yielding varieties. Pub. 3. Dept. of Genetics and Plt. Breed., Aristotelian Univ. of Thessaloniki, Greece. 42 pp. (1973). — FASOULAS, A.: The honeycomb field designs. Pub. 9. Dept. of Genetics and Plt. Breed., Aristotelian Univ. of Thessaloniki, Greece. 70 pp. (1979). — FLETCHER, A. M., HOWELL, R. S. and FAULKNER, R.: Problems associated with the layout of progeny-tests in Britain, with special reference to a recent plot-size experiment. Comptes Rendus 14th IUFRO-Kongress (Munich). Sec. 22: 426—434 (1967). — HANSON, W. D., BRIM, C. A. and HINSON, K.: Design and analysis of competition studies with an application to field plot competition in the soybean. Crop Sci. 1: 255—258 (1961). — HARPER, J. L., CLATWORTHY, J. N., MCNAUGHTON, I. H. and SAGER, G. R.: The evolution and ecology of closely related species living in the same area. Evolution 15: 209—227 (1961). — HARPER, J. L.: The regulation of numbers and mass in plant populations. *In:* Population biology and evolution. Ed. R. C. LEWONTIN. Syracuse Univ. Press, Syracuse, NY. pp. 139—158 (1968). — HELGASON, S. B. and CHEBIB, F. S.: A mathematical interpretation of inter-plant competition effects. p. 535—44. *In:* Statistical Genetics and Plant Breeding. *Eds.* W. D. HANSON and H. F. ROBINSON. NAS-NRC pub. 982. Wash. D. C. 623 p. (1963). — HEYBROEK, H. M.: Primary considerations: multiplication and genetic diversity. Unasylva 30 (119/120): 27—33; 49—50 (1978). — LEE, J. A.: A study of plant competition in relation to development. Evolution 14: 18—28 (1960) . — MATHER, K.: Response to selection. CSH Sym. Quant. Biol. 20: 158—65 (1955). — PEARCE, S. C.: Field experimentation with fruit trees and other perennial plants. Tech. Comm. 23, Commwealth. Bur. Hort. and Plantation crops. E. Malling, England (1953). — RIGHTER, F. I.: New perspectives in forest tree breeding. Science 104 (2688): 1—3 (1946). — SAKAI, K.-I.: Competition in plants and its relation to selection. CSH Sym. Quant. Biol. 20: 137—157 (1955). — SAKAI, K.-I. and HATAKEYAMA, S.: Estimation of genetic parameters in forest trees without raising progeny. Silv. Genet. 12: 152—157 (1963). — SALMON, S. C.: Random versus systematic arrangement of field plots. Agron. Jour. 45: 459—462 (1953). — SHIUE, C. and PAULEY, S. S.: Some considerations on the statistical design for provenance and progeny tests in tree improvement programs. For. Sci. 7: 116—122 (1961). — SKINNER, J. C.: Sugar cane selection experiments, 2. Competition between varieties. Bur. Sugar Expt. Sta. Tech. Comm. 1961—1. Brisbane, Australia. 26 p. (1961). — SMITH, H. F.: An empirical law describing heterogeneity in the yield of agricultural crops. Jour. Ag. Sci. 28: 1—23 (1938). — SNYDER, E. B.: Comments on "The divergent points of view of forest geneticists and agronomic and horticultural crop breeders". Jour. For. 57: 666—68 (1959). — STERN, K., HOLST, M., NILSSON, B., JOHNSSON, H., DIETRICHSON, J., GUNZL, L., NANSON, A., GONZALEZ, G., KING, H., and FERRANDES, P.: Design, layout and analyses of provenance experiments. p. 685—97. *In:* Standardization of Methods for provenance research and testing. *Comp.* R. Lines. Comptes Rendus 14th IUFRO-Kongress (Munich). Sec. 22: 672—718 (1967). — STONECYPHER, R. W.: The loblolly pine heritability study. Southlands Expt. For. (International Paper Co., Bainbridge, Ga.) Tech. Bull. 5. 128 p. (1966). — WRIGHT, J. W. and FREELAND, F. D.: Plot size and experimental efficiency in forest genetic research. Mich. State. Univ. Tech. Bull. 280. 28 p. (1960).

The Genetic Structure and Levels of Inbreeding in a Pinus radiata D. Don Seed Orchard

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Summary

Thirty clones of a seed orchard of *Pinus radiata* were assayed to determine their genotypes at 22 allozyme loci: 12 loci were monomorphic and 10 polymorphic. The average number of alleles per locus (A) was 1.54 and the estimate of total genetic diversity (H) for the seed orchard 0.108. In three annual seed crops of the orchard the allelic frequencies at eight polymorphic loci, both in the pollen and maternal components of the progeny, were in good agreement with the frequencies in the thirty parental clones. From the progeny arrays in the crops the overall rate of outcrossing in the orchard was at least 90 %. There were no significant differences between the rates of outcrossing in the three individual annual crops.

Key words: Pinus radiata D. DON, seed orchards, allozyme variation, genetic diversity, inbreeding levels.

Zusammenfassung

Dreißig verschiedene Klone aus einer Samenplantage von *Pinus radiata* wurden im Hinblick auf ihren Genotyp an 22 Allozymloci untersucht, wovon 12 Loci monomorph und 10 polymorph waren. Die durchschnittliche Anzahl der Allele pro Locus (A) betrug 1,54, die geschätzte genetische Gesamtvariation (H) für die Samenplantage 0.108. In den Ernten dreier verschiedener Jahre stimmten die Allelfrequenzen von acht polymorphen Loci sowohl beim Pollen als auch bei den mütterlichen Komponenten der Nachkommenschaften gut mit den Häufigkeiten in den 30 elterlichen Klonen überein.

Nach den Herkunftsreihen zu schließen, kann die Fremdbefruchtungsrate in der Plantage auf mindestens 90 % geschätzt werden.

Es ergaben sich keine signifikanten Unterschiede in den Fremdbefruchtungsraten zwischen den Erntejahren.

Introduction

An essential requirement for evaluating the status of the genetic resources of forest tree species under various strategies of domestication is the estimation of the genetic variability in populations within tree breeding programs. The better-performing clones in tree improvement programs are usually grown in seed orchards with the aim of producing superior seed for commercial plantations. The measurement of genetic variability in seed orchards would therefore seem a desirable first step in the monitoring of the genetic variability in current and future plantations of tree species. Such estimates would also enable comparisons to be made of the levels of genetic diversity between natural and planted populations of a species. The isozyme technique is the best currently available method for measuring genetic variation (BROWN and MORAN 1980).

Breeding programs commonly used for pine species assume random mating. In particular, in the case of clonal seed orchards the premise is that natural wind-pollination will give virtually complete outcrossing. Progeny tests

of wind-pollinated progenies are often analysed as half-sib families on the assumption of random mating. If this premise is invalid, such analyses can result in over-estimates of additive genetic variance and hence heritability and eventually genetic gain (SQUILLACE 1974), although the estimates can be appropriately adjusted if the level of inbreeding is known.

In conifer species estimates of the degree of self pollination in natural populations have been based on morphological markers in a few trees and have generally been less than 20 % (BANNISTER 1965, SQUILLACE 1974). Until recently, quantitative studies of inbreeding in seed orchards had not been made (HADDERS and KOSKI 1975) and even the estimates now available have been based on only a few clones, with unique alleles at isozyme loci (RUDIN and LINDGREN 1977, ADAMS and JOLY 1980). Thus, though some data exist on the variation in level of selfing between a few clones in seed orchards, there are almost no data on the overall level of selfing based on all the seed orchard clones. Selfing could be important in determining the genetic composition and size of commercial seed crops as it is generally agreed that selfing reduces seed yields following selfing compared to outcrossing (PAWSEY 1964, FRANKLIN 1970, SORENSEN 1971, KOSKI 1973). In a breeding program, reduced seed output represents a loss of efficiency. In addition, there is evidence of inbreeding depression in several pine species (PAWSEY 1954, SORENSEN and MILES 1974). The development of strategies to avoid inbreeding may quickly result in considerable gains, both in quantity and quality of seed produced.

Pinus radiata D. DON is the most widely planted pine species in the southern hemisphere. Of the approximate 22 000 ha planted in Australia per year at least 50 % is planted with seed orchard stock (PEDERICK and GRIFFIN 1978). Here we report studies of the mating system and the genetic structure of a *Pinus radiata* seed orchard and of the genetic composition of the seed crops using allozymes as genetic markers.

Material and Methods

The first *P. radiata* seed orchard in Australia was established in Tallaganda State Forest, N.S.W. in 1957. The total area of the seed orchard is 4.4 ha and it is situated at least 12 kilometres from the nearest plantation. Thirty clones are planted in 34 blocks with one ramet of each clone in each block (FIELDING 1964, BROWN 1971).

Open pollinated seed was collected from a ramet of the thirty different clones located in either of two of the central blocks in the orchard. In a few instances seed from some clones had to be collected in adjacent blocks. The collections were made after cones had ripened in October -November in each of the years 1974, 1977 and 1978. The seed in each crop results from pollination two years previously. For the 1974 crop, seed was available for only 27 clones.

Assays were performed on the tissues of five seedlings and the corresponding haploid megagametophytes from five seeds in each clone. An additional five seedlings were assayed per clone for five enzyme systems. The young seedlings and megagametophytes were crushed in one and two drops respectively of 0.1M phosphate buffer (pH 7.0) containing 1 mg per ml of dithiothreitol and the extracts absorbed onto paper chromatography wicks (6 mm × 5 mm). Samples were run in a horizontal electrophoresis system with 12.5 % starch gels. Two gel buffer systems were employed. In the first, gels were buffered with 5mM histidine titrated to pH 8.0 and electrophoresis was conducted for 4½ hours at 7v cm^{-1} with a connecting buffer of 0.41 M sodium citrate pH 8.0. On this gel system the enzyme systems assayed were isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGD), alcohol dehydrogenase (ADH), phosphoglucosisomerase (PGI), phosphoglucomutase (PGM), malate dehydrogenase (MDH), aconitase (AC), shikimate dehydrogenase (SDH) and menadione reductase (MDR). Standard assay methods (GURIES and LEDIG 1978, CONKLE 1971) were employed with slight modifications except for SDH and MDR where the assays were those used by BROUE (*et al.* 1977) and BURDON (*et al.* 1980) respectively. In the second system, a tris-citrate-lithium hydroxide — borate buffer (BREWBAKER *et al* 1986) was employed. The slices from these gels were assayed for glutamate — oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP). glutamate dehydrogenase (GDH), acid phosphatase (AP), and glucose 6 phosphate dehydrogenase (G6PD) by standard procedures (BREWBAKER *et al.* 1968, SHAW and PRASAD 1970).

The maternal genotypes of the thirty clones were determined from each array of 15 haploid megagametophytes (i. e. 5 seed from each crop for each clone). The allelic frequencies in the progeny were based on a minimum of 150 seed per crop. Mating system parameters were estimated by maximum likelihood procedures (BROWN *et al.* 1975, CLEGG *et al.* 1978).

Results

The thirty clones are monomorphic for the same genotypes at the following twelve loci: PGI-1, PGM-1, LAP-2, SDH-1, GOT-1, 6PGD-2, G6PD-1, MDR-1, MDR-5, and 3 MDH loci. However, the seed orchard clones are polymorphic at 10 loci *(Table 1)*. The level of polymorphism is low at five of these loci and in fact for three loci, only one of the thirty clones is heterozygous. For the 22 loci surveyed in the seed orchard, the average number of alleles per locus was 1.54 and the total genetic diversity, H, was .106 where H --is the mean expected panmictic heterozygosity (NEI 1975). The average number of heterozygous loci/clone was 2.23.

The allelic frequencies in the progeny of the three crops are very similar to those in the parental clones *(Table 2)*. The AP-2 and MDR-3 loci were not assayed in the progeny because of difficulty in reliably scoring genotypes in diploid material. The survey of 5 seeds/clone/crop for both haploid megagametophyte and the diploid embryo enabled direct allelic frequencies in the ovule and the pollen components to be determined *(Table 2)*. At the four loci for which the level of polymorphism is low, there was close

Table 1. — Genotypic arrays at ten polymorphic loci for the thirty clones in Tallaganda Seed Orchard.

Locus	Genotype						
	MM*	MS	SS			MV	Total
GDH-1	27	3	0				30
AP-2	11	14	4			1	30
IDH-1	28	2	0				30
PGI-2	22	6	2				30
	FF	FM	MM	MS	SS	FS	
GOT-2	0	1	29	-	-	-	30
6PGD-1	0	1	29	-	-	-	30
MDR-3	0	1	29	-	-	-	30
AC-1	3	12	15	-	-	-	30
LAP-1	0	7	19	4	0	0	30
ADH-2	3	4	9	8	4	2	30

* F, M, S and V refer to the fast, medium, slow and very slow alleles at each locus.

Table 2. — Allelic frequencies at eight polymorphic loci among the parental clones, progeny, and pollen and maternal components of progeny.

Locus	Allele	Parent	Progeny*			Pollen			Maternal		
			1974	1977	1978	1974	1977	1978	1974	1977	1978
GDH-1	M	0.95	0.95	0.90	0.96	0.97	0.93	0.97	0.92	0.96	0.95
	S	0.05	0.05	0.10	0.04	0.03	0.07	0.03	0.08	0.04	0.05
GOT-2	F	0.02	0.02	0.05	0.01	0.01	0.03	0.02	0.02	0.02	0.01
	M	0.98	0.98	0.95	0.99	0.99	0.97	0.98	0.98	0.98	0.99
IDH-1	M	0.97	0.97	0.95	0.96	0.96	0.98	0.98	0.98	0.96	0.94
	S	0.03	0.03	0.05	0.04	0.04	0.02	0.02	0.02	0.04	0.06
ADH-2	F	0.20	0.20	0.20	0.24	0.26	0.24	0.29	0.18	0.23	0.17
	M	0.50	0.46	0.49	0.44	0.42	0.42	0.36	0.51	0.51	0.53
	S	0.30	0.34	0.31	0.32	0.32	0.34	0.35	0.31	0.26	0.30
6PGD-1	F	0.02	0.02	0.03	0.01	0.02	0.03	0.00	0.02	0.03	0.02
	M	0.98	0.98	0.97	0.99	0.98	0.97	1.00	0.98	0.97	0.98
PGI-2	M	0.83	0.81	0.81	0.80	0.82	0.78	0.78	0.79	0.83	0.81
	S	0.17	0.19	0.19	0.20	0.18	0.22	0.22	0.21	0.17	0.19
LAP-1	F	0.13	0.11	0.13	0.13	0.10	0.11	0.14	0.12	0.13	0.13
	M	0.82	0.83	0.80	0.80	0.86	0.78	0.81	0.80	0.77	0.79
	S	0.05	0.06	0.07	0.07	0.04	0.11	0.05	0.08	0.10	0.08
AC-1	F	0.32	0.26	0.27	0.24	0.30	0.32	0.15	0.25	0.27	0.31
	M	0.68	0.74	0.73	0.76	0.70	0.68	0.85	0.75	0.73	0.69

* For the first five loci 300 progeny (10 embryos/clone) per crop were assayed; for the other three loci 150 progeny were assayed.

agreement in allelic frequencies between parental clones and ovule and pollen components of the progeny. Likewise, at the PGI-2 and LAP-1 loci the allelic frequencies in the parental, pollen and maternal samples are very similar. However, at the ADH-2 locus there is an apparent deficiency of the M allele in the progeny and this is mirrored by a significant deficiency of this allele in the pollen pool. Maximum likelihood (ML) estimates of allelic frequencies in the pollen pool of the total progeny confirm the significantly low frequency of the M allele in all crops (i.e. S.E's of $\hat{p}$ are $\pm$ .04 — .05). The deficiency of the M allele could be due to low pollen production or abnormal time of pollen shedding by some of the clones homozygous for the M allele. There is a marked deficiency of the F allele at the aconitase locus in the 1978 pollen pool.

There tends to be an excess of heterozygotes at the four most polymorphic loci in the progeny compared to those in the parental clones *(Table 3)*. The parental clones were part of a synthesised population and the observed heterozygote frequencies in the three crops agree closely with those expected after a cycle of random mating.

The ML estimates of the outcrossing rates ($\hat{t}$) from the progeny arrays of four loci are listed in *Table 4*. The $\hat{t}$ values indicate high levels of outcrossing in all crops as measured at the viable embryo stage. The overall level of outcrossing in the seed orchard was 90%. Most of the estimates of $\hat{t}$ for individual loci are not significantly different from $\hat{t} = 1.0$ given the size of the standard errors. Estimates of the mean outcrossing rates for the 3 crops show very close agreement which suggests that the frequency of outcrossing in different seasons is essentially the same.

Levels of selfing for individual clones in the orchard can be determined for those which have unique alleles. Thus the clone 12419 is heterozygous at GOT 2 (FM) whereas all the other clones are homozygous (MM). Homozygotes for the rare allele from open-pollinated progeny of this clone would arise from selfing events. 20 progeny from each of 10 ramets in the orchard for two crops, 1972 and 1978, were assayed for their genotype at this locus *(Table 5)*. The data indicate an average of 14% selfing for this clone in the two crops. This figure includes two components — (1) selfing within ramets and (2) selfing between ramets of clone 12419. The removal of the selfing component that would occur between ramets of the same clone due to ran-

Table 3. — Observed heterozygosity in the progeny and parental clones of four loci and the expected level after random mating (ERM).

Locus	Type	1974	1977	1978	Parents	ERM
PGI-2	MS	0.28	0.29	0.31	0.20	0.28
AC-1	FM	0.40	0.44	0.37	0.40	0.43
ADH-2	FM	0.18	0.21	0.18	0.13	0.20
	FS	0.13	0.11	0.13	0.07	0.12
	MS	0.30	0.30	0.34	0.27	0.36
Total		0.61	0.63	0.65	0.47	0.68
LAP-2	FM	0.20	0.19	0.22	0.23	0.21
	FS	0.01	0.03	0.02	0.00	0.01
	MS	0.09	0.11	0.11	0.13	0.08
Total		0.30	0.33	0.35	0.36	0.30

Table 4. — Estimates of the outcrossing rate ($\hat{t}$) in three seed crops of the Tallaganda Seed Orchard

Locus	Crop			
	1974	1977	1978	Total
ADH-2	0.82±0.08	1.00±0.07	0.92±0.08	0.93±0.07
PGI-2	0.92±0.12	0.96±0.12	0.81±0.14	0.92±0.07
LAP-2	1.02±0.16	0.66±0.19	0.93±0.17	0.72±0.10
AC-1	0.97±0.09	0.99±0.12	1.04±0.12	1.01±0.07
mean	0.93	0.90	0.92	0.90

Table 5. — GOT-2 genotypes of open-pollinated progeny of clone 12419 from Tallaganda Seed Orchard and level of selfing (s) for this clone

Year	FF	FM	MM	Total	s(%)
1972	9	91	100	200	18
1978	5	95	100	200	10

dom pollination leads to an average of 10% selfing within ramets of clone 12419. Unfortunately seed from ramets of the other clones with unique alleles was unavailable, so estimates of selfing for these clones could not be obtained.

Discussion

One objective of tree breeding programs should be to maintain as broad a genetic base as possible to allow changes of selection criteria, e.g. safeguard plantations against attacks of diseases and pests. Estimates of genetic diversity in seed orchards enable a monitoring of such a genetic base. Results from this study indicate that in the Tallaganda seed orchard of *Pinus radiata* the average number of allelles per locus (A) was 1.54 and the genetic diversity was .108. In marked contrast, ADAMS and JOLY (1980) found that in two loblolly pine seed orchards the levels of genetic variability for both diversity measures (A=3.4, H=.200) were twice those for *P. radiata*. In the light of these results the questions of how much genetic diversity is present in the natural populations of *P. radiata* and whether the levels of genetic diversity have changed with domestication of the species are of considerable importance. Studies are currently in progress to answer these questions. The genetic structure of the orchard, especially the low average number of heterozygous loci/clone, suggests that breeding strategies for *P. radiata* which use seed orchards consisting of a low number of clones, e.g. 2-clone orchard (WILCOX *et al.* 1975) may lead to a rapid loss of genetic variability in the plantations. As well such strategies imply an increase in the number of ramets/clone in an orchard which would result in more selfing between ramets of the same clone even with complete random wind pollination.

The overall level of selfing of 10% indicates that most viable embryos are the products of outcrossing. Likewise for one clone in the seed orchard 14% on average of the progeny were derived by self-fertilization. These data do not necessarily reflect how much selfing occurs at time of fertilization. It is generally conceded that there can be considerable embryo abortion beetween fertilization and germination and that many of the embryos which die are products of self-fertilization (SARVAS 1962, FRANKLIN 1970). There is independent evidence for this in *P. radiata* (MATHESON 1980). Variation in the size of the seed crop from year to year, both in total and within and between clones, could in part be due to variable rates of selfing and the abortion of resultant embryos. Whether the net selection against selfed seed is as strong in natural populations of *P. radiata* and other conifers as in seed orchards and plantations is unknown. In contrast to the evidence for eucalypts (MORAN and BROWN 1980) this study showed that there was no significant variation between 3 crops of the seed orchard in the level of inbreeding as measured at the viable embryo stage of the life cycle. Nevertheless the fact that nearly all the seedlings from the orchard crop are from outcrossing events is encouraging for the current tree breeding strategies of *Pinus radiata*.

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References

ADAMS, W. T., and JOLY, R. J.: Allozyme studies in loblolly pine seed orchards: Clonal variation and frequency of progeny due to self-fertilization. Silv. Genet., in press, (1980). — BANNISTER, M. H.: Variation in the breeding system of *Pinus radiata*. *In:* H. G. BAKER and STEBBINS, G. L. (ed.): The Genetics of Colonising Species, p. 353—374. Acad. Press (1965). — BREWBAKER, J. L., UPADHYA, M. D., MACKINEN, Y., and MACDONALD, T.: Isoenzyme polymorphisms in flowering plants. III. Gel electrophoretic methods and applications. Physiol. Plant. 21: 930—940 (1968). — BROUE, P., MARSHALL, D. R., and MULLER, W. J.: Biosystematics of subgenus *Glycine* (VERDE.): Isoenzymatic data Aust. J. Bot. 25: 555—556 (1978). — BROWN A. G.: Experience in management of a radiata pine seed orchard at Tallaganda State Forest, New South Wales. Aust. For. Res. 5: 15—30 (1971). — BROWN, A. H. D., MATHESON, A. C., and ELDRIDGE, K. G.: Estimation of the mating system of *Eucalyptus obliqua* L'Herit. using allozyme polymorphisms. Aust. J. Bot. 23: 931—949 (1975). — BROWN, A. H. D., and MORAN, G. F.: Isozymes and the genetic resources of forest trees. In press. USDA (1980). — BURDON, J. J., MARSHALL, D. R., and GROVES R. H.: Isozyme variation in *Chondrilla juncea* L., in Australia. Aust. J. Bot. In press. (1980). — CLEGG, M. T., KAHLER, A. L., and ALLARD, R. W.: Estimation of life cycle components of selection in an experimental plant population. Genetics 89: 765—792 (1978). — CONKLE, M. T.: Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in knobcone pine. For. Sci. 17: 190—194 (1971). — FIELDING, J. M.: Notes on a pine seed orchard on Tallaganda State Forest in New South Wales. Aust. For. 28: 203—6 (1964). — FRANKLIN, E. C.: Survey of mutant forms and inbreeding depression in species of the family Pinaceae. Southeast For. Exp. Stn., U. S. D. A. For. Serv. Res. Pap. SE-61, 21 p. (1970). — GURIES, R. P., and LEDIG, T.: Inheritance of some polymorphic isozymes in pitch pine (*Pinus rigida* MILL.). Heredity 40: 27—32 (1978). — HADDERS, G., and KOSKI, V.: Probability of inbreeding in seed orchards. *In:* R. FAULKNER (ed.): Seed Orchards. Bull For. Comm. Lond. 54: 108—117 (1975). — KOSKI, V.: On self-pollination, genetic load, and subsequent inbreeding in some conifers. Commun. Inst. For. Fenn. 78 (10): 42 p. (1973). — MATHESON, A. C.: Frequencies of outcrossed seedlings higher than expected among offspring from mixtures of self and cross pollen in *Pinus radiata* D. DON. Aust. For. Res. 10. In press (1980). — MORAN, G. F., and BROWN, A. H. D.: Temporal heterogeneity of outcrossing rates in alpine ash (*Eucalyptus delegatensis* R. T. BAK.). Theor. Appl. Genetics. In press. (1980). — MULLER, G.: A simple method of estimating rates of self- fertilization by analysing isozymes in tree seeds. Silvae Genet. 25: 15—17 (1976). — NEI, M.: Molecular population genetics and evolution — Elsevier, New York (1975). — PAWSEY, C. K.: Inbreeding radiata pine. Leafl. For. Timb. Bur. Aust. 87 (1964). — PEDERICK, L. A., and GRIFFIN, A. R.: The genetic improvement of radiata pine in Australasia. Proc. 3rd World Consult For. Tree Breeding, Canberra p. 562—571 (1977). — RUDIN, D., and LINDGREN, D.: Isozyme studies in seed orchards. Studia Forestalia Suecica Nr. 139: 23 p. (1977). — SARVAS, R.: Investigations on the flowering and seed crop of *Pinus silvestris*. Commun. Inst. For. Fenn. 53 (4): 198 p. (1962). — SHAW, C. R., and PRASAD, R.: Starch gel electrophoresis of enzymes — a compilation of recipes. Biochem. Genet. 4: 297—320 (1970). — SORENSEN, F. C.: Estimate of self-fertility in coastal Douglas-fir from inbreeding studies Silvae Genet. 20: 115—120 (1971). — SORENSEN, F. C., and MILES, R. S.: Self-pollination effects on Douglas-fir and ponderosa pine seeds and seedlings. Silvae Genet. 23: 135—138 (1974). — SQUILLACE, A. E.: Average genetic correlations among offspring from open-pollinated forest trees. SilvaeGenet. 23: 149—156 (1974). — WILCOX, M. D., SHELBOURNE, C. J. A., and FIRTH, A.: General and specific combining ability in eight selected clones of radiata pine. N. Z. J. For. Sci. 5: 219—225 (1975).