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POPULATION VARIATION IN THE ENDEMIC *PINUS CULMINICOLA* DETECTED BY RAPD

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ABSTRACT

Pinus culminicola, the dwarf pinyon, is an endangered species endemic to northeastern Mexico, where it grows at the highest altitude of any of the Cembroides group. In order to determine the degree of genetic isolation between populations of *P. culminicola* and the amount of gene flow between them, samples were obtained from Cerro El Potosi and Sierra La Viga, two localities within its restricted area of distribution in the Sierra Madre Oriental, and analyzed using random amplified polymorphic DNA (RAPD). The five primers tested for the analysis showed banding patterns with very high reproducibility and clear band resolution. These five primers produced a total of 72 distinct bands, 52 of which were polymorphic across the whole sample. The genetic diversity in the two populations was high with a percentage of polymorphism of 53.7% and degree of diversity measured by the Shannon index of 56%. The total variation found between the two populations was 5.98% (P = 0.0001). Most of the variation was found within populations (94.02%). Contrary to expectations, the level of genetic variation found in the two isolated populations was high but differentiation between them low. This suggests that even though this tree-line pine has a restricted and fragmented distribution through the Sierra Madre Oriental, gene flow between populations has been sufficient to prevent a dramatic loss of genetic variation and genetic drift.

Key words: *Pinus culminicola*, genetic variation, RAPD.

RESUMEN

Pinus culminicola, piñonero enano que crece en las partes más elevadas que cualquier pino del grupo Cembroides, es endémico del Noreste de México y está enlistado como especie amenazada. Con la intención de examinar los niveles y patrones de variación genética entre poblaciones de *P. culminicola* para determinar su grado de aislamiento genético y la ocurrencia de flujo genético entre poblaciones, se tomaron muestras en dos localidades de su área de distribución restringida en la Sierra Madre Oriental y se analizaron utilizando RAPDs.

Los cinco primers probados para el análisis de *P. culminicola* mostraron patrones de bandeo con un alto grado de

reproducibilidad y una resolución clara de las bandas. Estos primers arrojaron un total de 72 bandas distintas, de las cuales 52 fueron polimorfas a través de todas las muestras. La diversidad genética fue alta en las dos poblaciones con un porcentaje de polimorfismo de 53.7% y un grado de diversidad medido por el índice de Shannon de 56%. El total de la variación encontrada entre las dos poblaciones fue de 5.98% (P = 0.0001). La mayor variación fue dentro de poblaciones (94.02%). Contrario a lo esperado, los niveles de variación entre las poblaciones aisladas de *P. culminicola* fue baja. Esto sugiere que a pesar de que la especie presenta una distribución restringida y aislada en la Sierra Madre Oriental, el flujo genético entre las poblaciones ha sido suficiente para prevenir una pérdida dramática de variación genética y una deriva.

Palabras clave: *Pinus culminicola*, variación genética, RAPD.

INTRODUCTION

Molecular studies have revealed that conifers have high levels of genetic variation and relatively little genetic differentiation among populations (Ledig, 1998). The common explanation for the low genetic differentiation found in conifers species is the reproductive system: seed and pollen are basically wind dispersed and this allows a more efficient gene flow among distant populations (Delgado *et al.*, 1999). Levels of diversity in Mexican conifers are generally twice as those in species from northern temperate latitudes (Ledig *et al.*, 2000). The most obvious reason for higher levels of differentiation in Mexican species is that they are more highly fragmented than those in northern latitudes (Ledig *et al.*, 2000). However, this pattern can be affected by historical events. Many conifers of the northern latitudes expanded into their present range within the last 10 000 years, leaving little time for differentiation to occur. The ancestors of the present-day *Pinus banksiana* and *Pinus contorta*, for example, were in contact in the geologically recent past; the lack of differentiation within these species reflects recent gene flow (Ledig *et al.*, 2000).

The geographical range of a species is considered to be one of the best predictors of the level of genetic variation found in natural populations (Hamrick *et al.*, 1992). An example is provided by the genus *Pinus*. Pine species that are distributed as scattered isolated populations have more genetic diversity among populations, while more widespread and continuous distributed pines have less among-population diversity (Hamrick *et al.*, 1992, Ledig *et al.*, 2001, Molina-Freaner *et al.*, 2001).

Fragmentation into small, scattered populations is expected to lead to genetic isolation, loss of genetic diversity, differentiation of the populations and increased probability of extinction (Ledig *et al.*, 2001). Thirty five of 47 pine species reported to Mexico are endemic to this country (Perry *et al.*, 1998). Many of these species are represented by small, scattered populations and also are presumed threatened or endangered. Nine of these Mexican pine species are listed by the International Union for Conservation of Nature and Natural Resources (IUCN, 2001) as species of concern (Farjon and Page, 1999).

Pinus culminicola, the dwarf pinyon, grows at the highest altitude of any of the Cembroides group, in the Northeast of Mexico. It occurs at the top of the two highest mountains of the Sierra Madre Oriental, separated from each other by about 72 km (straight-line distance) and is listed as an endangered species. It grows in an altitudinal range of 3300-3 650 m. It is a shrub or small tree 1-5 m high, commonly multi-stemmed from the base, usually spreading with branches extending outward from the base from 3-4 m, forming dense vegetation ("matorral"). The soils are shallow, rocky, gravely limestone. Rainfall is high since rain, sleet and snow occur frequently throughout the year. Unfortunately at the very high, isolated locations where this taxon grows, temperatures and rainfall measurements are not available. At the summit of Cerro el Potosi, Nuevo León, one of the populations where this species grows, it occurs with *Pinus hartwegii*. In Sierra La Viga, Coahuila, *P. culminicola* has been found with *P. hartwegii* and also with *P. strobiformis* and in association with other conifer species (*Pseudotsuga menziesii* and *Abies vejarii*).

Pinus culminicola is an interesting pine because it is so different from the other pinyon pines species. The ecological conditions of moist, cool, high-altitude environments is in marked contrast to the semiarid conditions generally associated with the other pinyon pines species. *P. hartwegii* and *P. strobiformis*, the associated species, are well adapted to the high altitude conditions; however, they do not have "close relatives" that are adapted to warmer semiarid and arid conditions. The taxon *P. cembroides* grows around the base of Cerro el Potosi under hot and dry conditions. One question is how *P. culminicola* adapted to such different conditions; or alternatively whether it represents a relict of an ancient plant community.

Pinus culminicola has a very restricted range and during dry periods is very susceptible to fire. In 1975 more than 5 ha of a large community on the northern slope of Cerro el Potosi were completely destroyed by fire. In 1996 another big fire destroyed more than the 40% of the remaining community in Cerro el Potosi. *P. culminicola* is classified as "EN" (A3c + B2a,b(i-v)) on the IUCN Red List, and is considered endangered based principally on its low area of occupancy and estimated continuing decline of at least 50% within 10 years or three generations (IUCN, 2001).

The aim of this study is to use RAPD markers to examine the levels and patterns of genetic variation between two populations of *Pinus culminicola* to determine their degree of genetic isolation and if there is evidence that gene flow occurs between the two populations.

MATERIAL AND METHODS

Sample collection

Two populations were sampled from their restricted distribution in the Sierra Madre Oriental (Fig. 1, Table 1). Within each population, 30 individuals were randomly selected and 10 g of mature needle samples collected from each tree. These were dried and stored in plastic bags containing 10 g of silica gel.

DNA extraction

DNA was extracted from 0.5g of dried needles following the method of Doyle

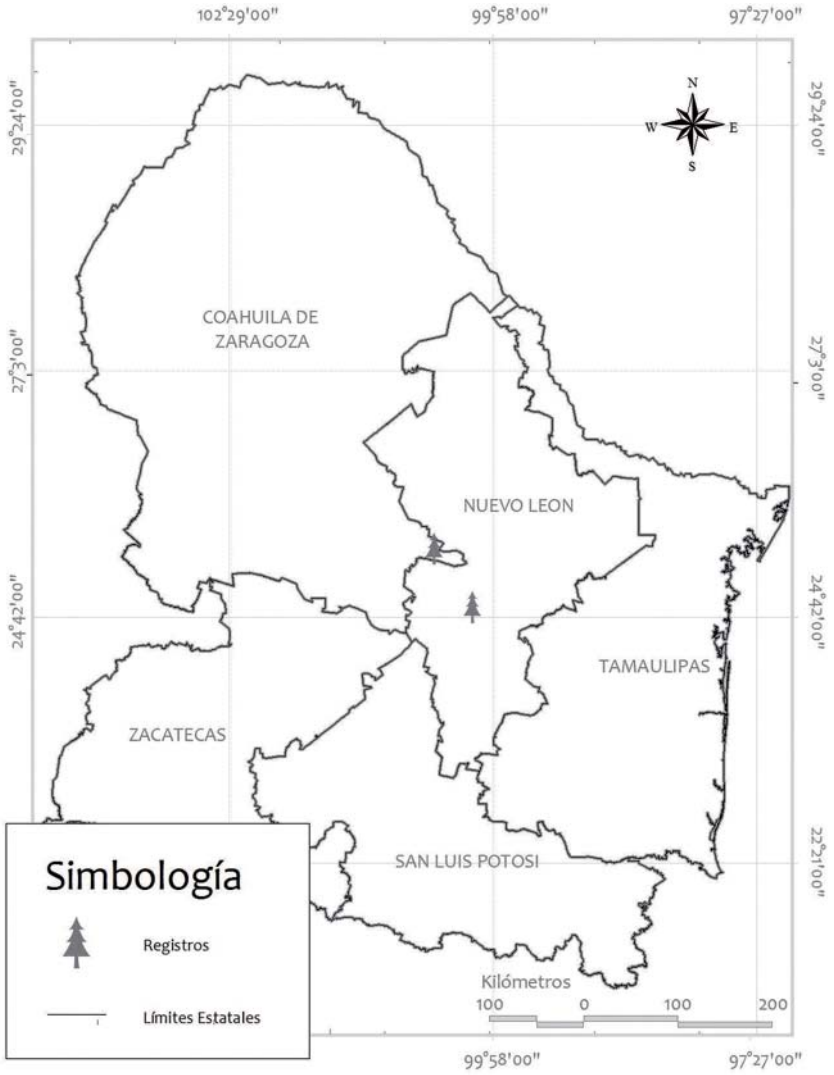

Fig. 1. Populations of *Pinus culminicola* sampled in the Northeast of Mexico.

Table 1. Sampled populations of *P. culminicola* in the Northeast of Mexico.

Species	Coordinates	Population number/Locality	Altitude
P. culminicola	25º 21' 25"	**1**. Sierra La Viga, Ramos	3 240 m
	100º 31' 59"	Arizpe, Coahuila	
P. culminicola	24º 47' 20"	**2**. Cerro El Potosí, Galeana,	3 450 m
	100º 11' 38"	Nuevo León*****	

and Doyle (1990) with the addition of an ammonium acetate wash to remove excess carbohydrates (Hollingsworth *et al.*, 1999). DNA extractions were further purified using a DNeasy plant mini kit (QIAGEN) with slight modifications to the published instructions. The quality and quantity of DNA was assessed by running samples alongside a HyperLadder 1 concentration marker (Bioline UK) on 1.0% agarose in TBE Buffer. DNA was visualized via ethidium bromide staining (0.1 mg/ml) under UV light and images captured using Genesnap 4.0 (Syngene UK). DNA samples were stored at -20°C.

RAPD analysis

Primer selection

From an initial screen of 54 10mer RAPD primers (Operon RAPD kits OPC, OPG, OPP technologies, Alameda), were used to screen a subset of samples. Five primers were chosen that gave clear and reproducible banding pattern (OPC-06, OPG-05,OPG-09,OPP-12 and OPP-14). All 60 samples were screened for genetic variation using these primers. RAPD products were separated alongside 1 Kb ladder on 1.6% agarose gels, stained with ethidium bromide (0.5 µg/ml) gel in TBE buffer and visualized using a Genesnap 4.0 (Syngene UK).

PCR conditions

25µl PCR reactions were prepared with the following reagents: 1X reaction buffer (16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20); 2.5 mM $MgCl_2$, 0.2 mM dNTP's; 0.5 µM primer 2% formamide, 1Unit BioTaq (Bioline), ~10 ng template DNA. Amplification was carried out using the following PCR profile: 2 min at 95ºC; 2 cycles of 30 sec at 95 ºC, 1 min at 37ºC, 2 min at 72ºC; 2 cycles of 30 sec at 95ºC, 1 min at 35ºC, 2 min at 72ºC; 41 cycles of 30 sec at 94ºC, 1 min at 35ºC, 2 min at 72ºC; and 5 min at 72ºC, on a MJ Research PTC 200 DNA Engine. Negative controls lacking template DNA were included in each PCR run.

PCR products were separated alongside 1Kb ladder on 1.6% agarose gels, stained with ethidium bromide (0.5 µg/ml) gel in

TBE buffer and visualized using a Genesnap 4.0 (Syngene UK).

Data analysis

RAPD bands were scored visually as either present (1) (all dominant homozygotes (AA) and heterozygotes (Aa)) or absent (0) (recessive homozygotes (aa)). A binary matrix of band presence and absence was constructed and then used to measure the genetic variability and structure of species and populations. Samples that failed to amplify for specific primers were scored as missing data.

Measures of genetic variation

The Shannon diversity index (Lewontin, 1972) was used to quantify levels of genetic variation within each population. Estimates of variation were calculated using the formula:

$$S = -\sum p_i \log_2 p_i$$

where p_i is the frequency of presence or absence of each RAPD band (RAPD phenotypes). The Spop was calculated as the mean value of S over the entire population sample. This analysis was calculated manually in an Excell spreadsheet.

The percent polymorphic RAPD loci (%P) were calculated for each population, as well as the mean value for all populations.

Measures of genetic distance

Based on the phenotypic data, a pairwise genetic distance measure (Jaccard's) was calculated using the following formula (Sneath and Sokal, 1973):

$$D = 1 - (S_{ij}/T_{ij})$$

where S is the total number of shared present band positions and T is the total number of band positions shared between the ith and the jth individuals. The genetic distance matrix generated was subjected to a Principal Coordinate Analysis (PCO) (R package, Legendre and Vaudor, 1991), which produce a visual representation (principal coordinate plot (PCO)) of the genetic relationship among individuals in the sample (Hollingsworth and Ennos, 2004).

Measures of genetic structures

In order to analyse the population structure of the taxa, analysis of molecular variance (AMOVA) was calculated from the pairwise genetic distance using Jaccard distance measure to examine variation (i) between populations and (ii) within populations. The Arlequin programme generates Φ statistics (Excoffier *et al.*, 1992), which is analogous to Wright's FST (Wright, 1951). This approach has been widely adopted in the analysis of RAPD data (Allnut *et al.*, 1999, Diaz *et al.*, 2001, Bekessy *et al.*, 2002, Newton *et al.*, 2002, Nkongolo *et al.*, 2002, Castro-Felix *et al.*, 2008). This analysis was conducted using the Software program Arlequin (Schneider *et al.*, 2000).

RESULTS

Genetic diversity in populations of *Pinus culminicola*

The five primers tested for the analysis of *Pinus culminicola* showed banding patterns with very high reproducibility and clear band resolution. These five primers pro-

Table 2. Shannon diversity index (*S*) and percent polymorphic RAPD loci (%P) of *Pinus culminicola*.

Population	Shannon Index (*S*)	% Polymorphism	Mean S_{pop}	Mean % polymorphism
Sierra La Viga	0.5692	57.8		
Cerro El Potosí	0.5690	56.8	0.569	57.3

duced a total of 72 distinct bands, 52 were polymorphic across the whole sample.

The percentage of polymorphism found in *P. culminicola* was 57.3%. The degree of diversity in this taxon, measured by Shannon index was 56% (Table 2).

Principal coordinate analysis (PCO) was conducted to examine the relationship among the two populations of *Pinus culminicola* (Fig. 2). The two principal coordinates of RAPD distance described 7.78% and 7.57% of the variation, respectively. Individual samples from each population plotted as a continuous scatter, with the two populations overlapping.

The genetic variation between the two populations of *Pinus culminicola* was conducted by AMOVA analysis (Table 3).

The total variation found between the two populations was 5.98 % and this value was highly significant (P = 0.0001, tested using 1000 replications). However most of the variation was found within populations (94.02 %).

DISCUSION

Genetic diversity between populations of *Pinus culminicola*

The percentage of polymorphism of RAPD loci was relatively low (57.3%) compared with the other species of Cembroides group analysed (*Pinus remota* 84.4% and *P. cembroides* var. *bicolor* 69.9%, Favela, 2005) and also slightly low when is compared with other pine and tree species. A reduction in the proportion of polymorphic loci has been previously reported for endemic species (Hamrick and Godt, 1996). Even though this value is reported as low it is not as low as has been reported in other isolated population of Mexican pine species (*P. chiapensis* 24.5%, *P. maximartinezii* 30.3%, *P. greggii* 31.9% and *P. rzedowskii* 46.8%) (Newton *et al.*, 2002, Ledig *et al.*, 1999, Parraguirre *et al.*, 2002, Delgado *et al.*, 1999).

Diversity estimates indicated by Shannon index (0.56) did not differ between populations of *Pinus culminicola*. This value is comparable with other species of conifers

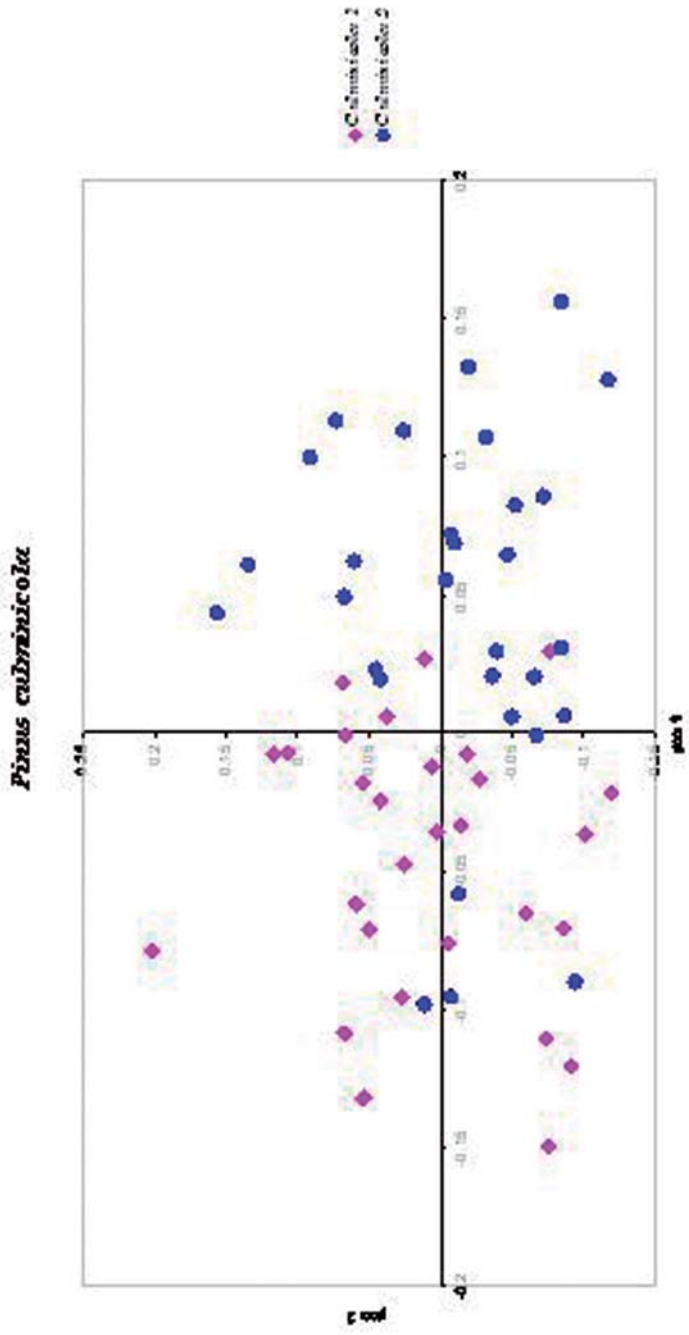

Fig. 2. Plot of the first two principal coordinates of pairwise distance derived from RAPD analysis from two populations of *Pinus culminicola* (describing 7.78% for *P. culminicola* 1 and 7.57% for *P. culminicola* 2 of the variation).

Table 3. AMOVA of RAPD variation among two populations of *Pinus culminicola***.**

	d.f.	Sum of squares	Variance components	% of total variation	P value
Among populations	1	20.7	0.45	5.98	0.0001
Within populations	58	414.3	7.14	94.02	

(0.53) (*Araucaria*, *Fitzorya* and *Cedrela*) survey by Bekessy *et al.* (2002), Allnut *et al.* (1999) and Gillies *et al.* (1997), respectively.

Genetic diversity within populations of ***Pinus culminicola***

Most of the variation in *Pinus culminicola* (94.02%) was recorded within populations, a result consistent with those from most of the other pine species (Ledig, 1998) and woody plant species. Hamrick *et al.* (1992) indicated that long-lived woody plant species tend to maintain higher levels of allozyme variation within populations. This is also comparable with results obtained from RAPD data where most of the tree species examined showed high levels of variation within populations (Gillies *et al.*, 1997, Allnut *et al.*, 1999, Bekessy *et al.*, 2002, Newton *et al.*, 2002).

Genetic diversity among populations of *Pinus culminicola*

The degree of genetic variation among populations found in *Pinus culminicola* was small (5.9%), but significant. For instance, when the genetic distance matrix was subjected to a PCO analysis (Fig. 2), the pattern showed evidence of gene flow between populations due to pollen interchange. However the genetic variation among populations for long-lived species is strongly influenced by geographical distribution (Hamrick *et al.*, 1992). Among the many species of pines studied previously, some endemic or isolated scattered distributions were found to retain higher gene differentiation among populations (e.g. *Pinus rzedowskii* (0.17) (Delgado *et al.*, 1999); *P. pinceana* (0.15) (Ledig *et al.*, 2001); *P. lagunae* (0.18), *P. muricata* (0.16) (Molina-Freaner *et al.*, 2001) and P. *strobiformis*, *P. ayacahuite*, *P. lambertiana* and *P. chiapensis* (0.5) (Castro-Felix *et al.*, 2008)). In contrast pine species with widespread and continuous distributions have retained lower gene differentiation among populations (e.g. *P. albicaulis* (0.034) (Jorgensen and Hamrick, 1997); *P. banksiana* (0.03) (Dancik and Yeh, 1983); *P. ponderosa* (0.015) (Hamrick *et al.*, 1989) and *P. oocarpa* (0.0054) (Sáenz *et al.*, 2003).

The low differentiation between the two populations of *Pinus culminicola* was par-

ticularly unexpected, given the restricted and isolated geographical range of distribution. However, Hamrick and Godt (1996) counted 274 studies in Pinaceae alone. One of the emerging generalities was that conifers had substantial genetic diversity within population and only low levels of differentiation among populations. In a review of 195 isozyme studies of long-lived perennial woody taxa, specifically in gymnosperms, Hamrick *et al.* (1992) recorded an overall mean Gst value of 6.5% in the genetic variation among populations. Such differentiation suggests that even though populations of *P. culminicola* have restricted and isolated distribution, gene flow among populations has been sufficient to prevent dramatic loss of genetic variation. Another point to consider is that these two populations may have been in historical contact in the geologically recent time, and hence the similarity may merely reflect insufficient time for differentiation to occur. However, there is no clear evidence to support this last point due to the lack of studies related to the postglacial history of the species. Information does exist in *Pinus banksiana* and *Pinus contorta* which can be used as the base for future studies in pinyon pine species from the northeast of Mexico.

CONCLUSION

The genetic variation found within two populations of *Pinus culminicola* was high and in concordance with previous studies where pine and tree species have been included. The degree of genetic differentiation found among populations of *P. culminicola* was low but consistent with values recorded for other conifers species. The extent of differentiation suggests that even though populations of *P. culminicola* have a restricted and isolated distribution, gene flow among populations has, at least presently, not lead to dramatic loss of genetic variation and population differentiation. Future work on the structure of population in pinyon pine species is needed in order to have a better understanding of the history of the species in the mountain range "Sierra Madre Oriental" where these species grows.

LITERATURE CITED

Allnut, T.R., A.C. Newton, A. Lara, A. Premoli, J.J. Armesto, R. Vergara and M. Gardner, 1999. "Genetic Variation in Fitzroya cupressoides (alerce), a threatened South American conifer". *Molecular Ecology*, **8**: 975-987.

Bekessy, S. A.,T.R.Allnut, A.C. Premoli, A. Lara, R.A. Ennos, M.A. Burgman, M. Cortes and A.C. Newton, 2002. "Genetic variation in the vunerable and endemic Monkey Puzzle tree, detected using RAPDs". *Heredity*, **88**: 243-249.

Castro-Felix, P., J.A. Pérez de la Rosa, G. Vargas Amado, S. Velázquez Magaña, A. Santerre, F. López-Dellamary Toral, A.R. Villalobos-Arámbula, 2008. "Genetic relationship among Mexican white pines (*Pinus*, Pinaceae) based on RAPD markers". *Biochemical Systematics and Ecology*, **36**: 523-530.

Dancik, B.P. & F.C. Yeh, 1983. "Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*Pinus banksiana*) in Alberta". *Can. J. Genet. Cytol.*, **25**: 57-64.

Delgado, P., D. Piñero, A. Chaos, N. Pérez-Nasser and E.R. Álvarez-Buylla, 1999. "High population differentiation and genetic variation in the endangered Mexican pine *Pinus rzedowskii* (Pinaceae)". *American Journal of Botany*, **86**: 669-676.

Diaz, V., L.M. Muñiz, and E. Ferrer, 2001. "Random amplified polymorphic DNA and amplified fragment length polymorphism assessment of genetic variation in Nicaraguan populations of *Pinus oocarpa"*. *Molecular Ecology*, **10**: 2593-2603.

Doyle, J. J. & J. L. Doyle, 1990. "Isolation of plant DNA from fresh tissue". *Focus*, **12**: 13-15.

Excoffier, L., P.E. Smouse and J.M. Quattro, 1992. "Analyses of molecular variance inferred form metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data". *Genetics*, **131**: 479-491.

Farjon, A. & C.N. Page, 1999. *Conifers. Status Survey and Conservation Action Plan*. IUCN/SSC Conifer Specialist Group, Gland, Switzerland and Cambridge, UK.

Favela, L.S., 2005. "Taxonomy and genetic variation of pinyon pines (*Pinus* subsection Cembroides) in the Northeast of Mexico". Unpublished doctoral disertation, University of Edinburgh, Scotland.

Gillies, A.C.M., J.P. Cornelius, A.C. Newton, C. Navarro and M. Hernández, 1997. "Genetic variation in Costa Rican populations of the tropical timber species *Cedrela odorata* L., assessed using RAPDs". *Molecular Ecology*, **6**: 1133-1145.

Hamrick, J.L., H.M Blanton, J.K. Hamrick, 1989. "Genetic structure of geographically marginal populations of ponderosa pine". *American Journal of Botany*, **76**: 1559-1568.

Hamrick, J.L. & Godt, M.J.W., 1996. "Conservation genetics of endemics plant species". In: J.C. Avise & J.L. Hamrick (Eds.). *Conservation Genetics: Case Histories from Nature*. Chapman & Hall. New York.

Hamrick, J.L., M.J.W. Godt, S.L. Sherman-Broyles, 1992. "Factors influencing levels of genetic diversity in wood plant species". *New Forests*, **6**: 95-124.

Hollingsworth, M.L., J.P: Bailey, P.M. Hollingsworth and C. Ferris, 1999. "Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae)". *Botanical Journal of the Linnean Society*, **129**: 139-154.

Hollingsworth, P.M. & R.A.Ennos, 2004. "Neighbour joining trees, dominant markers and population genetic structure". *Heredity*, **92**: 490-498.

Isabel, N., J. Beaulieu, and J. Bousquets, 1995. "Complete congruence between gene diversity estimates derived from genotypic data at enzyme and random amplified polymorphic DNA loci in

black Spruce". *Proc. Natl. Acad. Sci. USA*, **92**: 6369-6373.

IUCN (2001). *IUCN Red List Categories and Criteria*: version 3.1. Gland, Switzerland and Cambridge, UK, IUCN Species Survival Commission, IUCN.

Jorgensen, S.M. & J.L. Hamrick, 1997. "Biogeography and population genetics of whitebark pine, *Pinus albicaulis*". *Can. J. For. Res.*, **27**: 1574-1585.

Ledig, F.T., 1998. "Genetic variation in *Pinus*". In: D.M. Richardson, (Ed.) *Ecology and Biogeography of* Pinus. Cambridge University Press, Cambridge, UK. pp. 251-280.

Ledig, F.T., M.A. Capo-Arteaga, P.D. Hodgskiss, H. Sbay, F.L. C., M.T. Conkle, and B. Bermejo-Velázquez, 2001. "Genetic diversity and the mating system of a rare Mexican pynon, *Pinus pinceana*, and a comparison with *Pinus maximartinezii* (Pinaceae)". *American Journal of Botany*, **88**: 1977-1987.

Ledig, F.T., B. Velázquez-Bermejo, and J. Vargas-Hernández, 2000. *Genetic differentiation in Mexican Conifers*. The 16th North American Forest Biology workshop & The Western Forest Genetic Association. Merida, Mexico. July 2000.

Legendre, P., & A. Vaudor, 1991. *The R Package: Multidimensional analysis, spatial analysis*. Département de sciences biologiques. Université de Montreal: iv +124.

Lewontin, R.C., 1972. "The apportionment of human diversity". *Evolution Biology*, **6**: 381-398.

Molina-Freaner, F., P. Delgado, D. Piñero, N. Perez-Nasser, and E.R. Alvarez-Buylla, 2001. "Do rare pines need different conservation strategies? Evidence from three Mexican species". *Can. J. Bot.*, **79**: 131-138.

Newton, A.C., T.R. Allnut, A.C.M. Gillies, A. Lowe, and R.A. Ennos, 1999. "Molecular phylogeography, intraspecific variation and the conservation of tree species". *Trends in Ecology and Evolution*, **14**: 140-145.

Newton, A.C., T.R. Allnut, W.S. Dvorak, R.F. Del Castillo, and R.A. Ennos, 2002. "Patterns of genetic variation in *Pinus chiapensis*, a threatened Mexican pine, detected by RAPD and mitochondrial DNA RFLP markers". *Heredity*, **89**: 191-198.

Nkongolo, K.K., P. Michael, and W.S. Gratton, 2002. "Identification and characterization of RAPD markers inferring genetic relationships among Pine species". *Genome*, **45**: 51-58.

Parraguirre, L.C., J.J. Vargas Hernández, P. Ramírez Vallejo, H.S. Azíproz Rivero y J. Jasso Mata, 2002. "Estructura de la diversidad genética en poblaciones naturales de *Pinus greggii* Engelm". *Revista Fitotecnia Mexicana*, **25**(3), 279-287.

Perry, J.P., A. Graham, and D.A. Richardson, 1998. "The history of pines in

Mexico and Central America". In: D.M. Richardson (Ed.), *Ecology and Biogeography of* Pinus. Cambridge University Press. Cambridge, UK. pp: 137-152.

Sáenz-Romero, C. and B.L. Tapia-Olivares, 2003. "*Pinus oocarpa* isoenzymatic variation along an altitudinal gradient in Michoacán, México". *Silvae Genetica*, **52**(5-6): 237-240.

Sneath, P.H. A. & R.R. Sokal., 1973. *Numerical Taxonomy*. W.H. Freeman, San Francisco, USA.

Schneider, S., D. Roessli and L. Excoffier, 2000. *Arlequin: A software for population genetics data analysis*. University of Geneva.

Wright, S., 1951. "The genetical structure of populations". *Annals Eugenics*, **15**: 323-354.

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