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Conservation and Restoration of Pine Forest Genetic Resources in México

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Abstract

Deforestation rates in México are about 670,000 ha/year. This threatens the richness of forest genetic resources in México, causing the disappearance of locally adapted populations and rare and endangered pine species. México is one of the six megadiverse countries in the world, with half of the world's *Pinus* species. *Pinus* is one of the most economically and ecologically important forest genera in México. We suggest that delineation of seed zones and the establishment of a network of Forest Genetic Resource Conservation Units (FGRCUs), linked with forest management and ecological restoration programs will protect this valuable resource. We estimate that FGRCUs should include 25 to 50 ha each, with at least one FGRCU for each priority species in each seed zone. We highlight the need for studies of adaptive genetic variation among pine populations and for new methodologies and techniques to suit ecological restoration under Mexican forest conditions. We briefly describe ongoing research on these topics on forests owned by a well-organized indigenous community in Nuevo San Juan Parangaricutiro, Michoacán, western México.

Key words: *Pinus*, genetic resources, adaptive genetic variation, conservation, biodiversity, reforestation, ecological restoration, seed zones, México.

Introduction

Despite their importance in terms of biodiversity, ecology, and economy, Mexican forests are threatened by an alarming rate of deforestation. In the mid 1980's, loss of forest cover amounted to an estimated 668,000 hectares per year, which is equivalent to the removal of 1.29% of the forest cover (MASERA *et al.*, 1997). Most deforestation occurs in tropical forests, where 501,000 hectares are lost each year (1.90% in deciduous and 2.00% in evergreen forest; MASERA *et al.*, 1995; MASERA *et al.*, 1997). Today losses in temperate forests are also high and amount to 167,000 hectares per year (0.67% per year for coniferous forests and 0.64% for broadleaf forests; MASERA *et al.*, 1997). For temperate forests, most land conversion has been due to deliberate forest fires, clearing to open agricultural lands and pastures, and for fuel-wood harvest (CAIRNS *et al.*, 1995; MASERA *et al.*, 1997).

Biodiversity in México is great (MITTERMEIER, 1988), with an estimate of 22,800 vascular plant species (RZEDOWSKI, 1993). In the mid- 1980s, 51.1 million hectares (26% of the land area in México) were covered by mostly undisturbed forests (tropical humid, tropical dry and temperate forests). Temperate coniferous forests represent 16.9 million hectares (33% of all forests) and are mostly dominated by pine species (MASERA *et al.*, 1997).

México contains the greatest diversity of pine and oak species in the world, with 49 pine taxa representing about half of all known pine species (STYLES, 1993) and between 135 to 150 oak species (NIXON, 1993). Pine species in México cover a very wide elevational ranges, from near sea level (*Pinus caribaea* var. *hondurensis* in the southwestern state of Quintana Roo in the Yucatan Peninsula; DVORAK *et al.*, 2000), to timberline on high mountains (*Pinus hartwegii* occurring at 4300 meters above sea level on the Mexican Volcanic Axis; FARJON and STYLES, 1997). Mexican pines also occur from semitropical climates (*Pinus oocarpa*) to vast semiarid regions (*Pinus cembroides*). Most of the pine species are distributed in temperate forests, where there are several species with major economic importance, like *Pinus engelmannii*, *P. montezumae*, *P. patula* and *P. pseudostrobus*. In addition, México contains a diverse group of endemic, very rare, and highly endangered pine species such as *Pinus culminicola*, *P. maximartinezii*, *P. rzedowskii*, and *P. pinceana*, (PERRY, 1991; STYLES, 1993; FARJON and STYLES, 1997).

Pinus is the forest tree genus with the greatest economic importance in México. Commercial harvesting for timber and cellulose (10.1 million m³ per year; MASERA *et al.*, 1997) is mostly based on species belonging to this genus; 85% of commercial wood comes from pines (MASERA *et al.*, 1992). Pines also account for a large and unknown proportion of the mostly non-commercial harvesting of wood for fuel, which is a fundamental activity in rural México, representing a wood harvest of 37.2 million m³/year. Approximately 25 million Mexicans use fuelwood for domestic use (e.g. cooking) and/or for small family industries (e.g. pottery; MASERA *et al.*, 1997).

Deforestation causes losses in biodiversity and genetic diversity within species that can have serious consequences. For example: Endemic and very rare Mexican pine species, such as *Pinus culminicola*, *P. maximartinezii*, *P. rzedowski* and *P. pinceana*, exist in a few unique natural populations; losing one or a few populations threatens the whole species with extinction (PERRY, 1991). Within species, certain endangered provenances have economically valuable characteristics, like *P. radiata* from Guadalupe Island, Baja California, México, which is unique for its resistance to western gall rust (*Endocronartium hatknessii*) and to red-band needle blight (*Scirrhia pini*) (COBB and LIBBY, 1968; OLD *et al.*, 1986; LEDIG *et al.*, 1998). In addition, severe forest fragmentation is causing elimination of locally adapted populations (LEDIG, 1988b; LEDIG, 1992a), leading to losses of within-species genetic variation, which can reduce the chances of pine species to adapt to future changes in the environment such as global warming (LEDIG and KITZMILLER, 1992; REHFELDT, 2000). Disgenic selection by cutting of good-quality and superior tree phenotypes has occurred in pine stands in México for many years, either by illegal cuttings and change of use of the land, or by the application of an old – now discontinued – application of a Mexican silvicultural method that prescribed selective cuttings (JASSO, 1970; JASSO-MATA *et al.*, 1978; EGUILUZ-PIEDRA, 1984).

In summary, conservation of genetic diversity of natural populations of Mexican pines should be given priority for three main reasons: a) *Pinus* is the most important genus – ecologi-

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cally and economically – in the coniferous forests b) México is a diversity center for the genus; c) some pine species and locally-adapted populations that occur in México are endangered by land use change. Therefore, forest conservation practices are urgently needed before current rates of deforestation cause further forest fragmentation and depletion of forest genetic resources.

We will discuss the importance of a program of conservation of among and within pine species genetic diversity, the research needed to establish such a program, delineation of seed zones, and development of new approaches for ecological restoration of temperate forests in México. Although we elaborate on the case of Mexico, we believe that the described situations and suggestions apply to other countries such as Brazil, Colombia, and Indonesia which, like México, are countries with large forest genetic resources, high deforestation rates, megabiodiversity (MITTERMEIER, 1988) and the need to conserve and to restore valuable forest genetic resources.

Forest Genetic Resource Conservation Units

One strategy to protect at least part of the richness of genetic diversity among and within Mexican pine species *in situ* is to establish a network of Forest Genetic Resource Conservation Units (FGRCUs), also called gene resource management units (LEDIG, 1988a; MILLAR and LIBBY, 1991). A FGRCU is a representative natural stand of any species with a management priority to maintain genetic diversity as well as to allow natural evolutionary forces to mold the population's genetic structure (LEDIG, 1988a; MILLAR and LIBBY, 1991; LEDIG, 1992b).

Conservation of the original population genetic structure can be achieved in a relatively simple way by: a) regeneration within the FGRCU obtained either by natural regeneration or by reforestation using the local seed source (LEDIG, 1988a), and b) when needed, by protecting the population from incoming genetically alien pollen. This can be done, for example, by planting or designating trees in the perimeter of the FGRCU as a belt or buffer to attenuate pollen from neighboring plantations made with distant provenances (MILLAR and LIBBY, 1991). Timber harvest can be allowed within the units (DANIDA, 1984), as long as it would not change the original genetic structure and would not threaten the main objective of conservation of forest genetic resources (LEDIG, 1988a; MILLAR and LIBBY, 1991).

The timber harvesting within a FGRCU is a very important management feature, because this makes the FGRCU a viable option for *in situ* conservation in the Mexican context, where there is a chronic lack of government funding for biological conservation of forest resources (CONRAD and SALAS, 1993). FGRCUs could be partially self-funded by moderate timber harvesting and by using them as seed stands to support reforestation programs. Therefore, FGRCUs can be part of regular commercial forest management plans. Initially, subsidies may encourage stand owners to create FGRCUs by compensating for short-term losses of reduced timber extraction due to the management restrictions imposed by making conservation of genetic resources a primary goal.

Size and placement of Forest Genetic Resources Conservation Units

We roughly estimate that a FGRCU should have an average size of approximately 25 ha (without buffer zone) to 50 ha (with buffer zone), in order to maintain an average value of heterozygosity (which is an indicator of genetic diversity). Estimation was made as follows: (1) Effective population sizes required to maintain a given value of heterozygosity depend on the muta-

Table 1. – Estimations of mean expected heterozygosity (H_e) of several Mexican pines.

Species	H_e	Source
<i>P. engelmannii</i>	0.10	Bermejo-Velásquez 1993
<i>P. ayacahuíte</i>	0.12	Hernández 1990
<i>P. maximartinezii</i>	0.12	Ledig <i>et al.</i> 1999
<i>P. tecunumanii</i>	0.16	Ramírez-Herrera <i>et al.</i> 1997b
<i>P. greggii</i>	0.17	Ramírez-Herrera <i>et al.</i> 1997a
<i>P. pinceana</i>	0.17	Ledig <i>et al.</i> 2001
<i>P. pringlei</i>	0.17	Ramírez-Herrera <i>et al.</i> 1997b
<i>P. oocarpa</i>	0.18	Ramírez-Herrera <i>et al.</i> 1997b
<i>P. patula</i>	0.19	Ramírez-Herrera <i>et al.</i> 1997b
<i>P. rzedowskii</i>	0.22	Delgado <i>et al.</i> 1999
Average	0.16	

tion rate and on the original expected heterozygosity (MILLAR and LIBBY, 1991). (2) For our estimation purposes, we assumed an average mutation rate of $\mu = 10^{-5}$ (MILLAR and LIBBY, 1991). (3) We estimated a rough average of expected heterozygosity of $H_e = 0.16$, based on several studies on pine populations in Mexico (Table 1). (4) We compared such parameters ($H_e = 0.16$ and $\mu = 10^{-5}$) with estimates of effective population sizes required to maintain heterozygosity on populations of *Pinus ponderosa* and *Pseudotsuga mensiezii* made by MILLAR and LIBBY (1991), who based their estimations on formulae developed by CROW and KIMURA (1972). We found that approximately 4,660 trees of reproductive age are needed for maintaining a heterozygosity level of 0.16 in a pine population. (5) Considering that a well-preserved and fully stocked mature stand of Mexican pines might have around 200 adult trees per ha, the minimum size of a FGRCU would be 23.3 ha, or roughly 25 ha. (6) Where there are risks of pollen contamination from neighboring plantations of distant provenances, a buffer zone (natural or planted) would be needed, with a minimum width of 100 m (DANIDA, 1984; AYLING and MUBITA, 1988). This would increase the minimum size to approximately 50 ha per FGRCU.

The number and placement of FGRCUs should aim to cover the natural range of genetic variation within each species. The best strategy is to choose the number and placement of FGRCUs based on knowledge of the distribution of genetic variation within and among populations of each species (MILLAR and LIBBY, 1991). Although Mexican pines have been tested outside México (CAMCORE 2000), very little is known about the genetics of Mexican pine species in their native habitats (SAENZ-ROMERO *et al.*, 1994), due to the lack of sufficient provenance and common garden tests.

The Mexican government and forest organizations, with the help of Mexican Universities, should develop a provisional system of forest seed zones (NIENSTAEDT *et al.*, 1990; SAENZ-ROMERO and CLAUSEN, 1991; CONKLE, 1997), and then place at least one FGRCU for each priority pine species in each seed zone (SAENZ-ROMERO, 1990). Forest seed zones are regions or subregions that include forest populations with genotypic and phenotypic similarities growing on similar ecological conditions (BARNER and WILLAN, 1983). Seed zones typically follow clinal or ecotypic patterns of ecological variation and are used as guidelines for seed collection and seed movement to prevent maladaptation of seedlings to the planting sites (NIENSTAEDT, 1979; BARNER and WILLAN, 1983; SAENZ-ROMERO and CLAUSEN, 1991). The zones might differ from one species to the next.

Seed zones should ideally be based on results of provenance tests, but provisionally they can be based on ecological data, assuming that similarity in ecological conditions implies similarity in genetic constitution (BARNER and WILLAN, 1983). Thus, initially seed zone delineation can be done in México based on climatic, topographic, species distribution, and administrative data (NIENSTAEDT *et al.*, 1990; SAENZ-ROMERO and CLAUSEN, 1991). Alternatively, short-term common garden tests can be used to provisionally delineate seed zones (REHFELDT, 1983). Information from neutral or almost neutral to selection traits, such as isoenzymes and DNA fragments, could also be helpful to understand the genetic architecture within species (MILLAR and LIBBY, 1991).

The need of forest restoration rather than simply reforestation

Forest restoration of deforested areas and disturbed forests as well as sensible forest management of stands under commercial timber harvesting are needed to preserve biodiversity and within-species genetic diversity. Unlike other forest related activities, such as reforestation or commercial forest plantations, forest restoration should aim at recreating, whenever possible, the full spectrum of ecosystem attributes, structure, and functions of the original forest. If not possible, a reasonable goal is to maximize the number of native species and self-sustainability of the forest, particularly when the level of degradation or lack of resources prevents full recovery. In this context, FGRCUs could provide the appropriate seed to be used in forest ecological restoration programs to prevent the introduction of foreign provenances.

Reforestation programs in México

Government sponsored reforestation programs in México, in general, do not constitute forest restoration and do not foster native biodiversity. Although the National Reforestation Program (PRONARE by its abbreviation in Spanish), sponsored by the Mexican federal and state governments, is a large and laudable effort, it has several limitations. First, the total reforested area per year (approximately 200,000 ha) (SHEINBAUM and MASERA, 2000; CONAFOR, 2002) is less than a third of the total deforested area per year (668,000 ha; MASERA *et al.*, 1997), which means that the forest is still disappearing. Second, seedling survival according to official reports is poor, around 34% for the first year (SHEINBAUM and MASERA, 2000). Third, some reforested sites are already too eroded to support trees. Fourth, the choice of species and provenances is inadequate (NIENSTAEDT, 1994; SAENZ-ROMERO and MARTINEZ-PALACIOS, 2000; CONAFOR 2002).

Causes of low seedling survival are due to many cases: a) insufficient protection of the plantations against grazing, forest fires and competition with natural vegetation; b) late planting (planting operations frequently continue even when the rainy season is too advanced and seedlings will have insufficient time for establishment before the dry season) and; c) poor quality of the seedlings (NIENSTAEDT, 1994; SAENZ-ROMERO and MARTINEZ-PALACIOS, 2000; CONAFOR 2002).

Pine species are largely over-represented in the reforestation programs relative to the land surface appropriate for pine forest. For example, in the state of Michoacán (western México) 74% of total planted seedlings are pines, whereas pine and oak-pine forest represent only 30% of the forested land (COFOM 2001). Consequently, pine seedlings are sometimes planted on sites where, even without disturbance, pine species hardly would be able to establish successfully.

Data recording and planning processes needed to adequately match seed provenance and ecological characteristics of planta-

tion sites are almost non-existent, due to two main reasons: a) It is difficult to match seed provenances to the ecological conditions of the planting site. Available studies of seed provenance variation and adaptation are simply not enough. b) The important steps of reforestation programs *i.e.* seed collection and the actual planting are not adequately planned. Currently, the first step is to determine the total number of seedlings to be produced. Next, enough seed is collected. Frequently, where to plant is decided when seedlings are ready to leave the nursery, sometimes when the planting season has started already. We strongly stress that the first planning step should be to decide where to plant, then what (species and provenances) to plant, then where to collect seeds, then to produce the seedlings, to protect the sites to be planted and only then to plant the seedlings, followed by an appropriate maintenance of the site and evaluation of the results. Such planning should start perhaps three years in advance before the targeted planting date.

In practice, PRONARE has fostered reforestation without considering other management practices needed to attain restoration. Overlooked are: restoration of fire regimes (WILSON, 1994; ANGELSTAM, 1998; FRIES *et al.*, 1997), planting of understory species (WALTERS, 1997) and management of woody debris for wildlife habitat (PONGE, 1998).

It must be stressed that it would be better to increase the quality and complexity of the reforestation programs, even if this would downsize the area reforested.

Research priorities for improving the conservation and restoration of forest genetic resources in México

Two specific issues should be addressed to improve the conservation and restoration of forest genetic diversity in México. The first is to investigate the patterns of adaptive genetic variation among populations of Mexican pines. The second is to investigate methods and techniques necessary to attain ecological restoration of pine forests and pine-oak forests in Mexico, rather than the current simple approach of reforestation.

Studies of genetic variation among pine populations in México are key to deciding the number and placement of FGRCUs and to delineate seed zones. Seed zones would provide a reference needed to decide the appropriate movement of seed and seedlings, aiming to match seedling genotypes with ecological conditions of the reforested sites.

Currently, research is starting to address these issues. For example, the authors have a research project under way in Michoacán. The goal is to explore if significant adaptive genetic variation exists between populations of *Pinus oocarpa* and *P. pseudostrobus* along altitudinal gradients in the Neo Volcanic Axis, within the state Michoacán in western México. Common garden tests, provenance tests and progeny tests are being established on sites at contrasting altitudes, from seed collections made along the same altitudinal transects, following the methodology of REHFELDT (1988). Conifer populations genetically differentiate in response to selection intensities imposed by environmental variables, as has been shown for *Pinus contorta* (REHFELDT, 1988), *Pinus ponderosa*, (REHFELDT, 1991; REHFELDT, 1993), *Pseudotsuga menziessii* (REHFELDT, 1989) and other species in the Rocky Mountains, USA.

In our research in Michoacán, we are also estimating the isoenzymatic variation within and among *P. oocarpa* populations. This would give us an estimate of the gene flow among populations in the relatively narrow altitudinal range of 400 m for *P. oocarpa*.

The research is being conducted in the community forests and neighboring forests of a very well organized indigenous community of the "Purepecha" ethnic group at Nuevo San Juan

Parangaricutiro, Michoacán. The community has a certified sustainably managed forest and a large communally owned and operated timber and furniture industry (SANCHEZ-PEGO, 1995; JAFFEE, 1997).

A second ongoing research project is seeking new methods and techniques for ecological restoration on former forests disturbed by agriculture and volcanic ash deposition at Nuevo San Juan Parangaricutiro (LINDIG-CISNEROS *et al.*, 2002). We are testing the effect of including understory species (*Lupinus sp.* and *Eupatorium sp.*) in plantings with pines.

Conclusions

Biodiversity in México, particularly diversity within *Pinus* species, is threatened by an alarming rate of deforestation that will deplete genetic variation of forest resources and eventually cause local population or species extinctions. The establishment of Forest Genetic Resource Conservation Units (FGRCUs) is a necessary step to preserve the genetic variation among and within populations of the diverse, economically and ecologically important pines of México. The highest priority is to study the adaptive genetic variation among pine populations in order to decide the number and placement of such FGRCUs and to develop seed zones, which would provide much needed information for forest restoration. We estimate that FGRCUs should be between 25 and 50 ha each, depending on local conditions, and to that at least one FGRCU for each pine species would be needed in each seed zone. Current practices of government sponsored reforestation programs have several limitations and, therefore, a new approach of ecological restoration rather than reforestation is needed. To achieve this goal, research is needed to develop new methodologies and techniques suitable for the conditions in Mexico. Unless a sound policy that preserves what is left of natural forests is implemented, coupled with the creation of FGRCUs and forest ecological restoration programs, Mexican pine genetic diversity will be lost in the coming decades.

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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 tran-

sect 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 panmictic 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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