

Table 5. — Increase in profit (in %) due to greenhouse accelerated breeding compared to field breeding orchard (two-step selection at 2 and 8 years).

model	Selection intensity							
	0.3	1.2	1.6	2.0	2.4	2.8	3.2	3.6
A	23.7	23.0	22.9	22.8	22.8	22.8	22.8	22.8
B	24.5	23.1	23.0	22.9	22.9	22.8	22.8	22.8

the greenhouse installation and management, differential costs for pollination, cone collection, etc. It was therefore necessary to examine whether or not the time saving permitted by these new methods was not offset by these new costs or costs which are at least differently distributed over time. Detailed description of these techniques can be found in GREENWOOD (1982, 1983) and costs figures are evaluated in PAQUES (1984).

The major result we observe is that an increase of profit of at least 20% can be obtained by these new techniques, compared to field breeding (Table 5). Moreover, only the magnitude and not the nature of the response (E.A.R.) over selection intensities is changed.

These results are given for a simplified situation in which the genetic gain remains unchanged between both alternatives. It was assumed that the number of parent trees was fixed over generations, so that the selection process does not interfere with the breeding phase. Possibility of increasing the number of clones and parent-trees in the breeding orchard opened by accelerated methods should be tested. Its interest is obviously in the larger population leading to a higher number of crosses: selection could be more severe and somewhat correct the reduced gain due to an excessively early selection or by a poorer, early juvenile selection.

IV. Conclusions

Tree improvement programs present an increasing number of technical and management alternatives which require analysis in an integrated study combining both genetic and economic parameters. Efficiency coefficients, as currently described in the literature are particularly well suited for the study and comparison of single options (such as selection processes but are not well adapted to deal with the existing level of complexity of complete cycles of improvement programs.

The complexity of the economic effects of different tree breeding options revealed in this research indicates a need to more closely examine the effects of even small variations in breeding techniques. The results obtained were based on certain assumptions of genetic parameters and

operational efficiencies and the effects of variations in these should also be further examined.

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Breeding Systems and Genetic Structure in some Central American Pine Populations

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Abstract

The breeding systems, genetic structure and identity of populations of *Pinus caribaea* var. *bahamensis*, *P. caribaea* var. *hondurensis*, *P. oocarpa* and *P. maximinoi* were in-

investigated using seed samples from a total of eleven stands in Central America. Allozyme variation in embryo and gametophyte tissue of newly-germinated seeds was used to estimate allele frequencies and outcrossing rates and to examine relationships between populations. Estimates of the outcrossing rates varied considerably between populations and species, but averaged 80% to 90%. Some evidence is presented on the relationships between stands whose taxonomic status is still unclear, particularly with respect to *P. patula* ssp. *tecunumanni*. In particular, the Yucul and Mountain Pine Ridge stands of *P. oocarpa* seem to have affinities, but the allozyme evidence is equivocal on the inclusion of the Rafael stand in *P. patula* ssp. *tecunumanni*.

Key words: *Pinus caribaea*, *Pinus oocarpa*, *P. patula* ssp. *tecunumanni*, genetic structure, outcrossing.

Introduction

This paper presents the results of a study of the relationships between and mating systems within populations of three Central American species, *Pinus caribaea* MORELET, *P. oocarpa* SCHEIDE and *P. maximinoi* H. E. MOORE. *Pinus caribaea* is probably the most economically important tropical pine species. It grows naturally in the Bahamas, Cuba and the mainland of Central America. Commercial plantations have been established in 37 countries in Africa, South America, the Caribbean Islands, Asia, the Pacific Islands and in Australia. There are three varieties of *P. caribaea*; var. *caribaea* (Cuba), var. *hondurensis* BARR and GOLF. (Central American mainland) and var. *bahamensis* BARR and GOLF. (Bahama Islands).

P. oocarpa has a wider distribution on the mainland of Central America although it does not occur on the Caribbean Islands. Where the ranges overlap, *P. oocarpa* occupies the drier and poorer ridges whereas *P. caribaea* occupies the valleys. *P. oocarpa* may become an important plantation species for sites which are too dry or the soil too poor for *P. caribaea*.

There is still some difficulty over the classification of several provenances (particularly Yucul) of *P. oocarpa*. BARNES and STYLES (1983) have suggested the renaming of *P. tecunumanni* SCHWERDTF. as *P. patula* SCHEIDE and DEPPE ssp. *tecunumanni* (EQUILUZ and PERRY) Styles and including the Yucul provenance in this subspecies. Several collections (including Yucul) from this group are included in the present study and, for the purposes of this paper, are still included as *P. oocarpa* although some evidence is presented that some provenances are different from 'pure' *P. oocarpa*.

The collection at La Fortuna in Honduras was thought to be *P. pseudostrobus* LINDL. at the time of collection. However, it is presently thought to be *P. maximinoi* STYLES and HUGHES, 1983), although it has also been known as *P. tenuifolia* BENTH.

All three species are in the section *Pinus* of the genus (LITTLE and CRITCHFIELD (1969), but appear in different subsections. *P. caribaea* is in the subsection *Australes*, *P. oocarpa* in *Oocarpae* and *P. maximinoi* (as *P. pseudostrobus*) in *Ponderosae*.

Pine species are monoecious, wind-pollinated and generally regarded as predominantly outcrossing although self-pollination is usually both possible and easy to carry out artificially. Consideration of breeding systems of economically important forest trees is valuable because different domestication and improvement strategies must be applied to outcrossing as opposed to inbreeding species. In forest trees, inbreeding usually results in loss of wood

production; that is, inbred trees do not grow as well as those that are outcrossed. However the effects of inbreeding on growth vary a great deal between species (FRANKLIN, 1970) from being very severe in old *Picea abies* KARST (ERIKSSON *et al.*, 1973) to effectively absent during the first year of growth of *Picea glauca* VOSS (MERGEN *et al.*, 1965). Inbreeding effects vary from tree to tree both in numbers of seed set (GRIFFIN and LINDGREEN, 1985) and subsequent growth in *Pinus radiata* D. DON (MATHESON, unpublished). Thus the use of open-pollinated seed orchards for a species in which there is an appreciable amount of self-fertilization and in which there is inbreeding depression may not be appropriate.

The quantitative genetic resources of pines have been extensively studied by provenance trials of many species in many countries. Establishment and maintenance costs usually mean that such trials are planted on few sites and not all seedlots are represented at all sites. In addition, data are expensive to collect. BROWN and MORAN (1981) have argued that because isozyme data evaluate genetic variation close to the DNA level, they should be used for the study of the genetic resources of a species, or a sample of species. They are much easier to collect than morphological data and population genetic parameters such as overall genetic diversity, heterozygosity and effective population size may be estimated.

Knowledge of the genetic structure of natural populations is important more for the strategy of collecting seeds from the wild for introduction as trials or plantations than for subsequent breeding strategy. Populations which are fragmented or differentiated will require a different collection strategy than if they were highly uniform. Where there are appreciable amounts of neighbourhood inbreeding in the wild, a generation of outcrossing is advisable before beginning a breeding program. The objective of this paper is to use isozyme data to examine the inter-relationships between populations of the three species as represented by the provenance collections and to present estimates of their outcrossing rates.

Materials and Methods

Seeds collected from eleven natural stands in Central America were available for this study. Seed source locali-

Table 1. — List of populations of *P. caribaea*, *P. oocarpa*, *P. patula* ssp. *tecunumanni* and *P. maximinoi*. Collections were carried out by the Oxford Forestry Institute (see GREAVES, 1978, 1979). Locations of collections are given in *Figure 1*.

Species	CFI No.	No. trees	Seed source
<i>P. caribaea</i> var. <i>bahamensis</i>	39/77	10	Little Abaco, Bahamas
<i>P. caribaea</i> var. <i>bahamensis</i>	7/78	10	High rock, Grand Bahama
<i>P. caribaea</i> var. <i>hondurensis</i>	36/73	10	Mountain Pine Ridge, Belize
<i>P. caribaea</i> var. <i>hondurensis</i>	10/76	5	Mountain Pine Ridge, Belize
<i>P. caribaea</i> var. <i>hondurensis</i>	8/78	10	Melinda, Belize
<i>P. oocarpa</i>	6/77	10	Rafael, Nicaragua
<i>P. oocarpa</i>	3/76	10	Dipilto, Nicaragua
<i>P. oocarpa</i> *	9/78	10	Mountain Pine Ridge, Belize
<i>P. oocarpa</i>	10/78	10	Cusmapa, Nicaragua
<i>P. oocarpa</i> *	1/76	10	Yucul, Nicaragua
<i>P. maximinoi</i>	5/78	10	La Fortuna, Honduras

* possibly *P. patula* ssp. *tecunumanni* — see text

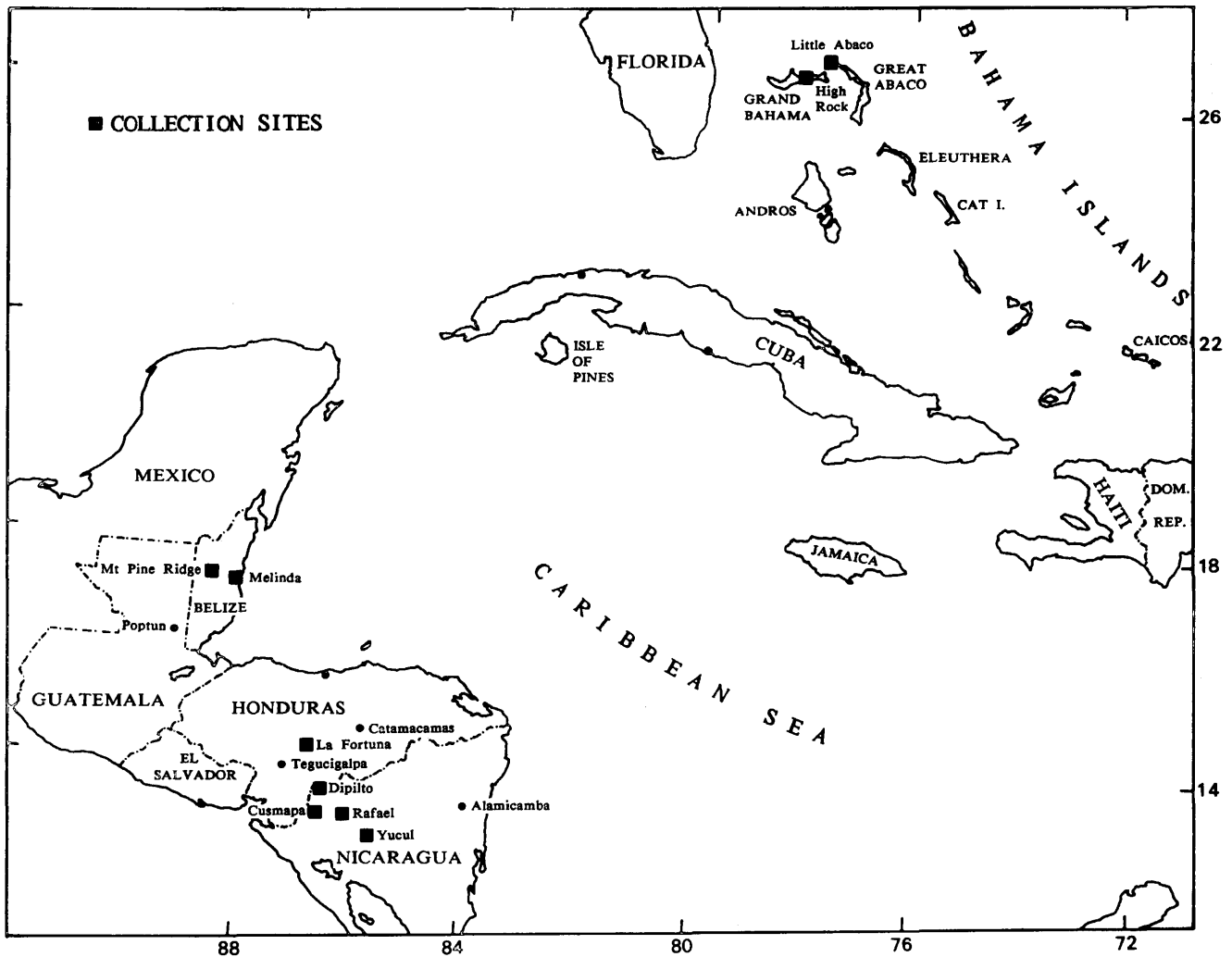


Figure 1. — Map of collection sites for populations of *P. caribaea* var. *bahamensis*, *P. caribaea* var. *hondurensis*, *P. oocarpa*, *P. patula* ssp. *tecunumannii* and *P. maximinoi* (after GREAVES, 1978).

ties for the collections and numbers of trees are presented in Table 1 and Figure 1. The seed collection strategy was to use mature unexploited stands where possible, to collect seed from dominant or codominant trees every 100 m or so and to avoid the inclusion of the same (pollen) parents as far as possible. The furthest distance apart of sampled trees varied from stand to stand, nearly 20 km at Mountain Pine Ridge but only 286 m at Dipilto. Collections 36/73 and 10/76, from the same place in different years, were combined for this study.

A total of 1939 seeds of the 10 populations were assayed for their genotypes at each of 16 isozyme loci. Seeds were germinated on moist filter paper in petri dishes and grown for about one week. Five endosperms and up to 25 embryos per mother tree were then ground singly in a 0.1 M phosphate buffer solution (containing 1 mg/ml dithiothreitol). Standard starch gel electrophoresis techniques (see BREWER and SING, 1970) were used to separate enzymes of thirty-five samples of homogenate per gel. Methods for preparing specimens and stain solutions were taken from BREWER and SING (1970), BROWN *et al.* (1978) and SHAW and PRASAD (1970). For this study the following enzymes were studied: Phosphogluconate dehydrogenase (PGD) [E.C. 1.1.1.44] (Leucine) aminopeptidase (LAP) [E.C. 3.4.11.1] Aspartate aminotransferase (AAT) [E.C. 2.6.1.1] Glutamate dehydrogenase (GDH) [E.C. 1.4.1.2]

Phosphoglucose mutase (PGM) [E.C. 2.7.5.1]
Malate dehydrogenase (MDH) [E.C. 1.1.1.37]
Aconitate hydratase (AC) [E.C. 4.2.1.3]
Shikimate dehydrogenase (SDH) [E.C. 1.1.1.25]
Glucosephosphate isomerase (GPI) [E.C. 5.3.1.9]

In several cases, the enzyme was produced by more than one gene locus (e.g. PGD-1, PGD-2; LAP-1, LAP-2 etc) and thirteen variable loci were scored (three were invariant). Inheritance of allozymes was assumed to be similar to that of other pine species (e.g. CONKLE, 1971; RUDIN, 1975; RUDIN, 1977; GURIES and LEDIG, 1978; O'MALLEY *et al.*, 1979; ADAMS and JOLY, 1980) and it was assumed that migrating bands in different species represented the same alleles. Banding patterns were similar to those observed in other pine species where mendelian inheritance has been verified (e.g. radiata pine) and hybrid studies have shown that similar bands in different pine species are allelic (JOLY and ADAMS, 1983). The genetic basis of the allozyme variation was supported by segregation patterns in open-pollinated progeny arrays and the occurrence of hybrid bands in putative heterozygotes.

The endosperm of pine seeds is haploid megagametophyte tissue, so scoring them permits estimation of the maternal genotype. Five endosperm were scored per mother tree giving a probability of $1-(\frac{1}{2})^4 = 0.9375$ per locus of correct identification of maternal heterozygotes.

Embryos from these and a further 20 seeds were used where possible. For populations where 20 seeds per mother tree were not available, equal numbers of embryos were used. Multi-locus estimates of outcrossing rates were obtained using the methods and computer program of RITLAND and JAIN (1981) in which maternal genotypes were estimated by megagametophyte analysis and individual paternal contributions estimated rather than directly counted as in BERGMANN (1973) and MÜLLER (1976).

The genetic structure among populations was examined using the computer program BIOSYS I (SWOFFORD and

Table 2. — Frequency of the most common allele (p), observed heterozygosity (H), fixation index (F), sample sizes (n), and chi-square (X^2 , 1 df) for testing deviations from Hardy-Weinberg expectations. Eleven polymorphic loci were assayed in ten Central American pine populations. Two loci (AAT-1 and AAT-2) were fixed for one allele in all populations except *P. maximinoi* in which they were fixed for another.

Locus/allele	Species/population ¹										
	P.c.b.		P.c.h.		P.o.			P.m.			
	39/77	7/78	36/73	8/78	1/76*	9/78*	3/76	6/77	10/78	5/78	
PGD-1/1	p	0.62	0.67	0.57	0.53	0.16	0.10	0.02	0.07	0.03	0.71
	H	0.43	0.38	0.38	0.42	0.25	0.37	0.12	0.13	0.05	0.35
	F	0.16	0.21	0.33	0.31	0.19	-0.02	0.03	0.06	0.22	0.16
	n	230	143	144	201	200	134	208	212	239	160
	Chi-sq	3.7	13.4*	25.6*	15.1*	6.6*	0.6	0.1	0.7	13.0*	4.1*
PGD-2/2	p	0.99	1.00	0.93	0.96	1.00	0.98	0.97	0.99	0.96	1.00
	H	0.02	0.00	0.12	0.06	0.00	0.04	0.05	0.01	0.09	0.00
	F	-0.01	-	0.04	0.16	-	-0.02	-0.03	-0.01	-0.05	-
	n	233	144	145	202	199	138	207	212	239	162
	Chi-sq	0.0	-	0.2	26.5*	-	0.1	0.1	0.0	0.5	-
LAP-1/2	p	0.75	0.76	0.75	0.76	1.00	0.91	0.97	0.93	0.91	0.00
	H	0.24	0.20	0.25	0.27	0.00	0.10	0.07	0.12	0.18	0.11
	F	0.38	0.46	0.06	0.30	-	0.39	-0.03	0.01	-0.10	0.65
	n	230	138	144	201	200	99	199	203	123	152
	Chi-sq	30.7*	29.4*	0.4	14.9*	-	16.0*	0.2	0.0	1.1	65.6*
LAP-2/2	p	0.96	0.96	0.77	0.81	0.43	0.40	0.91	0.70	0.90	1.00
	H	0.07	0.08	0.27	0.26	0.24	0.48	0.14	0.23	0.18	0.00
	F	0.09	-0.04	0.22	0.16	0.52	0.12	0.16	0.47	0.07	-
	n	208	140	146	197	200	138	202	198	239	151
	Chi-sq	1.5	0.2	7.1*	5.2*	54.9*	4.7*	8.2*	45.0*	1.0	-
AAT-3/2	p	0.84	0.77	0.74	0.72	0.77	0.83	0.85	0.86	0.92	0.00
	H	0.29	0.37	0.32	0.39	0.25	0.24	0.22	0.23	0.14	0.25
	F	-0.05	-0.02	0.17	0.03	0.29	0.19	0.16	0.07	0.10	0.32
	n	227	141	138	189	187	135	186	196	230	138
	Chi-sq	1.6	0.1	4.3*	0.2	15.5*	3.2	4.9*	0.5	1.9	14.9*
GDH/1	p	1.00	1.00	0.78	0.80	0.40	0.36	0.69	0.55	0.59	0.00
	H	0.00	0.00	0.35	0.34	0.40	0.46	0.38	0.43	0.49	0.47
	F	-	-	-0.03	-0.05	0.22	0.12	0.12	0.21	0.00	0.03
	n	232	128	114	190	189	132	171	202	229	144
	Chi-sq	-	-	0.0	0.5	114.2*	2.3	2.4	16.0*	0.1	0.2
PGM/3	p	0.21	0.31	0.72	0.51	0.95	0.98	0.79	0.96	0.82	0.19
	H	0.38	0.43	0.47	0.49	0.10	0.04	0.21	0.07	0.24	0.32
	F	0.10	0.08	-0.09	0.10	-0.04	-0.02	0.37	-0.03	0.17	0.02
	n	217	120	116	194	189	79	202	203	218	147
	Chi-sq	0.5	1.2	1.1	2.1	0.5	0.0	28.2*	0.3	6.1*	0.0
MDH/1	p	0.67	0.47	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	H	0.28	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F	0.36	0.17	-	-	-	-	-	-	-	-
	n	107	68	64	108	78	79	192	158	163	81
	Chi-sq	14.5*	2.2	-	-	-	-	-	-	-	-
AC/2	p	0.90	0.93	0.88	0.96	0.86	0.84	0.98	0.96	0.89	0.94
	H	0.17	0.15	0.21	0.08	0.26	0.26	0.05	0.09	0.23	0.08
	F	0.11	-0.08	0.01	-0.04	-0.07	0.07	-0.03	-0.05	-0.13	0.27
	n	150	62	67	131	106	58	102	104	123	114
	Chi-sq	1.7	0.3	0.0	0.2	0.6	0.2	0.1	0.2	2.0	8.9*
SDH/2	p	0.32	0.44	0.52	0.53	0.72	0.56	0.70	0.69	0.74	0.95
	H	0.42	0.57	0.42	0.34	0.45	0.64	0.35	0.38	0.43	0.10
	F	0.11	-0.12	0.22	0.40	-0.02	-0.27	0.23	0.13	-0.12	-0.05
	n	137	61	65	126	104	56	97	104	123	74
	Chi-sq	1.3	0.8	2.8	18.5*	0.0	3.8	4.0*	1.8	2.0	0.2
GPI/2	p	0.64	0.73	0.33	0.13	0.79	0.83	0.86	0.94	0.89	0.00
	H	0.45	0.37	0.21	0.27	0.26	0.24	0.24	0.13	0.19	0.08
	F	0.12	0.09	0.53	-0.15	0.20	0.15	0.01	-0.06	0.04	-0.04
	n	145	62	67	129	106	58	101	104	123	13
	Chi-sq	1.3	0.2	19.2*	3.1	4.4*	1.6	0.0	0.4	0.3	0.0

*) Chi-square significant at 5% level.

¹) species designations as follows: P.c.b. (*Pinus caribaea* var. *bahamensis*), P.c.h. (*P. caribaea* var. *hondurensis*), P.o. (*P. oocarpa*), P.m. (*P. maximinoi*).

²) possibly *P. patula* ssp. *tecunumannii*.

Table 3. — Average sample size (n), mean numbers of alleles per locus (A), percentage of loci polymorphic (P), mean observed heterozygosity (H_o), Hardy-Weinberg expected heterozygosity (H_e) and multilocus estimate of outcrossing rate (t) for ten Central American pine populations. Standard errors are given in brackets.

Population	n	A	P (%)	H_o	H_e	t
<i>P. caribaea</i> var. <i>bahamensis</i>						
39/77	199	2.4 (0.2)	77	0.21 (0.05)	0.25 (0.06)	0.93 (0.02)
7/78	115	2.1 (0.2)	69	0.23 (0.06)	0.26 (0.06)	0.85 (0.04)
<i>P. caribaea</i> var. <i>hondurensis</i>						
36/73	116	2.3 (0.2)	77	0.24 (0.05)	0.29 (0.06)	0.89 (0.03)
8/78	175	2.3 (0.2)	77	0.23 (0.05)	0.27 (0.06)	0.92 (0.06)
<i>P. oocarpa</i>						
1/76*	166	2.1 (0.3)	62	0.17 (0.05)	0.21 (0.06)	0.94 (0.02)
3/76	176	2.2 (0.2)	77	0.14 (0.04)	0.17 (0.05)	0.83 (0.04)
6/77	179	2.2 (0.2)	77	0.14 (0.04)	0.17 (0.05)	0.81 (0.04)
9/78*	106	2.3 (0.2)	77	0.22 (0.06)	0.24 (0.06)	0.83 (0.04)
10/78	195	2.2 (0.2)	77	0.17 (0.04)	0.17 (0.17)	0.96 (0.03)
<i>P. maximinoi</i>						
5/78	128	1.7 (0.2)	62	0.14 (0.04)	0.17 (0.05)	0.65 (0.06)

*) possibly *P. patula* ssp. *tecunumannii*

SELANDER, 1981) which calculated genetic distances (N_{EI} , 1975) between stands from the progeny array data. These genetic distances are thought to represent the degree of genetic differentiation in terms of genetic code differences in the DNA base sequence. These distance measures were used as the dissimilarity measures in an agglomerative polythetic classification (WILLIAMS, 1976).

Results

Details of alleles scored, heterozygosity, fixation indices and deviation from Hardy-Weinberg expectations are given in Table 2.

Most loci had at least three alleles although AAT-1, AAT-2 and MDH had two. However, not all alleles were present in all populations. For example, all populations of *P. caribaea* and *P. oocarpa* were fixed for the same alleles at the AAT-1 and AAT-2 loci but the *P. maximinoi* population was fixed for a different allele at both AAT loci. The average number of alleles per locus varied from 2.4 in *P. caribaea* var. *bahamensis* at Little Abaco (39/77) and 2.3 in *P. caribaea* var. *hondurensis* at Melinda (8/78) and in *P. oocarpa* at Mountain Pine Ridge (9/78) to 1.7 in *P. maximinoi* at La Fortuna (5/78) (Table 3). It seems unlikely that there are any real differences between the populations for this measure of diversity except for 5/78 which is significantly less diverse than all other populations (judged from the standard errors).

The expected mean heterozygosity within populations (H_e) varied from 0.29 in *P. caribaea* var. *hondurensis* at Mountain Pine Ridge (36/73) to 0.17 in *P. maximinoi* at La Fortuna (5/78) and *P. oocarpa* at Cusmapa (10/78) Rafael (6/77) and Dipilto (3/76). These values are similar to those obtained in comparable studies in other coniferous species (ADAMS, 1983), but are higher than those obtained for *Pinus radiata* and *P. rigida* (BROWN and MORAN, 1981) and *P. contorta* (HAMRICK, 1983; see also LOVELESS and HAMRICK, 1984). Populations of *P. oocarpa* seemed to have lower observed heterozygosities than those of *P. caribaea*, but on the whole, these levels of heterozygosity were similar to their Hardy-Weinberg expectations. The total heterozygosity ($H_T = 1 - \sum p_i^2$ where p_i is the mean frequency of the i th allele at a locus) is the sum of H_S (mean heterozygosity within populations) and the heterozygosity between populations (D_{ST}). The ratio D_{ST}/H_T is called G_{ST} and expresses the diversity between populations as a proportion of the total diversity (N_{EI} , 1975). These parameters are presented for each species and subspecies category in Table 4.

The populations of *P. caribaea* in this study represented two of the three varieties of the species. The ability of

Table 4. — Genetic diversity between and within groupings of species and populations. Comparison of overall *P. caribaea* with two of its varieties and overall *P. oocarpa* with two possible groupings of populations (*P. oocarpa* and *P. patula* ssp *tecunumannii*).

Grouping	Parameter ¹			
	H _T	H _S	D _{ST}	G _{ST}
<i>P. caribaea</i> 39/77, 7/78, 36/73, 8/78	0.297	0.259	0.039	0.131
<i>P. caribaea</i> ssp <i>bahamensis</i> 39/77, 7/78	0.257	0.253	0.003	0.013
<i>P. caribaea</i> ssp <i>hondurensis</i> 36/73, 8/78	0.282	0.279	0.003	0.011
<i>P. oocarpa</i> 1/76, 9/78, 3/76, 6/77, 10/78	0.207	0.186	0.021	0.104
1/76, 9/78 ²	0.172	0.169	0.004	0.023
3/76, 6/77, 10/78 ³	0.227	0.222	0.006	0.025

¹) H_T is the weighted average of values of H_T for each locus and population; it represents the total heterozygosity. It is the sum of H_S (the heterozygosity within populations) and D_{ST} (the heterozygosity between populations). G_{ST} expresses the heterozygosity between populations as a proportion of the total.

²) group of populations possibly *P. patula* ssp *tecunumannii*.

³) group of populations possibly 'pure' *P. oocarpa*.

genetic diversity measures to detect such varietal classification was tested by comparison of genetic diversity within *P. caribaea* as a whole and within the two varieties taken separately. Values of G_{ST} for the subspecies of *P. caribaea* taken separately were rather smaller than the average for wind-pollinated tree species (see BROWN and MORAN, 1981). When var. *bahamensis* and var. *hondurensis* were combined, they showed much more variation between populations (about twice BROWN and MORAN's 1981 average) than when they were kept separate (because this combination also contains the variation between subspecies).

This technique was also used to examine whether populations of *P. oocarpa* could be separated into true *P. oocarpa* and putative *P. patula* ssp *tecunumannii*. If diversity within *P. oocarpa* as a whole was much the same as diversity within the two groups of populations taken separately, this would suggest that the two groups are not differentiated and that the species (as represented by the five stands) is homogenous. The diversity between populations in *P. oocarpa* dropped to about the same level as each subspecies of *P. caribaea* when Yucul (1/76) and the Mountain Pine Ridge (9/78) (i.e. *P. patula* ssp *tecunumannii*) were excluded. In addition, Yucul and Mountain Pine Ridge taken together provide a G_{ST} estimate about the same as the three remaining *P. oocarpa* populations sampled. This indicates that the variation between Yucul and Mountain Pine Ridge on the one hand and the other populations of *P. oocarpa* on the other is about the same as that found between the subspecies of *P. caribaea*.

Outcrossing rates (Table 3) varied from 0.96 in *P. oocarpa* at Cusmapa (10/78) to 0.65 in *P. maximinoi* at La Fortuna (5/78). Six of the populations had outcrossing rates less than 0.9, but the only population with an outcrossing rate less than 0.8 was the *P. maximinoi* population at La Fortuna. Single-locus estimates of outcrossing rates were calculated for several populations and when averaged

Table 5. — Unbiased genetic distances between populations (NEI 1978).

Population ¹	7/78	36/73	8/78	1/76	3/76	6/77	9/78	10/78	5/78
39/77	0.12	0.071	0.073	0.226	0.142	0.184	0.212	0.161	0.778
7/78		0.079	0.089	0.202	0.123	0.160	0.197	0.139	0.702
36/73			0.009	0.100	0.084	0.103	0.097	0.094	0.905
8/78				0.142	0.113	0.147	0.146	0.128	0.857
1/76					0.042	0.034	0.007	0.039	1.155
3/76						0.010	0.045	0.002	1.038
6/77							0.030	0.007	1.114
9/78								0.040	1.222
10/78									1.060

across loci, were almost identical to the multi-locus estimates.

The genetic distance (NEI, 1978) between populations are presented in Table 5. It is clear that the *P. maximinoi* population (5/78) is the most different from the other populations. This was already evident in its levels of heterozygosity and outcrossing rate as well as the fact that at several loci, it had alleles not present in other populations. When these genetic distances were used in a polythetic agglomerative classification (WILLIAMS, 1976), the closest populations to each other were those populations of the same species as described by LITTLE and CRITCHFIELD (1969). Figure 2 shows the relationships between populations as expressed by a minimum spanning tree (MST — see GOWER and ROSS, 1969).

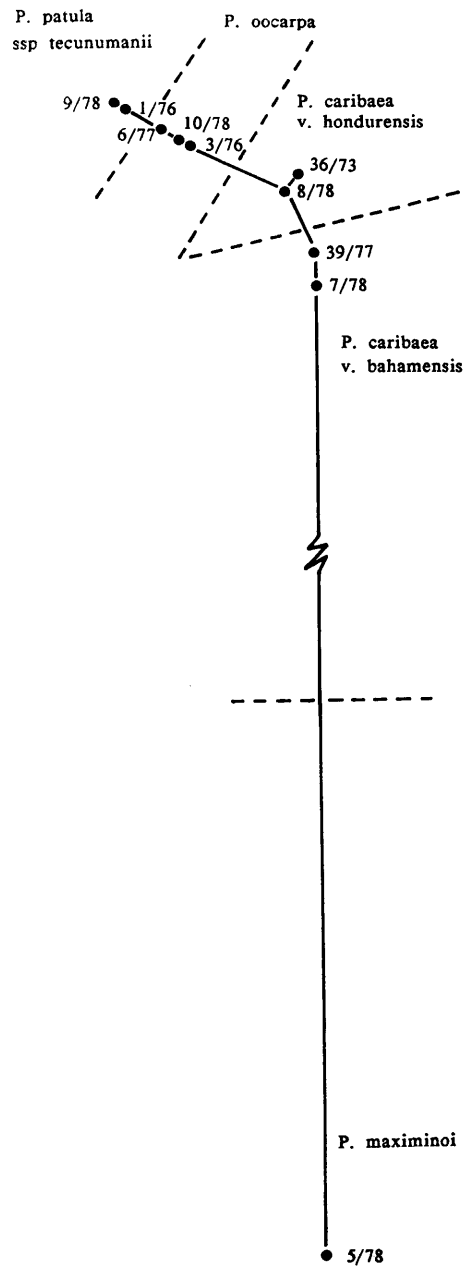


Figure 2. — Genetic distances between populations expressed as a Minimum Spanning Tree.

The closest populations in genetic terms to the *P. maximinoi* population were those of *P. caribaea* var. *bahamensis*. The three populations of *P. caribaea* var. *hondurensis* were grouped together between var. *bahamensis* and *P. oocarpa* which was furthest removed from the *P. maximinoi* population. Interestingly, the putative *P. patula* ssp. *tecummannii* populations (Yucul — 1/76 and Mountain Pine Ridge — 9/78) appeared to be separate from the *P. oocarpa* populations, although not quite as different from them as the *P. caribaea* varieties were from each other. This fits very well with the diversity measure G_{ST} above which also suggested that Yucul and Mountain Pine Ridge were together somewhat different from the *P. oocarpa* populations. The Rafael population (6/77) appears intermediate in the MST, falling between *P. oocarpa* and the *P. patula* ssp. *tecummannii*.

Cluster analysis based on NEI's (1978) and ROGERS' (1972) genetic distance agreed with the genetic diversity measures and the agglomerative procedure concerning the relationships within *P. oocarpa*. The cluster analyses, like the minimum spanning tree, separated *P. maximinoi* first from the others. The differences between the two subspecies of *P. caribaea* were slightly greater than those between the two groups of *P. oocarpa* populations.

Discussion

Allelic differences between and within the population collections reported here reflect the generally accepted phylogenetic relationships between species and subspecies of the taxa examined (LITTLE and CRITCHFIELD, 1969). Additionally, it is suggested that the Yucul and Mountain Pine Ridge (9/78) populations of *P. oocarpa* are almost as different from the other populations of *P. oocarpa* as the two subspecies of *P. caribaea* are from each other. Whether these differences are sufficient to suggest, as do BARNES and STYLES (1983), that the Yucul population should belong to another species is another matter. Such a decision should not be made on isozyme evidence alone, but on morphological and other evidence as well. The distribution of the proposed *P. patula* ssp. *tecummannii* given by BARNES and STYLES (1983) does not extend into Belize. But the evidence presented here suggests that there are affinities between the Yucul population and the Mountain Pine Ridge population (both collected as *P. oocarpa*) in their isozyme patterns which agree very well with morphological patterns observed by McCARTER and BIRKS (1985) and growth patterns reported by BIRKS and BARNES (1985). Both McCARTER and BIRKS (1985) and BIRKS and BARNES (1985) classify the Mountain Pine Ridge provenance as *P. patula* ssp. *tecummannii*.

The taxonomic status of the Rafael provenance of *P. oocarpa* (6/77) is unclear. McCARTER and BIRKS (1985) classified Rafael as *P. patula* ssp. *tecummannii* based on numerical taxonomy. BIRKS and BARNES (1985) presented confirmatory evidence from a multivariate analysis of growth performance in international provenance trials. However, in the minimum spanning tree analysis of genetic distances presented here the Rafael population fell into neither the *P. oocarpa* nor the *P. patula* ssp. *tecummannii* group, but between them and slightly closer to *P. oocarpa*.

Estimates of outcrossing rates were generally slightly lower than for natural populations of other conifers (e.g. SHAW and ALLARD, 1981, in Douglas-fir; and MITTON *et al.* 1977, 1981, in *P. ponderosa*), and artificial populations of

pinus (MORAN *et al.*, 1980). Single-locus estimates of outcrossing usually vary somewhat even in the same population (BROWN *et al.*, 1975) because not all outcrosses are detected with each estimate. The rarer the maternal genotype, the more easily are outcrosses detected. Any particular outcross event is more likely to be detected if many loci are examined together. The average of a series of single locus estimates should therefore be less than a multilocus estimate (SHAW and ALLARD, 1981) although this may not be appreciable. When the mean of single-locus estimates is substantially lower it may suggest that inbreeding other than selfing may be occurring.

When comparing estimates of outcrossing from natural and artificial stands, SHAW and ALLARD (1981) proposed that any difference between the estimates would be due to neighbourhood inbreeding implying the existence of microstructure or microdifferentiation. However, their estimates were very similar for both artificial and natural stands, suggesting there was no microstructure. Undetected genetic neighbourhoods could have led to lower estimates of outcrossing in this study but the multilocus and average of the single-locus estimated of outcrossing were very similar. The lowest multilocus estimate obtained was for *P. maximinoi* at La Fortuna (0.65). Although it was considerably different in terms of allele frequencies from the other populations, there is no *a priori* reason to suppose that this species is any different in its pollination biology. Possible causes for the lower outcrossing rate include higher selfing rate, lower inbreeding depression (higher inbreeding depression may lead to higher mortality among selfed embryos in other populations) as well as other types of inbreeding (such as mating between relatives in neighbourhood groups), crown structure and stand density. There is no collection map, the locality is remote and difficult of access and there is little chance of obtaining more information on this population to permit further study. There are other areas in Central America where the species occurs. The question of microstructure could be studied by subsampling large population samples to see whether inbreeding estimates differed between subsamples or whether allele frequencies varied sufficiently between subsamples to inflate inbreeding estimates.

Conclusion

The inbreeding levels of 10% to 20% estimated by the methods in this paper are rather higher and more variable than for other coniferous species. Whether this is due to neighbourhood inbreeding between relatives, greater true self-pollination or less inbreeding depression is unclear.

The classification of Yucul (1/76) and Mountain Pine Ridge (9/78) as *P. patula* ssp. *tecummannii* by McCARTER and BIRKS (1985) and by BARNES and BIRKS (1985) agrees with the evidence presented in this paper. There is no doubt that these two populations have different allozyme patterns than other populations of *P. oocarpa*, under which name they were collected.

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Variation in Fruitfulness in a Douglas-fir Seed Orchard and its Effect on Crop-Management Decisions

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Abstract

Genetic variation in seed-cone production among 37 open-pollinated families and 63 clones was studied in a coastal clonal/seedling Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] seed orchard. Individual-tree (seedling or ramet) cone counts collected for a period of eight years (1976 to 1983) were analyzed to assess the variation and to

provide information for comparisons between clonal and seedling trees. The seedling trees' cone crops surpassed those of the clonal trees in spite of the physiological maturity of the latter. Open-pollinated families and clones varied in their seed cone production rhythms and were classified as good, poor, and inconsistent cone producers. Parental balance in the resultant cone crop was greatly affected by the size of the crop. Clonal crops in good and poor cone years showed consistent parental imbalance when compared to the seedling cone crops. Heritability estimates for cone production varied among years and, contrary to expectations, the seedlings gave higher estimates than the clones. Several managerial options are proposed to alleviate the variation in cone crop production and produce seed crops with similar representation of all parents.

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