variances in Sitka spruce in Britain. Silvae Genet. 36 (5—6): 189—194 (1987). —— Hallauer, A. R. and Miranda, J. B.: Quantitative Genetics in Maize Breeding. Iowa State Univ. Press (1981). —— Harter, H. L.: Order statistics and their use in testing and estimation. Vol. II. Documents, U.S. Govt. Print. Office, Washington, D. C. (1970). —— Harvey, W. R. and Townsend, A. M.: Selection on a combination of individual, family, and stand merit in provenance tests. Forest Sci. 31: 813—821 (1985). —— Hühn, M., Kleinschmit, J. and Svolba, J.: Some experimental results concerning age dependency of different components of variance in testing Norway spruce (*Picea abies* (L.) Karst.) clones. Silvae Genet 36: 68—71 (1987). —— Jackson, N.: Effect of ignoring full sib relationships when making half sib estimates of heritability. Theor. Appl. Genet. 65: 61—66 (1983). —— Kiss, G. and Yeh, F. C.: Heritability estimates for height for young interior spruce in British Columbia. Can. J. For. Res. 18: 158—162 (1988). —— Klein, J. I.: Development of a jack pine seed orchard by mass selection. For. Chron. 62: 143—146 (1986). —— Klein, J. I.: Genetically improved jack pine for Manitoba and Saskatchewan. Environ. Can., Can. For. Serv.. North For. Res. Centre, Edmonton, Alberta. Forest Manage. Note 18. (1982). —— Land, S. B., Bongarten, B. and Toliver, J.: Genetic parameters and selection indices from provenance/progeny tests. Proc. Workshop on Statistical Considerations in Genetic Testing. Univ. Florida, Gainesville. June 25, 1986. (1986). —— Lin, C. Y.: Index selection for genetic improvement of quantitative characters. Theor. Appl. Genet. 52: 49—56 (1978). —— Lindgren, D.: Cost-efficient number of test sites for ranking entries in field trials. Biometrics 41: 887—893 (1985). —— Magnussen, S.: Minimum age-to-age correlation in early selections. Forest Sci. 34: 928—938 (1988). —— Magnussen, S. and Yeatman, C. W.: Accelerated testing of jack pine progenies: A case study. Page 107—121. *In:* Proc. Conf. on Breeding Theory/Progeny Testing/Seed Orchards. IUFRO/ North Carolina State Univ., Oct. 13 to 17, 1986, Williamsburg, Va. (1987a). —— Magnussen, S. and Yeatman, C. W.: Early testing of jack pine. I. Phenotypic response to spacing. Can. J. For. Res. 17: 453—459 (1987b). —— Magnussen, S. and Yeatman, C. W.: Early testing of jack pine. II. Variance and repeatability of stem and branch characters. Can. J. For. Res. 17: 460—465 (1987). —— Magnussen, S. and Yeatman, C. W.: Height growth and survival of within and between provenance crosses in jack pine. Can. J. For. Res. 18: 1145—1151 (1988). —— Magnussen, S. and Yeatman, C. W.: Provenance hybrids in jack pine: 15-year results in eastern Canada. Silvae Genet. 37: 206—218 (1988). —— Martell, D. L. and Fullerton, J. M.: Decision analysis for jack pine management. Can. J. For. Res. 18: 444—452 (1988). —— Matheson, A. C. and Raymond, C. A.: A review of provenance × environment interaction: Its practical importance and use with particular reference to the tropics. Commonw. For. Rev. 65: 283—302 (1986). —— Namkoong, G., Snyder, E. B. and Stonecypher, R. W.: Heritability and gain concepts for evaluating breeding systems such as seedling orchards. Silvae Genet. 15: 76—84 (1966). —— Namkoong, G.: Introduction to Quantitative Genetics in Forestry. USDA For. Serv. Techn. Bull. No. 1588, Washington, D. C. (1979). —— Namkoong, G. and Conkle, M. T.: Time trends in genetic control of height growth in ponderosa pine. Forest Sci. 22: 2—12 (1976). —— Namkoong, G., Usanis, R. A. and Silen, R. R.: Age-related variation in genetic control of height growth in Douglas-fir. Theor. Appl. Genet. 42: 151—159 (1972). —— Ontario Ministry of Natural Resources: Forestry Statistics 1986. (1986). —— Ontario Ministry of Natural Resources: Tree Improvement Master Plan. Northern Dev. Group., Timmins. (1987). —— Polk, R. B.: Heritabilities of some first-order branching traits in *Pinus banksiana* Lamb. Proc. 8th Central States For. Tree Improv. Conf. Ed. R. B. Polk. School For. Fish. Wildlife, Univ. Missouri (1972). —— Ponzoni, R. W. and James, J. W.: Possible biases in heritability estimates from intraclass correlation. Theor. Appl. Genet. 53: 25—27 (1978). Ralston, R. A.: Some effects of spacing on jack pine development in lower Michigan after twenty-five years. Mich. Acad. Sci. Arts Letters 38: 137—143 (1953). —— Rehfeldt, J.: Veracity of short-term testing. page 118—146. *In:* Proc. USDA Forest Serv. Servicewide Genetics Workshop, Charleston, SC. (1983). —— Riemenschneider, D. E.: Jack pine: Creating an alternative for the future. Eds.: G. D. Mroz and J. F. Berner, *In:* Artifical Regeneration of Conifers in the upper Great Lakes Region. Mich. Tech. Univ., Houghton, Mich. (1982). —— Rudolph, T. D. and Yeatman, C. W.: Genetics of jack pine. USDA For. Serv., Res. Pap. WO-38, 60 pp. (1982). —— Sattertwaite, F. F.: An approximate distribution of estimates of variance components. Biometrics. Bull. 2: 110—114 (1946). —— Searle, S.: Matrix Algebra Useful for Statistics. John Wiley and Sons (1982). —— Sittmann, K. and Tyson, H.: Estimates of inbreeding in *Pinus banksiana*. Can. J. Bot. 49: 1241—1245 (1971) —— Skrøppa, T.: A critical evaluation of methods available to estimate the genotype x environment interaction. Stud. For Suecica 166: 3—14 (1984). —— Smyth, J. H., and Ramsay, K. L.: Selected forestry statistics — Ontario, 1984. Can. For. Serv. Great Lakes For. Res. Cent. Misc. Rep. No. 13. (1984). —— Snedecor, G. W. and Cochran, W. G.: Statistical Methods. Sixth Ed. Iowa Univ. Press (1971). —— Snyder, T. P., Stewart, D. A. and Strickler, A. F.: Temporal analysis of breeding structure in jack pine (*Pinus banksiana* Lamb.). Can. J. For. Res. 15: 1159—1166 (1985). —— Tai, G. C. C.: A method to construct confidence interval for expected response to multi-trait selection. Theor. Appl. Genet. 71: 595—599 (1986). —— Tallis, G. M.: Sampling errors of genetic correlation coefficients calculated from analyses of variance and covariance. Aust. J. Statistics 1: 35—43 (1959). —— Westcott, B.: Some methods of analyzing genotype-environment interaction. Heredity 56: 243—253 (1986). —— Yamada, Y.: Genotype by environment interaction and genetic correlation of the same trait under different environments. Jap. J. Genet. 37: 498—509 (1962). —— Yeatman, C. W.: The jack pine genetics program at Petawawa Forest Experimental Station 1950 to 1970. Pub. no. 1331. Dept. Environment. Can. For. Ser., Ottawa (1974). —— Yeatman, C. W.: A progeny test of Ottawa valley jack pine — 6-year results. Proc. 9th Central States Tree Improv. Conf., Cct. 1974, pp 71—84 (1975). —— Yeh, F. C. and Heaman, J. C.: Estimating genetic parameters of height growth in seven-year old coastal Douglas-fir from disconnected diallels. Forest Sci. 33: 946—957 (1987). —— Ying, C. C. and Morgenstern, E. K.: Correlations of height growth and heritabilities at different ages in white spruce. Silvae Genet. 28: 181—185 (1979).

Inheritance and Linkage of Isozyme Variants from Seed and Vegetative Bud Tissues in Coastal Douglas-fir [Pseudotsuga menziesii var. menziesii (Mirb.) Franco][1])

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Summary

Isozyme variants of 19 enzyme systems coded by 28 genetic loci from seed [megagametophyte (1n) and embryo (2n)] and dormant vegetative bud tissues of coastal

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Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (MIRB.) FRANCO] were assayed by electrophoresis. Mendelian inheritance was confirmed for isozymes at 27 loci by testing the fit of band-pattern segregations in megagametophytes (egg gametes) or pollen gametes from heterozygous parent trees to the expected 1:1 ratio. Comparing isozyme band patterns in bud tissue from seed orchard clones with expected patterns from megagametophyte analysis confirmed that 20 loci of the 27 loci are also expressed in dormant vegetative bud tissues. The 28th locus (*Sdh*), which expressed only in bud tissue, was inferred by comparing band patterns in buds with those in needle tissues, where genetic control had been previously determined. Allelic pairs at three loci showed segregation distortion in the gametes of one sex, but not in the other sex, suggesting that selection leading to segretation distortion occurred at the gametic stage rather than among zygotes. Linkage relationships were examined for 155 pairs of loci showing joint segretation. Four linkage blocks, involving 19 of the loci, were identified.

Key words: Isozymes, inheritance, Douglas-fir, linkage, electrophoresis, seed tissues, bud tissues.

Introduction

Verification of the inheritance of isozymes is a prerequisite to their use in forest genetic studies. Mendelian inheritance has been demonstrated for a large number of species, including Douglas-fir *[Pseudotsuga menziesii* var. *menziesii* (MIRB.) FRANCO] (EL-KASSABY *et al.*, 1982a, 1982b; NEALE *et al.*, 1984). Although the tissues used in isozyme studies are mostly from newly germinated seeds (megagametophyte and embryo), tissues from young seedlings (YANG *et al.*, 1977), mature needles (MITTON *et al.*, 1979; CHELIAK and PITEL, 1984a; NEALE *et al.*, 1984; MORAN and ADAMS, 1989), dormant vegetative buds (CHELIAK and PITEL, 1984a; NEALE, 1985; MORAN and ADAMS, 1989), bark (COPES, 1979; PITEL and CHELIAK, 1982), and roots (WEBER and STETTLER, 1981) have also been used. Very few studies, however, have demonstrated interitance of isozymes from seeds and mature tissues of the same genetic materials (CHELIAK and PITEL, 1984a; *et al.*, 1984).

We have employed electrophoretic analysis in a range of studies of genetic variation in Douglas-fir in natural populations (MERKLE and ADAMS, 1987; LI and ADAMS, 1989; MORAN and ADAMS, 1989), managed stands (NEALE, 1985) and seed orchards (SMITH and ADAMS, 1983; OMI and ADAMS, 1986). We used seed tissues unless trees were too young to produce seed or there were no cones. In such cases mature tissues were used. When combining or comparing genetic data collected from different tissue types, it is important to be sure that isozyme variants from different tissues are indeed coded by the same alleles.

Our objectives in this study were to examine the genetic control of isozymes in seed and bud tissues in Douglas-fir, and to compare our findings to earlier reports on the inheritance of seed (EL-KASSABY *et al.*, 1982a, 1982b) and needle tissue (NEALE *et al.*, 1984) isozymes in this species. In this paper we: 1) describe electrophoretic procedures for assaying isozymes from seeds and dormant vegetative buds in Douglas-fir, 2) present evidence for the inheritance of isozyme variants from 19 enzyme systems, including additional information on 18 loci identified earlier and new evidence for 10 loci not previously verified, and 3) identify some linkage relationships among loci.

Materials and Methods

Tissue Preparation and Enzyme Extraction

Seed and bud tissues were obtained from individual trees or clones which had been used in previous studies, including: wind-pollinated seeds from a large number of trees in stands in southwest Oregon (MERKLE and ADAMS, 1987); wind-pollinated seeds from over 200 clones in a seed orchard near Corvallis, Oregon (ADAMS, 1983; SMITH and ADAMS, 1983); dormant vegetative buds from 80 of the clones in this orchard; and control-pollinated seeds from crosses of parent trees in Western Oregon and Washington breeding programs (ADAMS *et al.*, 1988).

Seeds were stratified for 48 hr in 1% H_2O_2 and then placed on moist filter paper in petri dishes in a seed germinator (12-hr photoperiod, 26° C day, 21° C night) until germinated radicles were 3 mm to 10 mm long, which normally occurred within 3 to 5 days. Megagametophytes and embryos were excised from seed coats and separated for analysis. In wind-pollinated seedlots, only the haploid (1n) megagametophytes were assayed, but in control-pollinated seedlots, the megagametophyte and embryo of each seed were assayed side-by-side on the same gel. Extracts were prepared by crushing seed tissues in two to three drops of 0.20 M phosphate buffer (ph 7.5) (CONKLE *et al.*, 1982).

Small branches with dormant vegetative buds, collected from individual orchard ramets, were stored in plastic bags at 4° C. Three bud primordia, excised from bud scales, were crushed in three drops of the extraction buffer given by CHELIAK and PITEL (1984a). Buds could be collected from December to mid-April and refrigerated for up to 90 days without affecting band-pattern expression. In both the seed and bud tissues, enzyme activity was best maintained when extracts were kept on ice during processing.

Electrophoresis

Seed and bud tissue extracts were absorbed onto 3.5-mm wide filter paper wicks (Whatman chromatography no 3 mm), which were then inserted into a vertical slice in 11% starch gels (Sigma Chemical Co., St. Louis, MO, USA). Gel preparation and dimensions were nearly identical to those described in CONKLE *et al.* (1982). We loaded 44 wicks per gel for seed tissue assays and, because of slightly poorer resolution, 36 wicks for bud tissues. Gels were placed on plexiglass forms which contained the tray buffers (see CONKLE *et al.*, 1982). Gel and tray buffers are given in *Table 1*. Gels were run under refrigeration (4° C) for 15 minutes before wicks were removed. Electrophoresis continued until a dye marker (dilute red food coloring) had migrated 8 cm from the origin for buffer systems A and B, and 6 cm for buffer systems C, D, and E. Gels were run at constant amperages (see footnote 1, *Table 1*), with the exception that they were not allowed to exceed 320 V. Gels were then cut horizontally into four to six slices, stained, and incubated in the dark at 37° C. Stain recipes in *Table 1* are modifications of those in CONKLE *et al.* (1982) and CHELIAK and PITEL (1984b).

Determination of Genetic Control

Evidence for the single-locus inheritance of isozymes from seed tissues was determined by assaying 10 to 60 ($\bar{n}$ = 24.6) megagametophytes from each of 82 individual parent trees or clones, which together were known to include heterozygotes for 27 putative loci. Parent trees

segregating at the same locus were first tested for heterogeneity of allelic segregation by a chi-square test. If this test was nonsignificant ($P > 0.05$), data from individual trees were combined and a chi-square goodness-of-fit test was performed to test the expected 1:1 ratio for segregating isozymes (Adams and Joly, 1980a). Further evidence for the genetic control of isozymes was obtained by assaying embryos resulting from control pollinations. The isozyme genotypes of the male and female parents were already known (Adams *et al.*, 1988). Thus, single-

Table 1. — Enzyme systems, gel and tray buffers, and staining recipes for starch gel electrophoresis of seed and vegetative bud tissue isozymes in coastal Douglas-fir.

Enzyme	Gel and tray buffers[1]	Staining recipe[3] Buffer[2]	Substrate	Other	
F-EST[4]	C	0.07 M Na Acetate pH 5.0 (30 ml)	4-methylumbelliferyl acetate 1.5 mg	Acetone	10 ml
PGM	A (seeds) E (buds)	0.10 M Tris pH 8.0	α-D-glucose-1-phosphate 0.140 g	NADP	0.020 g
				MTT (seeds) or NBT (buds)	0.025 g
				PMS	0.010 g
				$MgCl_2$ (10%)	2.0 ml
				G-6-PD	100 units
				α-D-glucose-1,6-diphosphate	0.002 g
PGI	A	0.10 M Tris pH 8.0	D-fructose-6-phosphate 0.036 g	NADP	0.014 g
				MTT	0.015 g
				PMS	0.003 g
				$MgCl_2$ (10%)	1.0 ml
				G-6-PD	50 units
LAP	A	0.1 M Tris-maleate pH 3.8 adjust pH to 8.0 with 1 N NaOH	L-leucine β-napthylamide 0.02 g	Black K salt	0.020 g
PEP	A	0.20 M Tris pH 8.0	L-leucylglycyl-glycine 0.080 g	Crude peroxidase	0.020 g
				Snake venom, (Crotalus atrox)	0.020 g
				O-Dianisidine DiHCl	0.100 g
MPI	B	0.04 M Tris pH 8.0	D-mannose-6-phosphate 0.050 g	NADP	0.010 g
				NBT	0.010 g
				PMS	0.006 g
				G-6-PD	80 units
				PGI	100 units
GDH	B	0.05 M Tris pH 8.5	L-glutamic acid 5.500 g	NAD	0.028 g
				NBT	0.028 g
				PMS	0.009 g
SOD	B	0.05 M Tris pH 8.5	--	NAD	0.028 g
				NBT	0.028 g
				PMS	0.009 g
GOT	B	0.20 M Phosphate buffer pH 7.5 (66 ml)	0.5% Pyroxidol-5-phosphate 2.1 ml	Fast blue BB salt	0.320 g
			3.0% Bovine albumim 4.2 ml	H_2O	21.0 ml
			0.2 M L-aspartic acid 18.2 ml		
			0.1 M α-ketoglutaric acid 5.4 ml		
G-6-PD	B	0.20 M Tris pH 8.0	D-glucose-6-phosphate 0.020 g	NADP	0.014 g
				MTT	0.015 g
				PMS	0.006 g
				$MgCl_2$ (10%)	0.400 ml
GLYD	B	0.10 Tris pH 8.5	DL-glyceric acid hemiCa salt 0.500 g	NAD	0.020 g
				MTT	0.020 g
				PMS	0.009 g
CAT	B	0.26 M Phosphate buffer pH 6.5	Potassium iodide solution (2%) 100 ml		
			H_2O_2 (0.03%) 100 ml		

Enzyme	Gel and tray buffers[1]	Staining recipe[3] Buffer[2]	Substrate	Other	
F-HEX[4]	C	0.05 M Citrate/ phosphate buffer pH 4.0 (15 ml)	4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide 0.030 g		
6-PGD	C	0.10 M Tris pH 8.0	Na_3-6-phosphogluconic acid 0.024 g	NADP MTT PMS $MgCl_2$ (10%)	0.010 g 0.015 g 0.004 g 0.500 ml
IDH	C (seeds) E (buds)	0.10 M Tris pH 8.0	DL-isocitric acid 0.020 g	NADP MTT PMS $MgCl_2$ (10%)	0.010 g 0.015 g 0.004 g 0.500 ml
SDH	C	0.10 M Tris pH 8.0	Shikimic acid 0.200 g	NADP MTT PMS	0.020 g 0.020 g 0.006 g
ACO	A	0.20 M Tris pH 8.0	Cis-aconitic acid 0.100 g	NADP NBT PMS $MgCl_2$ (10%) IDH	0.020 g 0.020 g 0.009 g 1.00 ml 80 units
DIA	C	0.20 M Tris pH 8.0	2,6-dichlorophenol-indophenol 0.004 g	β-NADH MTT	0.020 g 0.020 g
MDH	D (seeds) E (buds)	0.20 M Tris pH 8.0 (50 ml)	0.5 M DL-malic acid pH 7.0 50 ml	NAD NBT PMS	0.020 g 0.020 g 0.009 g

[1]) Gel and electrode buffers.
A Stock a: 1.8 g LiOH, 11.89 g H_3BO_3, H_2O to 1 liter., pH 8.3.
Stock b: 6.2 g TRIS, 1.6 g citric acid (monohydrate), H_2O to 1 liter, pH 8.3.
Gel: mix 1 part stock a and 9 parts stock b.
Electrode: straight stock a.
Running conditions: 65 mA for 12-mm thick gels; 50 mA for 9-mm thick gels.
B Gel: 12.11 g TRIS, 1.45 g citric acid (monohydrate), H_2O to 1 liter., pH 8.8.
Electrode: 18.55 g H_3BO_3, 4.0 g NaOH, H_2O to 1 liter., pH 8.6.
Running conditions: 70 mA for 12-mm thick gels; 50 mA for 9-mm thick gels.
C Stock: 7.69 g citric acid (anhydrous), H_2O to 1 Liter., adjust pH to 6.1 with N-(3-aminopropyl) morpholine.
Gel: 50 ml stock, 950 ml H_2O.
Electrode: straight stock.
Running conditions: 50 mA for 12-mm thick gels, 45 mA for 9-mm thick gels.
D Gel: 0.96 g DL-histidine HCl, adjust to pH 7.0 with NaOH, H_2O, to 1 liter.
Electrode: 105.82 g sodium citrate, adjust to pH 7.0 with 0.41 M citric acid, H_2O to 1 liter.
Running conditions: 60 mA for 12-mm thick gels, 45 mA for 9-mm thick gels.
E Same as buffer D except gel and electrode buffers adjusted to pH 8.0.
[2]) All buffers 100 ml unless otherwise noted.
[3]) Stain recipes for four gel slices.
[4]) Score bands on gels within five minutes under longwave UV light.

locus segregation of isozymes in pollen gametes from heterozygous male parents could be determined and tested for fit to the expected 1:1 ratio.

Evidence for the inheritance of isozymes from bud tissues was determined indirectly. Multilocus isozyme genotypes of 209 seed orchard clones had been previously inferred from samples of at least 10 megagametophytes per clone (Adams, 1983; Smith and Adams, 1983). The probability of correctly inferring a clones's genotype at any one locus from a sample of this size is > 0.99 (Morris and Spieth, 1978). Preliminary analysis indicated that isozyme bands coded by 20 of the loci identified in seeds could also be scored in bud tissues (*Table 2*). Buds were then assayed from 80 to of the orchard clones and their band pattern phenotypes compared with those expected based on the genotypes of the 20 loci determined from megagametophyte samples.

All segregating pairs of loci for which there was a total sample of at least 40 megagametophytes from heterozygous mother trees or clones, were included in the linkage analysis. Fifty-one parents, with samples of 15 to 40 megagametophytes from each, were involved. Chi-square tests for linkage were performed following the procedures described in Adams and Joly (1980b) on 155 of the 351 possible pairs of the 27 loci identified in seed tissues. Recombination fractions (r) were estimated for all locus pairs with joint segregations significantly different from expectation ($P < 0.05$).

Table 2. — List of alleles at 28 loci observed in one or more of three tissue types [seeds (S), dormant vegetative buds (B), needles (N)] of coastal Douglas-fir, and relative positions of isozyme bands they code.

Locus	Allele[1] number	Relative band positions (Rf x 100)[2] S[3]	B	N[4]
F-est2	1	54‡	54	Not
	2*	53	42	scorable
	3	46	--	
Pgm1	1	--	88	--
	2	64	85	70
	3*	61	82	66
	4	60	77	62
	5	58	73	--
Pgm2	1	40	47	--
	2	36	40	42
	3*	33	35	38
	4	30	30	--
Pgi1	1	55‡	Not	Not
	2*	53	scorable	scorable
	n	null		
Pgi2	1	29[5]	29[5]	32[5]
	2	26	--	--
	3*	23	23	24
	4	16	--	21
	5	11	11	--
Lap1	1	66	--	Not
	2*	64 or 64/68	65	scorable
	3	61 or 61/64	62	
	4	60 or 60/64	60	
	n	null	--	
Lap2	1	59	--	Not
	2	58	58	scorable
	3*	56	56	
	4	55	55	
	5	54	--	
	n	null	--	
Pep2	1	36‡	Not	Not
	2*	35	scorable	investigated
	n	null		
Mpi1	1*	89	Not	Not
	2	84	scorable	investigated
	n	null		
Gdh	1	35	--	35
	2*	33	33	34
	3	29	--	--
	4	26	--	--
Sod	1	74	76	Not
	2*	61	61	scorable
	3	49	--	
	4	38	40	
	5	24	26	
	n	null	--	
Got1	1	64	--	No[6]
	2*	60	60	activity
	3	54	56	
Got2	1	51	51	Not
	2*	46	46	scorable
	3	38	40	
	n	null	--	
Got3	1	31/41/51	--	
	2	21/33/39	21/39	No[6]
	3	16/26/43	--	activity
	4*	10/25/35	10/35	
	5	1/15/28	1/28	
	n	null	--	
G-6pd	1	63	--	--
	2	56	--	--
	3	54	--	--
	4*	51	51	58
	5	46	46	51
	6	40	40	45
	n	null	--	--

Locus	Allele[1] number	Relative band positions (Rf x 100)[2] S[3]	B	N[4]
Glyd	1	31	No	--
	2*	24	activity	26
	3	15		23
	4	5		--
Cat	1	20	No	Not
	2	15	activity	investigated
	3*	9		
	4	1		
F-hex	1	13‡	Not	Not
	2*	3	scorable	investigated
6-Pgd2	1	42	42	35
	2*	37	37	30
	3	33	33	--
	4	27	27	18
Idh	1	27	--	--
	2	23	40	35[7]
	3	20	--	
	4	17	--	25[7]
	5*	15	30	
	6	10	23	12[7]
	7	5	17	
	n	null	--	--
Sdh	1	Not	30	32
	2*	scorable	23	26
	3		20	24
Aco1	1	46	45	Not
	2*	44	44	investigated
	3	40	40	
Aco2	1	41	--	Not
	2*	39	39	investigated
	3	36	34	
	n	null	--	
Dia2	1	47	--	--
	2	43	43	40
	3	40	--	37
	4*	37	37	33
	5	33	33	--
Mdh1	1	60	60	58
	2*	53	53	50
	3	42	42	37
Mdh2	1	62‡	--	No
	2	57	67	activity
	3*	47	47	
	4	33	33	
	5	28	--	
Mdh3	1	47	47	No
	2*	33	33	activity
	3	26	--	
	4	23	20	
	5	13	--	
	6	7	3	
Mdh4	1	22‡	No	No
	2*	13	activity	activity
	3	10		
	4	7		
	n	null		

[1]) The asterisk (*) indicates the allele most commonly observed in the samples of materials used in this study.

[2]) Rf is the migration distance of the isozyme band(s) coded by the corresponding allele, relative to the migration distance of the marker dye (8 cm from origin). A dash indicates that an isozyme band corresponding to the particular allele was not observed in the sample of materials for that tissue type. Isozyme bands in italics are those for which allelism has not been confirmed either by segregation tests or by comparison of band pattern phenotypes in one tissue with phenotypes expected from genotypes confirmed in another tissue (see text).

[3]) ‡ indicates loci where bands were scorable only in the megagametophyte tissue of seeds. In all other cases, bands were scorable in both embryos and megagametophytes.

[4]) Information on needle tissue isozymes comes from Neale *et al., 1984.*

[5]) *Pgi2* variants were multi-banded (*Figure 1*). The Rf value for the slowest (most cathodal) band is given.

[6]) Bands were found in GOT1 and GOT3 zones of needle tissue gels, but were not controlled by the same loci coding GOT1 and GOT3 bands in seed and bud tissues (see text).

[7]) Because of poor resolution of close migrating bands in needle tissue, each IDH variant that could be identified in needles corresponded to a pair of allozymes expressed in seeds.

Nomenclature

An enzyme and its band phenotypes were designated by the enzyme's abbreviation in capital letters; each locus of an enzyme was specified by the enzyme's abbreviation in italics, with only the first letter capitalized. Many of the enzymes assayed exhibited more than one zone of activity. For such systems, the fastest migrating zone (locus) was designated by a 1 following the enzyme abbreviation, the second zone by a 2, etc. The same convention was used for numbering variants (alleles) within each zone. "Nulls", isozymes that lacked staining activity under the conditions of our assays, were specified by a lowercase "n".

Table 3. — Observed allozyme segregation in megagametophytes of heterozygous parent trees and goodness-of-fit to the expected 1:1 ratio.

Locus	Allelic[1] combination F	S	Observed no. F	Total	Deviation $X^2(1)$	P	Heterogeneity X^2 (df)	P
F-est2	1	2	106	226	0.75	.25-.50	7.64(6)	.25-.50
	2	3	13	20	1.25	.10-.25	--	--
Pgm1	2	3	236	450	0.98	.25-.50	16.78(15)	.25-.50
	2	4	20	40	0.03	.75-.90	--	--
	3	4	87	192	1.51	.10-.25	7.21(6)	.25-.50
	3	5	9	19	0.00	>.95	--	--
Pgm2	1	3	38	88	1.38	.10-.25	2.79(2)	.10-.25
	2	3	64	137	0.47	.25-.50	8.66(3)	.01-.05
	3	4	9	24	1.04	.25-.50	--	--
Pgi1	1	2	8	15	0.00	>.95	--	--
Pgi2	1	3	21	40	0.03	.75-.90	--	--
	2	3	26	40	3.03	.05-.10	--	--
	3	4	26	47	0.34	.50-.75	2.24(2)	.25-.50
	3	5	22	48	0.19	.50-.75	1.89(2)	.25-.50
Lap1	1	2	6	15	0.27	.50-.75	--	--
	2	3	151	300	0.00	>.95	2.71(8)	>.95
	2	4	106	222	0.37	.50-.75	5.09(7)	.50-.75
	2	n	22	44	0.02	.75-.90	0.00(1)	>.95
	3	4	197	370	1.43	.10-.25	12.90(11)	.25-.50
Lap2	1	3	19	40	0.03	.75-.90	--	--
	3	4	34	93	6.19	.01-.05	0.16(2)	.90-.95
	3	5	32	60	0.15	.50-.75	2.14(1)	.10-.25
	3	n	23	40	0.63	.25-.50	--	--
Pep2	2	n	18	33	0.12	.50-.75	0.61(1)	.25-.50
Mpi1	1	2	10	19	0.00	>.95	--	--
	1	n	11	19	0.21	.50-.75	--	--
Gdh	2	3	39	80	0.01	.90-.95	0.45(1)	.25-.50
	2	4	3	12	2.08	.10-.25	--	--
Sod	1	2	9	20	0.05	.75-.90	--	--
	2	4	37	65	0.99	.25-.50	2.43(3)	.25-.50
Got1	1	2	14	27	0.00	>.95	--	--
Got2	1	2	146	295	0.01	.90-.95	11.43(12)	.25-.50
	2	3	43	85	0.00	>.95	2.68(1)	.10-.25
Got3	2	4	75	146	0.06	.75-.90	6.19(5)	.25-.50
	3	4	14	31	0.13	.50-.75	--	--
	4	5	52	104	0.01	.90-.95	0.27(2)	.75-.90
	4	n	11	24	0.04	.75-.90	--	--
G-6pd	2	4	14	28	0.04	.75-.90	--	--
	3	4	359	709	0.09	.75-.90	28.80(23)	.10-.25
	3	6	25	60	1.35	.10-.25	0.14(1)	.50-.75
	4	5	24	52	0.17	.50-.75	0.09(1)	.75-.90
	4	6	11	20	0.05	.75-.90	--	--
	4	n	11	20	0.05	.75-.90	--	--

Locus	Allelic[1] combination F	S	Observed no. F	Total	Deviation $X^2(1)$	P	Heterogeneity X^2 (df)	P
Glyd	1	3	16	38	0.66	.25-.50	--	--
	2	3	321	711	6.50	.01-.05	35.63(25)	.05-.10
	2	4	21	33	1.94	.10-.25	0.89(2)	.50-.75
Cat	1	3	342	712	1.02	.10-.25	20.16(23)	.25-.50
	1	4	13	24	0.04	.75-.90	--	--
F-hex	1	2	255	508	0.00	>.95	14.67(14)	.25-.50
6pgd2	1	2	60	128	0.38	.50-.75	6.56(3)	.05-.10
	2	3	30	66	0.38	.50-.75	0.00(2)	>.99
	2	4	44	88	0.01	.90-.95	0.01(1)	.90-.95
Idh	1	5	9	18	0.06	.75-.90	--	--
	2	5	152	306	0.00	>.95	13.93(9)	.10-.25
	3	5	43	89	0.05	.75-.90	1.04(2)	.50-.75
	4	5	11	20	0.05	.75-.90	--	--
	5	6	18	40	0.23	.50-.75	--	--
	5	7	12	27	0.15	.50-.75	--	--
	5	n	10	20	0.05	.75-.90	--	--
Aco1	1	2	11	20	0.05	.75-.90	--	--
	2	3	61	118	0.08	.75-.90	5.26(2)	.05-.10
Aco2	1	2	73	128	2.26	.10-.25	3.01(2)	.10-.25
	2	3	74	143	0.11	.50-.75	0.40(3)	.90-.95
	2	n	9	21	0.19	.50-.75	1.29(1)	.25-.50
Dia2	1	2	11	24	0.04	.75-.90	--	--
	2	4	205	420	0.19	.50-.75	12.85(14)	.50-.75
	3	4	25	44	0.57	.25-.50	2.75(2)	.25-.50
	4	5	33	60	0.42	.50-.75	0.30(1)	.50-.75
Mdh1	1	2	9	18	0.06	.75-.90	--	--
	2	3	56	132	2.74	.05-.10	3.84(3)	.25-.50
Mdh2	2	3	17	36	0.03	.75-.90	--	--
	3	4	15	30	0.03	.75-.90	0.60(1)	.25-.50
	3	5	19	40	0.03	.75-.90	--	--
Mdh3	1	4	26	55	0.07	.75-.90	0.87(1)	.25-.50
	2	3	12	20	0.45	.50-.75	--	--
	2	4	212	391	2.62	.10-.25	11.37(12)	.25-.50
	2	6	10	20	0.05	.75-.90	--	--
Mdh4	1	2	6	20	2.43	.10-.25	--	--
	2	4	9	20	0.05	.75-.90	--	--
	2	n	23	40	0.63	.25-.50	--	--

[1]) Alleles coding faster (F) and slower (S) migrating isozyme bands of each allelic pair. "n" designates a null allele.

Results

Single-Locus Segregation

Isozyme band patterns (*Figure 1*) and evidence for their inheritance (*Table 3, Table 4*) will be presented separately for each enzyme. Unless otherwise stated, the isozymes in each zone of activity segregated according to the expected 1:1 ratio, indicating single-locus control (*Table 3, Table 4*), supporting results of EL-KASSABY *et al.* (1982b) and NEALE *et al.* (1984) for 18 of the enzymes. All data on needle tissue isozymes presented in *Table 2* and discussed in the text are from the work of NEALE *et al.* (1984). The term "seed" in this paper refers to both embryo and megagametophyte tissues.

Isozyme band pattern phenotypes scorable in buds were alyays those excepted given the tree genotypes inferred from megagametophyte analysis. With the exception of GOT, the same results were found when needle and seed tissue banding patterns were compared. Thus, for most enzymes in which comparisons were possible, isozymes in a particular zone appear to be under the control of the same gene-locus in seeds, buds, and needles. Since isozyme expression was similar in seeds and buds, only seed tissue band patterns are illustrated in *Figure 1*. In most cases, the only difference observed in the expression of the same isozyme in different tissues was in the absolute distances bands migrated on the gel, and these differences were generally minor (*Table 2*). Because dif-

ferent parent trees or clones were assayed for the different tissue types, isozymes observed in one tissue were not always detected in the other tissue.

Fluorescent esterase, F-EST1, F-EST2: Gels stained for fluorescent esterase displayed two zones of activity in seeds and buds, but did not give readable band patterns in needles. F-EST1 was not scorable in seed tissues, so segregation data were not available to validate genetic control. This zone, however, was variable among clones in bud tissue and had band-pattern phenotypes compatible with single-locus inheritance and single-banded isozymes.

F-EST2 was scorable in both megagametophytes and buds, but was not resolved well enough to be readable in embryos. Three alleles were detected at the *F-est2* locus in megagametophyte samples (*Figure 1*, *Table 3*), two of which were also detected in buds (*Table 2*).

Heterozygotes had triple-banded phenotypes in buds, with the slowest and fastest migrating bands in the same positions as the single-banded phenotypes of homozygotes for the corresponding alleles. These band patterns indicate that F-EST2 has a dimeric subunit structure, a conclusion reached for F-EST expressed in needle tissues of ponderosa pine (*Pinus ponderosa* DOUGL. ex LAWS) (MITTON *et al.*, 1979).

Phosphoglucomutase, PGM1, PGM2: Both zones of activity gave clear banding patterns in seeds, buds, and needles (*Figure 1*, *Table 2*). All four alleles of *Pgm1* found in seeds (*Table 3*, *Table 4*) were also detected in buds, but only three were observed in needles (*Table 2*). Isozyme band PGM1-1 was found only in buds of clones with no corresponding megagametophyte samples, so it was not possible to confirm that it is coded by an allele *Pgm1-1* at the same locus, but this is the most likely explanation.

Although, in all cases, the combined data over parent trees indicated that allozymes of *Pgm2* segregate according to the 1:1 expectation, significant heterogeneity of segregation ratios among parent trees was found for allelic combination *Pgm2-2/3* in both megagametophytes (*Table 3*) and pollen gametes (*Table 4*). This heterogeneity appeared to be due to sampling error. In only one of the four individual tree samples of megagametophytes making up the *Pgm2-2/3* data set was a significant deviation from the 1:1 ratio detected, and this sample involved only 20 seeds. Pollen gamete segregation for this allelic pair

Table 4. — Observed allozyme segregation in pollen gametes of heterozygous parent trees and goodness-of-fit to the expected 1:1 ratio.

Locus	Allelic[1] combination F	S	Observed no. F	Total	Deviation $X^2(1)$	P	Heterogeneity X^2 (df)	P
Pgm1	2	3	134	259	0.25	.50-.75	5.93(7)	.50-.75
	3	4	38	74	0.01	.75-.90	0.13(2)	.90-.95
Pgm2	2	3	24	52	0.17	.50-.75	4.79(1)	.01-.05
Lap1	2	3	78	156	0.01	.90-.95	12.33(5)	.01-.05
	2	4	108	228	0.53	.25-.50	4.39(7)	.50-.75
	3	4	10	24	0.38	.50-.75	--	--
Lap2	3	4	41	85	0.05	.25-.50	4.11(2)	.10-.25
Got3	2	4	33	52	3.25	.05-.10	0.02(1)	.50-.75
Cat	1	3	145	278	0.44	.50-.75	11.68(9)	.10-.25
Glyd	2	3	82	147	1.74	.10-.25	9.43(5)	.05-.10
6-Pgd2	1	2	23	28	10.32	<.01	--	--
	2	4	12	24	0.04	.50-.75	--	--
Idh	2	5	101	162	9.39	<.01	2.22(4)	.50-.75
	5	7	22	48	0.19	.50-.75	--	--
Dia2	2	4	150	318	0.91	.25-.50	6.65(10)	.75-.90

[1]) Alleles coding faster (F) and slower (S) migrating isozyme bands of each allelic pair.

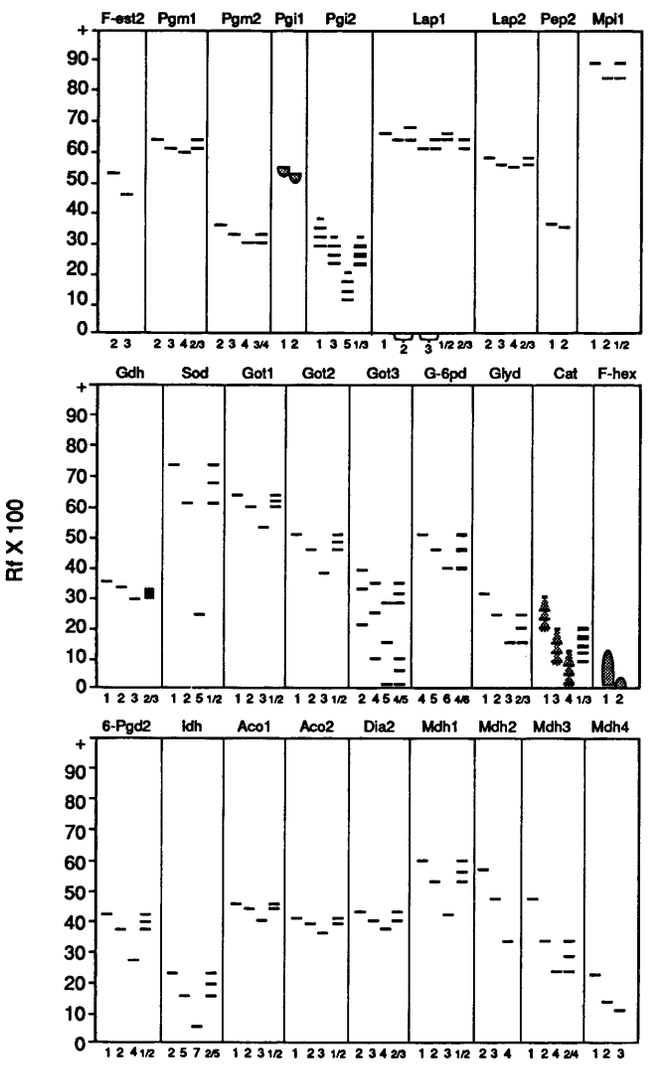

Figure 1. — Representative examples of allozyme band patterns for 27 loci observed in seed tissues of coastal Douglas-fir. Numbers on horizontal axis refer to genotypes of haploid megagametophytes (single number) or diploid embryos (paired numbers). Only band patterns for heterozygous embryos are given; however, with the exception of *Lap2* and *Got3* (see text), megagametophyte and homozygous embryo expression for the same allele are identical. Rf refers to the migration distance of bands relative to that of the marker dye.

was based on samples from two males. Neither sample taken separately revealed significant deviation from the expected ratio.

El-Kassaby *et al.* (1982b) reported that the PGM2 zone in Douglas-fir was actually IDH bands appearing on gels stained for PGM. Previously, we provided evidence that this condition is true only when gels are run on low pH (< 7.0) buffer systems and *Pgm2* is a distinct locus on high pH buffers (Neale *et al.*, 1984) such as the "A" and "E" buffer systems used in this study. Heterozygotes at PGM loci produce double-banded phenotypes (*Figure 1*; El-Kassaby *et al.*, 1982b; Neale *et al.*, 1984) indicating that PGM is a monomeric enzyme.

Phosphoglycose isomerase, PGI1, PGI2: PGI1 was clearly resolved only in megagametophyte tissue, with two of the three single-banded variants (PGI1-1 and PGI1-3) occurring only rarely, as has been observed in population samples of coastal Douglas-fir (Merkle and Adams, 1987). Segregation was consistent with single locus inheritance (*Table 3*), but the sample tested was small.

Three of the five *Pgi2* alleles detected in seeds were observed in clones assayed for bud tissues, and three were observed in needle samples (*Table 2*). In all three tissues, *Pgi2* allozymes were multibanded, consisting of 3 to 4 closely spaced bands that stained almost equally (*Figure 1*). Although somewhat confounded by the multibanded nature of allelic products, heterozygotes at *Pgi2* produced band patterns consistent with a dimeric subunit structure (Neale *et al.*, 1984). El-Kassaby *et al.* (1982b) also concluded that PGI is functionally dimeric in this species.

Leucine aminopeptidase, LAP1, LAP2: One-and two-banded variants of *Lap1* were observed in megagametophytes, embryos, and buds, but only the slower migrating band of two-banded allozymes in megagametophytes was expressed in diploid tissues (*Figure 1*). Furthermore, all but one of the single-banded allozymes detected in megagametophytes (LAP1-1) had a migration distance which corresponded to the slower migrating band of a two-banded variant. Since an allele coding a double-banded variant in megagametophytes would be indistinguishable in diploid tissue from an allele coding a single isozyme with the same migration distance as the slower band, all allelic pairs of this type were bulked, resulting in five alleles (or allelic classes) being recognized in seeds (*Table 2*). Three of these alleles were also observed in the sample of clones assayed for bud tissue.

Significant heterogeneity among parent trees was observed in the segregation ratios of alleles *Lap1-2* and *-3* in pollen gametes (*Table 4*); but, as in the two previous cases of heterogeneity, this result is probably due to sampling error, since only one of the six individual tree samples showed significant deviation from the expected 1:1 ratio.

Allozymes of *Lap2* were single-banded in both seeds and buds (*Figure 1, Table 2*) and segregated in all but one case according to the 1:1 ratio expected for single-locus inheritance (*Table 3, Table 4*). Significant deviation from the 1:1 expectation was found for combinations of alleles *3* and *4* in segregating megagametophytes (egg gametes) (*Table 3*). Although sample sizes were generally small (15, 18, and 60), the deficiency of allele *3* was consistent for three parent trees (with different genetic backgrounds), suggesting that selection caused the distortion by acting against allele *3*, or against one or more alleles with which it is tightly linked. The same combination of alleles did not show segregation distortion in pollen gametes (*Table 4*), nor did allele *3* appear to be selected against in segregating megagametophytes involving any other combination of alleles (*Table 3*). Since allele *4* occurred only in the *3/4* combination, it is not known whether alleles other than 3 have a selective disadvantage in segregating gametes of heterozygotes involving allele *4*.

Heterozygotes at both *Lap1* and *Lap2* produced two-banded phenotypes in embryos and buds. This indicates that LAP is functionally monomeric, as has been reported for other conifers (Conkle, 1971; Adams and Joly, 1980a, Neale and Adams, 1981; Millar, 1985).

Peptidase, PEP1, PEP2: Although two zones of activity were detected in seeds and buds, PEP was clearly resolved

only in megagametophyte tissue, and only PEP2 could be scored consistently. This contrasts with three invariant zones in Douglas-fir reported by EL-KASSABY *et al.* (1982b). When scorable, band patterns at PEP1 appeared to be the same as LAP2. Mendelian inheritance was confirmed for two of the three variants of *Pep2* (*Table 3*).

Mannose-6-phosphate isomerase, MPI1, MPI2: Although MPI2 could not be clearly resolved, *Mpi1* allozymes were scorable in both embryo and megagametophyte tissues (*Table 2, Figure 1*). EL-KASSABY *et al.* (1982b) reported two invariant zones for MPI in seed tissues of Douglas-fir. Two-banded heterozygotes were observed in embryos, indicating that MPI1 is monomeric (*Figure 1*).

Glutamate dehydrogenase, GDH: Variants were clearly resolved in seeds, buds and needles, but only a single variant was observed in buds (*Table 2, Table* 3). Heterozygotes produce a single broad band which occurs midway between the positions of allozymes of the component alleles (*Figure 1*). This pattern has been observed for *Gdh* heterozygotes in other conifers as well, and presumably indicates that this enzyme has a multimeric subunit structure (MITTON *et al.*, 1979; ADAMS and JOLY, 1980a; CHELIAK and PITEL, 1984a; PITEL *et al.*, 1987).

Superoxide dismutase, SOD: This enzyme appeared as clear bands against a blue background in both seeds (*Figure 1*) and buds. Heterozygotes were triple-banded, suggesting that SOD is dimeric (*Figure 1*; EL-KASSABY et *al.*, 1982b).

Glutamate-oxaloacetate transaminase, GOT1, GOT2, GOT3 (also called Aspartate aminotransferase, AAT): All three zones of activity gave clear banding in seeds and buds (*Figure 1, Table 2*). EL-KASSABY *et al.* (1982b) also reported three zones of activity for GOT in Douglas-fir, but GOT1 was invariant in their materials and, thus, inheritance could not be confirmed. We found segregation at this locus in megagametophytes from only a single mother tree (*Got1-1/2, Table 3*).

Allozymes coded by *Got1* and *Got2* were single-banded and heterozygotes had triple-banded phenotypes, indicating GOT has a dimeric subunit structure (*Figure 1*; EL-KASSABY *et al.*, 1982b). Allozymes of *Got 3* have been found to be two- or three-banded in a number of conifers (GURIES and LEDIG, 1978; O'MALLEY *et al.*, 1979; ADAMS and JOLY, 1980a; ECKERT *et al.*, 1981; NEALE and ADAMS, 1981; BOYLE and MORGENSTERN, 1985; MILLAR, 1985; PITEL *et al.*, 1987). Under our electrophoretic conditions, variants of *Got3* were triple-banded in megagametophytes, but in both embryos and buds, homozygotes were double-banded; the intermediate band of the megagametophyte variants did not express in diploid tissue (*Figure 1*). Heterozygotes contained six bands; four corresponded to the isozymes occurring in homozygotes of the component alleles, one band was intermediate to the two faster isozymes coded by each allele, and one was intermediate to the two slower isozymes.

Three zones of GOT activity were also found in needle tissue, but GOT1 and GOT3 banding patterns were not those expected from genotypes based on megagametophyte analysis, indicating that these zones are not under the control of the same loci active in seeds and buds. GOT2 banding patterns, however, did appear to be consistent with expectation, and thus, this zone may be controlled by the same locus in all three tissues. Unfortunately, GOT2 does not stain consistently enough to be reliably scored in needles.

Glucose-6 phosphate dehydrogenase, G-6PD: This enzyme was clearly resolved in seeds, buds and needles (*Figure 1, Table 2*). As found for jack pine (*Pinus banksiana* LAMB.) (CHELIAK *et al.*, 1984), G-6PD banding patterns in Douglas-fir indicate that this enzyme has a dimeric subunit structure (*Figure 1*; EL-KASSABY *et al.*, 1982b). Although G-6PD-3 and -4 are easily identified in megagametophytes, the resolution of these bands in diploid tissue is not clear enough to allow *G-6pd-3/3* homozygotes and *G-6pd-3/4* heterozygotes to be unambiguously assigned. Thus, it is probably best to bulk these two alleles for analyses involving embryo, bud or needle tissues.

Glycerate dehydrogenase, GLYD: The same locus appears to code GLYD variants in seeds and needles (*Table 2*; NEALE *et al.*, 1984), but no activity for this enzyme was found in buds.

Glyd-2/3 heterozygotes, represented by a particularly large sample (N = 711) of segregating megagametophytes, gave an observed ratio of 0.90:1.10, significantly different from the 1:1 expectation (*Table 3*). This combination, however, failed to produce significant segregation distortion in pollen gametes (*Table 4*), or in progenies of two-parent crosses in which both male and female parents were heterozygous for *Glyd-2/3* (NEALE *et al.*, 1984). The observed deficiency of *Glyd-2* megagametophytes was either due to sampling error or because the selective disadvantage of *Glyd-2* (or the linkage block it marks) is slight and can only be detected statistically in large samples.

Glyd heterozygotes were triple-banded, indicating GLYD is functionally dimeric (*Figure 1*). DAVIS (1981) reached the same conclusion for Rocky Mountain Douglas-fir (var. *clauca*).

Catalase, CAT: When seed tissues were assayed, gels stained for CAT produced a single zone of clear bands on a dark blue background (*Figure 1*). This enzyme was not active in buds and was not investigated in the earlier study of needle tissue. Products of individual alleles (in megagametophytes or homozygous embryos) were multibanded, with the slowest band being the most intensely stained and several (2 to 4) faster migrating bands accompanying it (*Figure* 1). Heterozygotes were also multibanded, and included the slowest band from each allelic product, plus several intermediate bands. CAT has been reported to have a tetrameric subunit structure in maize (SCANDALIOUS, 1969).

Fluorescent hexaminase, F-HEX (also called Hexoseaminidase, HA): Clear banding for F-HEX could only be obtained in megagametophytes (*Table 2, Figure 1*).

6-Phosphogluconate degydrogenase, 6-PGD1, 6-PGD2: EL-KASSABY *et al.* (1982b) reported two zones of activity for 6-PGD in coastal Douglas-fir (6-PGD1 and 6-PGD2). Under our electrophoretic conditions (*Table 1*), the two zones overlapped, with 6-PGD1 so poorly resolved that only 6-PGD2 could be reliably scored. 6-PGD2, however, was readable in seeds, buds and needles (*Table 2*). Triple-banded heterozygotes indicate that 6PGD is functionally dimeric (*Figure 1*; EL-KASSABY et al., 1982b).

Significant deviation from the 1:1 expectation was observed in pollen gametes segregating for alleles *6-Pgd2-1* and *-2* (*Table 3*). Although the sample was small and was derived from only a single male, the observed deficiency of *6-Pgd2-2* is likely due to selection against this allele or against some closely linked gene or genes, since allele *2* was also deficient in the seedling progeny of a controlled cross involving another male parent

heterozygous for *6-Pgd2-1/2* (NEALE *et al.*, 1984). The frequency of allele *2* was not observed to be deficient in segregating megagametophytes of *6-Pgd2-1/2* heterozygotes (*Table 3*). In fact, when the same parent tree that produced the segregation distortion observed in *Table 4* was used as a female parent in the reciprocal cross, the ratio of *6-Pgd2-1* to *6-Pgd2-2* in megagametophytes was 15:13 (W. T. ADAMS, unpublished). A similar situation of segretation distortion in pollen but not in egg gametes was observed for *Pgi2* alleles in loblolly pine (*Pinus taeda L.*) (ADAMS and JOLY, 1980a). *6-Pgd2-2* was the most common allele observed in the materials sampled in this study (*Table 2*), and there is no evidence that this allele is selectively inferior in combinations of alleles other than *6-Pgd2-1/6-Pgd2-2* (*Table 3, Table 4*). Allele *1*, however, occurred only in the *1/2* combination, so whether other alleles have a selective disadvantage when segregating with allele *1* is unknown.

Isocitrate dehydrogenase, IDH: The eight distinct single-banded variants detected in the megagametophytes were, with the exception of IDH-n (a null), also observed in embryos. Because of poor resolution, which made clear separation of close migrating bands impossible, only three variants could be distinguished in needles, each of which corresponded to a pair of allozymes expressed in seeds (*Table 2*). Four isozymes were observed in the bud samples assayed, but in each case the isozymes corresponded to a single isozyme found in seeds. Band patterns in diploid tissues indicate IDH has a dimeric subunit structure (*Figure 1*; EL-KASSABY *et al.*, 1982b).

In all but one allelic combination (*Idh-2/Idh-5*), alleles segregated according to the expected 1:1 ratio (*Table 3, Table* 4). As observed for 6-*Pgd2-1/6-Pgd2-2, Idh-2/Idh-5* produced significant segregation distortion in pollen, but not in egg gametes. The deficiency of allele *5* in this combination, however, was consistent over five male parents, suggesting that selection caused the observed distortion.

Shikimate dehydrogenase, SDH: Bands in seeds did not stain consistently enough to be scored. NEALE *et al.* (1984) provided evidence that the single zone of SDH activity in needles is controlled by a single locus, and identified three single-banded allozymes. By assaying both needles and buds in the same orchard clones, we found in this study that the same three alleles code allozyme variants in bud tissues (*Table 2*). *Sdh* heterozygotes are double-banded (NEALE *et al.*, 1984), indicating that SDH is functionally monomeric in coastal Douglas-fir, as has been observed in the Rocky Mountain variety of this species (DAVIS, 1981), and in other conifers (LINHART *et al.*, 1981; BOYLE and MORGENSTERN, 1985).

Aconitase, ACO1, ACO2: Both ACO zones gave clear band patterns when seed and bud tissues were assayed on A buffer gels (*Table 1, Figure 1*). EL-KASSABY *et al.* (1982b), however, were able to score only ACO2 in seeds when a low pH (6.1) buffer system was utilized. A single zone of ACO activity has been reported for several pine and spruce species (GURIES and LEDIG, 1978; ADAMS and JOLY, 1980a; KING and DANCIK, 1983; MILLAR, 1985; STRAUSS and CONKLE, 1986; MUONA *et al.*, 1987; PITEL *et al.*, 1987), but two zones were observed in eastern larch (*Larix laricina*) (CHELIAK and PITEL, 1985). Band patterns of heterozygotes indicate that both ACO1 and ACO2 are structurally monomeric in coastal Douglas-fir (*Figure 1*). ACO has also been found to be monomeric in black spruce (*Picea marina*) (PITEL *et al.*, 1987), but MUONA et al. (1987) reported that this enzyme is dimeric in Norway spruce (*Picea abies*).

Diaphorase, DIA1, DIA2: As reported by EL-KASSABY *et al.* (1982b), we found two zones of activity in seeds tissues assayed for DIA, but only DIA2 resolved clearly enough to be scored under our electrophoretic conditions (*Figure 1*). Furthermore, only DIA2 was expressed in buds and needles (*Table 2*). Band patterns in diploid tissue indicate this enzyme has a monomeric subunit structure (*Figure 1*; NEALE *et al.*, 1984).

Malate dehydrogenase, MDH1, MDH2, MDH3, MDH4: While all four zones of activity were readable in megagametophytes, only three (MDH1, MDH2, MDH3) produced bands in embryos and buds, with only MDH1 and MDH3 resolved clearly enough to be read in embryos (*Figure 1, Table 2*). Only MDH1 isozymes were active in needles.

Although allozyme variants were single-banded at all four *Mdh* loci (*Figure 1*), hybrid isozymes were formed between the allelic products of *Mdh2* and *Mdh3*, so that an interlocus heterodimer always occurred midway between the MDH2 and MDH3 variants in megagametophyte assays (EL-KASSABY, 1981; EL-KASSABY *et al.*, 1982b). The presence of interlocus heterodimers complicated the interpretation of diploid band patterns for *Mdh2* and *Mdh3*, but with experience we were able to score both loci in buds, and *Mdh3* in embryos. When scorable, heterozygotes at *Mdh1, Mdh2* and *Mdh3* were triple-banded, indicating MDH is dimeric in coastal Douglas-fir. EL-KASSABY *et al.* (1982b) also reported that MDH3 is dimeric in Douglas-fir, but concluded that MDH1 is monomeric.

Linkage Relationships

Four of the 27 loci identified in seed tissues (*Pgi1, Mpi1, Sod, Got1*) did not occur in any two-locus combination with sufficient joint segregation data (i. e., $N \geq 40$) for linkage analyses. All other loci were found in at least six combinations where linkage could be assessed. In the interest of space, we report only on the 17 locus pairs which showed significant deviation from independent assortment (*Table 5*). A complete list of the 155 pairs analyzed can be obtained from W. T. ADAMS. Estimated rates of recombination in 12 of the 17 locus pairs indicated only weak linkage between loci ($\hat{r} \geq 0.28$). The remaining pairs appeared to be tightly linked: *Pgm2/Glyd* ($\hat{r} = 0.02$), *Pgm2/Mdh3* ($\hat{r} = 0.10$), *Pgi2/Got2* ($\hat{r} = 0.05$), *Got3/Cat* ($\hat{r} = 0.02$), and *Glyd/Mdh3* ($\hat{r} = 0.06$). Tight linkage has also been observed between *Pgi2* and *Got2* in eastern larch (*Larix laricina* (DUROI) K. KOCH) (CHELIAK and PITEL, 1985), balsam fir (*Abies balsamea* (L.) MILL.) (NEALE and ADAMS, 1981), and incense-cedar (*Calocedrus decurrens* (TORR.) FLORIN) (HARRY, 1986); and between *Pgi2* and *Got1* in various *Pinus* and *Picea* species (see *Table 3* in CHELIAK and PITEL, 1985). Comparison of GOT banding patterns in different conifers suggests that *Got1* in pines and spruces is functionally the same as *Got2* in Douglas-fir, eastern larch, balsam fir, and incense-cedar (CHELIAK and PITEL, 1985; HARRY, 1986). O'MALLEY *et al.* (1986) found *Pgm2* and *Mdh3* to be very tightly linked ($\hat{r} = 0.01$) in pitch pine (*Pinus rigida* MILL.). The other three locus pairs found to be tightly linked in this study have apparently not been investigated in other conifer species. Where comparisons were possible, our results were quite similar to those of EL-KASSABY *et al.* (1982a)

for Douglas-fir. They also found *Pgi2/Got2* to be tightly linked ($\hat{r}$ = 0.02) and weak linkage between *Got2/Mdh3* ($\hat{r}$ = 0.40), *G6pd/Idh* ($\hat{r}$ = 0.33), and *Dia2/Mdh3* ($\hat{r}$ = 0.33).

In five of the 17 locus pairs showing evidence of linkage (*Pgm2/Glyd*, *Lap2/Glyd*, *Got3/Cat*, *G-6pd/Idh*, *Glyd/Mdh3*), frequencies of joint segregation were significantly ($p < 0.05$) heterogeneous among mother trees. This may be due to differences in r among mother trees, or to alleles being in coupling arrangement in some mother trees and in repulsion in others (Adams and Joly, 1980b). A chi-square test of homogeneity of $\hat{r}$ over mother trees was significant for only one of the five combinations (*Glyd/Mdh3*), supporting the latter explanation. Furthermore, for *Glyd/Mdh3*, one tree of the seven sampled contributed over half of the homogeneity chi-square, with an observed r ($\hat{r}$ = 0.18), which was much higher than that of the others. Given the low sample sizes for each tree (15 to 45), chance sampling could explain the heterogeneity of $\hat{r}$ in this case. Thus, there is no evidence in this study, that recombination rates vary substantially among individual trees.

Based on the observed recombination values for the 17 locus pairs, four linkage blocks can be constructed (*Figure 2*). The exact order of tightly linked genes must be consisdered tentative until verified by additional data, and the ordering of genes in blocks *c* and *d* was not possible because of the lack of joint segregation data for *Pep2/Gdh* and *Aco2/Mdh4*. In general, linkages between any of the weakly linked pairs must also be considered tentative; however, three of the weakly linked pairs in block *a* (*Got2/Mdh3*, *G6pd/Idh*, *Dia2/Mdh3*) are supported by the results of El-Kassaby *et al.* (1982a). If the map for block a is correct, *Lap2* should be fairly tightly linked

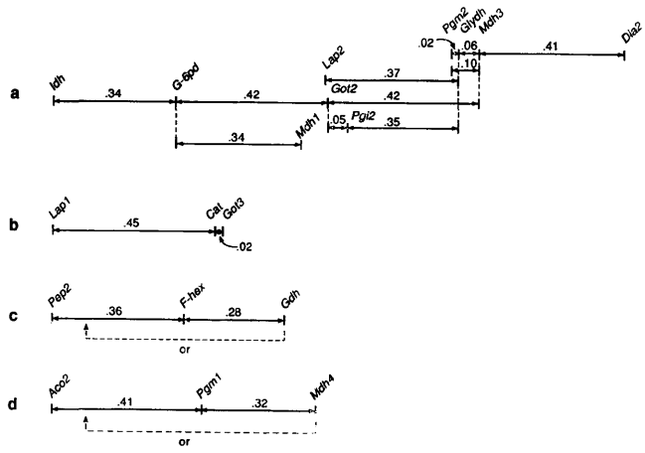

Figure 2. — Four linkage blocks identified in coastal Douglas-fir along with estimated recombination rates ($\hat{r}$) between pairs of loci.

Table 5. — Recombination estimates ($\hat{r}$) for pairs of loci with significant ($p < 0.05$) joint segregation chi-square values.

Locus combination	Sample: Parent trees	Sample: Total mega-gametophytes	$\hat{r}$	SE ($\hat{r}$)
Pgm1/Aco2	4	135	.41	.04
Pgm1/Mdh4	1	40	.32	.07
Pgm2/Glyd	4	114	.02	.01
Pgm2/Mdh3	4	127	.10	.03
Pgi2/Got2	1	40	.05	.03
Pgi2/Glyd	1	40	.35	.08
Lap1/Cat	13	409	.45	.02
Lap2/Glyd	4	115	.37	.04
Pep2/F-hex	2	67	.36	.06
Gdh/F-hex	1	40	.28	.07
Got2/G-6pd	9	223	.42	.03
Got2/Mdh3	8	212	.42	.03
Got3/Cat	4	137	.02	.01
G-6pd/Idh	9	259	.34	.03
G-6pd/Mdh1	2	58	.34	.06
Glyd/Mdh3	7	181	.06	.02
Dia2/Mdh3	6	161	.41	.04

with both *Got2* and *Pgi2* in Douglas-fir. *Lap2* and *Pgi2* appear to be linked on the same block in several pine species (Conkle, 1981; Eckert *et al.*, 1981; Szmidt *et al.*, 1984; Strauss and Conkle, 1986), but have high recombination values ($\hat{r} = 0.30$ to 0.50).

Discussion

Genetic Control of Isozymes in Different Tissues

Our data provide evidence for Mendelian inheritance of 10 enzymes not previously verified in coastal Douglas-fir (PGI1, LAP1, LAP2, PEP2, MPI1, GDH, GOT1, CAT, ACO2, MDH2), and additional support for the inheritance of 18 enzymes identified in earlier reports (El-Kassaby *et al.*, 1982b; Neale *et al.*, 1984). Twenty-seven of the 28 loci (*Sdh* not included) can be scored in megagametophyte tissue, where the clearest banding patterns are normally found. Of these 27 loci, six (*F-est2, Pgi1, Pep2, F-hex, Mdh2, Mdh4*) could not be scored in embryos because of poor band resolution, and seven could not be scored in dormant buds (*Pgi1, Pep2, Mpi2, Glyd, Cat, F-hex, Mdh4*), either due to poor band resolution or to the absence of isozyme expression altogether. Of the 27 loci which could be read in megagametophytes, 21 can be scored in embryos, 20 in buds, and 19 are common to all three tissues (*Table 2*). In addition, 10 loci identified in needle tissues by Neale *et al.* (1984) were found to be scorable in both seed tissues and buds. *Sdh* can be read in buds and needles, but not in seeds.

When banding patterns were clear, isozymes in a particular zone of activity were found to be under the control of the same gene locus in different tissues, except for GOT1 and GOT3, which appeared to be controlled by the same two loci in seeds and buds, but are coded by different loci in needles. Where isozyme zones were poorly resolved, inheritance could not be determined, and so there may be other exceptions. The complete lack of banding for some isozymes in buds (i. e., GLYD, CAT, MDH4) and needles (MDH2, MDH3, MDH4), that expressed clearly in seeds, may mean that the loci coding these enzymes are "turned-off" in the older tissues, or that the enzymes have been inactivated by the extraction procedures.

Although our results show that some allozymes can be scored reliably in both seeds and mature tissues, allelism of isozymes from different tissues should not be assumed without genetic tests.

Segregation Distortion

Significant ($p < 0.05$) deviation from Mendelian expectation was observed in only 4 of the 95 cases (*Table 3, Table 4*), which could be accounted for by chance. Nevertheless, in three of the significant cases [*Lap2-3/4* (*Table 3*); *6Pgd2-1/2* and *Idh-2/5* (*Table 4*)], the segregation distortion was consistent in more than one parent tree, suggesting that selection may be involved. Two observations common to all three of these allelic pairs are of interest: 1) segregation distortion occurred in the gametes of one sex, but not in those of the other; and 2) each pair included the most commonly observed allele in the study, and that allele was always deficient in gamete segregation.

Since both male and female gametes are assayed from viable seeds, it is usually unclear whether selection leading to segregation distortion occurs among gametes, or among the zygotes they form (Cheliak *et al.*, 1984; Strauss and Conkle, 1986). The presence of segregation distortion in the gametes of one sex and not in those of the other suggests that selection occurred at the gametic stage in the above examples. Selection may be acting on the allozymes themselves or on genes located at nearby loci.

Earlier workers have found common alleles to be overrepresented, not deficient, when segregation distortion is found in offspring of heterozygous parent trees (Strauss and Conkle, 1986). This observation led Strauss and Conkle to suggest that overrepresentation (i. e., superior fitness in segregating gametes) may in part explain the high frequency of common alleles in populations. On the other hand, segregation distortion favoring low frequency alleles as observed in this study, may in part explain the maintenance of these alleles in populations, even if they are at selective disadvantage in other life cycle stages.

Our results, like those for other conifers (Guries and Ledig, 1978; O'Malley *et al.*, 1979. Adams and Joly, 1980a; Neale and Adams, 1981; King and Dancik, 1983; Millar, 1985; Harry, 1986; Strauss and Conkle, 1986; Muona *et al.*, 1987), indicate that segregation distortion occurs relatively infrequently. It is, however, difficult to detect statistically (Strauss and Conkle, 1986), so that milder forms of distortion may go unnoticed. In most cases, inheritance of isozymes meets, or at least closely approximates, Mendelian expectation, but the rare occurrence of extreme segregation distortion, such as observed for *6pgd2-1/2* in this study, emphasizes the need for caution in interpreting the results of genetic analyses. For example, mating system estimates based on individual allozyme loci could be biased if segregation distortion is present, but not accounted for (Brown *et al.*, 1985). Mating system estimates based on multilocus methods are less likely to be biased by occasional (undetected) segretation distortion (Shaw *et al.*, 1981; Brown *et al.*, 1985; Neale and Adams, 1985; Ritland and El-Kassaby, 1985), but it would still be better to determine beforehand whether any loci are likely to be a problem in this respect.

Linkage

Multilocus estimators for mating system parameters assume the loci included in the analysis are randomly associated. Significant bias in estimation, however, will occur only if linkage disequilibrium is strong (Shaw et al., 1981), and this is unlikely if loci are not closely linked (Epperson and Allard, 1987). Tightly linked locus pairs were found to be infrequent in Douglas-fir, as in other conifers (e. g., O'Malley *et al.*, 1979; Adams and Joly, 1980b; Neale and Adams, 1981; King and Dancik, 1983; Harry, 1986). Thus, a large number of independent (or nearly independent) loci are readily available in a wide variety of species for multilocus mating system analysis.

More than cursory comparison of linkage relationships in Douglas-fir with those reported in other conifer species is nearly impossible due to: 1) the variation in electrophoretic methods and isozyme terminology used by different authors; 2) the lack of commonality in the enzymes that are assayed; 3) the fact that locus pairs with non-significant independence of joint segregation are frequently not reported; and 4) the possibility that the same zone of isozyme variation in different species may not be coded by the same (homologous) gene. One closely linked pair found in coastal Douglas-fir, *Pgi2/Got2*, appears to be common to a wide variety of conifers

(CHELIAK and PITEL, 1985). It would be interesting to determine whether the other two closely linked groups identified in this study (*Pgm2-Glyd-Mdh3 and Got3-Cat*) occur in other conifer species, as well.

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Literature Cited

ADAMS, W. T.: Application of isozymes in tree breeding. *In*: Isozymes in plant genetics and breeding. Part A. S. D. TANKSLEY and T. J. ORTON (eds). Elsevier Scientific Publ. B. V., Amsterdam. 381—400 (1983). —— ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. J. Hered. **71**: 33—40 (1980a). —— ADAMS, W. T. and JOLY, R. J.: Linkage relationships among twelve allozyme loci in loblolly pine. J. Hered. **71**: 199—202 (1980b). —— ADAMS, W. T., NEALE, D. B. and LOOPSTRA, C. A.: Verifying controlled crosses in conifer tree-improvement programs. Silvae Genet. **37**: 147—152 (1988). —— BOYLE, T. J. B. and MORGENSTERN, E. K.: Inheritance and linkage relationships of some isozymes of black spruce in New Brunswick. Can. J. For. Res. **15**: 992—996 (1985). —— BROWN, A. H. D., BARRETT, S. C. H. and MORAN, G. F.: Mating system estimation in forest trees: models, methods, meanings. *In*: Population genetics in forestry. H.-R. GREGORIUS (ed). Lecture Notes in Biomathematics **60**. Springer-Verlag, New York. 32—49 (1985). —— CHELIAK, W. M., MORGAN, K., DANCIK, B. P., STROBECK, C. and YEH, F. C. H.: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* LAMB. Theor. Appl. Genet. **69**: 145—151 (1984)). —— CHELIAK, W. M. and PITEL, J. A.: Genetic control of allozyme variants in mature tissues of white spruce trees. J. Hered. **75**: 34—40 (1984a). —— CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Inf. Rep. PI-X-2, Petawawa Nat. For. Inst., Canadian For. Serv., Agric. Canada (1984b). —— CHELIAK, W. M. and PITEL, J. A.: Inheritance and linkage of allozymes in *Larix laricina*. Silvae Genet. **34**: 142—148 (1985). —— CONKLE, M. T.: Inheritance of alcohol dehydrogenase and leucine aminopepdidase isozymes in knobcone pine. For. Sci. **17**: 190—194 (1971). —— CONKLE, M. T.: Isozyme variation and linkage in six conifer species. *In*: Isozymes of North American forest trees and forest insects. M. T. CONKLE (Tech. Coord.). USDA For. Serv. Gen. Tech. Rep. PSW-48, Pac. Southwest For. Range Exp. Stn., Berkeley, CA. 11—17 (1981). —— CONKLE, M. T., HODGSKISS, P. D., NUNNALLY, L. B. and HUNTER, S. C.: Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For. Serv. Gen. Tech. Rep. PSW-64, Pac. Southwest For. Range Exp. Stn., Berkeley, CA (1982). —— COPES, D. L.: A genetic analysis of aminopeptidase and peroxidase isoenzymes in Douglas-fir parent trees and seedling progeny. Can. J. For. Res. **9**: 189—192 (1979). —— DAVIS, M. L.: Habitat diversity and its effects on the genetic and ecological structures of two successional populations of Douglas-fir. Ph. D. Diss., Univ. Colorado, Boulder. 97 p. (1981). —— ECKERT, R. T., JOLY, R. J. and NEALE, D. B.: Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can. J. For. Res. **11**: 573—579 (1981). —— EL-KASSABY, Y. A.: Genetic interpretation of malate dehydrogenase isozymes in some conifer species. J. Hered. **72**: 451—452 (1981). —— EL-KASSABY, Y. A., SZIKLAI, O. and YEH, F. C.: Linkage relationships among 19 polymorphic allozyme loci in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Genet. Cytol. **24**: 101—108 (1982a). —— EL-KASSABY, Y. A., YEH, F. C. and SZIKLAI, O.: Inheritance of allozyme variants in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Genet. Cytol. **24**: 325—335 (1982b). —— EPPERSON, B. K. and ALLARD, R. W.: Linkage disequilibrium between allozymes in natural populations of lodgepole pine. Genetics **115**: 341—352 (1987). —— GURIES, R. P. and LEDIG, F. T.: Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* MILL.). Heredity **40**: 27—32 (1978). —— HARRY, D. E.: Inheritance and linkage of isozyme variants in incense-cedar. J. Hered. **77**: 261—266 (1986). —— KING, J. N. and DANCIK, B. P.: Inheritance and linkage of isozymes in white spruce (*Picea glauca*). Can. J. Genet. Cytol. **25**: 430—436 (1983). —— LI, P. and ADAMS, W. T.: Range-wide patterns of allozyme variation in Douglas-fir [*Pseudotsuga* menziesii (MIRB.) FRANCO]. Can. J. For. Res. **19**: 149—161 (1989). —— LINHART, Y. B., DAVIS, M. L. and MITTON, J. B.: Genetic control of allozymes of shikimate dehydrogenase in ponderosa pine. Biochem. Genet. **19**: 641—646 (1981). —— MERKLE, S. A. and ADAMS, W. T.: Patterns of allozyme variation within and among Douglas-fir breeding zones in southwest Oregon. Can. J. For. Res. **17**: 402—407 (1987). —— MILLAR, C. I.: Inheritance of allozyme variants in Bishop pine (*Pinus muricata* D. DON). Biochem. Genet. **23**: 933—946 (1985). —— MITTON, J. B., LINHART, Y. B., STURGEON, K. B. and HAMRIK, J. L.: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. **70**: 86—89 (1979). —— MORAN, G. F. and ADAMS, W. T.: Microgeographical patterns of allozyme differentation in Douglas-fir from southwest Oregon. For. Sci. **35**: 3—15 (1989). —— MORRIS, R. W. and SPIETH, P. T.: Sampling strategies for using female gametophytes to estimate heterozygosity in conifers. Theor. Appl. Genet. **51**: 217—222 (1978). —— MUONA, O., YAZDANI, R. and LINDQUIST, G.: Analysis of linkage in *Picea abies*. Hereditas **106**: 31—36 (1987). —— NEALE, D. B.: Genetic implications of shelterwood regeneration of Douglas-fir in southwest Oregon. For. Sci. **31**: 995—1005 (1985). —— NEALE, D. B. and ADAMS, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Can. J. Bot. **59**: 1285—1291 (1981). —— NEALE, D. B. and ADAMS, W. T.: The mating system in natural and shelterwood stands of Douglas-fir. Theor. Appl. Genet. **71**: 201—207 (1985). —— NEALE, D. B., WEBER, J. C. and ADAMS, W. T.: Inheritance of needle tissue isozymes in Douglas-fir. Can. J. Genet. Cytol. **26**: 459—468 (1984). —— O'MALLEY, D. M., ALLENDORF, F. W. and BLAKE, G. M.: Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. Biochem. Genet. **17**: 233—250 (1979). —— O'MALLEY, D. M., GURIES, R. P. and NORDHEIM, R. V.: Linkage analysis for 18 enzyme loci in *Pinus rigida* MILL. Theor. Appl. Genet. **72**: 530—535 (1986). —— OMI, S. K. and ADAMS, W. T.: Variation in seed set and proportions of outcrossed progeny with clones, crown position, and top pruning in a Douglas-fir seed orchard. Can. J. For. Res. **16**: 502—507 (1986). —— PITEL, J. A. and CHELIAK, W. M.: Extraction and characterization of isoenzymes from vegetative tissues of five conifers. *In*: Proc. 7th North Amer. For. Biol. Workshop, July 26 to 28, Lexington, KY. 312—326 (1982). —— PITEL, J. A., CHELIAK, W. H. and BARRET, J.: Inheritance of allozymes in a black spruce diallel cross. Silvae Genet. **36**: 149—153 (1987). —— RITLAND, K. and EL-KASSABY, Y. A.: The nature of inbreeding in a seed orchard of Douglas-fir as shown by an efficient multilocus model. Theor. Appl. Genet. **71**: 375—384 (1985). —— SCANDALIOUS, J. G.: Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Genet. **31**: 37—39 (1969). —— SHAW, D. V., KAHLER, A. L. and ALLARD, R. W.: A multilocus estimator of mating system parameters in plant populations. Proc. Natl. Acad. Sci. U.S.A. **78**: 1298—1302 (1981). —— SMITH, D. B. and ADAMS, W. T.: Measuring pollen contamination in clonal seed orchards with the aid of genetic markers. *In:* Proc. 17th South. For. Tree Improv. Conf., June 6 to 9, Univ. Georgia, Athens, GA. 69—77 (1983). —— STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483—493 (1986). —— SZMIDT, A. E., MUONA, O. and YAZDANI, R.: Linkage relationships in Scots pine (*Pinus sylvestris* L.). *In:* Genetic studies of Scots pine (*Pinus sylvestris* L.) domestication by means of isozyme analysis. Pap. IV, Swed. Univ. Agric. Sci., Dept. For. Genet. and Plant Physiol., Umea, Swed. (1984). —— WEBER, J. C. and STETTLER, R.: Isoenzyme variation among ten populations of *Populus trichocarpa* TORR. et. GRAY in the Pacific Northwest. Silvae Genet. **30**: 82—87 (1981). —— YANG, J.-CH., CHING, T. M. and CHING, K. K.: Isoenzyme variation of coastal Douglas-fir. I. A study of geographic variation in three enzyme systems. Silvae Genet. **26**: 10—18 (1977).