ASSESSING THE SAMPLING EFFICIENCY OF *EX SITU* GENE CONSERVATION EFFORTS IN NATURAL PINE POPULATIONS IN CENTRAL AMERICA

W. S. Dvorak[1], J. L. Hamrick[2] & G. R. Hodge[1]

[1)] Department of Forestry, CAMCORE Cooperative, Box 7626, North Carolina State University, Raleigh, NC 27695, USA
[2)] Departments of Botany and Genetics, University of Georgia, Athens, GA 30602, USA

Received October 25, 1998; accepted December 9, 1998

ABSTRACT

Seeds were collected from 39 phenotypically good and 70 randomly chosen trees in two small fragmented stands (< 7 ha in size) and one large population (>200 ha) of *Pinus tecunumanii* in the mountains of central Guatemala. Twelve seedlings from each of the 109 half-sib families were examined by starch gel electrophoresis for 27 allozyme loci to determine whether fragmented stands contributed private or unique alleles to the gene pool not found in the large population. The large population was separated from the two smaller populations by 65 km. Furthermore, it was important to determine whether allele frequencies in selected and randomly chosen trees varied greatly since plus tree selection is sometimes used to accommodate both gene conservation and breeding objectives during seed collections. Information was also required to determine how tree sample size varied in fragmented and relatively intact stands when the goal was to capture alleles with a frequency of 5% or greater. The results indicated that 25 of 27 loci analyzed were polymorphic in *P. tecunumanii* when all progeny were pooled. There were on average 3.04 alleles per polymorphic locus and mean heterozygosity was 0.142. A total of 78 alleles were observed at these 27 loci, 75/78 alleles in the large population and 58–60/78 alleles in the two small populations. Among population genetic diversity (G_{ST}) was 0.014. Alleles that were missing from the various populations were those with overall frequencies of 1% or less. The difference in total number of alleles between selected and randomly chosen trees was greatest in the smaller populations but involved alleles at very low frequencies. Alleles that were rare in one population were often present at higher frequencies in other populations. To sample all alleles with frequencies of 5% or greater, seed samples from approximately 10 and 20 trees would be needed in the large and small populations, respectively.

Key words: rare alleles, allozymes, plus trees, *Pinus tecunumanii*, sampling strategies

INTRODUCTION

Pinus tecunumanii Eguiluz & J. P. Perry is a closed-cone pine in the Oocarpa subsection of the Pinaceae (PERRY 1991) that occurs in a series of disjunct populations from southern Mexico to central Nicaragua. The species is usually found on fertile, well drained soils at altitudes between 450 and 2900 m in areas with more than 1200 to 1500 mm of annual rainfall. As late as the mid 1980s, it was possible to find trees in old growth stands in the humid montane cloud forests of Guatemala and Honduras that reached heights of 55 m and diameters of 120 cm. However, most of these magnificent specimens have now been harvested. The remaining stands of *P. tecunumanii* range from 2 to 300 hectares in size in Mexico and Central America and are being intensely exploited by local sawmillers, fuelwood cutters and farmers.

As part of an international *ex situ* gene conservation and testing effort, the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), North Carolina State University, in collaboration with the government forestry organizations in the donor countries in Belize, Guatemala, Honduras, El Salvador, Mexico and Nicaragua, began explorations and seed collections for *P. tecunumanii* in natural stands in the region. Between 1980 and 1996, CAMCORE sampled 45 populations and 2000 mother trees in Mexico and Central America, and had established nearly 100 provenance/progeny tests in 10 countries (DVORAK *et al.* 1996).

Ex situ conservation efforts have often been considered a complementary approach to *in situ* conservation of forest species in the tropics. In most *ex situ* gene conservation efforts in Central America and Mexico, organizations sample 10 to 75 trees per population from a large number of provenances to ensure that genes for broad adaptability and important metric traits are represented. Some organizations like CAMCORE place mild selection pressure on trees with large volume and stem form in natural stands when stands are sufficiently large (>20 ha) but relax selection standards when stands are extremely small or degraded. Essentially, when natural populations are < 20 hectares, trees

selected for the *ex situ* conservation program are chosen at random. CAMCORE's goal is to sample all alleles that occur in populations at frequencies of 5% or greater. The question remains of how effective such seed collections are in wild populations of tropical forest species for the purpose of *ex situ* conservation. Do small, fragmented stands contribute unique genes to the breeding pool or could these genes be sampled elsewhere in larger, more accessible populations at less cost?

Tables have been derived using theoretical genotype and gene frequencies to estimate the number of trees per species or population that should to be sampled to capture alleles at a given frequency (NAMKOONG *et al.* 1980). However, these tables can not take into account the landscape dynamics of the target populations, their proximity to neighboring populations, the degree and rate of fragmentation, and the influence of introgression with related taxa. Furthermore, field seed collections in geographically isolated locations in the tropics and subtropics seldom have the luxury of *a priori* assessments of clinal and ecotypic variation to know where to most effectively sample within population variation.

The purpose of this preliminary study was to assess levels of genetic diversity in large and small stands of *P. tecunumanii* subjected to different degrees of fragmentation and to determine the sampling efficiency of an applied gene conservation program like CAMCORE. Recommendations are made on the number of trees to sample in populations of both sizes using actual allele frequency data. The value of continued seed collections in highly fragmented populations for *ex situ* conservation purposes are discussed.

MATERIAL AND METHODS

To determine how population size and levels of fragmentation affect sampling strategy for *ex situ* conservation, one large and two small stands of *P. tecunumanii* were chosen for the study in Guatemala. San Jeronimo is one of the largest populations of *P. tecunumanii* in Guatemala that covers nearly 200 ha. It occurs from 1600 to 1800 m elevation and has been subdivided by farmers into clusters of small stands that range in size from 2 to 20 hectares. At higher elevations at San Jeronimo, small pockets of *P. maximinoi* H.E. Moore occur sympatrically with *P. tecunumanii*. At the lower elevations near 1600 m altitude, *P. tecunumanii* and *P. oocarpa* Schiede ex Schlechtendal occur together. *Pinus maximinoi* and *P. tecunumanii* do not naturally cross but *P. tecunumanii* and P. oocarpa are known to hybridize (DVORAK & RAYMOND 1991; SQUILLACE & PERRY 1992). As part of its normal conservation and testing program, CAMCORE selected 44 trees at San Jeronimo for volume and stem form between 1980 and 1985 and distributed the seeds to its members for field tests.

Sixty-five km southeast of San Jeronimo, two small stands of *P. tecunumanii* exist at El Ingenio and Anshigua and are separated from each other by 3.5 km. The *P. tecunumanii* trees at El Ingenio and Anshigua occur from 1850 to 2020 m elevation (Table 1). At El Ingenio, only approximately 40 mature trees of *P. tecunumanii* remain and all are located in a 7 hectare area on a mountain ridge. The surrounding pine and oak (*Quercus* sp) forests have been completely destroyed and were converted to corn (*Zea mays*) and bean (*Phaeselosis vulgare*) fields as long as 50 years ago. Some *Pinus oocarpa* trees can be found on the borders of the *P. tecunumanii* stand at El Ingenio and appear to be gradually invading the site. The Anshigua site contains approximately 65 trees spread over a distance of 1 km. Fifty percent of the trees at Anshigua are found on a hilltop and represents old growth forest, uncut by farmers most likely because of the steepness of the terrain and as a means to reduce soil erosion. The hilltop covers an area of approximately 4 hectares. Within the small forest on the hilltop are also several P. maximinoi trees. The pine forests at Anshigua were probably at one time connected with those at El Ingenio. In 1993, CAMCORE collected seeds from three trees at El Ingenio and eight trees at Anshigua and distributed them as one provenance for the purpose of establishing field conservation plantings.

To determine how mild selection pressure affected

Table 1. Location of *Pinus tecunumanii* populations sampled in Guatemala.

Populations	Department	Latitude	Longitude	Altitude	Annual rainfall (mm)	Forest stand (ha)	Age (yrs)
San Jeronimo	Baja Verapaz	15° 03' N	90° 18' W	1620–1850	1200	200	30–60
El Ingenio	Jalapa	14° 43' N	90° 02' W	1850–1920	1400	5	20–40
Anshigua	Jalapa	14° 43' N	90° 02' W	1930–2025	1500	7	30–50

* values estimated

allele frequencies in the large and small stands of *P. tecunumanii*, 28 of the original 44 CAMCORE trees at San Jeronimo were re-sampled in 1995. This represented the "selected population". To obtain a population-wide estimate of allele frequencies at San Jeronimo, seeds from an additional 40 trees were selected at random along an elevation gradient from 1610 to 1820 m elevation. This group represented the "random population" at San Jeronimo.

At El Ingenio and Anshigua, the 11 CAMCORE trees were re-sampled and seed from an additional 21 trees were collected to represent the random population. The random population probably sampled 65% of all the mature *P. tecunumanii* trees remaining at El Ingenio and Anshigua (Table 2).

Allozyme study

An allozyme analysis was initiated to determine levels of genetic diversity among and within the three sites, and between the CAMCORE selected and randomly chosen trees (six populations). Twelve-hundred and fifteen seedlings from 109 mother trees (11.1 seeds /seed tree) were grown at the Department of Botany, University of Georgia, and assessed electrophoretically at one month. Enzyme extraction and electrophoretic procedures generally followed those of PARKER *et al.* (1997). Each seedling was analyzed for 27 allozyme loci and the percent of polymorphic loci (*P*), mean number of alleles per polymorphic locus (*AP*), total number of alleles (*TA*), genetic diversity or the proportion of loci heterozygous per individual (H_e), and mean observed heterozygosity (H_o) were calculated using standard approaches (HAMRICK *et al.* 1992, BERG & HAMRICK 1997). The proportion of total genetic diversity at polymorphic loci that was found among populations (G_{ST}) was also calculated as $G_{ST} = (H_T - H_S)/H_T$ where H_T and H_S were total genetic diversity and mean diversity within populations, respectively. H_T and H_S were estimated from the polymorphic loci (NEI 1975).

Sampling Efficiency

A second analysis was initiated to determine the number of trees needed to be collected in large and small populations to sample alleles at frequencies of 5% or greater using actual allozyme data. Such an analysis has much practical significance since it would determine how much genetic diversity is lost by lowering the number of maternal trees from which seeds were collected. Allozyme data from the two small populations El Ingenio and Anshigua were pooled because both were probably part of one continuous population in the recent past. Data from selected and randomly chosen trees described in the first study were also combined to give a larger population from which to sample. Therefore, for this study, the large San Jeronimo population was made up of 68 trees (40 random trees + 28 selected trees) and the smaller pooled population of Ingenio and Anshigua consisted of 41 trees (30 random trees + 11 selected trees).

To determine sampling efficiency, 50 of the 68 trees from the San Jeronimo population were sampled without replacement 50 times using a computerized algorithm. Mean values for total number of alleles (*TA*), percent of polymorphic loci (*P*) and genetic diversity values (H_e) were calculated. Parameters were also estimated for sample sizes of 40, 30, 25, 20, 15, 10, 8, 6, and 4 trees, each sample drawing replicated 50 times. Population parameters were plotted against sample size to observe trends. The same procedure was also used for the smaller pooled populations of El Ingenio and Anshigua beginning with a sample size of 40 seed trees.

RESULTS

Genetic Diversity

Genetic diversity values for pooled samples of the CAMCORE selected trees and trees and randomly are presented in Table 3. The results indicate that 25 of the

Table 2. Summary of the number of selected *P. tecunumanii* trees sampled by the CAMCORE Cooperative for *ex situ* conservation and additional trees selected at random within the populations.

Population	Population size * (no. of trees)	No of trees selected	Number of trees chosen at random	Total o. of trees sampled
San Jeronimo	15000	28	40	68
El Ingenio	60	3	17	20
Anshigua	55	8	13	21

* values estimated

Table 3. Levels of genetic diversity for selected and randomly chosen trees in *Pinus tecunumanii* populations at San Jeronimo, El Ingenio, and Anshigua, Guatemala. *N* = number of seedlings analyzed and (number of maternal trees); *P* = percent of polymorphic loci; *AP* = number of alleles per polymorphic locus; *TA* = total number of allelles; H_e = genetic diversity=proportion of loci heterozygous per individual; H_o = observed heterozygosity.

Population	*N*	*P*	*AP*	*TA*	H_e	H_o
S. Jeronimo (selected)	309 (28)	85.2	2.70	66	0.142	0.134
S. Jeronimo (random)	469 (40)	85.2	2.83	69	0.136	0.126
S. Jeronimo(pooled)	778 (68)	92.6	2.92	75	0.139	0.129
Ingenio (selected)	34 (3)	55.6	2.27	46	0.142	0.142
Ingenio (random)	170 (17)	77.8	2.48	58	0.145	0.137
Ingenio (pooled)	204 (20)	77.8	2.48	58	0.148	0.137
Anshigua (selected)	90 (8)	74.1	2.50	57	0.132	0.128
Anshigua (random)	143 (13)	74.1	2.45	56	0.141	0.131
Anshigua (pooled)	233 (21)	77.8	2.57	60	0.139	0.130

Table 4. Levels of genetic diversity in *Pinus tecunumanii* pooled over six "populations". *P* = percent polymorphic loci; *AP* = number of alleles per polymorphic locus; H_e = genetic diversity = proportion of loci heterozygous per individual; G_{ST} = proportion of the total genetic diversity at polymorphic loci that is found among populations.

Plant type	*P*	*AP*	H_e	G_{ST}
P. tecunumanii (pooled over 6 populations)	92.6	3.04	0.142	0.014
P. tecunumanii (means within populations)	75.3	2.54	0.140	–
All *Pinus* *				
within species	–	–	0.157	0.065
within populations	–	–	0.136	–
Woody Plants				
within species	65.0	2.88	0.177	0.084
within populations	49.3	2.54	0.148	–

* from HAMRICK *et al.* 1992

27 (92.6%) loci analyzed were polymorphic when the progeny from San Jeronimo, El Ingenio, and Anshigua, were pooled (Table 4). There were on average 3.04 alleles per polymorphic locus and the overall genetic diversity was 0.142. A total of 78 alleles were observed at these 27 loci. The mean levels of polymorphism for the "six populations" (selected and randomly chosen trees at three sites) was 75.3% and there were 2.54 alleles per polymorphic locus. Mean heterozygosity across the six populations was 0.140. However, since the six populations do not represent actual biological entities, pooled values of these statistics were also calculated for each site and averaged: *P* = 82.7, *AP* = 2.66 and H_e = 0.142.(Table 4).

The largest population, San Jeronimo, had the most polymorphic loci and alleles per polymorphic locus but had a genetic diversity value that was somewhat below the species mean (Table 3). The Anshigua population had intermediate levels of polymorphism and alleles per polymorphic locus and less genetic diversity (Table 3). The small El Ingenio population had moderate amounts of polymorphism, the fewest alleles per polymorphic locus but the highest overall genetic diversity. This seeming contradiction is due to El Ingenio having more even allele frequencies at its polymorphic loci.

Genetic differences among the three collection sites were quite small (G_{ST} = 0.014) indicating that almost all of the genetic diversity present in Central Guatemala can be found in an individual collection site. Each site captured somewhat less of the overall allelic diversity with San Jeronimo having 75 of the 78 alleles observed (96.1%), Anshigua having 60 alleles (76.9%) and El Ingenio having 58 alleles (74.4%). Alleles missing from the various populations were those with overall frequencies of 1% or less. The mean genetic identity among the three locations was quite high (0.993).

Comparisons between the CAMCORE selected trees and trees chosen at random at each site indicated that the CAMCORE collections captured the vast majority of genetic variation present. At San Jeronimo, the selected trees had 66 total alleles, 6 of which were not found in the randomly chosen trees. The San Jeronimo non-selected population had 69 alleles, 9 of which were not in the selected population. The alleles not present in one or the other population at San Jeronimo all had frequencies of 1% or less. In the case of all three locations, however, the non-selected population had equal or larger values of *P* and *AP* and nearly equal or more total alleles (*TA*). The greatest discrepancy between the CAMCORE collections and non-selected trees came at El Ingenio where CAMCORE collections represented only 3 trees. The CAMCORE selected trees had fewer polymorphic loci, lower AP values and somewhat lower He values. The CAMCORE selections also contained 12 fewer alleles than the randomly chosen trees (59.0% vs 74.4%). The mean frequency of these 12 alleles in the randomly chosen populations was 0.014 and only one of these had a frequency > 0.04. Thus, it appears that the majority of the biologically significant genetic diversity was captured in the progeny arrays of the three seed trees.

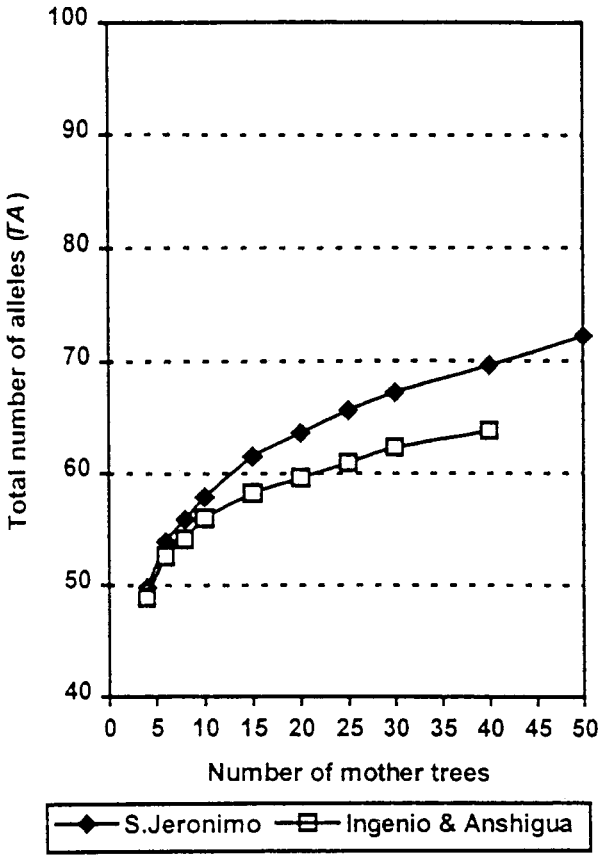
Sampling Efficiency

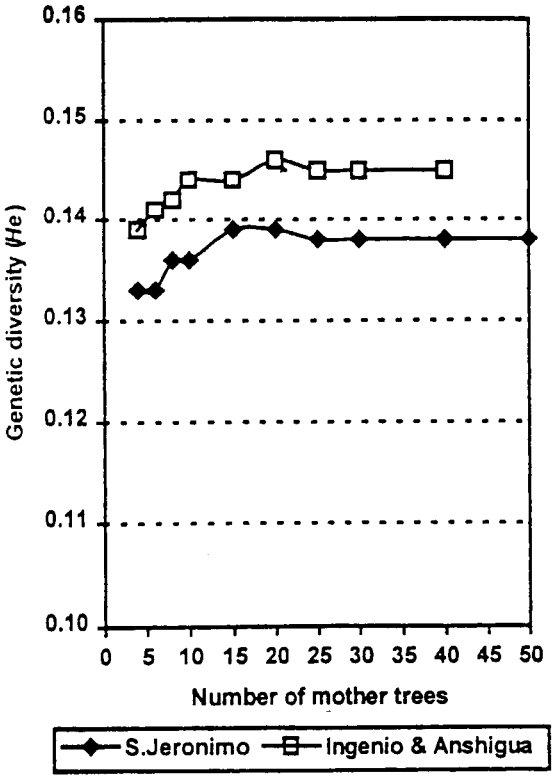
Changes in *TA*, *P*, and H_e with changes in the number of seed trees is shown in Figures 1a–1c and Table 5. At a sample size of 40 trees , San Jeronimo and the pooled small stands, El Ingenio and Anshigua, had 69.6 and 63.8 total alleles, respectively. By selecting only four trees in each population, the total number of alleles were reduced 33.6% (to 49.8 alleles) at San Jeronimo and 23.7% (to 48.8 alleles) at El Ingenio and Anshigua. The percent of polymorphic loci (*P*) was greater at San Jeronimo than at El Ingenio and Anshigua (pooled) regardless of the number of trees sampled. However, genetic diversity was greater in the smaller stands than in the larger stand since El Ingenio and Anshigua had more even allele frequencies at its polymorphic loci. At sample sizes of four trees, the genetic diversity of El Ingenio and Anshigua was 0.139 vs 0.133 for San Jeronimo.

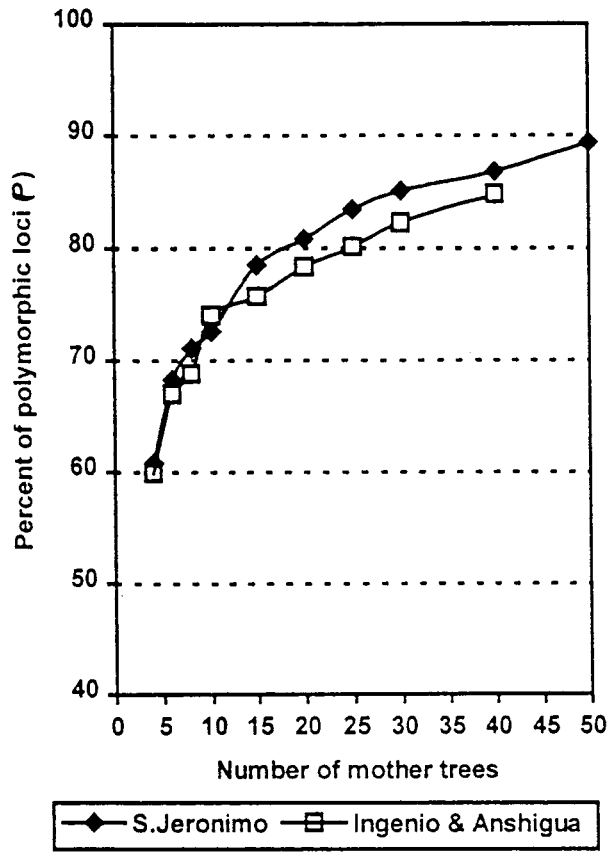
In the small stands, one allele at a frequency of 5% was lost at a sample size of 15 trees. This apparently was a unique occurrence because it occurred only once in 50 replications. No alleles at frequencies of 5% or greater were lost at sample size 10 trees. Two such alleles were lost at sample size of eight trees. One of these alleles was lost once, the other twice, in 50 runs.

Table 5. Changes in total number of alleles (*TA*), percent of polymorphic loci (*P*) and genetic diversity (H_e) with changes in number of mother trees selected in a large (San Jeronimo) and small (Ingenio & Anshigua) population of *P. tecunumanii*. Values are means based on 50 samples, with standard errors in parentheses.

Population	Number of mother trees	*TA*	*P*	H_e
S. Jeronimo	50	72.2 (.221)	.894 (.004)	.138 (.0002)
	40	69.6 (.223)	.868 (.004)	.138 (.0003)
	30	67.2 (.236)	.851 (.005)	.138 (.0004)
	25	65.6 (.326)	.834 (.006)	.138 (.0005)
	20	63.6 (.260)	.808 (.006)	.139 (.0006)
	15	61.5 (.339)	.785 (.007)	.139 (.0008)
	10	57.9 (.335)	.726 (.007)	.136 (.0011)
	8	55.9 (.404)	.711 (.009)	.136 (.0011)
	6	53.9 (.368)	.683 (.010)	.133 (.0014)
	4	49.8 (.428)	.608 (.011)	.133 (.0016)
Ingenio & Anshigua	40	63.8 (.052)	.848 (.001)	.145 (.0000)
	30	62.3 (.179)	.823 (.003)	.145 (.0003)
	25	60.9 (.179)	.801 (.004)	.145 (.0003)
	20	59.6 (.193)	.783 (.005)	.146 (.0005)
	15	58.2 (.257)	.757 (.006)	.144 (.0006)
	10	56.0 (.245)	.740 (.007)	.144 (.0007)
	8	54.1 (.295)	.688 (.006)	.142 (.0009)
	6	52.6 (.301)	.670 (.007)	.141 (.0012)
	4	48.8 (.373)	.599 (.009)	.139 (.0013)







Figures 1a–1c. Changes in genetic diversity parameters with changes in the number of mother trees sampled in large and small populations of *Pinus tecunumanii.* (a) Number of alleles (*TA*); (b) percent polymorphic loci (*P*); (c) genetic diversity (H_e). All values based on a mean of 50 samples.

At a sample size of six trees more alleles with frequencies of 5% or greater were lost. In the large stand, San Jeronimo, two alleles with a frequency of 5% or greater were lost at a sample size of six trees. One of these alleles was lost once, the other twice, in 50 runs. The results suggest that for conservation purposes more trees need to be sampled in the smaller fragmented stands than in the larger, more intact, populations to ensure the capture of alleles at frequencies of 5% or greater.

DISCUSSION

The results from this study apply to isozyme variation and its sampling. Other marker methods would show different patterns of genetic variation and presumably produce different results. Regardless of the marker system chosen, financial restrictions generally limit the size of *ex situ* conservation seed collections to ~25 trees per population and ~30 populations. Given the financial restraints, could conservation sampling be made more effective?

The provenances examined in this study all come

from an altitude above 1500 m and have unique RAPD markers and subtle morphologic differences that distinguish them from sources that occur below 1500 m elevation in Belize, Honduras and Nicaragua (FURMAN *et al.* 1996, DVORAK *et al.* 1989). For several years, foresters have referred to these two groups as high and low elevation populations of *Pinus tecunumanii* and have, in some cases, kept breeding orchards separate (DVORAK *et al.* 1989). Within population genetic diversity levels for San Jeronimo, Anshigua and El Ingenio found in this study were similar to those obtained by MATHESON *et al.* (1989) in an allozyme study of low elevation populations of *P. tecunumanii* from Mt. Pine Ridge, Belize and San Rafael and Yucul, Nicaragua ($P = 71.4$, $AP = 2.20$, $H_e = 0.165$). Genetic diversity among the low elevation populations appears to be slightly higher than among high elevation populations with (G_{ST}) values of 0.023 to 0.014, respectively. When the genetic diversity values of the high elevation populations used in this study are compared to means for pine species and for woody plants in general, *P. tecunumanii* maintains close to average levels of genetic diversity (Table 4). If a larger portion of the geographic range of *P. tecunumanii* was represented in the analysis, it seems likely that the species would exhibit somewhat higher levels of overall genetic diversity compared to most other pine species.

One of the questions raised by the study deals with the efficacy of placing mild selection pressure on trees for important metric traits in natural stands > 20 ha in size. Number of alleles were about the same in the "random" and "select" populations at San Jeronimo, and alleles present in one group and absent in the other all had frequencies of less than 1.0%. Since CAMCORE has a dual objective of both gene conservation and tree breeding, we believe that the selection process employed has merit in large natural stands. First, the allelic patterns emerging from the San Jeronimo random and select populations represent neutral genes that are not probably related to metric performance. At best, the selection procedure can produce small improvement in highly heritable traits like wood density (DVORAK & WRIGHT 1994) and possibly height and stem straightness. At worst, it causes no harm to either gene conservation or tree breeding efforts in the CAMCORE approach because hundreds of trees are being sampled across the entire species' range to ensure that genes for broad adaptability have been included in the sample.

Gene conservation efforts in the small degraded stands of El Ingenio and Anshigua did not add any alleles of frequencies greater than 0.4% to the gene pool sampled at San Jeronimo. Furthermore, alleles at frequencies of 1% or less in the small populations were sometimes found at higher frequencies in the large population. From a gene conservation standpoint, seed collections could have occurred in either type of population and would have captured the majority of the allozyme diversity. However, from a productivity standpoint, the San Jeronimo population is one of the fastest growing sources of high elevation *P. tecunumanii* across almost all sites in the tropics and subtropics (HODGE & DVORAK 1999) and should receive conservation priority. Unfortunately, the *P. tecunumanii* forests at San Jeronimo have been greatly reduced since the early 1980s and only about 30% of the original stands remain. No growth information is yet available for El Ingenio and Anshigua. Trees were being cut by local farmers in both populations at the time of seed collections.

Should seed collections be continued in small, degraded stands like El Ingenio and Anshigua in the future if their contribution to the *ex situ* gene pool is minimal? Until we better know what the allelic patterns represent in terms of adaptability and productivity, the answer is yes. One of the advantages of the cooperative approach to field testing is that adaptability and productivity information is now available for many of the Mexican and Central American pines.

Based on our results, a sample of size 10 and 20 trees would be adequate to capture most of the genetic diversity in large and small populations of *P. tecunumanii*, respectively. Estimates of sample size numbers were based on an assessment of 12 progeny per mother tree. In practice, as many as 300 to 500 offspring per family are represented in CAMCORE genetic tests and conservation banks. Thus, the actual sample size needed in natural stands to capture alleles at frequencies 5% or greater is presumably smaller than 10 trees.

The success of *ex situ* conservation can not be assessed by the number of trees sampled in natural stands but by the percentage of progeny that survive in field tests and conservation banks. Taken across all sites, more than 1500 half-sib families from more than 30 provenances may be included in a CAMCORE genetic testing program (DVORAK *et al.* 1996). The number of trees to sample in natural stands presented here are only guidelines but do suggest that the CAMCORE sampling approach is effective. Results from electrophoresis studies such as these must be used in conjunction with provenance field trials to choose provenances to target for *ex situ* conservation.

ACKNOWLEDGMENTS

The authors would like to thank CAMCORE Field Technician

Elmer Gutiérrez for his assistance in the seed collections in Guatemala and the support received from the National Seed Bank, BANSEFOR. In addition, we would like to thank Melinda Burke for the technical help in the laboratory.

REFERENCES

BERG, E. E. & HAMRICK, J. L. 1997: Quantification of genetic diversity at allozyme loci. *Can. Journ. For. Res.* **27**:415 –424.

DVORAK, W. S., BALOCCHI, C. E. & RAYMOND, R. H. 1989: Performance and stability of provenances and families of *P. tecunumanii* in the tropics and subtropics. *In:* Breeding Tropical Trees. Proc. IUFRO Conference, Pattaya, Thailand, Nov. 1988, (Gibson *et al.* eds.) Oxford Forestry Institute, Oxford, UK and Winrock International, Arlington, Va. pp. 187–196.

DVORAK, W. S., DONAHUE, J. K. & HODGE, G. R.. 1996: Fifteen years of *ex situ* gene conservation of Mexican and Central American forest species by the CAMCORE Cooperative. Forest Genetics Resources, FAO, Rome. **24**:15–21.

DVORAK W. S. & RAYMOND, R. H. 1991. The taxonomic status of closely related closed-cone pines in Mexico and Central America. *New Forests* **4**:291–09.

DVORAK, W. S. & WRIGHT, J. A. 1994: Off-spring-parent correlations for wood density in *P. tecunumanii* between native and exotic environments. *Can. Journ For. Res.* **24**: 1593–97

FURMAN, B. J., DVORAK, W. S., SEDEROFF, R. R. & O'MALLEY, D. M. 1996: Molecular markers as a diagnostic tool to identify species, hybrids and introgression; a study of the Central American and Mexican pines. Proc. QFRI – IUFRO Conf., Caloundra, Queensland, Australia (eds. Dieters *et al.*), 27 Oct–1 Nov. pp. 485–491.

HAMRICK, J. L., GODT, M. J. W. & SHERMAN BROYLES, S. L. 1992: Factors influencing levels of genetic diversity in woody plant species. *New Forests* **6**:95–124.

HODGE, G. R. & DVORAK, W. S. 1999: The genetics of *P. tecunumanii* (in preparation)

MATHESON, A. C., BELL, J. C. & BARNES, R. D. 1989: Breeding systems and genetic structure in some Central American pine populations. *Silvae Genetica* **38**(3–4): 107–113.

NAMKOONG, G., BARNES, R. D. & BURLEY, J. 1980: A philosophy of breeding strategy for tropical forest trees. Tropical Forestry Papers No. 16, Oxford Forestry Institute, University of Oxford, UK. 67 p.

NEI, M. 1975: Molecular Genetics and Evolution. North Holland. Amsterdam.

PARKER, K. C., HAMRICK, J. L., PARKER, A. J. & STACY, E. A.. 1997: Allozyme diversity in *Pinus virginiana* (Pinaceae): intraspecific and interspecific comparisons. *Am. J. Bot.* **84**:1372–1382

PERRY, J. P. Jr. 1991. The Pines of Mexico and Central America. Timber Press Inc. 231 p.

SQUILLACE, A. E. & PERRY, JR., J. P. 1992: Classification of *Pinus patula, P. tecunumanii, P. oocarpa, P. caribaea* var. *hondurensis* and related taxonomic entities. USDA Forest Service, S. E. Forest Expt. Station. Research Paper SE–285. 23 p.