gas-liquid chromatographic screening test for the chemotaxonomy of *Populus* species. J. Chromatog. **84**, 315—318 (1973). —— Steele, J. W., Ronald, W. and Bolan, M.: Phytochemistry of the *Salicaceae*. V. The use of a gas-liquid chromatographic screening test to detect phytochemical variations in *Populus deltoides* Marsh. J. Chromatog. **84**, 309—314 (1973). —— Wollenweber, E. and Egger, K.: Die lipophilen Flavonoide des Knospenöls von *Populus nigra*. Phytochem. **10**, 225—226 (1971). —— Wollenweber, E. and Weber, W.: Die farbgebenden Chalkone des Pappelöls. Z. Pflanzenphysiol. **69**, 125—128 (1973). —— Wollenweber, E.: Flavonoidmuster als systematisches Merkmal in der Gattung *Populus*. Biochem. Syst. Ecol. **3**, 35—45 (1975).

Reduction in Levels of Inbreeding in a Seed Orchard of Eucalyptus regnans F. Muell. compared with natural Populations

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(Received 20th November 1987)

Summary

The 1983 seed crop of a *Eucalyptus regnans* seed orchard was assayed for allozyme genotypes at 10 loci to determine multilocus estimates of the mating system parameters. Outcrossing was high with an overall estimate for the whole orchard of 91% at the germinated seedling stage. However, both for the overall seed orchard and the individual blocks, estimates showed significant departure from random mating (t = 1.0). There was also significant variation in outcrossing between the 29 open pollinated families sampled. The level of outcrossing was significantly higher in the seed orchard (t = 0.91) compared to a nearby natural population (t = 0.74). This increase in outcrossing could have been due to a reduction in neighbourhood inbreeding, or differences in the size and density of trees and hence fecundity. This reduction in inbreeding with the use of seed orchards in eucalypt breeding programs could result in significant genetic gains for characters such as growth rate.

Key words: Eucalypts, seed orchards, mating system, outcrossing rates.

Introduction

In natural populations of eucalypts a significant fraction of the viable seed originates from inbreeding. A mixed mating system, predominantly outcrossing but with significant inbreeding, has been shown to be common to a number of species (Moran and Bell, 1983). For instance, in a population of *E. delegatensis* R. T. Bak. the level of outcrossing was 77% with a significant amount of inbreeding (23%) (Moran and Brown, 1980). This inbreeding may be largely due to mating between relatives in neighbourhoods within the population. The markedly detrimental effects of selfing on the growth, seed set and other characters in eucalyptus have been well documented and currently the evidence suggests that the mixed mating system is maintained under natural conditions by selection against inbreds through self-thinning of the population (Hodgson, 1976; Eldridge and Griffin, 1983; Griffin *et al.*, 1987).

In Australia, eucalypt breeding programs are at an early stage of development. Current strategy calls for the commercial production of improved seed for plantations from selected superior families in seedling seed orchards. A similar strategy in conifers appears to be soundly based because the levels of selfing in both seed orchards and natural populations has been found to be less than 10% (Moran *et al.*, 1980; Shaw and Allard, 1982; Freidmann and Adams, 1985; Ritland and El-Kassaby, 1985; Neale and Adams, 1985; Muona and Harju, 1989). In effect, in conifer orchards, the seed crops can potentially reflect the genetic superiority of the orchard families to non-selected stock. In comparison, such gains may not be realised in eucalypts if the outcrossing rates in seed orchards mirror that in natural populations and significant selfing occurs. However, differences in outcrossing rates between seed orchards and natural populations could occur if the assumptions underlying the mixed mating model (Clegg, 1980), on which the estimation procedure is based, are met in one but not both places. For instance, one of the assumptions of the model is that pollen gene frequencies are uniform over maternal plants within a population (Brown *et al.*, 1985). In particular, the pollen involved in each outcrossing event is assumed to come randomly from a homogenous pollen pool encompassing the whole population. In fact spatial heterogeneity in the pollen pool could be expected if neighbourhoods of related individuals occur in a natural population. As a result estimates of the outcrossing rate may be lower since both selfing and neighbourhood inbreeding could occur.

There are currently seed orchards of 5 eucalypt species in Australia. For one of these, *E. regnans* F. Muell., which is one of the three major timber species in southeastern Australia, genetic improvement commenced in Victoria in 1970 (Eldridge, 1971). In this paper we report estimates of the mating system parameters in a 13 year old seedling seed orchard of this species and compare them with those from a nearby natural population.

Materials and Methods

An open-pollinated seedling seed orchard was established at Silver Creek near Thorpdale in Gippsland, Victoria in 1970 by APM Forests Pty Ltd (Cameron and Kube, 1983). Seed was collected in 1969 from plus trees selected in natural populations in the Traralgon Creek area of southern Gippsland. The orchard contained 40 families laid out in a square of 4 × 4 (= 16) randomised complete blocks over an area of 2.4 ha. Initially each family was represented within each block by a plot of five trees in a closely spaced line with 0.6 m between trees. These family plots, spaced at 6 m × 6 m centres, were culled to one vigorous tree during the first 3 years.

In April 1983 seed was collected from all trees that had seed in the four central blocks of the orchard. Since

establishment 10 families had been culled and varying numbers of deaths had occurred in each block. At the time of seed collection the mean number of trees per central block ranged from 23 to 29 and the mean number of trees per block from which seed was collected ranged from 15 to 24. The trees were about 20 m high.

From a nearby natural population of *E. regnans* at Narracan (Griffin, 1980; Griffin *et al.*, 1987) open-pollinated seed was collected from 41 trees to enable a comparable estimate of the mating system to be made.

Isozyme assays by starch gel electrophoresis were performed on 20 open pollinated seedlings from each of the 81 trees collected in the four central blocks of the orchard. Ten seedlings from each of the 41 families of the Narracan population were assayed. Individual seedlings were squashed in one drop of 0.05 M, pH 9.0 borate buffer containing 1 mg/ml dithiothreitol and 20 mg/ml polvinyl pyrrolidone. Each sample was stained for 8 enzyme systems and scored for electrophoretic variants at a total of 10 isozyme loci (*Table 1*). The enzyme systems were as follows: aspartate amino-transferase (AAT,E.C.2.6.1.1); leucine aminopeptidase (LAP,E.C.3.4.11.1); glutamate dehydrogenase (GDH,E.C.1.4.1.2); acid phosphatase (AP,E.C.3.1.3.2); glucosephosphate isomerase (GPI,E.C.5.3.1.9); glycerate dehydrogenase (GLY, E.C.1.1.1.29); malate dehydrogenase (MDH, E.C.1.1.1.37) and shikimate dehydrogenase (SDH, E.C.1.1.1.25). Starch gel electrophoresis procedures and enzyme staining techniques were as described previously (Moran and Bell, 1983; Moran and Hopper, 1983). GLY has not been reported previously in eucalypts and the staining method is the same as for GDH, except glutamate is omitted and DL-glycerate included (2 mg/ml, final concentration). Normal mendelian segregation has been shown for these loci (Moran and Bell, 1983; unpublished) from assays of controlled pollinated seedlots of *E. regnans*.

Multilocus estimates across 10 loci of mating system parameters ($\hat{t}$, the outcrossing rate and $\hat{p}$, the pollen gene frequency) and their variances were calculated by the method of Ritland and Jain (1981). The heterogeneity between outcrossing estimates was tested by Chi-square test of homogeneity (Rao, 1973). Test of significance between pairs of means were made using the t-test. Statistical tests of significance were conducted at the $\alpha = 0.05$ level. For the analysis of variance an arcsine transformation of the data was carried out because of the upper bound on t of 1.0 due to the estimation procedure (Ritland and Jain, 1981). Since this gave essentially the same result as untransformed data the latter data was used in the analysis presented. Fixation indices ($F = 1-H_o/H_e$) for progeny and adult trees were used as a measure of the total deviation in heterozygosity from random mating expectations.

Results

For the whole seed orchard the allelic frequencies in the parental trees and in the progeny are based on 81 parental and at least 1600 progeny genotypes. The allelic frequencies in the progeny were very similar to those in the parental trees (*Table 1*). In the estimation procedure for pollen allele frequencies (Ritland and Jain, 1981) a maximum of 3 alleles per locus could be used. For the 3 loci at which more than 3 alleles were detected the data set was reduced to 3 alleles by combining the rarer alleles with the next most frequent allelic class. Therefore at these 3 loci only pollen gene frequencies of the two most common alleles are presented (*Table 1*). At the other 7 loci only 3 alleles were detected and pollen gene frequencies are given for all alleles. The frequencies of alleles in the pollen pool correspond cosely to those in the parental and progeny pools. In fact, there were no changes of more than 6% in gene frequency across the three classes for the orchard as a whole. Pollen gene frequencies in the four blocks (data not presented) were essentially homogenous with the maximum difference (between any two blocks) in frequency for the two most common alleles at all loci being 10%.

Table 1. — Estimates of allele frequencies in maternal trees, progeny and pollen for the seed orchard of *Eucalyptus regnans*.

Locus	Allele	Maternal	Progeny	Pollen
Aat-1	1	0.085	0.091	
	2	0.604	0.592	0.605
	3	0.293	0.298	0.290
	4	0.018	0.019	
Aat-2	1	0.091	0.112	0.127
	2	0.775	0.766	0.791
	3	0.134	0.122	0.082
Aat-3	1	0.043	0.038	0.026
	2	0.957	0.961	0.972
	3	0.000	0.001	0.002
Mdh-2	1	0.689	0.703	0.712
	2	0.311	0.296	0.287
	3	0.000	0.001	0.001
Gpi-2	1	0.024	0.045	0.068
	2	0.866	0.849	0.839
	3	0.110	0.106	0.093
Sdh-1	1	0.006	0.012	
	2	0.805	0.784	0.768
	3	0.183	0.191	0.188
	4	0.006	0.013	
Gly-1	1	0.000	0.006	0.013
	2	0.848	0.848	0.861
	3	0.152	0.146	0.126
Gdh-2	1	0.012	0.008	0.005
	2	0.805	0.813	0.863
	3	0.183	0.179	0.131
Ap-1	1	0.927	0.923	0.921
	2	0.067	0.070	0.067
	3	0.006	0.007	0.011
Lap-2	1	0.415	0.421	0.414
	2	0.012	0.017	
	3	0.360	0.342	0.357
	4	0.189	0.186	
	5	0.024	0.034	

Estimates of the multilocus outcrossing rates for each of the blocks and for the seed orchard overall are given in *Table 2*. The overall level of outcrossing in the seed orchard was 91% but with a significant level of 9% selfing. Outcrossing rates for individual blocks were calculated on the basis of two types of gene pools. In the first method gene pools for individual blocks were used in the estimation procedure and in the second the gene pool used was for the whole orchard i.e. all central blocks combined (*Table 2*). There was significant heterogeneity (as indicated by the Chi-square test) between t values when individual block gene pools were used but not when the overall gene pool was used. The heterogeneity was primarily due to the low t value for block 6. Use of the gene pool of the whole orchard, compared to the gene pool of the actual block, to obtain t values for individual blocks could lead to a lowering of apparent outcrossing rates if any heterogeneity is present in the larger pollen pool. Comparison of t estimates within blocks did indeed show a reduction in 3 out of the 4 blocks as predicted but the differences were not significant.

There was significantly less selfing in the seed orchard compared to that in the nearby population of Narracan (*Table 2*). The estimate of 74% outcrossing in the Nar-

Table 2. — Estimates of outcrossing rates* for (a) individual blocks of the seed orchard using pollen gene pools at (1) the block level and (2) the whole seed orchard level and (b) the natural population of Narracan.

Seed orchard	Pollen Gene Pool	
Block Number	Block	Overall
6	0.855 ± 0.020	0.882 ± 0.016
7	0.929 ± 0.013	0.919 ± 0.015
10	0.937 ± 0.017	0.928 ± 0.019
11	0.925 ± 0.015	0.915 ± 0.016
$\chi^2_{(3)}$	10.80[1]	4.43
Mean	0.912 ± 0.008	0.911 ± 0.008
Natural Population		
		0.735[2] ± 0.025

*) All outcrossing rates significantly different from t = 1.0 at the 0.1% level.
1) $P < 0.05$.
2) t-test for difference between outcrossing rate in the seed orchard and outcrossing rate in the natural population is significant at 5% level.

racan stand was very similar to the overall mean of 78% outcrossing calculated from earlier estimates of ten eucalypt species (Moran and Bell, 1983).

An important component of the breeding systems of eucalypts which has not been previously estimated, is the extent of variation in outcrossing rates between individuals. Individual-tree multilocus estimates of the outcrossing rate were calculated by joint estimation of t and p for each tree (*Table 3*). Overall the t estimates for individual trees were high but there is much greater variation between individual trees than between blocks. Thus for 11 out of the 81 trees estimates were below 0.8 and the range in t values was from 0.42—1.00. Since the outcrossing rate is essentially a quantitative character, and blocks can be treated as replicates of a uniform environment, the distribution of variation within and among families can be compared. The one-way analysis of variance of outcrossing rates gave a significant F ratio for variation among families compared to within families (*Table 3*). It was noticeable that of the 11 trees with estimates of outcrossing less than 0.80, six were in block 6. One of the assumptions of the mixed-mating model is that the outcrossing rate is independent of the maternal genotype. There was a very low and non-significant correlation (r = 0.113) between the number of heterozygous loci per maternal tree and the outcrossing rate.

Table 3. — Estimates of multilocus outcrossing rates (t_m) for individual trees of the 29 families in the four central blocks of the seed orchard.

family	Block 6	7	10	11	mean
1	0.999	0.802	1.000	1.000	0.950
2		0.999	0.999	0.890	0.963
3	0.873	0.900	1.000	0.999	0.943
4	0.577	0.893			0.735
5	0.913	0.958		0.935	0.935
6	0.501	0.849			0.675
11	0.941	1.000	0.812	0.921	0.918
12		0.859		0.999	0.929
13		1.000			1.000
15		0.999		0.999	0.999
16	0.672	0.797	0.917	0.959	0.836
17	0.941	0.922	0.936		0.933
20	0.598	0.999	1.000		0.866
21	0.999	1.000	1.000	0.841	0.960
23	1.000	1.000	0.866		0.955
24				0.999	0.999
26	0.942	1.000		0.765	0.902
28	0.928	0.781			0.854
30	0.999			0.999	0.999
31	0.422	0.768	0.999	0.938	0.782
32	0.755		0.744	1.000	0.833
33	0.913	1.000	0.888	1.000	0.950
34	0.999	0.878		1.000	0.959
35	1.000	0.999	1.000		1.000
36				0.988	0.988
37	0.898	1.000		0.999	0.966
38	0.999			0.718	0.858
39	1.000	0.954	0.928	0.999	0.970
40		0.877	0.999		0.938
mean	0.858	0.926	0.939	0.946	0.917
SE	0.020	0.013	0.016	0.014	

ANOVA	DF	mean squares	F
between families	28	0.0270	1.89*
within families	52	0.0142	

*) significant at the 5% level.

In contrast to previous studies of eucalypt mating systems in natural populations, the mean observed heterozygosity in the progeny was not significantly less than in the parental trees (*Table 4*). Similarly, the observed and expected heterozygosities are not significantly different in either the progeny or parents. This was confirmed by the small departures of the fixation indices from zero

Table 4. — Mean estimates across 10 loci of the observed (H_o) and expected (H_e) heterozygosities, the fixation indices (F)[1] and the equilibrium coefficient (F_e) in the seed orchard.

	H_o	H_e	F	F_e
Progeny	0.324 ± 0.053	0.343 ± 0.056	0.062[2]	0.078
Maternal	0.348 ± 0.057	0.338 ± 0.056	-0.042	-

1) $F = 1 - H_o/H_e$, $F_e = (1 - t)/(1 + t)$.
2) Rejection of the null hypothesis that F = 0 at the 5% level.

(*Table 4*). The mean fixation index across 10 loci was just significant at the 5% level for the progeny, but not for the parents. The negative parental F value raises the question of whether this occurred by chance or is a reflection of some selection against selfed individuals in the culling process in early establishment. The predicted equilibrium inbreeding coefficient, F_e, which assumes that self-fertilization is the only cause for departure from random mating, showed good agreement with the fixation index for the progeny. This suggests that the small deficiency in heterozygotes compared to that expected under random mating can largely be accounted for by the breeding system.

Discussion

The overall estimate of outcrossing in the Silver Creek seed orchard of *E. regnans* was 91%. The level of non-random mating although low was also significant. Moreover, the seed orchard was found to have a significantly higher level of outcrossing than occurs in a nearby population of *E. regnans* (t = 0.74). This estimate for the local population was similar to estimates of outcrossing previously reported for natural populations of eucalypts (Brown *et al.*, 1975; Phillips and Brown, 1978 Moran and

BROWN, 1980; MORAN and BELL, 1983). In all these studies of natural populations a mixed mating system was found with predominant outcrossing but also with a substantial fraction of inbreeding in the order of 22%. The increase in the level of outcrossing for the orchard has considerable practical significance for plantation forestry. GRIFFIN and COTTERILL (1987) have shown a 12% increase in growth rate for controlled outcrosses among a sample of the Narracan trees compared to open-pollinated progeny from the same trees at age 4 years. Generally such marked differences in outcrossing between seed orchards and natural populations have not been demonstrated for other types of trees such as gymnosperms. For instance, in Douglas-fir (SHAW and ALLARD, 1982; NEALE and ADAMS, 1985; RITLAND and EL-KASSABY, 1985) and Scots pine (RUDIN *et al.*, 1986; MUONA and HARJU, 1989) outcrossing rates are 0.90 or higher in both natural stands and seed orchards.

The estimates calculated are actually effective outcrossing rates and include a number of possible causes of departure from panmixia such as gametic selection, zygotic lethality and varying levels of self fertility. Also, one of the assumptions of the mixed mating model is that there is a homogenous pollen pool in the population across maternal parents (BROWN *et al.*, 1985). If marked subpopulation structure, such as neighbourhoods of related individuals, exists within a population then heterogeneity can arise in the pollen pool leading to downward biases in the level of outcrossing (ENNOS and CLEGG, 1982). Inbreeding in such a situation arises not only through self-fertilization but also from mating between relatives. This is the most likely explanation for the marked differences in outcrossing between natural populations of eucalypts and the seed orchard. Neighbourhoods may be more pronounced in natural populations of eucalypts compared to conifers because both pollen and seed dispersal is generally more limited in eucalypts. In the orchard, with its random distribution of unrelated genotypes, there is no neighbourhood structure. This is reflected in the essentially homogenous pollen pools determined for each block. Within the orchard there was only one member of each open pollinated family per block and this artificial structure probably minimizes matings between relatives in a similar fashion to the overdispersed treatment used by ELLSTRAND and FOSTER (1983). The extent to which heterogeneity in pollen pools occurs within eucalypt populations, because of spatial clumping of relatives, needs to be examined. Temporal heterogeneity in pollen allele frequencies was found within the Narracan population of *E. regnans* as a result of differences in flowering time between trees (FRIPP *et al.*, 1987). Of course, it is possible that in natural populations the differences in outcrossing between conifers and eucalypts is not due to the absence of neighbourhoods in conifers but rather that selection against inbreds at the viable embryo stage (i.e. before the assay point) is stronger in conifers.

Other environmental and biological factors could substantially affect outcrossing rates in the seed orchard. Like many heritable quantitative characters the mating system is plastic and subject to environmental influences. For instance both spatial and temporal variation in the mating system can occur in tree species (MORAN and BELL, 1983; BROWN *et al.*, 1985; CHELIAK *et al.*, 1985). Of more immediate relevance is the evidence that variation in outcrossing can be related to the density and size of plants in a stand. SHEA (1987) reported that in ENGELMANN spruce the highest outcrossing rates are expected in trees that are medium to large rather than the largest. In the *E. regnans* seed orchard trees were fairly uniform in size and about 20 m high such that fecundity was fairly uniform between trees. In contrast, large differences in fecundity and presumably pollen production were found associated with the size of trees in the Narracan stand (FRIPP *et al.*, 1987). An inverse relationship between plant density and outcrossing rates has been reported in natural populations of the animal-pollinated *Helianthus annus* (ELLSTRAND *et al.*, 1978). Whether this relationship is linear or even holds generally for animal pollinated plants such as eucalypts is unclear. It is clear however that stand density in the orchard is probably more uniform and lower than in natural eucalypt populations of comparable age. This could contribute to the higher outcrossing rates. The effect of density on outcrossing in natural and artificial stands of eucalypts needs to be examined, since several factors which may be contributing to the higher outcrossing rates in the orchard could not be separated in this study.

In effect the question is the relative importance of intrinsic the variation in t-effects as distinct from p-effects (BROWN *et al.*, 1985). The t-effects concern outcrossing rates and p-effects the gamete allele frequencies. Thus the above discussion about subpopulation structure and heterogenous pollen pools constitutes p-effects. In contrast variation between individuals in outcrossing rates may be a major source of t-effects.

In this study evidence was presented of variation in outcrossing for individual trees both within and between families and that there was significant variation between families when compared to variation within families. Even so, two thirds of the families had mean outcrossing rates over 90%. Assuming that a substantial component of the variance in outcrossing is genetic, selecting for outcrossing rate is an option which can be considered in the development of a eucalypt breeding strategy (see VAN WYK, 1978). This selection might be applied in either direction, either for highly self-sterile lines or for self-fertility in conjunction with lower levels of inbreeding depression (ELDRIDGE and GRIFFIN, 1983). Line breeding may be a realistic option since controlled self pollination is technically easier than outcrossing, especially if this would lead to the removal of semi-lethals from the breeding population.

The results of this study lend encouragement to the use of seed orchards as a means of realising genetic gain in eucalypt improvement programs. It is an implicit assumption that the trees in a seed orchard will freely interbreed but, prior to this study, it was by no means clear that this was valid for such moderately self-fertile species as eucalypts. In the orchard used in this study, trees were from populations geographically close together. Whether outcrossing rates will be as high when select material incorporated in a seed orchard comes from more widely separate populations with different flowering times is something that has not been addressed in this study. Nevertheless, this study has shown that in this seed orchard the objective of freely mixing the genes from superior families for the production of commercial seed is being substantially met.

Acknowledgements

We thank CHRISTINE TOMPKINS for excellent assistance with the laboratory work. We are grateful to APM Forests Pty Ltd for allowing us to use their seed orchard for this study and to K. W. JOHNSON for collecting the seed.

References

Brown, A. H. D., Barrett, S. C. H. and Moran, G. F.: Mating system estimation in forest trees: models, methods and meanings. *In:* H. R. Gregorius (ed.). Population Genetics in Forestry. p. 32—49. Springer-Verlag, Berlin (1985). — 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). — Cameron, J. N. and Kube, P. D.: Management of seedling seed orchards of *Eucalyptus regnans*. 1. Selection, strategy and flowering studies. Silvicultura (Sao Paulo) **32**: 765—768 (1983). — Cheliak, W. M., Dancik, B. P., Morgan, K., Yeh, F. C. H. and Strobeck, C.. Temporal variation of the mating system in a natural population of jack pine. Genetics **109**: 569—584 (1985). — Clegg, M.T.: Measuring plant mating systems. Biosciene **30**: 814—818 (1980). — Eldridge, K. G.: Genetically improved eucalypt seed for Australian pulpwood forests. Appita **25**: 105—109 (1971). — Eldridge, K. G. and Griffin, A. R.: Selfing effects in *Eucalyptus regnans:* Silv. Genet. **32**: 216—221 (1983). — Ellstrand, N. C. and Foster, K. W.: Impact of population structure on the apparent outcrossing rate of grain sorghum (Sorghum Bicolor). Theor. Appl. Genet. **66**: 323—327 (1983). — Ellstrand, N. C., Torres, A. M. and Levin, D. A.: Density and the rate of apparent outcrossing in *Helianthus annuus* (Asteraceae). Syst. Bot. **3**: 403—407 (1978). — Ennos, R. A. and Clegg, M. T.: Effect of population substructuring on estimates of outcrossing rates in plant populations. Heredity **48**: 283 292 (1982). — Friedman, S. T. and Adams, W. T.: Levels of outcrossing in two loblolly pine seed orchards. Silv. Genet. **34**: 157—162 (1985). — Fripp, Y. J., Griffin, A. R. and Moran, G. F.: Variation in allele frequencies in the outcross pollen pool of *Eucalyptus regnans* F. Muell. throughout a flowering season. Heredity **59**: 161—171 (1987). — Griffin, A. R.: Floral phenology of a stand of mountain ash in Gippsland, Victoria. Aust. J. Bot. **28**: 393—404 (1980). — Griffin, A. R., Moran, G. F. and Fripp, Y. J.: Preferential outcrossing in *Eucalyptus regnans* F. Muell. Aust. J. Bot. **35**: 465—475 (1987). — Griffin, A. R. and Cotterill, P. P.: Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. Silv. Genet. **37**: 124—131 (1988). — Hodgson, L. M.: Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* at J. D. M. Keet Research Station. 3. Relative yield, breeding systems, barriers to selfing and general conclusions. S. A. For. Journ. **99**: 53—58 (1976). — Moran, G. F. and Brown, A. H. D.: Temporal heterogeneity of outcrossing rates in alpine ash (*Eucalyptus delegatensis* R. T. Bak.). Theor. Appl. Genet. **57**: 101—105 (1980). — Moran, G. F. and Bell, J. C.: Eucalyptus. *In:* Tanksley, D. S. and Orton, T. J. (eds.). Isozymes in Plant Genetics and Breeding. Part B. Elsevier, Amsterdam. p. 423—441 (1983). — Moran, G. F. and Hopper, S. D.: Genetic diversity and the insular population structure of the rare granite rock species, *Eucalyptus caesia* Benth. Aust. J. Bot. **31**: 161—172 (1983). — Moran, G. F., Bell, J. C. and Matheson, A. C.: The genetic structure and levels of inbreeding in a *Pinus radiata* D. Don seed orchard. Silv. Genet. **29**: 190—193 (1980). — Muona, O. and Harju, A.: Genetic variability and the mating system in natural stands and seed orchards of *Pinus sylvestris*. Silv. Genet., in press (1989). — 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). — Phillips, M. A. and Brown, A. H. D.: Mating system and hybridity in *Eucalyptus pauciflora*. Aust. J. Biol. Sci. **30**: 337—344 (1978). — Rao, C. R.: Linear statistical inference and its applications. 2nd Ed. Wiley, N. Y. (1973). — Ritland, K. and Jain, S.: A model for the estimation of outcrossing rate and gene frequencies using n independent loci. Hered. **47**: 35—52 (1981). — 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). — Rudin, D., Muona, O. and Yazdani, R.: Comparison of the mating system of *Pinus sylvestris* in natural stands and seed orchards. Hereditas **104**: 15—19 (1986). — Shaw, D. V. and Allard, R. W.: Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theor. Appl. Genet. **62**: 113—120 (1982). — Shea, K. L.: Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. Evolution **41**: 124—136 (1987). — Van Wyk, G.: Progress with the *Eucalyptus grandis* breeding programme in the Republic of South Africa. Proc. Third World Cons. Forest Tree Breeding, Canberra. Vol. 2. pp. 639—643 (1978).

Buchbesprechung

Cell and Tissue Culture in Forestry. Three Volumes. Edited by J. M. Bonga and D. J. Durzan. 1987. Martinus Nijhoff Publishers, a Member of Kulwer Academic Publishers Group, Dordrecht, Boston and Lancaster. Hardcover. Volume 1: Genetic Principles and Biotechnology. 422 pages. US $ 78.50. Volume 2: Specific Principles and Methods: Growth and Development. 447 pages. US $ 82.50. Volume 3: Case Histories: Gymnosperms, Angiosperms and Palms. 416 pages. US $ 76.50. Three Volume set: US $ 210.—.

In 1982 Jan Bonga and Don Durzan edited a volume 'Tissue Culture in Forestry' published by Martinus Nijhoff Publishers. Since then there has been remarkable progress in tissue culture of forest trees. In order to update the 1982 edition, the editors embarked on a new project. The outcome has been not one volume but three volumes with a slightly different title: Cell and Tissue Culture in Forestry, published in 1987. The three volumes present a broad spectrum of forest biotechnology.

Volume 1 deals with general principles and biotechnology. It is divided in three Sections: I. Media and Physical Environment (9 chapters); II. Clonal Propagation (5 chapters); and III. Genetic Variation and Ultrastructure (8 chapters). Section I contains chapters on general media, micronutrients, growth regulators, nitrogen and carbohydrate metabolism, polyamines, pH, temperature, and vitrification in tissue culture of forest tree species. Section II contains chapters on testing and deployment of genetically engineered trees, potential genetic gain through tissue culture, juvenility, maturity and rejuvenation, and micropropagation of mature trees. Section III contains chapters on somaclonal variation, measurement and origin of genetic variation in tissue culture systems, DNA in tree species, application of recombinant DNA techniques in pines, cytogenetic manipulations in forest trees through tissue culture, and developmental ultrastructure and role of mitochondria in organogenesis.

Volume 2 deals with specific principles and methods in growth and development of the tree species. This volume contains 22 chapters. The topics discussed in this volume include protoplast culture in conifers and hardwoods, biochemistry of forest trees, somatic embryogenesis, in vitro control of morphogenesis, embryo culture, root formation, haploids, induction of androgenesis, triploids, mycorrhizae, cold storage of tissue cultures, cryopreservation, tissue culture application to forest pathology and pest control, tumors, correlations within the trees, cell senescence, nursery handling of propagules, and metabolic phenotypes in embryonic development of tree species.

Volume 3 deals with case histories of gymnosperms, angiosperms and palms. There are 30 chapters in this volume. The coverage of tree species is broad and varied. This volume includes chapters on tissue culture of Norway spruce, Sitka spruce, European pines, loblolly pine, Douglas fir, Eastern and Western North American conifers, *Cryptomeria, Cunninghamia lanceolata,* radiata pine, *Sequoia,* Himalayan conifers and allied gymnosperms, *Araucaria,* South American conifers, and conifer micropropagation — applied research and commercial aspects. Tissue culture of hardwoods includes poplar and aspen, European hardwoods, North American hardwoods, Juglans, mulberry, *Leucaena, Liquidambar,* teak, tamarind, *Hevea,* tallow, Eucalyptus, palms, and *Casuarina.*

The three volumes represent a compilation of 75 chapters contributed by international experts. These constitute a comprehensive up-to-date works in the area of genetics and biotechnology of forest tree species. The three volumes are useful references for students and researchers interested in the area of cell and tissue culture of tree species. G. H. Melchior (Grosshansdorf)

Herausgeberin: Bundesforschungsanstalt für Forst- und Holzwirtschaft: Schriftleitung: Institut für Forstgenetik und Forstpflanzenzüchtung, Siekerlandstraße 2, 2070 Großhansdorf 2-Schmalenbeck (Holstein). — Verlag: J. D. Sauerländer's Verlag, 6000 Frankfurt a. M., Finkenhofstraße 21. — Anzeigenverwaltung: J. D. Sauerländer's Verlag, Frankfurt am Main. — Satz und Druck: H. Robert, 6310 Grünberg, Hess. 1. — Printed in Germany.

ISSN 0037-5349