summarized in *table 2*. In addition, the phenotypes of variants at each locus are presented in *figure 1*. If more than 1 isozyme zone was observed on gels stained for a given enzyme, the slowest migrating zone was labeled 1, the next zone 2, etc., for convenient descriptions of banding patterns. The nomenclature of loci and alleles follows that used by SHIRAISHI (1988b).

Alcohol dehydrogenase (ADH)

Three active zones were observed on gels stained for ADH. Two kinds of single-banded phenotype were found in zone 1, and 3 kinds of single-banded phenotype and 1 non-active phenotype were found in zone 3 (*Fig. 1*). The segregation of bands in zone 1 and zone 3 was investigated and no significant deviations from Mendelian segregation were apparent (*Table 3*). The phenotypes in zone 1 and zone 3 are controlled by *Adh-1* with 2 alleles and by *Adh-3* with 4 alleles, respectively. By contrast, only 1 single-banded phenotype appeared in the middle zone (zone 2) generated by all the families tested. Although no segregation tests were performed, we inferred that this zone corresponded to a single locus (*Adh-2*). The isozyme phenotypes in the 3 zones resembled those found in a close related species, *Pinus thunbergii* (SHIRAISHI, 1988b). In particular, the masson pine had 3 loci, and 1 single-banded phenotype was recognized in the case of *Adh-2*.

Shikimate dehydrogenase (ShDH)

Two active zones (zone 1 and zone 2) appeared on gels stained for ShDH (*Fig. 1*). One invariant band and 4 single-banded phenotypes were observed in zone 1 and zone 2, respectively. The bands in zone 2 segregated according to the expected ratio of 1:1 in 4 families (*Table 3*). Four phenotypes are encoded by the 4 alleles of *Sdh-2*. Although no variations appeared in zone 1, the zone was inferred to correspond to a single locus (*Shd-1*; *Fig. 1*), as observed in many *Pinus* species, for example, *P. thunbergii* (SHIRAISHI, 1988b), *P. attenuate* (STRAUSS and CONKLE, 1986), P. *koraiensis* (TOMARU *et al.*, 1990) and *P. densiflora* (NA'IEM *et al.*, 1989).

Glycerate dehydrogenase (G2DH)

An active zone was observed in gels stained for G2DH. One phenotype in this zone consisted of 1 intense band and 1 less intense band. No segregation was observed in

all the families studied. The double-banded phenotype was inferred to correspond to an allele at a single locus (*G2d*). This phenotype was consistent with those of other species of conifer (SHIRAISHI, 1988b; NA'IEM *et al.*, 1989; TOMARU et al. 1990; and SUYAMA *et al.*, 1992).

Malate dehydrogenase (MDH)

Two single-banded phenotypes could be recognized in zone 1 and in zone 2; there was only 1 band in zone 3; and 3 single-banded phenotypes were found in zone 4 (*Fig. 1*). Results of the χ^2-test revealed that the phenotypes in zone 1 and zone 2 were controlled by *Mdh-1* and *Mdh-2*, respectively, with 2 alleles each; the phenotype in zone 3 was controlled by *Mdh-3* with 1 allele; and the phenotypes in zone 4 were controlled by *Mdh-4* with 3 alleles (*Table 3*).

For MDH in masson pine, interlocus heterodimers could be observed consistently midway between the 2 allozymes that corresponded to *Mdh-1* and *Mdh-4*. Interlocus heterodimers of MDH were also reported in *Pseudotsuga menziesii* (EL-KASSABY, 1981), *Pinus ponderosa* (O'MALLEY et al., 1979), and *Pinus koraiensis* (TOMARU et al., 1990).

6-Phosphogluconate dehydrogenase (6PGD)

A total of 5 bands appeared in 2 zones that overlapped each other (*Fig. 1*). Since it was difficult to assign 1 of the 5 bands to either zone, analysis of this band was omitted from the present analysis. In zone 1, there was significant heterogeneity between the segregation in 2 families (*Table 3*). One (Y2SS1) of the 2 families showed segregation that deviated from the expected ratio. However, the segregation of bands of the other family (Y1P3) in zone 1 and the pooled segregation of bands in zone 2 fit well with the expected ratio. The phenotypes in the 2 zones are controlled by *6Pg-1* and *6Pg-2*, respectively, with 2 alleles each.

Glucose-6-phosphate dehydrogenase (G6PD)

There were 3 kinds of single-banded phenotype (*Fig. 1*). Two bands, designated Rf 19 and Rf 19H, had the same mobility, but the intensities of staining were quite different. Based on band segregation (*Table 3*), we concluded that 3 alleles were encoded at the *G6p* locus. In 2 other species, *P. thunbergii* and *P. densiflora*, that are closely

Table 3. — Segregation of allelic variants in megagametophytes of heterozygous families at 28 polymorphic loci.

Locus	Genotype	No. of family	Sample size	Segregation S : F	Heterogeneity χ^2 (df)	Heterogeneity P	Deviation χ^2 (1)	Deviation P
Adh-1	*a/b*	2	238	121 : 117	0.27(1)	0.60	0.07	0.80
Adh-3	*a/b*	1	117	60 : 57	–	–	0.08	0.78
	b/c	1	96	45 : 51	–	–	0.38	0.54
	b/o	1	18	9 : 9	–	–	0.00	–
Shd-2	*a/b*	1	34	19 : 15	–	–	0.47	0.49
	b/c	1	68	31 : 37	–	–	0.53	0.47
	c/d	1	240	122 : 118	–	–	0.07	0.80
	b/d	1	144	69 : 75	–	–	0.25	0.62
Mdh-1	*a/b*	3	239	124 : 115	1.24(2)	0.54	0.34	0.56
Mdh-2	*a/b*	3	229	108 : 121	0.07(2)	0.97	0.74	0.39
Mdh-4	*a/b*	1	30	15 : 15	–	–	0.00	–
	a/c	1	18	13 : 5	–	–	3.56	0.06
6Pg-1	*a/b*	2	132	42 : 90	7.39(1)	< 0.01	–	–
		(1	12	8 : 4	–	–	1.33	0.25) [1]
6Pg-2	*a/b*	4	407	195 : 212	2.51(3)	0.47	0.71	0.40
G6p	*a/b*	2	240	117 : 123	2.02(1)	0.16	0.15	0.70
	a/c	1	24	13 : 11	–	–	0.17	0.68
	b/c	1	120	64 : 56	–	–	0.53	0.47
Gdh	*a/b*	3	209	99 : 110	7.12(2)	< 0.05	–	–
		(2	66	40 : 26	0.36(1)	0.55	2.97	0.08) [1]
	b/o	1	30	15 : 15	–	–	0.00	–
Gr	*a/b*	3	296	150 : 146	2.95(2)	0.23	0.05	0.82
Dia-1	*a/c*	1	18	9 : 11	–	–	0.20	0.65
	b/c	3	299	153 : 146	0.66(2)	0.72	0.16	0.69
Dia-2	*a/b*	5	597	234 : 363	47.45(4)	< 0.01	–	–
		(3	281	142 : 139	3.32(2)	0.19	0.03	0.86) [1]
Dia-3	*a/b*	1	14	9 : 5	–	–	1.14	0.29
	b/c	1	110	58 : 52	–	–	0.33	0.57
	b/d	1	38	15 : 23	–	–	1.68	0.19
Dia-4	*a/b*	4	216	99 : 117	2.34(3)	0.50	1.50	0.22
	a/c	1	108	54 : 54	–	–	0.00	–
	b/o	1	12	4 : 8	–	–	1.33	0.25
Got-2	*a/b*	3	448	215 : 233	5.38(2)	0.07	0.72	0.40
	b/c	1	12	8 : 4	–	–	1.33	0.25
Got-3	*a/b*	3	401	205 : 196	2.26(2)	0.32	0.20	0.65
Pgm	*a/b*	2	154	77 : 77	2.79(1)	0.09	0.00	–
	a/c	3	404	214 : 190	1.43(2)	0.49	1.43	0.23
	a/d	2	132	74 : 58	0.12(1)	0.73	1.94	0.16
	b/d	2	192	89 : 103	0.62(1)	0.43	1.02	0.31
	c/d	1	30	17 : 13	–	–	0.53	0.47
Est-1	*a/b*	3	202	105 : 97	1.58(2)	0.45	0.32	0.57
	a/o	2	116	61 : 55	0.04(1)	0.84	0.31	0.58
	b/o	4	426	203 : 223	4.77(3)	0.19	0.94	0.33

Locus	Genotype	No. of family	Sample size	Segregation S : F	Heterogeneity χ^2 (df)	Heterogeneity P	Deviation χ^2 (1)	Deviation P
Est-2	*a/c*	2	140	68 : 72	0.01(1)	0.91	0.11	0.74
	a/o	1	14	6 : 8	–	–	0.29	0.59
	b/c	2	126	65 : 61	0.08(1)	0.78	0.13	0.72
	b/d	1	96	44 : 52	–	–	0.67	0.41
	b/e	2	174	78 : 96	2.05(1)	0.15	1.86	0.17
	b/o	1	30	14 : 16	–	–	0.13	0.72
	d/o	1	150	79 : 71	–	–	0.43	0.51
	e/o	1	24	11 : 13	–	–	0.17	0.68
Est-3	*a/c*	1	26	12 : 14	–	–	0.15	0.69
	b/c	1	25	11 : 14	–	–	0.36	0.55
	c/d	2	180	93 : 87	0.36(1)	0.55	0.20	0.65
	c/o	1	173	87 : 86	–	–	0.01	0.94
	d/o	1	94	43 : 51	–	–	0.68	0.41
Amy	*a/b*	2	59	32 : 27	2.03(1)	0.15	0.42	0.52
	a/c	2	120	63 : 57	1.88(1)	0.17	0.30	0.58
	a/e	1	125	66 : 59	–	–	0.39	0.53
	b/c	2	222	112 : 110	0.01(1)	0.93	0.02	0.89
	b/d	1	30	17 : 13	–	–	0.53	0.47
	b/e	1	96	44 : 52	–	–	0.67	0.41
	c/d	3	156	84 : 72	4.34(2)	0.11	0.92	0.34
	c/e	1	24	16 : 8	–	–	2.67	0.10
Aap-1	*a/c*	1	96	42 : 54	–	–	1.50	0.22
	a/d	2	114	46 : 68	1.80(1)	0.18	4.25	< 0.05
		(1	12	7 : 5	–	–	0.33	0.56) [1]
	b/c	2	53	21 : 32	0.73(1)	0.39	2.28	0.13
	b/d	2	153	79 : 74	0.01(1)	0.93	0.16	0.69
	c/d	1	120	63 : 57	–	–	0.30	0.58
Aap-2	*a/b*	1	29	14 : 15	–	–	0.03	0.85
	a/c	1	31	13 : 18	–	–	0.81	0.37
	c/d	1	102	48 : 54	–	–	0.35	0.55
Aap-3	*a/b*	1	31	13 : 18	–	–	0.81	0.37
	b/c	1	102	48 : 54	–	–	0.35	0.55
Fm	*a/b*	1	108	52 : 56	–	–	0.15	0.70
	b/c	1	125	62 : 63	–	–	0.01	0.93
Pgi-1	*a/b*	4	264	120 : 144	0.15(3)	0.99	2.18	0.14
	a/c	1	16	9 : 7	–	–	0.25	0.62
	b/c	1	16	7 : 9	–	–	0.25	0.62
Pgi-2	*a/b*	4	264	120 : 144	0.15(3)	0.99	2.18	0.14
	a/c	1	16	9 : 7	–	–	0.25	0.62
	b/c	1	16	7 : 9	–	–	0.25	0.62

[1] χ^2-tests for heterogeneity and/or goodness-of-fit to a ratio of 1:1 were made excluding data from families with significant segregation distortion.

related to the masson pine, 2 single-banded phenotypes with bands of quite different intensities were also observed in an analysis of G6PD (SHIRAISHI, 1988b; NA'IEM *et al.*, 1989).

Glutamate dehydrogenase (GDH)

One active zone with 3 single-banded phenotypes was evident on gels stained for GDH. The χ^2 value for heterogeneity for the observed segregation among 3 families was significant (*Table 3*). This heterogeneity was caused by 1 family (D1S1) with significant distortion of segregation from the expected ratio of 1:1. The segregation of bands showed no deviation from the expected ratio of 1:1 with this single exception. GDH was deduced to be encoded by a locus (*GdH*) with 3 alleles.

Glutathione reductase (GR)

The gels stained for GR had a single zone of activity (*Fig. 1*). Two phenotypes in this zone were observed as single-banded phenotypes. The observed segregation ratio was close to the expected ratio of 1:1 (*Table 3*), indicating that the phenotypes in this zone were controlled hy a locus (*Gr*) with 2 alleles. Two of the zones (zone 3 and zone 4, Rf 29—58) in gels stained for DIA could also be found in the gels stained for GR since the staining solution for GR also contained the substrate for DIA.

Diaphorase (DIA)

Four active zones (zones 1 through 4) were evident on gels stained for DIA (*Fig. 1*). In zone 1 and zone 2, 5 kinds of phenotype were observed, and each phenotype was represented by 1 single band. In zone 3 and zone 4, 8 kinds of phenotype were seen, and each phenotype was represented by more than 2 bands. In zone 2, there was significant heterogeneity among the segregation of families since 2 (Y2SS1 and D1S1) of the 5 families had significant segregation distortions. However, after excluding data for these 2 families, the χ^2 values for heterogeneity and the goodness-of-fit to the expected ratio of 1:1 were not significant (*Table 3*). Moreover, the results of the χ^2-test for the remaining three zones indicated that the segregation ratios agreed with the expected ratio of 1:1 for these phenotypes. Therefore, the phenotypes in zone 1 are encoded by *Dia-1* with 3 alleles; zone 2 by *Dia-2* with 2 alleles; zone 3 and zone 4 by *Dia-3* and *Dia-4*, respectively, with 4 alleles each.

The inheritance of DIA has been reported for many species of conifer. The numbers of loci that encode DIA differ, depending on the species, for example, there are 2 loci in *P. menziesii* (EL-KASSABY *et al.*, 1982b) and *P. densi-*

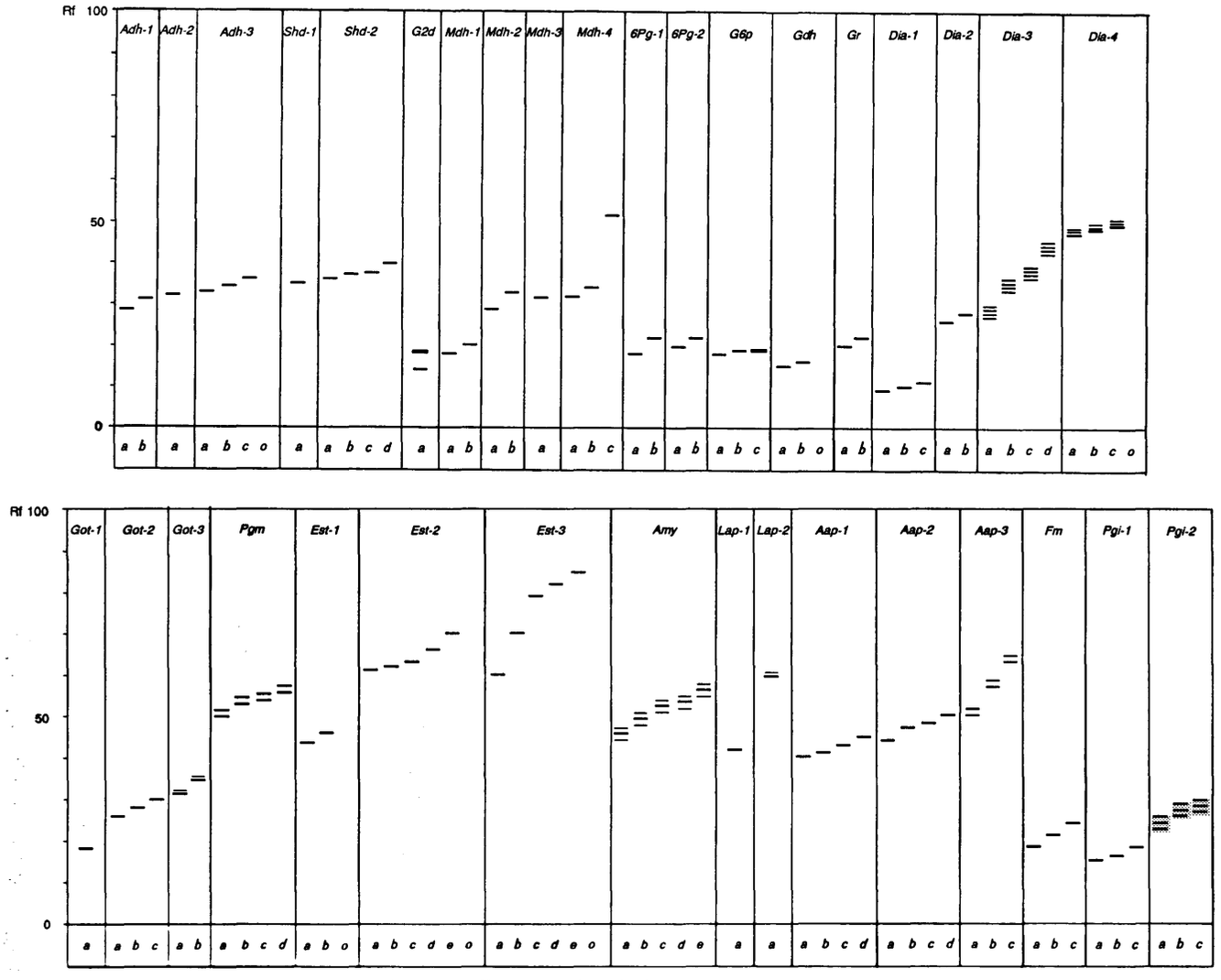

Fig. 1. — Banding patterns of allozymes at 35 loci for 17 enzymes systems in masson pine.

flora (NA'IEM *et al.*, 1989) and 3 loci in *Abies mariesii* (SUYAMA *et al.*, 1992). Phenotypes represented by 4 bands were also reported in *P. densiflora* (NA'IEM *et al.*, 1989) and *A. mariesii* (SUYAMA *et al.*, 1992).

Glutamate oxaloacetate transaminase (GOT)

There were 4 active zones on gels stained for this enzyme. In zone 1, an unstable double-banded phenotype was observed; this zone was excluded from consideration because it was difficult to analyze. No variations were observed in zone 2; 3 single-banded phenotypes appeared in zone 3; and 2 double-banded phenotypes were observed in zone 4 (*Fig. 1*). The results of χ^2-tests indicated that the phenotypes in zone 3 and zone 4 were controlled by *Got-2* and *Got-3*, respectively, and there were 3 and 2 alleles of *Got-2* and *Got-3*, respectively (*Table 3*). For zone 2, from the studies of *P. thunbergii* (SHIRAISHI, 1988b) and *P. densiflora* (NA'IEM *et al.*, 1989), the zone was inferred to correspond to *Got-1* with 1 single allele.

Phosphoglucomutase (PGM)

Two active zones were observed on gels stained for PGM. Zone 1 was excluded from the present analysis because of poor resolution. Four double-banded phenotypes appeared in zone 2 (*Fig. 1*). These 4 phenotypes are encoded by 1 locus (*Pgm*) with 4 alleles (*Table 3*). Double-banded phenotypes were also reported in *P. thunbergii* (SHIRAISHI, 1988b) and *P. densiflora* (NA'IEM et al., 1989).

Esterase (EST)

Many bands appeared on gels stained for EST. However, only 3 active zones were continuously detectable. All phenotypes observed in these zones were single-banded. Three kinds of phenotype were observed in zone 1; 6 kinds of phenotype appeared in zone 2 and in zone 3, respectively (*Fig. 1*). A non-banded phenotype was observed in each of zone 1, zone 2 and zone 3. The χ^2-tests showed that EST is encoded by at least 3 loci (*Est-1, Est-2 and Est-3; Table 3*). *Est-1* had 3 alleles; *Est-2* and *Est-3* each had 6 alleles.

Amylase (AMY)

Five triple-banded phenotypes were observed on gels stained for this enzyme (*Fig. 1*). All the families, having a total of 8 allelic combinations, segregated according to the expected ratio (*Table 3*), showing that the phenotypes in this zone were controlled by 5 alleles at 1 locus (*Amy*). Triple-banded phenotypes were also detected in *P. thunbergii* (SHIRAISHI, 1988b) and *P. densiflora* (NA'IEM, 1991).

Leucine aminopeptidase (LAP)

Two active zones (zone 1 and zone 2) appeared on gels stained for LAP. A single-banded phenotype and a double-

banded phenotype were detected in zone 1 and zone 2, respectively. According to the similar LAP phenotypes of *P. thunbergii* (SHIRAISHI, 1988b) and *P. densiflora* (NA'IEM *et al.*, 1989), the 2 zones in masson pine were inferred to correspond to *Lap-1* and *Lap-2*, respectively. Although many alleles of *Lap-1* and *Lap-2* were detected in *P. thunbergii* and *P. densiflora*, no allelic variations at the 2 loci were detected in masson pine.

Alanine aminopeptidase (AAP)

Three zones of activity were found on gels stained for AAP. In zone 1 and in zone 2, 4 single-banded phenotypes were found, and in zone 3, 3 double-banded phenotypes with low-intensity bands were recognized (*Fig. 1*). The χ^2-tests showed that the phenotypes in zone 1 and zone 2 were controlled by *Aap-1* and *Aap-2* with 4 alleles each, respectively, and the phenotypes in zone 3 were controlled by *Aap-3* locus with 3 alleles (*Table 3*). The segregation of bands of a heterozygous combination (*a* and *d*) in *Aap-1* deviated significantly from the expected ratio of 1:1, as being a single exception. Three loci encoding AAP were also reported in *P. densiflora* (NA'IEM et al., 1989). We observed another single band (Rf 42) that overlapped with the zone of *Aap-1*. This band corresponded to *Lap-1* and was generated as a result of the fact that LAP recognized the substrate for AAP (SCANDALIOS, 1969; NA'IEM *et al.*, 1989).

Fumarase (FM)

One active zone appeared on gels stained for FM (*Fig. 1*). Three phenotypes composed of a single band each were observed. The segregation ratios of haploid megagametophytes from 2 families corresponded closely to the expected ratio of 1:1 (*Table 3*). Thus, FM is encoded by 1 locus (*Fm*) with 3 alleles.

Phosphoglucose isomerase (PGI)

Two active zones were evident on gels stained for this enzyme (*Fig. 1*). In zone 1, 3 single-banded phenotypes were observed. Zone 2 was stained intensely and widely, and 3 phenotypes were observed in this zone also. Although we observed only 3 combined phenotypes in the 2 zones (that is, *a* in zone 1 and *a* in zone 2; *b*, *b*; *c*, *c*) and could not detect a recombinant phenotype, the phenotypes in each zone was inferred to be encoded by a separate locus (*Pgi-1* and *Pgi-2*). Because the segregation ratios for 6 families in each zone corresponded to the expected ratio of 1:1 (*Table 3*); and 2 closely linked loci for PGI were reported in *P. densiflora*, with phenotypes similar to those identified in masson pine (NA'IEM, 1991).

Table 5. — Recombination values, map distances, and assigned linkage groups for 2-locus (A-B) combinations.

Combination Locus *A* – Locus *B*	Sample size	No. of recomb.	Recombination value $R \pm SE_R$	Linkage group
Adh-1 – Dia-1	114	20	0.175 ± 0.036	B
Adh-1 – Pgi-1,2	142	31	0.218 ± 0.035	B
Mdh-1 – 6Pg-2	96	36	0.375 ± 0.049	D
G6p – Dia-4	119	45	0.378 ± 0.044	A
Gdh – Dia-2	144	56	0.401 ± 0.042	A
Gdh – Amy	119	47	0.395 ± 0.045	A
Dia-1 – Pgi-1,2	167	29	0.174 ± 0.029	B
Dia-2 – Fm	95	37	0.389 ± 0.050	A
Dia-3 – Est-2,3	144	26	0.181 ± 0.032	C
Est-1 – Pgi-1,2	96	38	0.396 ± 0.050	B
Est-2,3 – Aap-2,3	94	17	0.181 ± 0.040	C
Aap-2 – Aap-3	71	0	0.000 ± 0.000	C
Dia-4 – Amy	187	49	0.262 ± 0.032	A
Est-2 – Est-3	379	0	0.000 ± 0.000	C
Pgi-1 – Pgi-2	311	0	0.000 ± 0.000	B

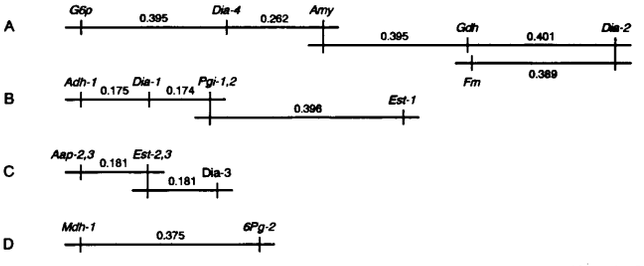

Fig. 2. — The 4 linkage groups of masson pine with estimated recombination values between loci.

Linkage relationships

We used those 26 of the 28 polymorphic loci in 13 families that had at least 1 doubly heterozygous locus for linkage analysis. The remaining 2 polymorphic loci, *Mdh-4* and *6Pg-1*, were not heterozygous in any family even when the other loci were heterozygous. Of 325 possible 2-locus combinations of loci, we were able to test linkage relationships with 187 combinations. Seventy-six combinations

Table 4. — Segregation in 2-locus (A-B) combinations with significant deviation from random joint segregation, χ^2 values for heterogeneity among families (χ^2H), deviation at locus A (χ^2A) and locus B (χ^2B), and deviation from random joint segregation (χ^2L).

Combination Locus *A* – Locus *B*	No. of families	Sample size	Segregation class *A1B1*	*A1B2*	*A2B1*	*A2B2*	Heterogeneity χ^2H (df)	*P*	Deviation χ^2A (1)	*P*	χ^2B (1)	*P*	χ^2L (1)	*P*
Adh-1 – Dia-1	1	114	9	44	50	11			0.56	0.45	0.14	0.71	48.04	<0.01
Adh-1 – Pgi-1,2	1	142	49	18	13	62			0.45	0.50	2,28	0.13	45.07	<0.01
Mdh-1 – 6Pg-2	1	96	17	23	37	19			2.67	0.10	1.50	0.22	6.00	<0.05
G6p – Dia-4	1	119	28	22	23	46			3.03	0.08	2.43	0.12	7.07	<0.01
Gdh – Dia-2	1	144	30	29	27	58			4.69	<0.05	6.25	<0.05	4.92	<0.05
Gdh – Amy	1	119	22	30	42	25			1.89	0.17	0.68	0.41	5.25	<0.05
Dia-1 – Pgi-1,2	1	167	12	75	63	17			0.29	0.59	1.73	0.19	71.14	<0.01
Dia-2 – Fm	1	95	19	33	25	18			0.85	0.36	0.52	0.47	4.64	<0.05
Dia-3 – Est-2,3	1	144	60	14	12	58			0.11	0.74	0.00	–	58.78	<0.01
Est-1 – Pgi-1,2	1	96	30	18	20	28			0.00	–	0.17	0.68	4.17	<0.05
Est-2 – Est-3	1	92	0	41	51	0			1.09	0.30	1.09	0.30	92.00	<0.01
Est-2,3 – Aap-2,3	1	94	9	41	36	8			0.38	0.54	0.17	0.68	38.30	<0.01
Aap-2 – Aap-3	1	71	35	0	0	36			0.01	0.91	0.01	0.91	71.00	<0.01
Dia-4 – Amy	2	187	69	17	32	69	7.32(3)	0.06	1.20	0.27	1.20	0.27	42.36	<0.01
Est-2 – Est-3	2	287	152	0	0	135	1.02(1)	0.31	1.00	0.32	1.00	0.32	287.00	<0.01
Pgi-1 – Pgi-2	3	311	155	0	0	156	0.11(2)	0.95	0.00	0.95	0.00	0.95	311.00	<0.01

occurred in 2 or more families, while 111 combinations were found in only 1 family. The sample size (the number of megagametophytes analyzed) in each combination ranged from 44 to 540.

Table 4 shows 2-locus segregations with non-random joint segregation for 16 combinations (including separate combinations for coupling and repulsion in *Est-2 — Est-3*). Three combinations in more than 1 family represented homogeneous joint segregations among families. For the *Gdh-Dia-2* combination, the single-locus segregation observed at both locus deviated significantly from the expected ratio of 1:1 ($P < 0.05$). A special test of linkage (Bailey, 1961; Rudin and Ekberg, 1978) was made in this case. All combinations except the *Gdh-Dia-2* combination segregated according to the expected ratio of 1:1 at each locus. All of the 16 combinations gave significant χ^2 values for joint segregation (at least, $P<0.05$). Therefore, we were able to detected 15 linkage relationships with 18 loci (*Table 4*). The recombination values for each linkage pair and the linkage map are presented in *table 5* and *figure 2*, respectively. Two (A and B) of 4 linkage groups were constructed by the 3-point mapping method; these groups were composed of 5 or more loci.

Group A

This group included 6 loci. The linear arrangement of the loci could not be determined because there were no available data with which the relationships between *Gdh* and *Dia-4* and between *Fm* and *Gdh* were examined. We did identify, however, 2 linear groupings, namely, *G6p — Dia-4 — Amy* and *Amy — Gdh — Dia-2*. The estimated recombination values for the 6 loci indicated moderate or weak linkage between loci ($R = 0.262$ to 0.401). The linkage of *Dia-4 — Amy* was identical to that of *Dia-2 — Amy* observed in *Pinus densiflora* (Na'iem *et al.*, 1993); *Dia-4* in this study corresponds to *Dia-2* in *P. densiflora*. In addition, the same linkage between *Gdh* and *G6p* was found as the moderate linkage reported in *Pinus taeda* (Conkle, 1981), *Larix decidua* subsp. *polonica* (Lewandowski and Mejnartowicz, 1991) and *Picea abies* (Muona et al., 1987).

Group B

Adh-1, Dia-1, Pgi-1,2 and *Est-1* belonged to this group. No recombinant was detected in 1 pair, *Pgi-1 — Pgi-2*, from 3 families. This pair was completely linked as it is in *P. densiflora* (Na'iem *et al.*, 1993). Although *Est-1* was weakly linked with *Pgi-1,2* ($R = 0.396$), we could not determine on which side of *Pgi-1,2 Est-1* was located. However, *Adh-1 — Dia-1* and *Dia-1 — Pgi-1,2* were strongly linked with recombination values of 0.175 and 0.174, respectively. Linkage similar to that detected in this study between *Adh-1* and *Pgi-1* has also been reported in many other members in Pinaceae, e.g., *P. taeda* (Conkle, 1981), *P. sylvestris* (Niebling *et al.*, 1987), *P. attenuata* (Strauss and Conkle, 1986), *P. albicaulis* (Furnier *et al.*, 1986), *P. ponderosa* (O'Malley *et al.*, 1979) and *P. densiflora* (Na'iem *et al.*, 1993). Furthermore, *Dia-1* was also found to be located between *Adh-1* and *Pgi-1* in *P. contorta* and *P. jeffreyi* (Conkle, 1981), and in *P. attenuata* (Strauss and Conkle, 1986). *Dia-3* (or *Mnr-3*), *Adh-2* and *Pgi-2* in these studies by others correspond to *Dia-1, Adh-1* and *Pgi-1* in the present study.

Group C

This group included *Aap-2,3, Est-2,3* and *Dia-3*. For 2 pairs, namely, *Aap-2 — Aap-3* and *Est-2 — Est-3*, since loci were completely linked, no recombinants were detected in the families studied. Two pairs (*Aap-2,3 — Est-2,3* and *Est-2,3 — Dia-3*) were found to be strongly linked with the same recombination value of 0.181, but no data were available for examination of the relationship between *Aap-2,3* and *Dia-3*. Na'iem *et al.* (1993) also detected that *Aap-2,3 — Est-2,3* linkage, in which *Aap-2 — Aap-3* and *Est-2 — Est-3* were linked completely.

Group D

Mdh-1 and *6Pg-2* were linked with a recombination value of 0.375. Similar linkage was reported in *P. sylvestris* (Niebling *et al.*, 1987), *P. ponderosa* (O'Malley et al., 1979) and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982b).

In summary the isozyme variations for 17 enzyme systems in the 43 heterozygous families studied were found to be controlled by 35 loci with 98 alleles. Out of these allozyme loci, 7 loci were found to be monomorphic, and 28 loci were found to be polymorphic. For the monomorphic loci, we postulated the genetic control of their single invariant phenotypes from comparisons with variant phenotypes reported for the closely related species *P. thunbergii* (Shiraishi, 1988b) and *P. densiflora* (Na'iem *et al.*, 1989) and for other species of conifer. Significant deviation from the expected ratio of 1:1 within a family was observed in 5 families. These deviations might have been due to causes such as meiotic drive, selection against a particular allele of a genotype or against some closely linked gene or genes around the isozyme gene, or sampling error (Adams and Joly, 1980a; Strauss and Conkle, 1986). Linkage relationships were found for a total of 18 polymorphic loci by conventional χ^2 analysis. Four linkage groups composed of 18 loci were identified, most of which were similar to those reported for many other species in Pinaceae. The linkage groups revealed in this study supported the proposed high degree of conservation of the Pinaceae genome.

The allozyme loci identified in this study will be useful as effective genetic markers in various aspects of research into the genetics of forest trees.

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Identification of Races in Eucalyptus globulus ssp globulus Based on Growth Traits in Tasmania and Geographic Distribution

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Summary

Canonical discriminant analysis of 432 open-pollinated families grouped into 46 collection localities covering the natural geographic range of *Eucalyptus globulus* ssp *globulus* was carried out on four year growth data from five trial sites in Northern Tasmania. Combining results from the analysis of growth responses with natural geographic boundaries and reported taxonomic groupings it was possible to identify 12 geographical races in the *E. globulus* ssp *globulus* base population. The three races from Victoria [(1) the Otway Ranges, (2) the Strzelecki Ranges and (3) South Gippsland], the (4) King Island, the (5) Furneaux Group, and (6) Central Western Tasmania races, showed relatively similar growth responses. Races from eastern Tasmania, [(7) Northeastern Tasmania, (8) Eastern Tasmania, (9) Jericho, and (10) Southeastern Tasmania] were distinct from the previous large group. Two small races, (11) Port Davey and (12) Lighthouse, Wilson's Promontory had very slow growth. The trees at the Lighthouse locality had a distinct shrub-like habit. The classification of four localities (South West Lavers Hill, Clarke Island, Dover and Recherche Bay) was doubtful. The variation in growth response across environments among the different races suggest that appropriate genetic groupings (tentatively the 12 geographic races identified here) should be made prior to further genetic analysis of *E. globulus* ssp *globulus* breeding populations.

Key words: *Eucalyptus globulus*, growth response, base population, canonical discriminant analysis, geographic variation.

FDC: 232.11/12; 165.52; 176.1 *Eucalyptus globulus*; (946).

Introduction

In breeding programmes, accurate predictions of parental breeding values require correct identification of races (genetically and phenotypically distinct groups within a species; King and Stansfield, 1990) when family or individual selections are carried out across these groupings. In particular, if there are fewer parents from superior races in the breeding population, or if there is unbalanced representation of races across trials, biased estimates can occur with less selection pressure being allocated to the best geographic races resulting in sub-optimal genetic progress (Quaas and Pollack, 1981). Significant gains from direct race selection have also been reported (e.g. in *E. globulus;* Orme, 1988; Almeida, 1993). Unfortunately, it is