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Linkage analysis for 18 enzyme loci in *Pinus rigida* Mill.*

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Summary. Estimates of recombination frequency among enzyme loci of pitch pine revealed two new linkages, *Mdh3 : Pgm2* ($\hat{\theta}=0.01$) and *Pep1 : Mdh4* ($\hat{\theta}=0.38$), and confirmed two previously established linkages. Tighter linkage ($\theta=0.30$) was ruled out for nearly all gene pairs examined. In general, the Bayesian approach used in this study to test for linkage performed better than alternative methods.

Key words: Recombination frequency – Linkage – Bayesian approach – *Pinus rigida* Mill.

Introduction

Allozyme surveys have been widely used in forestry to assess the extent and patterning of genetic variation in populations of forest trees (Guries and Ledig 1982; Steinhoff et al. 1983; Weber and Stettler 1980; Wheeler and Guries 1982). Conifer megagametophytes have facilitated such surveys because the segregation of allozymes in these haploid products of meiosis permits the documentation of inheritance without recourse to breeding (Adams and Joly 1980a; Bartels 1971; Eckert et al. 1981; Guries and Ledig 1978; King and Dancik 1983). In addition, individuals identified as being heterozygous at two or more loci also provide opportunities for studying linkage relationships among enzyme loci.

Typically, genotypic surveys of conifers involve analysis of 6–10 megagametophytes from a number of trees so that populations can be characterized in terms of allozyme frequencies; linkage studies involve analysis of 50–200 megagametophytes from one or a few trees so that departures from independent assortment can be identified. If segregation data are recorded during genotypic surveys of conifers, a Bayesian approach to estimating recombination frequency can provide an efficient alternative to studies aimed solely at determining linkage relationships (Nordheim et al. 1983). In addition to minimizing experimental effort, this approach yields linkage estimates based upon a large sample of genotypes rather than one or a few individuals. In situations involving weak linkage and/or small sample sizes, uncertainties regarding the identity of parental and recombinant genotypes become important in estimating recombination frequency (Nordheim et al. 1983).

Meiotic segregation in conifer megagametophytes follows a genetic model analogous to a testcross with parental phase unknown. This model has been used extensively in human genetic studies where parental and recombinant genotypes cannot be distinguished beforehand because of incomplete pedigree information (e.g., Haldane and Smith 1947). Forest trees present problems similar to those encountered in humans in that generation times are long and breeding is impractical. Thus, breeding histories are generally unknown, making it uncertain whether the parental category is associated with coupling or repulsion gametes except in cases of tight linkage.

In this paper, we use a Bayesian approach (Nordheim et al. 1983) to estimate recombination frequency and test for linkage among 18 enzyme loci in pitch pine (*Pinus rigida* Mill.).

Electrophoretic procedures

Guries and Ledig (1982) identified the genotypes of 694 pitch pine trees for 21 enzyme loci in a study of genetic differentiation among 11 natural populations. Details of the electrophoretic procedures can be found in Guries et al. (1978) and Marty et al. (1984). We selected trees heterozygous for several enzyme loci to conduct inheritance tests. Sufficient variability was present to test for linkage between nearly all pairwise combinations of 18 enzyme loci (Table 1).

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Table 1. Enzymes and loci examined in pitch pine

E.C. No.[a]	Enzyme name	Enzyme locus
4.2.1.3	Aconitate hydratase	*Aco*
3.1.3.2	Acid phosphatase	*Acp*
4.1.2.13	Fructose-biphosphate aldolase	*Ald2*
2.6.1.1	Aspartate aminotransferase	*Aat1*
		Aat3
1.1.1.49	Glucose-6-phosphate dehydrogenase	*Gpd*
1.1.1.42	Isocitrate dehydrogenase	*Idh*
1.1.1.37	Malate dehydrogenase	*Mdh1*
		Mdh3
		Mdh4
3.4.13.11	Dipeptidase	*Pep1*
		Pep2
2.7.5.1	Phosphoglucomutase	*Pgm1*
		Pgm2
1.1.1.44	6-Phosphogluconic dehydrogenase	*Pgd1*
5.3.1.9	Glucosephosphate isomerase	*Pgi1*
		Pgi2

[a] Enzyme Commission numbers given by Dixon and Webb (1979)

Estimation of recombination frequency and testing for linkage

A tree heterozygous for two genes, A and B, produces four kinds of gametes: a_1b_1, a_1b_2, a_2b_1, and a_2b_2, where the subscripts denote alleles. Parental and recombinant categories are associated with the numbers of gametes in 'coupling' (those in categories a_1b_1 and a_2b_2) and 'repulsion' (those in a_1b_2 and a_2b_1). Because the breeding history of a tree is unknown, it is not certain whether the parental category is associated with coupling or repulsion gametes, thereby complicating inference about the recombination fraction.

Several procedures exist for estimating recombination frequency. Rudin and Ekberg (1978) proposed a binomial-type estimator. Maximum likelihood procedures have been widely used in the human genetics literature (Haldane and Smith 1947; Morton 1955) but recently have been shown to perform poorly when the recombination frequency is close to 0.5 (Nordheim et al. 1984). A Bayesian procedure, using a prior distribution that places equal probability on all values of θ, has been developed for use in linkage studies with unknown parental phase (Nordheim et al. 1983; O'Malley 1984). This method is superior to the others in estimating recombination frequency involving moderate to weak linkage and will be used throughout this paper. Numerically, the estimates produced by the Bayesian likelihood, and Rudin-Ekberg methods are rather similar; for the pitch pine study, all estimates can be found in O'Malley (1984). A "confidence interval" corresponding to the Bayesian procedure can also be computed (Nordheim et al. 1983), and this will also be used in this manuscript.

Tests for linkage are made by testing the null hypothesis $\theta = 1/2$ against the alternative $\theta < 1/2$. Satisfactory procedures for testing must account for the low prior probability of linkage due to the fact that genes can be linked only if they occur on the same chromosome. Maximum likelihood methods were formalized by Morton (1955); The relevant test statistic is a Z-score which is the common log of the ratio of the likelihood evaluate at the maximum likelihood estimate to the likelihood evaluated at $\theta = 1/2$. A Z-score of 3.0 (corresponds to $P < 0.001$) or higher is generally interpreted as providing evidence to reject the null hypothesis. A Bayesian alternative incorporating the low prior probability of linkage was proposed by Smith (1959). The likelihood and Bayesian results for testing are very similar; we report Z-scores in the manuscript.

Estimation and testing using several trees requires an assumption of homogeneity of recombination fraction across all trees. This assumption is tested by a likelihood ratio test (effectively assessing the variability among Z-scores) suggested by Morton (1956). This test performs poorly when θ is near 1/2 and results in too few rejections (Rao et al. 1978; Nordheim et al. 1984). We report the chi-square value resulting from this test since there are no tractable alternatives.

Results

Single locus segregations

The single-locus individual tree segregation data were generally consistent with the expected 1 : 1 ratio; infrequently, deviations from 1 : 1 segregation were noted in *Aat1, Pep2, Pgd1* and *Idh*. Partitioning the chi-squares among genotypes and among populations did not reveal significant effects that could be attributed to either subdivision (O'Malley 1984).

Pairwise segregation and linkage

Trees were available to test for linkage among 144 of the 153 pairwise combinations for 18 enzyme loci studied. *Ald2* and *Pgi1* were rarely heterozygous and no data were obtained for 9 pairs involving these loci. Thus, the recombination fraction was estimated for 94% of the possible pairwise combinations, and testing revealed 11 pairs of loci where the recombination fraction was significantly less ($\alpha = 0.05$) than 1/2 (Tables 2–6). Tight linkage can be ruled out for most of the remaining locus pairs since the lower bound of the Bayes' 95% confidence interval was greater than $\theta = 0.30$ in nearly all cases.

Tight linkages

Two pairs of loci, *Aat1 : Pgi2* and *Mdh3 : Pgm2*, are tightly linked with recombination fraction 0.035 and 0.013, respectively (Table 2). The recombination values were highly significant in all families and were consistent across families.

Moderate linkages

Segregations from 6 trees argue strongly for linkage between *Gpd* and *Idh* (Table 3). Independence of seggregation was rejected for all families ($\alpha = 0.05$). The *Idh* locus departed significantly from 1 : 1 segregation in 3 families and homogeneity of segregation at *Idh* was

Table 2. Segregation analysis, estimate of recombination frequency (θ), and measures of significance for *Aat1:Pgi2* and *Mdh3:Pgm2*

Tree	*Aat1:Pgi2*							Bayes' estimate of θ	Z-score	Bayes' 95% CI
	Observed no.				Chi-square (1 df)					
	a1b1	a1b2	a2b1	a2b2	locus 1	locus 2	linkage			
BRD131	17	0	0	22	0.64	0.64	39.00***	0.014	11.439	0.000–0.081
CC438	18	1	0	15	0.47	0.12	30.12**	0.056	7.975	0.007–0.077
WA14	3	75	59	1	2.35	1.42	122.46***	0.036	33.378	0.015–0.049
90-10	63	4	3	90	4.22*	4.90*	133.22***	0.049	35.378	0.027–0.065
							Combined	0.035	87.469	0.023–0.044
								Likelihood ratio homogeneity chi-square: 3.23 with 3 df		
Tree	*Mdh3:Pgm2*							Bayes' estimate of θ	Z-score	Bayes' 95% CI
	Observed no.				Chi-square (1 df)					
	a1b1	a1b2	a2b1	a2b2	locus 1	locus 2	linkage			
BRN25	8	0	1	11	0.80	0.20	16.20***	0.018	5.418	0.000–0.036
EP100	28	0	0	32	0.27	0.27	60.00***	0.006	17.761	0.000–0.011
HE20	13	0	0	17	0.53	0.53	30.00***	0.011	8.730	0.000–0.023
SHA13	1	19	19	0	0.03	0.03	35.10***	0.049	9.419	0.006–0.067
							Combined	0.013	40.745	0.002–0.018
								Likelihood ratio homogeneity chi-square: 2.68 with 3 df		

CI = confidence interval
* Significant at 0.05 level; *** Significant at 0.005 level

Table 3. Segregation analysis, estimates of recombination frequency (θ), and measures of significance for *Gpd:Idh* linkage

Tree	Observed no.				Chi-square (1 df)			Bayes' estimate of θ	Z-score	Bayes' 95% CI
	a1b1	a1b2	a2b1	a2b2	locus 1	locus 2	linkage			
CC379	10	5	2	23	2.50	6.40*	16.90***	0.190	3.684	0.112–0.253
CC381	5	19	17	4	0.20	0.02	16.20***	0.231	3.466	0.136–0.276
CC414	17	7	6	9	2.08	1.26	4.33*	0.340	0.658	0.262–0.411
CC429	4	22	9	8	1.88	6.72**	8.40**	0.289	1.587	0.205–0.362
EP104	4	29	23	3	0.83	0.42	34.32***	0.131	8.127	0.075–0.174
WPB16	26	6	12	15	0.42	4.90*	8.97**	0.311	1.699	0.239–0.377
							Combined	0.233	17.004	0.203–0.262
								Likelihood ratio homogeneity chi-square: 10.21 with 5 df		

* Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.005 level

rejected ($P < 0.01$); however, estimation and testing for linkage are not affected unless both loci depart from 1 : 1 (Mather 1951). Homogeneity of recombination frequency across all trees could not be rejected.

Weak linkages

In 17 families segregating for the *Pep1 : Mdh4* gene pair (Table 4), the chi-square test for independence of segregation revealed significant deviations in only 7 families. However, the families appeared quite homogeneous and the combined confidence interval indicated a clear departure from $\theta = 1/2$.

Inconclusive evidence for linkage was found for another 6 locus pairs (Table 5). Linkage between *Pgi1* and *Pgd2* was indicated strongly in one family, but not in a second family. Single-locus segregations fit Mendelian expectations and the combined data argue strongly

Table 4. Segregation analysis, estimate of recombination frequency (θ), and measures of significance for *Pep1:Mdh4* linkage

Tree	Observed no.				Chi-square (1 df)			Bayes' estimate of θ	Z-score	Bayes' 95% CI
	a1b1	a1b2	a2b1	a2b2	locus 1	locus 2	linkage			
BRD30	11	10	15	3	0.23	4.33*	3.10	0.363	0.383	0.247–0.500
BRD134	12	5	10	18	2.69	0.02	5.00*	0.340	0.806	0.263–0.409
CC376	12	7	11	9	0.03	1.26	0.23	0.431	0.000	0.335–0.500
CC382	21	14	12	13	1.67	0.60	1.07	0.425	0.001	0.334–0.500
CC385	15	7	5	13	0.40	0.00	6.40*	0.309	1.128	0.224–0.384
CC414	5	17	8	9	0.64	4.33*	3.10	0.363	0.383	0.247–0.500
CC421	13	14	24	9	0.60	3.27	4.27*	0.370	0.637	0.307–0.430
CC423	9	17	20	14	1.07	0.07	3.27	0.385	0.415	0.288–0.500
EP44	19	13	8	20	0.27	0.60	5.40*	0.354	0.890	0.286–0.418
EP71	9	21	16	14	0.00	1.67	3.27	0.385	0.415	0.288–0.500
EP104	11	18	10	21	0.07	5.40*	0.27	0.443	0.000	0.363–0.500
EP145	7	11	12	10	0.40	0.10	0.90	0.414	0.000	0.306–0.500
HE54	11	12	7	10	0.90	0.40	0.10	0.436	0.000	0.345–0.500
LL327	12	16	10	7	2.69	0.02	1.09	0.414	0.002	0.310–0.500
NN3	13	19	21	7	0.27	1.07	6.67**	0.339	1.175	0.268–0.404
SHA10	28	10	7	15	4.27*	1.67	11.27***	0.290	2.228	0.219–0.354
WPA28	22	9	13	16	0.07	1.67	4.27*	0.307	0.637	0.307–0.430
							Combined	0.384	6.065	0.364–0.404
								Likelihood ratio homogeneity chi-square: 13.98 with 16 df		

* Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.005 level

Table 5. Segregation analysis for gene pairs not conclusively linked

Tree	Observed no.				Chi-square (1 df)			Bayes' estimate of θ	Z-score	Bayes' 95% CI
	a1b1	a1b2	a2b1	a2b2	locus 1	locus 2	linkage			
	Pgi1:Pgd2									
CC388	33	9	15	33	0.40	0.40	19.60***	0.272	4.125	0.214–0.324
EP148	14	7	7	12	0.10	0.10	3.60	0.355	0.493	0.241–0.500
							Combined	0.295	4.419	0.247–0.341
								Likelihood ratio homogeneity chi-square: 0.91 with 1 df		
	Gpd:Pgm1									
CC430	10	8	19	7	1.45	4.45*	2.27	0.387	0.201	0.277–0.500
EP49	11	15	13	6	1.09	0.20	2.69	0.380	0.291	0.270–0.500
WPA7	14	7	10	13	0.09	0.36	2.27	0.387	0.201	0.277–0.500
WPA22	5	17	10	9	0.22	2.95	4.12*	0.348	0.610	0.272–0.417
							Combined	0.379	1.245	0.333–0.419
								Likelihood ratio homogeneity chi-square: 0.26 with 3 df		
	Acp:Pgm1									
CC401	12	6	7	15	0.40	0.10	4.90*	0.33	0.786	0.252–0.405
	Mdh3:Pgm1									
WPA5	37	18	15	20	4.44*	2.18	6.40*	0.369	1.106	0.312–0.424
	Acp:Pgi1									
CC401	7	10	14	4	0.03	1.49	4.83*	0.323	0.773	0.237–0.399
	Aat3:Pgm2									
MX95	7	14	15	9	0.20	0.02	3.76	0.360	0.526	0.251–0.500

* Significant at 0.05 level; ** Significant at 0.005 level

Table 6. Segregation analysis, estimate of recombination fraction (θ), and measures of significance for *Mdh4:Pgm2*

Tree	Observed no.				Chi-square (1 df)			Bayes' estimate of θ	Z-score	Bayes' 95% CI
	a1b1	a1b2	a2b1	a2b2	locus 1	locus 2	linkage			
	Mdh4:Pgm2									
HE21	9	12	11	11	0.02	0.21	0.21	0.435	0.000	0.343–0.500
MX95	3	16	19	7	1.09	0.02	13.89***	0.234	2.893	0.155–0.300
							Combined	0.349	1.424	0.288–0.407
								Likelihood ratio homogeneity chi-square: 6.76* with 1 df		

* Significant at 0.05 level; ** Significant at 0.005 level

for linkage. Considering only the segregation data for this locus pair, we would conclude that linkage exists. However, in addition to *Pgi1:Pgd2,* segregation data for two other locus pairs which do not appear to be linked showed significant departure from independent segregation in family CC388. Data from more families would be desirable to confirm this possible linkage.

There was evidence for linkage between *Gpd* and *Pgm1* with an estimated recombination frequency of 0.379. Considered individually, the four families did not suggest linkage, but the combined data provided substantial evidence for linkage.

Data were available from only 1 family for the locus pair *Acp:Pgm1* and provided marginal support for linkage. The interrelationships of the 4 enzyme loci: *Acp, Gpd, Idh,* and *Pgm1* suggest a linkage block; the confidence levels of the intervals not including $\theta = 1/2$ are all high but no families were analyzed for more than one pair.

Locus pairs *Acp:Pgi1, Mdh3:Pgm1,* and *Aat3:Pgm2* each were analyzed in a single family. The results suggested weak linkage. Indirect inference for linkage for the pairs *Mdh3:Pgm1* and *Aat3:Pgm2* was provided by families heterozygous for the other member of the tightly linked locus pair *Mdh3:Pgm2*. Ignoring the small recombination fraction between *Mdh3:Pgm2* and combining the families, the subsequent estimates of recombination frequency for *Mdh3:Pgm1* and *Aat3:Pgm2* were 0.428 and 0.443, respectively, somewhat greater than the estimates given in Table 5.

Heterogeneous linkages

The segregation data for locus pair *Mdh4:Pgm2* (Table 6) suggest linkage but the test for homogeneity rejects the hypothesis of equal recombination frequency for the 2 families ($\theta = 0.01$). Single-locus segregation data fit Mendelian expectations; in one family, the genes assorted independently, while in the second they appeared moderately linked ($\theta = 0.234$).

For the 142 locus pairs (involving 751 segregations) not shown to be linked, 24 segregations (3.2%) led to rejection at the $\alpha = 0.05$ level of the null hypothesis that $\theta = 1/2$. While fewer than 5% of these 751 segregations showed significant departure from independence at the $\alpha = 0.05$ level, 5 of 80 trees provide one-half of these observations and two showed highly significant departure from independence for one locus pair and moderate departure for two other locus pairs. While segregation data overall indicate that rates of recombination are relatively homogeneous, infrequent trees, perhaps possessing chromosomal anomalies, may account for the majority of non-random segregations.

Discussion

Linkage arrangements found in pitch pine are in general agreement with those reported for other conifers. The structural loci (*Pgd1, Pgd2; Pgm1, Pgm2; Pgi1, Pgi2; Mdh1, Mdh3, Mdh4*) encoding isozymes presumably specific to different subcellular locations are not closely linked, nor are loci which are components of the same biochemical pathways (Gottlieb 1982). Conkle (1981) found that a disproportionately large number of enzyme loci mapped to a single chromosome in several pines. His sample of loci included enzymes which are important in anaerobic metabolism (alcohol dehydrogenase, peroxidase, esterases) so linkage might represent a functional arrangement. For the enzyme loci that we examined in pitch pine, almost all cases of linkage involved only a single pair of loci. Our data indicate that *Acp, Gdp, Idh* and *Pgm1* might map to a single chromosome, but only *Gpd* and *Idh* appear to be moderately linked.

The *Aat1:Pgi2* linkage (previously referred to as locus pair *Got1:Gpi2*) has been documented in *Pinus contorta* Dougl., *P. taeda* L. (Adams and Joly 1980b), *P. jeffreyi* Grev. and Balf. (Conkle 1981), *P. strobus* L. (Eckert et al. 1981), *Picea glauca* (Moench) (King and Dancik 1983) and was

reported previously for *P. rigida* by Guries et al. (1978). In every case, the recombination fraction was small. Several other loci including *Adh1, Adh2, Adh3, Per1, Per2, Est4, Dia3,* and *Gpd2* have been mapped to the linkage group to which *Aat1* and *Pgi2* belong (Conkle 1981). The only other locus of this group analyzed in pitch pine is *Pep2* but there was no evidence found to support close linkage between *Aat1* and *Pep2.* The lower bound of the Bayes' 95% CI for recombination fraction between *Aat1* and *Pep2* was 0.40 based on 7 families (312 megagametophytes). The linkage between *Gpd* and *Idh* has been reported for *Pinus jeffreyi, P. taeda,* ($\hat{\theta}=0.20$, Conkle 1981), and *Pseudotsuga menziesii* (Mirb.) Franco ($\hat{\theta}=0.33$, El-Kassaby et al. 1982).

The *Mdh3: Pgm2* and *Pep1: Mdh4* linkages, not previously reported, are firmly established for *Pinus rigida.* Conkle (1981) reported that a fluorescent esterase locus and *Pep1* (LAP-1) were linked in *Pinus contorta, P. taeda,* and *P. jeffreyi* with map distances of 35.6, 45.6, and 25.6 centimorgans respectively. El-Kassaby et al. (1982) found weak evidence for linkage between *Mdh-4* and the locus encoding malic enzyme (ME) in *Pseudotsuga menziesii.* In addition, an individual of *Pinus hartwegii* Lindl. provided strong evidence that *Me, Pep1,* and *Fle* are linked in that order, with the total map distance approximately 40 centimorgans and *Pep1* very near the middle (O'Malley, unpublished data). Thus, *Me, Pep1, Fle,* and *Mdh4* are part of the same linkage group.

References

Adams WT, Joly RJ (1980a) Genetics of allozyme variants in loblolly pine. J Hered 71:33–40

Adams WT, Joly RJ (1980b) Linkage relationships among twelve allozyme loci in loblolly pine. J Hered 71:199–202

Bartels H (1971) Genetic control of multiple esterases from needles and macrogametophytes of *Picea abies.* Planta 99:283–289

Conkle MT (1981) Isozyme variation and linkage in six conifer species. In: Conkle MT (ed) Isozymes of North American forest trees and forest insects. USDA Forest Ser Gen Tech Rep PSW-48:11–17

Dixon M, Webb EC (1979) Enzymes. Academic Press, New York

Eckert RR, Joly RJ, Neale DB (1981) Genetics of isozyme variants and linkage relationships among allozyme loci in eastern white pine clones. Can J For Res 11:573–579

El-Kassaby YA, Sziklai O, Yeh FC (1982) Linkage relationships among 19 polymorphic allozyme loci in coastal Douglas-fir (*Pseudotsuga menziesii* var. 'menziesii'). Can J Genet Cytol 24:101–108

Gottlieb LD (1982) Conservation and duplication of isozymes in plants. Science 216:373–380

Guries RP, Friedman ST, Ledig FT (1978) A megagametophyte analysis of genetic linkage in pitch pine (*Pinus rigida* Mill.). Heredity 40:309–314

Guries RP, Ledig FT (1978) Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* Mill.). Heredity 40:27–32

Guries RP, Ledig FT (1982) Genetic diversity and population structure in pitch pine. Evolution 36:387–402

Haldane JBS, Smith CAB (1947) A new estimate of the linkage between the genes for haemophilia and colour-blindness in man. Ann Eugen 14:10–31

King JN, Dancik BP (1983) Inheritance and linkage of isozymes in white spruce (*Picea glauca*). Can J Genet Cytol 25:430–436

Marty TL, O'Malley DM, Guries RP (1984) A manual for starch gel electrophoresis: microwave edition. Univ Wisconsin-Madison Staff Paper No 20

Mather K (1951) The measurement of linkage in heredity. Wiley and Sons, New York

Morton NE (1955) Sequential tests for the detection of linkage. Am J Hum Genet 7:277–318

Morton NE (1956) The detection and estimation of linkage between the genes of elliptocytosis and the Rh blood type. Am J Hum Genet 8:80–96

Nordheim EV, O'Malley DM, Chow SC (1984) On the performance of a likelihood ratio test for genetic linkage. Biometrics 40:785–790

Nordheim EV, O'Malley DM, Guries RP (1983) Estimation of recombination frequency in genetic linkage studies. Theor Appl Genet 66:313–321

O'Malley DM (1984) Population genetic studies in the *Pinaceae.* PhD Thesis, University of Wisconsin-Madison

Rao DC, Koats BJB, Morton NE, Yee S, Lew R (1978) Variability of human linkage data. Am J Hum Genet 30:516–529

Rudin D, Ekberg I (1978) Linkage studies in *Pinus sylvestris* L. using macrogametophyte allozymes. Silvae Genet 27:1–12

Smith CAB (1959) Some comments on the statistical methods used in linkage investigations. Am J Hum Genet 11:289–304

Steinhoff RJ, Joyce DG, Fins L (1983) Isozyme variation in *Pinus monticola.* Can J For Res 13:1122–1132

Weber JC, Stettler RF (1980) Isoenzyme variation among ten populations of *Populus trichocarpa* Torr. et Gray in the Pacific Northwest. Silvae Genet 30:82–87

Wheeler NC, Guries RP (1982) Population structure, genic diversity, and morphological variation in *Pinus contorta* Dougl. Can J For Res 12:595–606