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Pinus oocarpa Isoenzymatic Variation Along an Altitudinal Gradient in Michoacán, México.

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Abstract

Due to changes in land use, *Pinus oocarpa* Schiede stands are quickly disappearing in México, in particular in the region of Uruapan, in the western state of Michoacán. In order to understand the genetic variation along altitudinal gradients and to generate guidelines for the conservation of forest genetic resources, we investigated isoenzymatic genetic variation among populations of *P. oocarpa* Schiede along an altitudinal gradient. Open-pollinated seeds from individual trees were collected from five natural populations along an altitudinal transect from 1500 m to 1100 m, one population every 100 m of altitude, near of Uruapan city, in Michoacán. We found polymorphism in eleven of twelve examined loci. The average observed heterozygosity value (H_o = 0.1147) was above the average expected heterozygosity value (H_e = 0.1020), but it did not deviate significantly from Hardy Weinberg equilibrium. Genetic differentiation among populations (over loci F_{ST} = 0.0011) was not significantly different from zero ($p > 0.05$). Comparisons for allele frequencies indicated no significant difference for all the tested pairs of populations. Average genetic distance was very low: 0.0054, and genetic flow among populations very high (N_m = 227). Results suggest a small excess of heterozygotes within populations, and a lack of genetic differentiation among the five populations, which can be considered a single panmitic unit. Considering the alarming deforestation rate in the region of Uruapan, Michoacán, we suggest the selection of at least one stand for conversion to a gene resource management unit. We suggest to select the population at 1200 m of altitude, which has the highest number of polymorphic loci (9 of 12) and the highest average number of alleles per loci (1.92).

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Introduction

Pinus oocarpa Schiede has a very wide distribution, on the low coastal slopes of Sierra Madre Occidental and Sierra Madre Oriental and on the southern slopes of the Neovolcanic Axis in México; it ranges south to Nicaragua, in Central America (PERRY, 1991). This species is economically important in México, where it is used for sawtimber and fuel-wood (ZAMORA-SERRANO, 1981); in the western state of Michoacán, it is the most important species for resin production (COFOM, 2001). Commercial plantations of *P. oocarpa* are common in other parts of the world (GREAVES, 1982; DVORAK *et al.,* 2000; Forestry Compendium, 2003). Although *P. oocarpa* is not endangered as a species, locally adapted populations of important value as genetic resources are at present severely decimated by changes in land use, from forests to grasslands and agriculture (FARJON and STYLES, 1997; DVORAK *et al.,* 2000). In the region of Uruapan, Michoacán, *P. oocarpa* stands are converted to avocado orchards, a highly profitable product for exportation to the U.S.A. market (GUERRERO, 2002). Approximately a total of 30,000 to 40,000 ha of temperate forest and tropical-dry forest are deforested yearly in Michoacán alone (COFOM 2001).

Before it is too late the Mexican government needs to establish a program of forest genetic resource conservation. Such a program will require the protection of a number of stands representative of the genetic resources. To design the program and decide the placement, number and size of forest genetic resource management units (LEDIG, 1988; MILLAR and LIBBY, 1991), we need to know the pattern of genetic variation among and within populations.

Unfortunately, there are few studies on genetic variation among *P. oocarpa* populations on traits neutral or nearly neutral to selection. Some studies of isoenzymes (MILLAR *et al.,* 1988; MATHESON *et al.,* 1989; RAMÍREZ-HERRERA *et al.,* 1997b) and of DNA fragments (DIAZ *et al.,* 2001) have been published, and there are a few reports of isoenzyme variation of pine populations growing along altitudinal gradients (ETTL and PETERSON, 2001; MACDONALD *et al.,* 2001).

We studied the isoenzymatic variation among five natural populations of *P. oocarpa*, along an altitudinal gradient on the southern slope of the Neovolcanic Axis in Michoacán, México. The number of trees examined per population is small. Thus, results of this study should be considered only as indicative of possible data trends that might be found using larger sample sizes. However, considering the lack of information about genetic structure of Mexican pine populations, we believe that this report will help to understand that in some populations heterozygosity can be very low.

Methodology

Open-pollinated seed from randomly selected trees from five natural *P. oocarpa* populations were collected on an altitudinal transect along a southern slope of the Neovolcanic Axis, in Michoacán. The populations were located approximately every 100 m of altitude, from 1500 m (population "1"), near Uruapan city (19°21'57" N, 102°06'43" W) to 1100 m (population "5"), near the town of Charapendo, Municipality of Gabriel Zamora, Michoacán (19°16'33" N, 102°06'39" W). The number of trees collected from populations 1 through 5, was: 8, 13, 10, 10 and 8, respectively.

Six megagametophytes from germinated seeds from each tree were assessed for twelve loci (ACP-1, ACP-2, GDH, G6P, MDH-1, MDH-2, MDH-3, PGM, PGI-1, PGI-2, 6PG-1 and 6PG-2) in horizontal starch (12%) gels, following the methodology of YAMADA and GURIES (1989). Loci of the same enzyme system was labeled as locus 1 for the fastest migrating zone and then 2 and 3 for the slower zones. Alleles were scored and called by its relative mobility compared to *Pinus resinosa* megagametophytes which were used as the reference. Due to poor resolution of samples placed on the edges of some gels, actual number of scored trees per population per loci averaged 8.8 individuals.

We estimated the number of polymorphic loci, the average number of alleles per loci, allele frequencies and observed and expected heterozygosity. We tested departure from Hardy-Weinberg Equilibrium pooling alleles by the most common alleles, testing by locus using the exact test of HALDANE (1954) and over loci by Chi square test. We estimated Wright's F statistics by the WEIR and COCKERHAM's (1984) method, standard deviations jackknifing over loci and 95% confidence intervals by bootstrapping over loci with 10,000 iterations. We also estimated genetic flow (N_m) among populations ($N_m = ((1/F_{st}) - 1) / 4$), genetic distances in the sense of NEI (1972), a cluster analysis of genetic distances using the Unweighted Pair of Group Means Analysis (UPGMA) (SWOFFORD and OLSEN, 1990), and pairwise comparisons of allele frequencies over loci for all possible pairs of populations using Fisher's combined probability test (FISHER, 1954; MANLY, 1991; SOKAL and ROHLF, 1995). Genetic parameter estimations were conducted using the Tools for Population Genetics Analysis (TFPGA) software (MILLER, 2000).

Results

Considering results over populations, eleven of the twelve analyzed loci (91.2%) were polymorphic and a single locus (GDH) was monomorphic. However, using the 95% criterion, only three loci (25%) could be considered polymorphic (ACP-1, G6P and 6PG-1), whereas eight loci had the most-frequent-allele at frequencies above 95% (ACP-2, MDH-1, MDH-2, MDH-3, PGM, PGI-1, PGI-2, and 6PG-2). Over loci average number of alleles per locus was 2.4.

Considering populations individually, population 4 (1200 m of altitude, 19°17'22" N, 102°05'38" W) had the highest number of polymorphic loci (9 loci, 75%), and population 1 (1500 m of altitude) the lowest number of polymorphic loci (3 loci, 25%). Population 4 also had the highest average number of alleles per loci (1.92), whereas populations 1 and 3 (1300 m of altitude, 19°18'59" N, 102°05'20" W) both had the lowest average number of alleles per loci (1.33).

Observed heterozygosity over loci and over populations (H_o = 0.1147) was above expected heterozygosity (H_e = 0.1020), and suggests an excess of heterozygotes. However, testing for departure from Hardy-Weinberg equilibrium over populations by locus showed no significant difference between heterozygosity observed and heterozygosity expected for all twelve loci individually and over loci; this suggests that all the studied loci are in Hardy-Weinberg equilibrium.

Genetic differentiation among populations was not significantly different from zero (over loci F_{ST} = 0.0011, confidence interval from 0.0390 to –0.0147). Inbreeding coefficient over loci was a negative value and significantly different from zero (F_{IS} = –0.1139, confidence interval from –0.0389 to –0.1503); this supports the finding that there is an excess of heterozygotes within populations. Estimation of gene flow (N_m) based on estimated F_{ST} was very high: N_m = 227 migrants per generation.

Genetic distances among populations were very low, averaging 0.0054. None of the pairwise comparisons between popula-

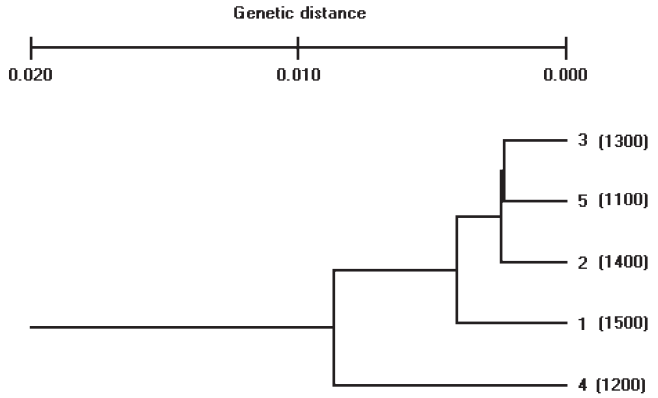

Fig. 1. – UPGMA phenogram based on genetic distances in the sense of NEI (1972). Population code number is indicated, with altitude above sea level in m in parenthesis.

tions for allele frequencies over loci were significant. Organization of the nodes of the UPGMA phenogram indicates that there is no particular arrangement of the populations following the altitudinal transect (*Figure 1*). In other words, the grouping of populations does not correspond to the populations´ altitude.

Results from Wright's F statistics, estimated genetic distances and pairwise comparisons among populations all are consistent with an apparent lack of genetic differentiation among populations.

Discussion

The average expected heterozygosity value seems to be relatively low (H_e = 0.1020), well below of what has been found previously (on isoenzyme loci) for *P. oocarpa*: H_e = 0.27 by MILLAR *et al.* (1988) and H_e = 0.18 by RAMÍREZ-HERRERA *et al.* (1997b). In comparison to other Mexican pines, our results are close to the lower reported values: H_e = 0.19 for *P. patula* (RAMÍREZ-HERRERA *et al.,* 1997a), H_e = 0.17 for *P. greggii* (RAMÍREZ-HERRERA et al., 1997a), *P. pringlei* (RAMÍREZ-HERRERA *et al.,* 1997b) and *P. pinceana* (LEDIG *et al.,* 2001), H_e = 0.12 for *P. ayacahuite* (HERNÁNDEZ, 1990), H_e = 0.11 for *P. maximartinezii* (LEDIG *et al.,* 1999), and H_e = 0.10 for *P. engelmannii* (BERMEJO-VELÁZQUEZ, 1993).

The low heterozygosity values that we found might be explained by one or a combination of the following reasons: a) We sampled a very small portion of the within-specie genetic variation, including only five populations distributed over an area of approximately 40 km^2 (with geographical distances between populations from 3 km to 10 km), whereas other studies on the same species sampled a much larger portion of the within-species genetic variation; MILLAR *et al.* (1988) included populations from two countries: México and El Salvador, and RAMÍREZ-HERRERA *et al.* (1997b) populations from three Mexican states: Estado de México, Michoacán and Veracruz (personal communication with RAMÍREZ-HERRERA and VARGAS-HERNÁNDEZ). b) We sampled too few trees per population, which reduced the likelihood of detecting rare alleles that might have increased the heterozygosity, or c) Our populations might have lower densities than the populations studied by MILLAR *et al.* (1988) and by RAMÍREZ-HERRERA *et al.* (1997b). Low population densities might be the long term result of grazing, which dramatically prevents regeneration. In the five studied populations we only observed a single 40 cm tall natural regeneration seedling. Similarly, deforestation by illegal logging, firewood extraction and change of use of land to agriculture might have caused loss of individuals carrying rare alleles and fixation or nearly fixation of the most common alleles.

The finding of observed heterozygosity above the expected heterozygosity, although not significatively different, is similar to what was found in *P. greggi* populations (RAMÍREZ-HERRERA *et al.,* 1997a). Probably homozygotes are at a selective disadvantage relative to heterozygotes (CHELIAK *et al.,* 1985). There is evidence of a linear correlation between the amount of heterozygosity and the percent of progeny survival (BUSH and SMOUSE, 1991). Also, in natural stands heterozygosity increases with the age of cohorts (FARRIS and MITTON, 1984; CHELIAK *et al.,* 1985; YAZDANI *et al.,* 1985; PLESSAS and STRAUSS, 1986; BALLAL *et al.,* 1994).

There is no indication of significant genetic differentiation among populations; consequently we conclude that there is no genetic differentiation along the altitudinal gradient, all the genetic variation is within populations. It would appear that the five studied populations can be considered a single panmitic unit.

The lack of altitudinal differentiation among populations is similar to results of high elevation and low elevation unmanaged *P. contorta* stands in Alberta, where there were no significant genetic differences between altitudes (MACDONALD *et al.,* 2001). Also for *Abies lasiocarpa* in the state of Washington, USA, there were no significant genetic differences among high, medium and low elevation stands on Blue Mountain (ETTL and PETERSON, 2001). It is possible that the active gene flow among *P. oocarpa* populations (estimated N_m = 227) overwhelms any forces like genetic drift promoting population differentiation (CHUNG and KANG, 1996). Also considering the overall lack of association between isoenzymatic variation and adaptive trait variation (HAMRICK and GODT, 1990), it is likely that the selective forces due to altitude (temperature, precipitation and others) are not strong enough to significantly differentiate the studied populations in terms of enzymatic loci.

The present trend of accelerate deforestation in Michoacán necessitates the conservation of the remaining forest genetic resources. One recommended strategy for *in situ* conservation in Michoacán, would be to manage some natural stands of *P. oocarpa* for conservation of the present natural genetic structure – so called "gene resource management units" (MILLAR and LIBBY, 1991).

In order to conserve at least the genetic diversity of neutral or nearly neutral to selection traits, we suggest two alternative strategies for preliminarily stand selection for conversion to gene resource management units: (a) Considering that there appears to be no significant differences in genetic structure among the five populations, any of the studied stands could be converted to gene resource management units; selecting one particular stands could be based on practical considerations, such as stand size, stand density, accessibility and land tenure status, or (b) To select at least population 4 (1200 m of altitude) for conversion to a gene resource management unit, because it is the population with the largest number of polymorphic loci (nine from a total of twelve), and the highest average number of alleles per locus (1.92). These values might indicate that population 4 is the stand with the largest genetic diversity among the five studied populations, even if statistical tests indicate lack of significant difference with the other stands.

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