CONSERVATION STRATEGIES FOR *PINUS MAXIMINOI* BASED ON PROVENANCE, RAPD AND ALLOZYME INFORMATION

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ABSTRACT

Pinus maximinoi is a subtropical pine native to Mexico and Central America. It is an aggressive pioneer species with a reproductive cycle of 12 to 14 months. Seed samples were collected from 25 populations of *P. maximinoi* from Guerrero, Mexico to central Nicaragua and established in provenance tests in Brazil, Colombia and South Africa. The trials were assessed for volume production at 3, 5 and 8 years of age. Subsets of 13 and 5 provenances were assessed using RAPD and allozyme markers, respectively, to determine patterns of genetic diversity and mating systems in natural stands. RAPD analyses indicated significant differences among provenances in percent polymorphism and observed heterozygosity. Geographical location of the population in Mesoamerica greatly influenced genetic diversity, with populations from Mexico and Guatemala exhibiting more diversity than those from Honduras and Nicaragua. Observed heterozygosity patterns detected in the RAPD analysis correlated reasonably well with provenance performance in Brazil ($r = 0.53, p = 0.06$), Colombia ($r = 0.48$, $p = 0.10$) and South Africa ($r = 0.43$, $p = 0.14$). Allozyme assessment showed *P. maximinoi* to be polymorphic for 22 of the 25 loci analyzed with an average of 2.86 alleles per polymorphic locus. Eleven of the 22 polymorphic loci had moderate or high levels of genetic diversity. The southernmost Nicaraguan population, Dantalí, had the lowest level of genetic diversity for these markers. Mean genetic diversity among populations (G_{ST}) was 0.047 and highly significant. The out crossing rate (t) in *P. maximinoi* was 0.904 vs. 0.975 for the *P. tecunumanii* control. There was also evidence of biparental inbreeding in the *P. maximinoi* populations. Provenances selected in trials for good volume production were generally the most genetically diverse based on biochemical and molecular marker assessment. Because of this relationship and the socio-economic needs of local people, *in situ* conservation programs for *P. maximinoi* in Mesoamerica should be based on securing the gene resources of populations that performed the best in well-replicated, international field trials.

Key words: *Pinus maximinoi,* alleles, provenance/progeny, genetic structure, evolutionary history

INTRODUCTION

Pinus maximinoi H. E. Moore is a 5-needle, subtropical Mesoamerican pine that is a member of the *Pseudostrobus* Group of the subgenus *Pinus* (PERRY 1991, PRICE *et al.* 1998). It is a medium to large tree, 15 to 42 m in height with diameter at breast height (outside bark) from 40 to 100 cm at maturity. *Pinus maximinoi* is relatively common, especially in Central America, where it is second only to *P. oocarpa* in abundance. Its geographic range extends 2250 km from north-central Mexico to northern Nicaragua across diverse environments and microclimates that include moist cloud forests and dry pine oak-ecosystems (DVORAK *et al.* 2000). It occurs in pure and mixed stands from 600 to 2800 m altitude in areas mostly free of winter frosts, but is most common between 1100 and 1800 m elevation throughout the region (DVORAK & DONAHUE 1992). Its distribution is not continuous throughout southern Mexico and Central America, but is broken into disjunct populations that range in size from only a few to several thousand hectares. The local people in Mesoamerica use the wood of *P. maximinoi* for construction purposes and firewood, but the species is not especially sought after for any specific use over other pines.

Recent research efforts by CAMCORE, North Carolina State University, have concentrated on learning more about the reproductive biology of the species. *Pinus maximinoi* sheds pollen from February to early April in southern Mexico and Central America and cones mature 12 to 14 months later in April of the following year just as the summer rains arrive (GUTIÉRREZ *et al.* unpublished). The length of reproductive cycle, which is the shortest of the Mesoamerican pines yet studied, is maintained when the species is planted as

an exotic in the Colombian highlands (ISAZA & ARCE 1997, ISAZA *et al.* 2000). *Pinus maximinoi* trees do not produce an abundance of cones in native environments, but yields of filled seeds per cone are often higher than that for other tropical pines such as *Pinus oocarpa* or *P. caribaea* var. *hondurensis* (ORDOÑEZ 1981). Significant clinal variation exists in seed size for the species, with seeds becoming progressively larger as one travels from west to east from southern Mexico into Honduras (DVORAK & DONAHUE 1988).

Little information is available on the genetic structure and breeding system of *P. maximinoi* in its native environment. Allozyme assessment of a natural population in Honduras by MATHESON *et al.* (1989) showed it to have fewer alleles per locus (1.7) and lower outcrossing rates (0.65) than either *P. oocarpa* or *P. caribaea* var. *hondurensis*. The authors concluded that the low outcrossing rate might result from higher selfing rates, related matings within neighborhood groups, and low stand density but suggested that there was no reason to assume that *P. maximinoi* was different in its pollination biology from other pines.

The potential value of *Pinus maximinoi* as a plantation species in the tropics and subtropics has been greatly enhanced by its excellent performance in the international series of provenance trials sponsored by CAMCORE Cooperative and summarized by GAPARE *et al.* (2001). Twenty-six populations were sampled from Guerrero, Mexico to northern Nicaragua and established at 47 locations in Brazil, Colombia, South Africa, Venezuela, and Zimbabwe (Table 1, Figure 1). Results from 29 of the oldest CAMCORE tests vary, but average productivity of unimproved *P. maximinoi* was nearly 30 % greater than improved *P. taeda* in south-central Brazil (FIER 2001), and 17 % better than improved *P. patula* in South Africa at 8 years of age (GAPARE *et al.* 2001).

Pinus maximinoi differs in two respects from other tropical and subtropical pines. First, despite the large number of provenances tested, provenance variation for basal area between the best and worst sources was about 15%, approximately half of for other tropical pines (GAPARE *et al.* 2001). Second, provenance/progeny trials are characterized by the occur

Table 1. Provenances of *Pinus maximinoi* that were sampled in natural stands in Mexico and Central America (amended from DVORAK 2000). Provenances with map code numbers that are shaded are those used in the RAPD study; those with * were used in the allozyme analysis.

Map Code	Provenance	State or Department	Country	Latitude	Longitude	Elevation Range (m)	Annual Rainfall (mm)
1*	Cobán	Alta Verapaz	Guatemala	15° 28′ N	90° 24′ W	1330–1440	2109
2*	San Jeronimo	Baja Verapaz	Guatemala	15° 04′ N	90° 14′ W	1280–1590	970
3*	San Juan Sacatepequez	Guatemala	Guatemala	14° 41′ N	90° 38′ W	1580–2000	1138
4*	Dulce Nombre de Copán	Copan	Honduras	14° 50′ N	88° 51′ W	1100–1300	1386
5	Marcala	La Paz	Honduras	14° 10′ N	88° 01′ W	1600–1800	1670
6	Tapiquil	Yoro	Honduras	15° 10′ N	86° 50′ W	1500–1769	1069
7	Tatumbla	Fco. Morazán	Honduras	14° 02′ N	87° 07′ W	1400–1600	1153
8	Altamirano	Chiapas	Mexico	16° 43′ N	92° 02′ W	1280–1350	1644
9	San Jerónimo	Chiapas	Mexico	17° 03′ N	92° 08′ W	940–1020	1417
10	Ciénega de León	Chiapas	Mexico	16° 41′ N	94° 00′ W	1050–1240	1078
11*	Coapilla	Chiapas	Mexico	17° 08′ N	93° 10′ W	1360–1510	1350
12	La Cañada	Chiapas	Mexico	16° 49′ N	92° 09′ W	1270–1360	1576
13	Monte Cristo	Chiapas	Mexico	15° 44′ N	92° 33′ W	750–900	2000
14	Valle de Angeles	Fco. Morazán	Honduras	14° 10′ N	87° 02′ W	1200–1600	1118
15	San Juan Copala	Chiapas	Mexico	17° 10′ N	97° 58′ W	1370–1560	1350
16	San Jerónimo	Oaxaca	Mexico	16° 10′ N	97° 00′ W	1220–1480	1350
17	Candelaria	Oaxaca	Mexico	16° 00′ N	96° 31′ W	1370–1480	1117
18	Las Compuertas	Guerrero	Mexico	17° 10′ N	99° 59′ W	1050–1200	1400
19	El Portillo	Ocotepeque	Honduras	14° 28′ N	89° 01′ W	1400–1600	1325
20	Yuscaran	El Paraiso	Honduras	13° 50′ N	86° 55′ W	1500–1700	1300
21	Minas de Oro	Comayagua	Honduras	14° 47′ N	87° 21′ W	950–1300	1067
22	La Lagunilla	Jalapa	Guatemala	14° 42′ N	89° 57′ W	1540–1860	1017
23	San Lorenzo	Zacapa	Guatemala	15° 05′ N	89° 40′ W	1900–2100	1500
24	San José Bayuncún	Nueva Segovia	Nicaragua	13° 45′ N	86° 20′ W	980–1240	1184
25	San Francisco Murra	Nueva Segovia	Nicaragua	13° 45′ N	86° 00′ W	930–1130	1410
26*	Datanlí	Jinotega	Nicaragua	13° 07′ N	85° 54′ W	980–1200	1213

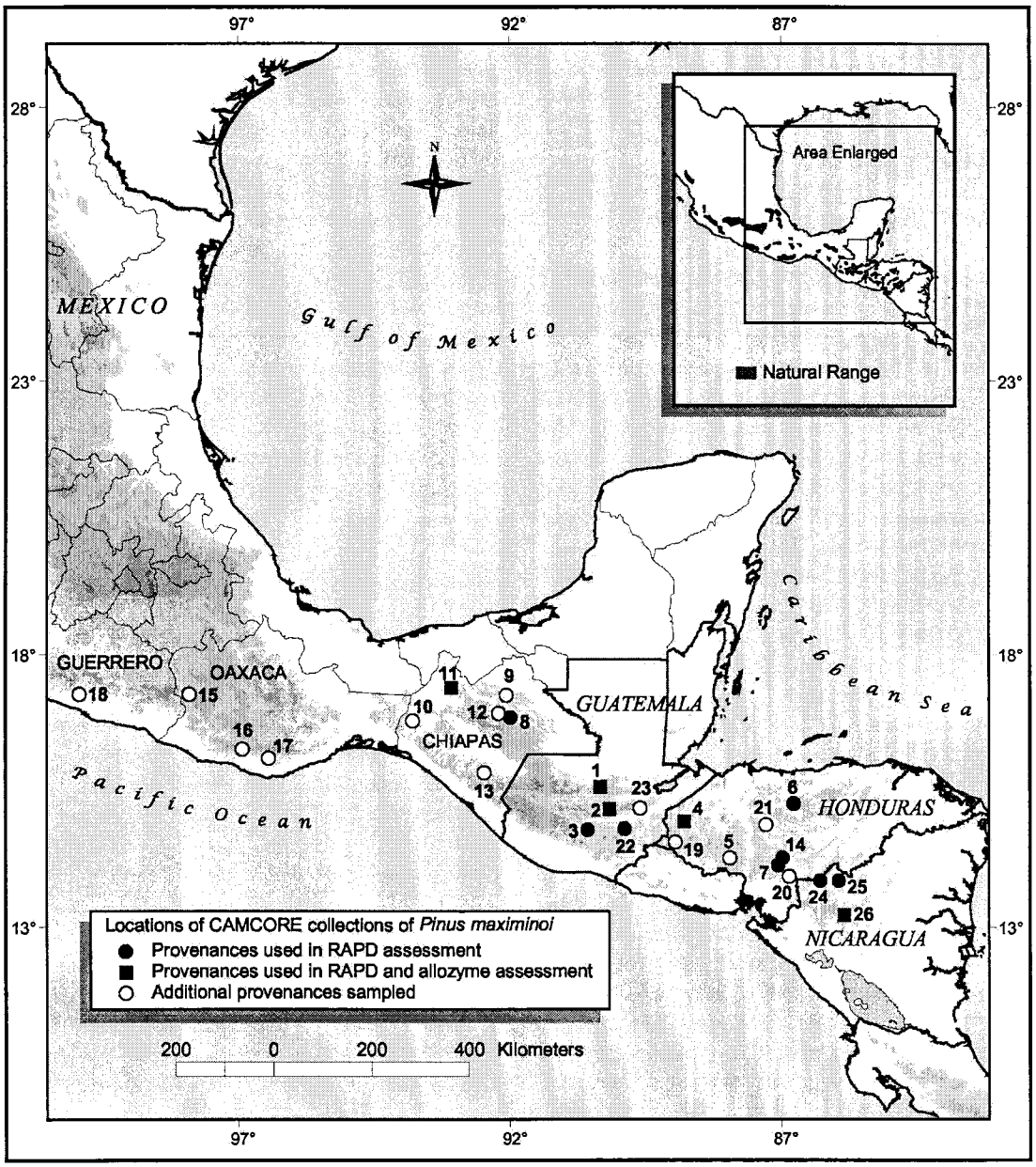

Figure 1. Map showing the locations of provenance collections for *Pinus maximinoi* in Mexico and Central America and the sample subsets used for the RAPD and allozyme analyses.

rence of individual, very large trees surrounded by clusters of much smaller and crooked trees. No explanation can be given for the sporadic occurrence of these "super trees" in the trials other than they might be the result of outcrossing vigor in what would normally be inbred natural populations.

The future of obtaining genetic material of *P. maximinoi* from genetically superior provenances in natural stands is in question, even though the species range is large. Many populations located on deep fertile soils in the highland cloud forests of Chiapas, Mexico and Central America range in size from only 2 to 20 hectares. They are fragmented and degraded, and their conservation status is vulnerable to critically endangered (DVORAK *et al.* 2000). These stands are being harvested to make way for high-income crops like coffee and cardamom. *In situ* conservation programs for *P. maximinoi* are needed in southern Mexico and Central America to complement existing *ex situ* efforts by international organizations.

In this paper we use allozyme and RAPD (random amplified polymorphic DNA) markers to examine genetic diversity patterns and the breeding system of *P. maximinoi*. International provenance trial results and

marker information are used in combination to quantify genetic structure of the species. Recommendations are then made for effective *in situ* gene conservation strategies.

MATERIALS AND METHODS

Provenance Collections and Testing

Seed collections were made in 26 populations of *P. maximinoi* from Guerrero, Mexico to central Nicaragua by CAMCORE from mid 1985 to 1993 (DVORAK *et al.* 2000). A total of 856 mother trees were sampled (Table 1, Figure 1). Provenance/progeny tests were established in Brazil, Colombia, South Africa, Venezuela and Zimbabwe using a randomized complete block design. Trees were measured at 3, 5, and 8 years of age. Results from 22 populations established on 29 test sites were reported by GAPARE *et al.* (2001).

In this study, we use the same data set but add measurements from trials of three of the southernmost provenances from Nicaragua and include the latest measurements from all the other trials. The data set comprises 25 provenances, 45 tests and 76,000 trees established in Brazil, Colombia and South Africa. Trials were assessed at either 3 years, 5 years or 8 years of age for height and diameter at breast height (dbh). Five and eight year measurements accounted for 73 % of the data. Individual tree volume for juvenile trees were calculated using the formula $V = d^2 \cdot h \cdot 0.00003$, where d is dbh (overbark) and h is total tree height. Not all provenances were established at all sites. To calculate provenance effects for volume across countries, age 3, 5, and 8-year data were standardized to a common mean and variance. Standardized data were then used to calculate family means in each test, which were used as units of observation to predict provenance effects. The PROC MIXED procedure of SAS® (LITTELL *et al.* 1996) was used to calculate country-specific best linear unbiased predictions (BLUPs) of the provenance effects assuming homogeneous genetic parameters across countries and ages. Provenance effects were expressed as a percentage gain above or below the mean for all provenances grown in each country. Provenance performance were presented on a country-by-country basis rather than combined across countries because of the differences in growing conditions among geographic regions.

RAPD study

Plant Material

A subset of 13 of the 25 populations (seeds from one population did not germinate) included in the provenance tests were chosen for RAPD molecular marker assessment to quantify genetic diversity within and among populations (Table 1). Provenances chosen were selected to represent extremes in the geographic range in our sample (western vs. eastern) or in vegetation types (cloud forests vs. pine-oak forests.) They were also chosen to represent good producers such as San Jeronimo, Coban, and Copan, and poor producers such as Coapilla and Tapiquil based on the original assessment by GAPARE *et al.* (2001).

A total of eight open-pollinated families per provenance were included in the study. Pine megagametophytes were collected from RAPD seedlings following greenhouse germination. After nucellus removal and embryo excision, the remaining haploid material was transferred to a microfuge tube and was stored at –80 °C.

DNA Extraction

Total genomic DNA was isolated from megagametophyte tissue using the DNeasy 96 well block plant kit (Qiagen Inc., Valencia CA). DNA concentration and size were monitored on a 0.8% agarose gel by comparison to lambda DNA standards. DNA preparations were then diluted to $1 ng \cdot \mu l^{-1}$ concentration with sterile distilled water.

Genetic Analyses

For each *P. maximinoi* family, six megagametophyte samples were analyzed to increase the probability of sampling both alleles possessed by the mother tree at each locus. DNA amplification for RAPD marker analysis was based on WILLIAMS *et al.* (1990). Decamer DNA primers were obtained from Genosys Beiotechnologies, Inc., The Woodlands, TX. Each amplification reaction contained 1.5 µl of 10X reaction buffer (100 mM Tris HCl, pH 8.8; 500 mM KCl; 1% Triton-X100; 25mM $MgCl_2$); 100 µM each of dATP, dCTP, dGTP, dTTP; 0.2 µM primer; 5 ng of genomic DNA template and 0.95 units of Taq DNA polymerase, in a total volume of 15 µl. Amplification was carried out in 96-well plates using a MJ Research PTC-100 thermal controller. The thermal program parameters were: 41 cycles of 1 min at 92 °C, 1 min at 35 °C and 2 min at 72 °C. A total of 50 primers were used to assay DNA amplification. Amplified DNA fragments were separated electrophoretically on ethidium bromide-stained agarose gels (1.5 %). DNA migrations were visualized under UV light on an Eagle Eye video imaging system. (Stratagene, La Jolla, CA).

Each family was scored for the presence/absence of

RAPD fragments. Because multiple megagametophytes were scored for each seed parent, it was possible to determine with reasonable certainty both alleles possessed by the seed parent, providing the equivalent of diploid data. Only RAPD markers of high amplification intensity were analyzed.

Bands that could not be scored with confidence due to failed or poorly amplified reactions were treated as missing data.

Data Analyses

Cluster analysis was used to identify natural groupings based on the RAPD markers. Unweighted pair-group method using an arithmetic average (UPGMA) (Sneath & Sokal 1973) was carried out using the computer program POPGENE v1.32 (YEH *et al.* 1999). UPGMA defines the inter-cluster distance as the average of all pairwise distances for members of two clusters (WEIR 1996). A dendrogram was created from the results of the UPGMA analyses. A matrix of genetic identities and distances was produced following procedures developed by NEI (1972). As clustering techniques produce clusters irrespective of any actual structure of the data, single linkage trees were compared with complete linkage trees (SNEATH & SOKAL 1973) generated using NTSYS-pc (ROHLF 2001). Robustness of the resulting groupings was qualitatively evaluated by producing a strict consensus tree, as clusters resolved in the consensus topology are likely to be well supported (ROHLF 2001). The matrix correlation between genetic distance based on RAPD data and geographic proximity (in kilometers) among sampling localities was examined using a Mantel test (MANTEL 1967). The normalized Mantel statistic computed from the original data was compared to a sample distribution based on 1000 permutations of the geographic matrix using NTSYS-pc (ROHLF 2001).

Data were then analyzed using POPGENE v1.32 (YEH *et al.* 1999) to obtain the proportion of polymorphic loci (P), the observed heterozygosity (H_o) and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$, where p_i is the frequency of the i^{th} allele) for each population, the groups obtained from the cluster analysis and all individuals combined. The degree of population structure among samples of *P. maximinoi* was estimated using Wright's F_{ST} (WEIR & COCKERHAM 1984). Populations of *P. maximinoi* were ranked overall and within groups according to observed heterozygosity.

Correlation Analysis

Population values for observed heterozygosity generated by the RAPD study were correlated to environmental variables collected in natural stands (provenances) and with volume performance of provenances established in tests in Colombia, Brazil and South Africa. Peasons's correlation coefficients (r) were calculated using the PROC CORR procedure of SAS® (SAS 1988). Environmental variables included total precipitation, winter precipitation (November through April), summer precipitation (May through October), as well as latitude, longitude and altitude of the collection site.

Allozyme Study

Plant Material

A subset of 5 of the 13 provenances (Coapilla, Mexico, Coban and San Jeronimo, Guatemala, Copan, Honduras, and Dantalí, Nicaragua) assessed in the RAPD study was analyzed using allozymes. Also included was a control lot of *Pinus tecunumanii* from San Jeronimo, Guatemala, a site where one of the *P. maximinoi* seed collections was also conducted. Since genetic diversity estimates from allozyme studies in pines are common (HAMRICK *et al.* 1992), results of the allozyme assessment would help benchmark results from RAPD analyses. Furthermore, since allozymes are co-dominant markers, outcrossing rates could be easily obtained. The advantages and disadvantages of using allozymes and RAPD markers in tree genetic diversity studies are summarized by WU *et al.* (1999).

Extraction

Thirty seeds from each maternal family were sown into standard greenhouse potting soil and allowed to germinate. After the resulting seedlings had grown for 4–6 weeks, twelve randomly chosen plants (tops and roots) per family were crushed using a mortar and pestle. Seedling enzyme extracts were stabilized by the addition of an extraction buffer (MITTON *et al.* 1979) and absorbed onto chromatography paper wicks that were stored at –70 °C until needed for electrophoresis.

Starch gel electrophoresis was used to analyze gene diversity. Fourteen enzyme systems were analyzed which resolved 25 allozyme loci. Four electrophoretic buffer systems were used to resolve these enzyme systems. Following the nomenclature of SOLTIS *et al.* (1983) alcohol dehydrogenase (*Adh–1*), diaphorase (*Dia–2*) and phosphoglucomutase (*Pgm–1, Pgm–2*) were resolved on buffer system 6. Amino acid transferase (*Aat–1, Aat–2, Aat–3*) and mendione reductase (*Mnr–1, Mnr–2*) were resolved on system 7. Fluorescent esterase (*Fe–1, Fe–2*), glutamate dehydrogenase (*Gdh*) and triose phosphate isomerase

(Tpi–1, Tpi–2) were resolved on a modified system 8. System 11 was used to resolve malate dehydrogenase (*Mdh–1, Mdh–2, Mdh–3*), phosphoglucoisomerase (*Pgi–1, Pgi–2*), shikimic dehydrogenase (*Skdh–1*), 6-phosphoglucoisomerase (*Pgd–1, Pgd–2*), isocitrate dehydrogenase (*Idh*), and UTP–glucose-1-phosphate (*Ugpp–1, Ugpp-2*). The genetic basis of the allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (WEEDEN & WENDEL 1989).

Genetic Analyses

Genetic diversity statistics – For the genetic diversity analyses, five seedlings were randomly selected from each maternal progeny array and pooled to produce a "population sample". Standard statistics of genetic diversity (BERG & HAMRICK 1997) were calculated for individual population samples and for the species as a whole for *P. maximinoi* by pooling over all five populations. For each population and for pooled "species level" sample, the proportion of polymorphic loci (*P*), the mean number of alleles per polymorphic locus (*AP*), the effective number of alleles per locus ($A_e = 1/\Sigma p_i^2$) and observed (H_o) and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$, where p_i is the frequency of the i^{th} allele) were determined. Statistics were calculated for each locus and then averaged over all loci. Observed and expected heterozygosities for each polymorphic locus in each population were compared by calculating Wright's fixation index (where $F = 1 - [H_o/H_e]$) to determine deviations from random-mating expectations. Mean inbreeding coefficients in each population were summarized across populations for each locus by calculating F_{IS} values. Deviations of F_{IS} from zero were tested using χ^2 (LI & HOROVITZ, 1953).

Differences in allele frequencies among populations were examined using a heterogeneity χ^2 analysis (WORKMAN & NISWANDER 1970). For each polymorphic locus, statistics of gene diversity were calculated (NEI 1973, 1977) including total gene diversity (H_T), mean gene diversity within populations (H_S), and G_{ST}, the proportion of the total gene diversity found among populations ($G_{ST} = (H_T - H_S)/H_T$). Overall means were calculated by averaging over all polymorphic loci. Nei's coefficients of genetic identity (NEI 1972) were also calculated between each pair of populations. The degree of isolation among populations was estimated by *Nm*, the number of migrants per generation, in two ways. First, from the number and frequency of unique alleles found in one population (SLATKIN 1985) and second, from the proportion of genic diversity among populations as $Nm = (1-G_{ST}/4G_{ST})$ following procedures in WRIGHT (1951).

Mating system estimation – Mating system parameters, including single locus (t_s) and multilocus outcrossing rates (t_m) were estimated using the program of RITLAND (1990) developed from the algorithms of RITLAND and JAIN (1981) which are based on the mixed-mating model of BROWN and ALLARD (1970). Assumptions of this model are given in CLEGG (1980) and SHAW *et al.* (1981). Maternal genotypes were inferred from progeny arrays by the method of BROWN and ALLARD (1970). Standard errors for outcrossing estimates are based on 200 bootstraps. BROWN (1988) has demonstrated that differences between single-locus and multi-locus (i.e. $t_m - t_s$) estimates of outcrossing are most likely due to biparental inbreeding. The expected fixation index at inbreeding equilibrium was calculated from the multi-locus outcrossing rate (FYFE & BAILEY 1951) by the equation $F_e = (1 - t_m)/(1 + t_m)$.

RESULTS

Provenance trials

The best performing provenances for volume production across all three countries were: La Cañada and San Jeronimo, Mexico, Cobán and San Jeronimo, Guatemala, Copán Honduras and San José Bayuncún, Nicaragua (Figure 1, Table 2). Some provenances performed well only in specific localities, e.g. Altamirano in Brazil and San Juan Sacatepequez and Candeleria in South Africa. Several of the Honduran and Nicaraguan sources performed poorly across all sites (Table 2). There was a general trend for populations from the western and central part of the geographic range of our sample to perform better than provenances at the southern end of the species' distribution.

RAPD

RAPD analysis provided 76 loci for evaluation. Natural clustering obtained from UPGMA analysis grouped provenances from geographic regions together, showing a distinct separation among groups identified. The four groups corresponded to the geographic distribution of the populations sampled in the four countries: Mexico, Guatemala, Honduras and Nicaragua (Figure 2). Genetic distances between populations were well defined. The strict consensus tree based on single linkage and complete linkage is nearly identical to and supports the UPGMA clustering. There was a strong correlation between genetic distance and geographic proximity of populations ($r = 0.671, p = 0.001$).

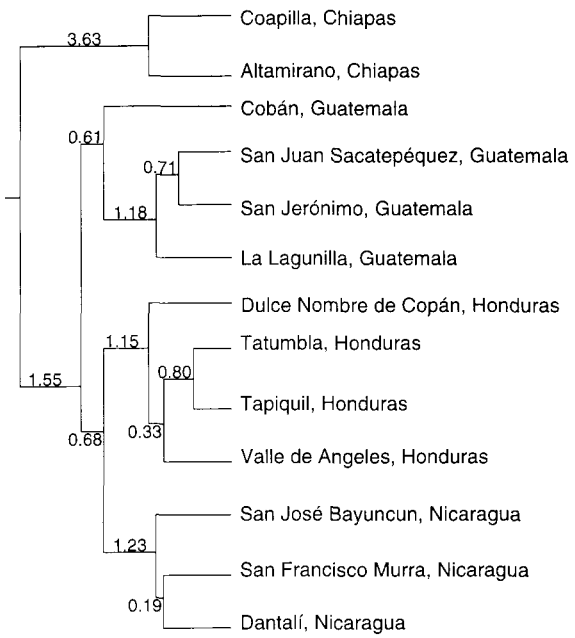

Figure 2. Dendrogram based on NEI's (1978) genetic distance using UPGMA. The diagram has been modified from NEIGHBOR procedure of PHYLIP version 3.5. Length is shown for each internal branch.

The percent polymorphic loci ranged from 26.3 for Dantali, Nicaragua to 51.3 for Altamirano (Chiapas), Mexico (Table 3). The ranking of top populations for percent polymorphic loci were consistent for observed heterozygosity Dantalí, Nicaragua exhibited the lowest observed heterozygosity of 0.101. Percent polymorphic loci found in the RAPD study were lower than that observed in the allozyme study but the ranks were approximately the same. Observed heterozygosity values for both markers systems were nearly identical for the San Jeronimo, Cobán and Dantalí populations but varied somewhat for Coapilla and Copán.

Mean genetic diversity among populations was (F_{ST}) 0.134 (Table 3). Analysis of population structure using RAPD suggests that there is substantially more variation among populations within regions than there is among regions. Genetic differentiation among populations within groups (F_{SG}) was 0.126 and represented approximately 93.5 % of the total genetic variation (F_{ST}).

Correlations between Provenance and RAPD data

Observed heterozygosity values generated for each population from the RAPD study were significantly correlated ((r = 0.61, p = 0.03) to the longitude of the collection sites in Mesoamerica and supported trends observed in the UPGMA analysis. Longitude and winter rainfall also were related (r = –0. 38, p = 0.05) and suggests that the severity of the dry season during the winter months diminishes from west to east. There was a noteworthy relation between observed population heterozygosity and volume performance by provenance in Brazil (r = 0.53, p = 0.06), Colombia (r = 0.48, p = 0.10) and South Africa (r = 0.43, p = 0.14). Provenances that exhibited good volume production often were the most genetically diverse. There were no significant correlations between observed heterozygosity and latitude, total or summer rainfall, and altitude.

Allozyme

Twenty-two of the 25 (88 %) allozyme loci were polymorphic for *P. maximinoi* (Table 4). However, only 11 of the 22 polymorphic loci have moderate to high levels of genetic diversity (H_T > 0.150, Table 5). Considering all five populations analyzed, there were 2.86 alleles per polymorpic locus (*AP*). Expected heterozygosity was 0.152 for the species. At the population level, the percent of polymorphic loci ranged from 52 % (Dantalí) to 76 % (San Jeronimo and Coban) with a mean P = 67.2 % (Table 4). The mean number of alleles per polymorphic locus within populations was 2.42 (range 2.23 – 2.56). Mean observed (H_o) and expected (H_e) heterozygosities within popula tions were identical (0.140). Coapilla (H_o = 0.177, H_e = 0.175) had the highest level of observed and expected genetic diversity while Dantalí had the lowest genetic diversity values (H_o = 0.109; H_e = 0.122). Unique alleles were found in Coapilla (2 alleles), Coban (2), San Jeronimo (2) and Copan (4) while no unique alleles were found in the southern-most population (Dantalí). Genetic diversity values for the Jeronimo population of *P. tecunumanii* were similar to those for *P. maximinoi* (Table 4).

Observed levels of heterozygosity within the five *P. maximinoi* populations were very close to values expected for a random mating population (mean F_{IS} = –0.002, Table 5). Approximately 95 % of the total genetic diversity at the polymorphic loci (H_T) occurred within populations of *P. maximinoi* (mean G_{ST} = 0.047). Such low levels of genetic differentiation among populations are consistent with high historical levels of gene flow. An estimate of gene flow (i.e. *Nm*) based on the estimated G_{ST} was 5.12 whereas *Nm* based on the 10 unique alleles (mean frequency 0.015) was 10.12.

Mean genetic identity among the five *P. maximinoi* populations analyzed was 0.982. Dantalí had the lowest mean identity with the other four populations (0.973) while San Jeronimo had the highest mean identity value (0.988).

Table 2. Volume performance of *Pinus maximinoi* provenances assessed in field trials in Brazil, Colombia (Col.) and South Africa (SA) at 3 to 8 years of age. Individual gains are expressed as percentages above or below the mean for all provenances in the test. The number of open-pollinated families (Fams) and trials (Tests) assessed in each country are recorded. Provenances with map code numbers that are shaded are those used in the RAPD study.

Map Code	Provenance	Brazil			Columbia			South Africa		
		Volume Gains	Fams	Tests	Volume Gains	Fams	Tests	Volume Gains	Fams	Tests
1	Cobán	8.9	20	4	3.1	24	4	8.9	24	5
2	San Jerónimo (Gua.)	4.3	25	8	11.4	19	6	4.1	19	15
3	San Juan Sacatepéquez	–0.5	21	9	–5.6	24	6	5.2	24	15
4	Copán	6.4	22	6	7.5	32	2	2.8	32	10
5	Marcala	–7.8	12	3	–5.7	15	4	–1.9	18	5
6	Tapiquil	–0.7	26	2	–1.4	13	1	–7.8	13	2
7	Tatumbla	–4.6	62	6	4.4	55	9	–1.6	43	6
8	Altamirano	6.3	9	2	3.1	13	3	2.9	13	2
9	San Jerónimo (Chis.)	5.8	23	7	6.3	23	2	12.1	23	10
10	Ciénega de León	–4.3	–	–	–4.7	11	3	–6.5	6	2
11	Coapilla	–3.4	7	2	–8.8	16	3	–7.9	21	3
12	La Cañada	11.1	11	4	9.1	16	2	11.9	16	4
13	Monte Cristo	–2.5	–	–	–6.0	13	3	–0.5	10	3
14	Valle de Angeles	–4.8	16	3	–1.3	6	1	–10.1	6	2
15	San Juan Copala	–5.6	1	2	–2.5	–	–	–0.8	1	2
16	San Jerónimo (Oax.)	–6.0	10	3	–2.8	–	–	–1.3	11	3
17	Candelaria	–0.7	13	3	2.2	–	–	6.4	13	4
18	Las Compuertas	1.6	10	3	1.3	–	–	1.9	8	4
19	El Portillo	–3.2	17	3	–4.8	–	–	–9.8	13	4
20	Yuscarán	3.2	23	3	–1.6	–	–	–7.5	9	2
22	La Lagunilla	3.2	–	–	4.3	7	1	4.8	6	2
23	San Lorenzo	0.7	–	–	–0.2	3	1	2.2	6	2
24	San José Bayuncún	5.8	16	1	10.8	16	2	4.4	–	–
25	San Francisco Murra	–10.4	11	1	–11.5	6	1	–9.1	–	–
26	Datanlí	–4.0	5	1	–6.9	14	2	–3.2	–	–

Multi-locus estimates of the outcrossing rates (t_m) in the *P. maximinoi* populations averaged 0.904 while that of the single *P. tecunumanii* population was 0.975 (Table 6). There was little variation in outcrossing rates among the five *P. maximinoi* populations (t_m range = 0.898 – 0.907). Little difference was detected in outcrossing rates between the genetically most diverse population (Coapilla, t_m = 0.907) and the least genetically diverse site (Dantalí, t_m = 0.904). These results also indicate that there is some biparental inbreeding in the *P. maximinoi* populations ($t_m - t_s$ = 0.090). If the populations were in inbreeding equilibrium a slight deficiency of heterozygotes would have been expected (F_e = 0.050). However, the observed inbreeding coefficient (F_{IS}) was –0.002, indicating somewhat more heterozygosity than expected based on the estimates of the mating system.

DISCUSSION

The amount and distribution of genetic diversity reflect the interaction of various evolutionary processes (WU *et al.* 1999). Based on its large geographic distribution, we can only speculate that *P. maximinoi* is of ancient rather than recent origin. Possibly it evolved from a *Pseudostrobus* ancestor in Central Mexico in the late Tertiary during the period of mountain uplifting and progressively drier climates. High levels of genetic diversity in Mexico and Guatemala with progressively lower levels in Honduras and Nicaragua suggest an origin in central Mexico with subsequent migration to the south and east into present-day Central America. Significant correlation between genetic diversity and geography have also been found using allozymes for several pine species in the United States (LEDIG 2000, SCHMIDTLING & HIPKINS 2000, SCHMIDTLING & HIPKINS 1998).

As *P. maximinoi* migrated from Mexico to the south and east it became broadly adapted to both mesic cloud forest and dry pine-oak habitats. Root morphology of the species varies greatly to accommodate these different environments depending on the population

Table 3. Hierarchical structure of *Pinus maximinoi* populations as defined by RAPD molecular marker assessment. N = sample size, H_o = observed heterozygosity and H_e = expected heterozygosity.

Country	Subpopulations Population	P (%)	H_o	H_e	Group H_e	Total H_e
Maxico	Altamirano	51.32	0.1363	0.1581		
	Coapilla	42.11	0.1336	0.1522		
					0.1581	
Guatemala	San Juan	44.74	0.1299	0.1495		
	Coban	42.11	0.1471	0.1311		
	San Jeronimo	43.42	0.1471	0.1572		
	La Lagunilla	39.47	0.1469	0.1561		
					0.1495	
Honduras	Copan	47.37	0.1548	0.1703		
	Tapiquil	35.53	0.1104	0.1316		
	Tatumbla	35.53	0.1177	0.1230		
	Valle Angeles	36.84	0.1320	0.1365		
				0.1316	0.1316	
Nicaragua	Murra	39.47	0.1198	0.1240		
	Bayuncun	36.84	0.1210	0.1243		
	Dantalí	26.32	0.1010	0.0959		
				0.1243	0.1243	
Average heterozygosity			$H_S = 0.1294$	$H_G = 0.1480$	$H_G = 0.1480$	$H_T = 0.1495$

$F_{SG} = 0.126$, $F_{GT} = 0.010$, $F_{ST} = 0.134$

Table 4. Levels of genetic diversity within populations of *Pinus maximinoi* and *P. tecunumanii* using allozymes. N = sample size; P = proportion of polymorphic loci; AP = number of alleles per polymorphic locus, A_e = effective numbers of alleles per locus; H_o = observed heterozygosity; H_e = Hardy-Weinberg expected heterozygosity. Standard deviations are in parentheses. See text for additional information.

Species	Provenance	Country	N	P(%)	A_P	A_e	H_o	H_e
P. tecunumanii	S. Jeronimo	Guatemala	65	76.0	2.47	1.27	0.140 ± 0.136	0.150 ± 1.040
P. maximinoi	Coapilla	Mexico	60	64.0	2.56	1.30	0.177 ± 0.042	0.175 ± 0.040
P. maximinoi	Cobán	Guatemala	59	76.0	2.37	1.20	0.142 ± 0.040	0.131 ± 0.031
P. maximinoi	S. Jeronimo	Guatemala	65	76.0	2.47	1.21	0.143 ± 0.038	0.138 ± 0.031
P. maximinoi	Copán	Honduras	60	68.0	2.47	1.20	0.129 ± 0.040	0.136 ± 0.031
P. maximinoi	Dantalí	Nicaragua	60	52.0	2.23	1.22	0.109 ± 0.034	0.122 ± 0.037
Population mean *P. maximinoi*			61	67.2	2.42	1.23	0.140 ± 0.017	0.140 ± 0.015
Pooled species value *Pinus maximinoi*			304	88.0	2.86	1.23	–	0.152

(DVORAK 1990). Seed dispersal in April occurs at the beginning of the rainy season so that seedling shoot and root growth is maximized before the onset of the dry season. Its rapid initial growth rate protects it from competing vegetation and minimizes the likelihood of complete destruction from ground fires. The 12 to 14 month reproductive cycle enables it to recover rapidly after flowering periods are disrupted by short term weather events (droughts, hurricanes etc.) and allows it to adapt more quickly to long term climatic changes.

Table 5. Distribution of genetic diversity within and among populations of *P. maximinoi*. H_T = total genetic diversity at polymorphic lcoi, H_S = mean genetic diversity within populations, G_{ST} = proportion of the total genetic diversity among populations, F_{IS} = mean inbreeding coefficient within populations. Values of G_{ST} with asterisks indicate significant heterogeneity in allele frequencies among populations.

Locus	H_T	H_S	G_{ST}	F_{IS}
Pgm-1	0.297	0.260	0.123***	-0.073
Tpi-1	0.023	0.023	0.014	-0.026
Tpi-2	0.445	0.355	0.201***	0.037
Ugpp-1	0.403	0.400	0.008	0.020
Ugpp-2	0.020	0.019	0.018*	-0.029
Aat-1	0.020	0.019	0.017*	-0.026
Aat-2	0.022	0.022	0.017*	-0.030
Aat-3	0.283	0.267	0.054***	0.012
Mnr-1	0.026	0.025	0.029***	-0.044
Mnr-2	0.228	0.220	0.038***	-0.037
Fe-1	0.305	0.276	0.094***	0.044
Fe-2	0.152	0.147	0.033***	0.010
Gdh	0.448	0.423	0.055***	0.025
Mdh-2	0.051	0.050	0.021*	0.084
Mdh-3	0.243	0.228	0.064***	-0.126
Pgi-2	0.020	0.019	0.017*	-0.024
Skdh	0.215	0.211	0.021***	0.028
6P-1	0.441	0.384	0.130***	0.035
6P-2	0.023	0.022	0.016*	-0.026
Idh	0.082	0.079	0.037***	-0.084
Adh-1	0.007	0.006	0.005	-0.008
Dia-2	0.058	0.057	0.010	0.076
Mean	0.173	0.160	0.047***	-0.002

* = P < 0.05; *** = P < 0.001

The genetic structure of a species is influenced by whether it moved steadily along an advancing front or in a series of long-distance colonization events that created permanently or temporarily isolated populations (LEDIG 2000). Most likely both evolutionary scenarios occurred in *P. maximinoi*. With the long history and destructive nature of volcanism in Mexico and Central America (EGUILUZ-PIEDRA 1985) the chance colonization of founder populations seems to be likely and undoubtedly explains the origin of some populations. The cluster of small pure stands of *P. maximinoi* at Dantalí (Nicaragua) that exhibited low levels of RAPD and allozyme, no unique alleles and poor growth in provenance trials, may be one such recently formed founder population. Subsequent spread of founder colonies would eventually reunite individual populations and replace lost alleles (LEDIG 2000). Other populations probably developed along a steadily moving front, which retreated and expanded over centuries in response to climatic changes and volcanism. The degree of genetic isolation among populations as measured by Nm was in the average range for most pines (see LEDIG 1998) and suggests relatively few recent barriers to gene flow. Reduction in genetic diversity in *P. maximinoi* is only noticeable near the species' southern periphery in Nicaragua where populations are presumably younger than in other areas of its natural distribution.

The genetic structure of *Pinus maximinoi* is typical of most pines. RAPD and allozyme assessment indicated important differences in genetic diversity among populations and geographic regions but there was no discernable relationship with regards to whether the provenance originated in cloud forest or pine oak ecosytems. Expected heterozygosity for *P. maximinoi* is very similar to the mean for a number of pine species (0.157) reported by HAMRICK *et al.* (1992). Mean genetic diversity (G_{ST}) values for *P. maximinoi* (0.047) were lower than the average (0.065) for other pines studied.

Mating system parameters of *P. maximinoi* are also typical of other pines despite its one-year reproductive cycle. Clinal trends in seed size may be related to winter rainfall amounts that increase from west to east in Mesoamerica. The high percentage of filled seeds per cone might reflect better synchronization between

Table 6. Multilocus (t_m) mean single locus (t_s) estimates of outcrossing and equilibrium inbreeding coefficients (F_e) for five population of *Pinus maximinoi* and one population of *P. tecunumanii*. Differences between t_m and t_s represent the level of biparental inbreeding. Standard deviations are in parentheses.

Species	Provenance	Country	Families	t_m	t_s	$t_m - t_s$	F_e
P. tecunimanii	S. Jeronimo	Guatemala	13	0.975±0.039	0.920±0.040	0.055	0.013
P. maximinoi	Coapilla	Mexico	12	0.907±0.060	0.815±0.070	0.092	0.049
P. maximinoi	Cobán	Guatemala	12	0.898±0.051	0.805±0.608	0.093	0.054
P. maximinoi	S. Jeronimo	Guatemala	13	0.907±0.052	0.821±0.067	0.086	0.049
P. maximinoi	Copán	Honduras	12	0.905±0.054	0.818±0.062	0.087	0.050
P. maximinoi	Dantalí	Nicaragua	12	0.904±0.053	0.813±0.063	0.090	0.050
Mean *P. maximinoi*				0.904	0814	0.090	0.050

pollen dispersal and receptivity of female strobili than either *Pinus oocarpa* or *P. caribaea* var. *hondurensis*. Lower outcrossing rates were found for *P. maximinoi* than for *P. tecunumanii* but not to the degree described by MATHESON *et al.* (1989) in their study of one population of *P. maximinoi* in Honduras. The observed inbreeding coefficient (F_{IS}) does not differ significantly from values expected if the populations were in inbreeding equilibrium (F_e).

In situ conservation strategies

Forestry institutions in developing countries have limited funds and are likely only to conserve tree populations that grow well so local people have an opportunity to improve their standard of living. Unlike most published genetic diversity in other studies, provenance rankings for volume based on field trials correlated reasonably well with genetic diversity patterns discerned from RAPD and allozyme studies in our work. Priorities for *in situ* conservation of *P. maximinoi* in Mexico and Central America should focus on the most productive populations as determined by international field-testing and also include occasional outlier populations identified as genetically diverse based on genetic markers. Based on the results of the provenance tests *in situ* conservation efforts should include the populations of La Cañada and San Jeronimo, Mexico, Cobán and San Jeronimo, Guatemala, Copán Honduras and San José Bayuncún, Nicaragua. Coapilla and Altamirano might also be added to the list if resources were available. These seed sources not only exhibit good growth (with the exception Coapilla) but also are broadly adapted to many sites because they represent the major ecotypes of *P. maximinoi* found in Mesoamerica. Of all the provenances listed, only Copán, Honduras is under little risk of being destroyed by agriculture and wood cutters (DVORAK *et al.* 2000). All the other populations are classified as vulnerable to critically endangered by CAMCORE. There is an urgent need to develop strong *in situ* conservation programs for *Pinus maximinoi*.

Because of the geographic distribution of genetic diversity for *P. maximinoi* in Mesoamerica, *in situ* conservation programs should be regionally organized, and not developed in an isolated manner on a country-by-country basis. As an example, Nicaragua is host to relatively few populations of *P. maximinoi*, most of which are genetically high graded and under severe pressure by human encroachment (DVORAK *et al.* 2000). However, these populations on average are less genetically diverse that those in other regions of Central America. Conservation of all three populations in Nicaragua is important locally, but a more efficient approach would be for neighboring countries to work together to allocate funds to conserve, *in situ,* those populations throughout Mesoamerica that are both productive and genetically diverse. Unfortunately, international collaborative efforts for the *in situ* conservation of pines are rare.

The development of sound *in situ* conservation strategies for tree species in Mesoamerica requires reliable information on both provenance performance and genetic diversity. Generally discussions center on what kinds of molecular markers should be used to generate genetic diversity values. However, for applied programs in developing countries, the results produced from field trials are equally important for designing conservation approaches. The CAMCORE international provenance test series for *P. maximinoi* is the most complete of its kind. For provenance information to be useful in the development of gene conservation programs, it must be based on well-designed field trials replicated dozens of times across a number of locations. Field results based on a single to a few tests probably might be of little value when developing conservation strategies for species that have broad geographic ranges.

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