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GENETIC DIVERSITY AND THE MATING SYSTEM OF A RARE MEXICAN PIÑON, *PINUS PINCEANA*, AND A COMPARISON WITH *PINUS MAXIMARTINEZII* (PINACEAE)[1]

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Weeping piñon (*Pinus pinceana*) has a restricted and fragmented range, trees are widely scattered within populations, and reproduction is limited. Nevertheless, genetic diversity was high; based on 27 isozyme loci in 18 enzyme systems, unbiased expected heterozygosity averaged 0.174. Differentiation also was high (F_{ST} = 0.152), reflecting isolation between southern, central, and northern fragments of the range. Among populations in the northern fragment, F_{ST} was only 0.056, and the number of migrants per generation (Nm) was 4.21, which should preclude fixation. Nm between central and southern populations or between them and populations in the northern fragment was lower, 0.99–1.66, indicating a degree of genetic isolation. Multilocus outcrossing rates (t_m) ranged from 0.836 in the south to 0.897 in the north. Therefore, selfing is low but statistically significant. The equilibrium inbreeding coefficient (F_e) calculated from t_m was in good agreement with observed inbreeding coefficients, suggesting that weeping piñon may be near equilibrium with respect to inbreeding and selection against selfed trees. Weeping piñon was variable at all loci polymorphic in maxipiñon (*Pinus maximartinezii*) and, therefore, qualifies as a possible progenitor of maxipiñon. Because of the high level of diversity, reasonable levels of gene flow within the northern fragment of weeping piñon's range, high rates of outcrossing, and, perhaps, only weak selection against inbred trees, protection in reserves would be a viable option for conservation.

Key words: endangered species; fitness; fragmentation; genetic distance; genetic structure; isozymes; pollen allele frequencies; selfing.

Fragmentation into small, scattered populations is expected to lead to genetic isolation, random genetic drift, inbreeding, loss of genetic diversity, differentiation of populations, and increased probability of extinction (Frankham, 1998; Saccheri et al., 1998; Westemeier et al., 1998). Many species in Mexico are represented by small, scattered populations, and also are presumed threatened or endangered. Of a total 22 000 plant species in Mexico, 52% are endemic (Rzedowski, 1993), and many endemics are among those endangered. Among 16 endemics in a total of 42 pines that occur in Mexico (Farjon and Styles, 1997), 7 (44%) are listed by the International Union for Conservation of Nature and Natural Resources (IUCN) as species of concern (Farjon and Page, 1999). Nevertheless, in anemophilous species with high pollen output, such as pines, mechanisms to promote outcrossing might offset the effects of fragmentation, reducing the loss of genetic diversity and the danger of extinction (Ledig, 1998). In Mexican pines, the net effect of fragmentation seems to have been speciation, since Mexico is a secondary center of diversity for the genus (Eguiluz-Piedra, 1988; Styles, 1993).

Weeping piñon (*Pinus pinceana* Gordon), a Mexican endemic, provides an excellent opportunity to study the effects of fragmentation and low density on patterns of genetic variation and inbreeding. Weeping piñon is a short tree or bush of the Sierra Madre Oriental, reaching from 4 m tall at maturity up to 10 or, perhaps, 12 m (Martínez, 1953; Perry, 1991; Farjon and Styles, 1997). It ranges from Coahuila in the north to Hidalgo in the south (Fig. 1), a distance of ~750 km, and is distributed between 1400 and 2700 m in elevation on calcareous slopes or in barrancas (Farjon and Styles, 1997). Of all the Mexican pines, it occupies the most arid sites, below or just overlapping the lower border of the true piñon–juniper zone (Passini, 1985). Annual precipitation is only ~300–400 mm, falling mostly in the summer months (Perry, 1991). Trees may be scattered far apart among Mexican piñon (*Pinus cembroides* Zuccarini) and drooping juniper (*Juniperus flaccida* Schlechtendal), or, at lower elevations, with a variety of sclerophyllous and deciduous shrubs of the desert (Farjon and Styles, 1997). Not only is weeping piñon scattered among oth-

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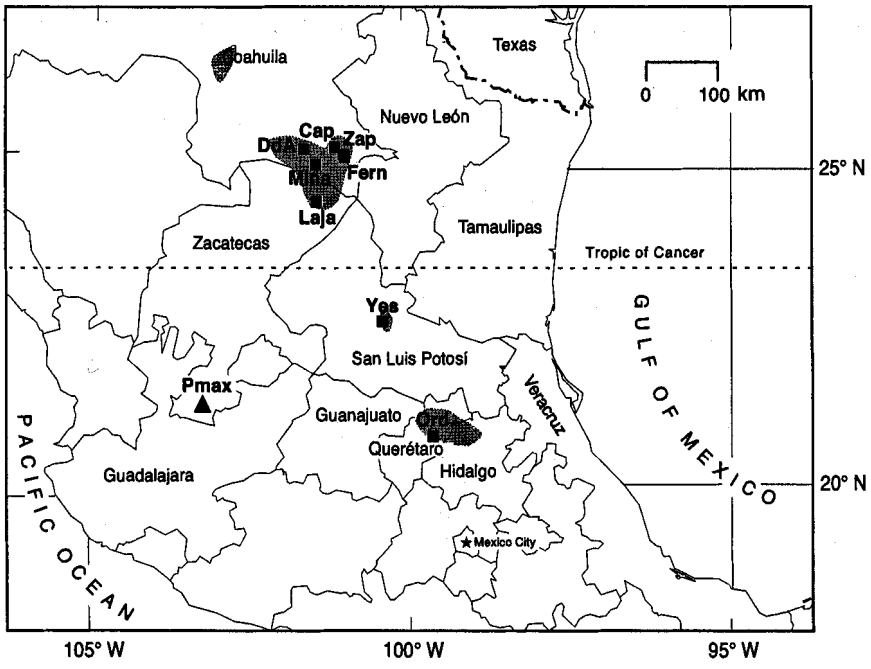

Fig. 1. The ranges of weeping piñon (shaded) and maxipiñon (▲) after Perry (1991), and the locations of weeping piñon populations (■) sampled for isozyme analysis. Abbreviations are: Cap = Ejido de Capulin, DdA = Dos de Abril, Zap = Sierra Zapalinamé, Fern = Jagüey de Ferniza, Mina = Sierra la Mina, Laja = Cañon de la Laja, Yes = Cañon la Yesera, Ord = Bajada al Arroyo de Orduña, Pmax = *Pinus maximartínezii.*

er tree and shrub species where it occurs, but its range is highly fragmented, especially in the south, in the States of San Luis Potosí, Querétaro, and Hidalgo. It is considered a paleorelict (Perry, 1991).

Weeping piñon is classified as "LRnt" on the IUCN Red List, which means that it is considered at "lower risk" than species in the Vulnerable or Endangered categories but is "near threatened," or close to qualifying for Vulnerable, probably based on its low area of occupancy and fragmented range (Farjon and Page, 1999). It was placed in the "lower risk" category in part because information about the species was lacking. However, Perry (1991) claimed it was "clear that this species is now rare and endangered" because in all areas that he visited, "reproduction was extremely limited" and mature trees were scarce. Grazing by goats, burros, and range cattle reduces or eliminates seedling reproduction, and large branches are removed for firewood, limiting even the capacity for reproduction. Seed harvest and consumption by the campesinos may also be a factor.

The taxonomy of several rare piñons, including weeping piñon, is still in flux. Weeping piñon was provisionally included in subgenus *Strobus,* section *Parryanae,* subsection *Nelsoniae* by Farjon and Styles (1997). The only other member of this subsection is Nelson piñon (*Pinus nelsonii* G. R. Shaw). In the most recent taxonomic treatment of the pines, Price, Liston, and Strauss (1998) included weeping piñon with several other piñons in subsection *Cembroides,* but it is certainly not closely related to other members of the group, such as the common Mexican piñon, and these authors call for further analysis of the species in their subsection *Cembroides.* Perry (1991) placed weeping piñon in a new subsection, subsection *Pinceana,* which included Nelson piñon, maxipiñon (*Pinus maximartinezii* Rzedowski), and Rzedowski piñon (*Pinus rzedowskii* Madrigal Sánchez & Caballero Deloya).

Most authors have felt that maxipiñon, which Farjon and Styles (1997) did not assign to any subsection, is closely related to weeping piñon (Rzedowski, 1964; Zavarin and Snajberk, 1987; Perry, 1991; Farjon, 1999). Maxipiñon is known from only a single location. Weeping piñon and maxipiñon are very similar in their monoterpene composition (Zavarin and Snajberk, 1987). Phylogenetic trees, constructed from variation in cpDNA, group weeping piñon and maxipiñon, but place Nelson piñon and Rzedowski piñon on distinctly different branches (Pérez de la Rosa, Harris, and Farjon, 1995). Cladograms based on morphology and phenology separate weeping piñon and maxipiñon from the true piñons of subsection *Cembroides* and place them on the same branch as Nelson piñon, Rzedowski piñon, and Asian nut pines of subsection *Gerardianae;* however, maxipiñon branches off first and is not closely associated with any of the other species (Malusa, 1992). By contrast, weeping piñon clustered with singleleaf piñon (*Pinus monophylla* Torrey & Fremont) and fourneedle piñon (*Pinus quadrifolia* Parlatore ex Sudworth) and not with maxipiñon, based on seed and cotyledon characteristics (Eguiluz-Piedra, Niembro-Rocas, and Pérez-Rodríguez, 1985). The most recent phylogeny, using the sequence of an internal transcribed spacer region of nuclear ribosomal DNA, strongly supported a clade of weeping piñon, Rzedowski piñon, and maxipiñon as a sister clade of piñons in the *Cembroides* group and placed Nelson piñon as an outgroup to the subsection *Cembroides* (Liston et al., 1999).

Very little has been published about the genetic structure of weeping piñon. In an analysis of the monoterpene composition in three populations of weeping piñon, Zavarin and Snajberk (1987) declared that the variability within and between populations was "exceptionally small," a characteristic weeping piñon shared with maxipiñon, which is also considered a relict. The lack of monoterpene variability suggested that weeping piñon may lack genetic diversity. However, recent analysis of

TABLE 1. Location of weeping piñon stands sampled for this study.

Stand	Property	Municipio[a]	State	Latitude	Longitude	Elevation (m)
Ejido[b] el Capulin	Ejido el Capulin	Parras	Coahuila	25°21′ N	101°11′ W	2700
Dos de Abril	Ejido Dos de Abril	Parras	Coahuila	25°19′ N	101°40′ W	1800
Sierra Zapalinamé	Ejido el Recreo	Saltillo	Coahuila	25°15′ N	101°03′ W	2060
Jagüey de Ferniza	Ejido Jagüey de Ferniza	Saltillo	Coahuila	25°14′ N	101°02′ W	2100
Sierra la Mina	Ejido Garambullo	Saltillo	Coahuila	25°05′ N	101°29′ W	2300
Cañon de la Laja	Ejido Pabellón	Concepción del Oro	Zacatecas	24°34′ N	101°28′ W	2500
Cañon la Yesera	Ejido Nuñez	Guadalcazar	San Luis Potosí	22°40′ N	100°26′ W	1900
Bajada al Arroyo de Orduña	Ejido San Joaquin	Cadereyta	Querétaro	20°55′ N	99°38′ W	2080

[a] A municipio is a political division of a state.
[b] Ejidos are lands that were given to groups of peasants after the 1910 revolution and usually held communally.

isozymes in five populations of weeping piñon suggested high variability (Molina-Freaner et al., 2001).

We undertook a survey of weeping piñon to determine its level of genetic diversity and its genetic structure as a step in planning conservation measures. Within stands, weeping piñon is so scattered that we felt inbreeding might be an important consideration in its conservation. Therefore, we investigated its mating system. We also compared gene frequencies in weeping piñon to those in maxipiñon to see how closely the two species were related and, specifically, whether weeping piñon could be a progenitor of maxipiñon.

MATERIALS AND METHODS

Cones were collected from eight populations of weeping piñon in 1992 and 1993 by the Centro de Genética Forestal (CGF) and the Universidad Autónoma Agraria Antonio Narro (UAAAN). The populations covered nearly the entire latitudinal range of weeping piñon (Fig. 1 and Table 1). At Bajada al Arroyo de Orduña and Cañon la Yesera, total height and diameter at breast height (dbh) were measured on the trees from which cones were collected, and 8-mm diameter increment cores were extracted at 1.5 m above the ground. Age of the trees was determined by ring counts on the increment cores.

Cones were maintained separately by trees within populations, and seeds were extracted at UAAAN or CGF and stored under refrigeration until shipped to the Institute of Forest Genetics (IFG), California. Isozymes were analyzed at IFG between June 1994 and January 1995, using starch gel electrophoresis of the megagametophytes and embryos. In pines, the nutritive tissue of the seed is a haploid megagametophyte that gives rise to, and is of the same genotype as, the egg.

Alleles at a locus can be detected by segregation among megagametophytes from a heterozygote. The genotype of the seed parent can be determined by analyzing a number of megagametophytes. When two different alleles at a locus are detected, the seed parent is unequivocally a heterozygote. When only one allele is detected, the tree is classified as a homozygote, although the possibility remains that it is a heterozygote and that by chance the sample included only one allele. The probability of misclassification decreases with increases in sample size. We assayed four megagametophytes per tree (or nine in Cañon la Yesera). With a sample of four, there is a probability of 0.125 of misclassifying a heterozygote as a homozygote at any one locus. That is, the probability that all four megagametophytes in a sample from a heterozygous tree carry the same allele is $2(½)^4 = 0.125$.

Seeds were germinated in petri dishes, and when radicles emerged, megagametophytes and embryos were dissected, separated, and extracted. For estimating allele frequencies, the number of parent trees per population, N, varied from 7 to 30 (a mean, $\bar{N}$, of 15.3, or $2N$ = 14–60 genomes). Because cones were absent on most trees in some populations, the number of trees sampled was small. Therefore, we also estimated allele frequencies and variability statistics from the pollen contribution to the embryos. Because we know the contribution of the egg (the haploid genotype of the megagametophyte) to the embryo, the pollen contribution can be deduced by subtraction. Allele frequencies from the pollen genotypes were based on an N of 44–123 per locus per population ($\bar{N}$ = 70.3 genomes per population).

We used techniques of starch gel electrophoresis based on the laboratory manual of Conkle et al. (1982) to assay enzyme systems. In the megagametophytes, we were able to consistently score 27 presumptive loci in 18 enzyme systems, and in the embryos, 24 loci in 16 enzyme systems. The enzymes MNR and TPI, scored in the megagametophytes, could not be consistently scored in embryos. The enzymes ADH, FEST, LAP, MNR, PGI, PGM, and TPI were run on a Tris citrate/lithium borate buffer (buffer "A" of Conkle et al., 1982); CAT, FDH, GDH, and GOT were run on a Tris citrate/sodium borate buffer (buffer "B" of Conkle et al., 1982); and ACO, ALD, CADH, IDH, MDH, 6PG, and SKD were run on a morpholine citrate buffer (buffer "E," a pH 8.0 version of buffer "D" of Conkle et al., 1982). We interpreted the number of loci and alleles by drawing on the experience gained in our laboratory from studies of allozymes of other conifer species (Conkle, 1981). Samples of red pine (*Pinus resinosa* Aiton), which has proven to be an almost invariably monomorphic species, were included on each gel to aid interpretation. Where several zones of activity were observed for a single enzyme, numerals following the enzyme abbreviation were used for identification.

To determine whether allele frequencies in the trees were similar to those in the pollen pool, we used a chi-square test:

$$\chi^2 = (2N_T + N_P)(x_{qT} + x_{qP})/q,$$

where N_T is the number of trees, N_P is the number of pollen gametes, q is the mean frequency of the common allele in the trees and the pollen pool, and x_{qT} and x_{qP} are the binomial variances of gene frequencies in the trees and the pollen pool, respectively (Snedecor, 1956; p. 228). The variances are $q_T(1 - q_T)/2N_T$ and $q_P(1 - q_P)/N_P$.

For trees and the pollen pool, we separately estimated percent polymorphic loci, alleles per locus, and heterozygosity for each population, and Nei's (1978) unbiased genetic distance and Rogers' (1972) distance between pairs of populations with BIOSYS (Swofford and Selander, 1981). For small samples such as ours, BIOSYS calculates unbiased heterozygosity (Nei, 1978). We tested the relationships between mean annual height and diameter increments and individual heterozygosity at Bajada al Arroyo de Orduña by regression. Individual heterozygosity was calculated as the number of loci heterozygous in a tree, divided by the number of polymorphic loci for which the tree was scored. All inferences apply to the mature, cone-bearing or pollen-producing trees in the populations.

For the trees, fixation indices (F) within populations were calculated as the mean of the deviations of observed homozygosity from Hardy-Weinberg expectations at each locus. It is not possible to calculate fixation indices from pollen allele frequencies. We also used BIOSYS to calculate Wright's (1965) F statistics. BIOSYS calculates F_{IS} and F_{IT}, the fixation indices of trees relative to the population and the meta-population, respectively, as weighted averages across alleles. F_{ST}, the proportion of the total genic diversity among populations, is calculated from the relationship

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST}).$$

Because stands of weeping piñon are clustered within regions that are widely separated, we divided populations into a northern region with six populations, and central and southern regions with one population each. "Step Wright78" of BIOSYS was used to apportion variation among regions, among populations within the northern region, and among trees within populations for the megagametophytes.

The degree of genetic isolation among populations was estimated by Nm, the number of migrants per generation, calculated by two methods. From Wright (1951) we have

$$Nm = (1 - F_{ST})/4F_{ST}.$$

Nm also can be calculated from the frequency of private alleles (unique alleles found in only one population), using simulations developed by Slatkin (1985):

$$\log_{10}(\bar{p}(1)) = \alpha \log_{10}(Nm) + \beta,$$

where $\bar{p}(1)$ is the mean frequency of private alleles and α and β are constants determined by fitting simulated data developed for sample sizes of 10 and 25 (from Barton and Slatkin, 1986). Estimates of Nm were corrected for actual mean sample size, 17.5, by factors of 10/17.5 and 25/17.5, respectively.

To test for a relationship of genetic distance to geographic distance between pairs of populations, we used Mantel's generalized regression procedure (Mantel, 1967; Liedloff, 1999). Geographic distance between populations was calculated from latitude and longitude using Kindred's (1997) distance calculator.

Populations were grouped into phylogenetic trees with distance Wagner procedures (Farris, 1972; Swofford and Selander, 1981). The nodes of the Wagner tree represent hypothetical common ancestors (HTUs, hypothetical taxonomic units) for the branches above them. The sum of the branch lengths that connect any two populations (OTUs, operational taxonomic units) is the patristic difference, which is an approximation of the number of evolutionary steps occurring in the phyletic lines connecting the OTUs. The root of the tree is the closest common ancestor of all the populations, and total branch length from this root approximates the divergence from this common ancestor. The distance Wagner procedure is preferable to the unweighted pair-group (UPGMA) method because the latter depends on the assumption that the rate of divergence between lines is homogeneous (Farris, 1972). When founder events or genetic drift are likely events in the divergence of OTUs, rates of divergence are probably heterogeneous, and phenetic-similarity clustering techniques to estimate evolutionary trees are likely to be misleading and inadvisable. Because Nei's (1978) genetic distance does not obey the triangle inequality, it is inappropriate for distance Wagner procedures (Swofford and Selander, 1981). Therefore, Rogers' (1972) distance measure was used.

To compare weeping piñon to maxipiñon, we used data from Ledig et al. (1999), reducing the data set for each species to 24 common loci in 16 enzyme systems. We calculated Nei's (1978) unbiased genetic distance between taxa based on the 24 loci in common.

For mating system analysis, we used Ritland's (1986, 1989, 1990a, b, 1994) generalized multilocus estimation (MLTF) and multilocus mating system (MLTR) programs to calculate the single- and multiple-locus estimates of outcrossing rate, t_s and t_m, in the samples from Sierra Zapalinamé, Cañon la Yesera, and Bajada al Arroyo de Orduña. Sierra Zapalinamé and Bajada al Arroyo de Orduña were among the largest samples because they had either the greatest number of families or the greatest number of progeny (120 and 100 megagametophyte and embryo pairs, respectively), and they represent the northern and southern portions of the weeping piñon range. Cañon la Yesera represents the central portion of the range, and the number of megagametophyte–embryo pairs was 63. Generalized multilocus estimation was used to estimate t_m because the probability of outcrossing becomes much more accurate when information on the egg genotype is used (Ritland, 1990a). Furthermore, smaller progeny arrays are adequate and bias is reduced when the megagametophyte genotype is known. The Newton-Raphson method, as explained in Ritland (1990a), was used for iteration, and p, the allele frequency, and t were estimated jointly. Standard errors for t at some loci in some populations were taken from the estimated values in 100 bootstraps using MLTR because the Newton-Raphson iterations failed to converge to reasonable values, a common occurrence. MLTR also was used to estimate the correlation of outcrossed paternity, rp, among progeny within a family. Although adequate for estimation of population outcrossing rates, samples were too small to estimate family outcrossing rates; estimates failed to converge for most families.

RESULTS

At every locus, the frequency of the common allele was in fair agreement between seed trees and pollen. None of the 107 possible comparisons between the frequencies of the common allele in the trees to that in the pollen pool indicated significant differences. The most deviant case was allele number 1 at *Pgi-2* in Cañon la Yesera ($q_T = 0.643$ and $q_P = 0.548$), where chi-square was 2.74; with 1 df, the probability of a larger value is ~0.10. Because seed-tree and pollen allele frequencies were similar, only the frequencies for the seed-trees are presented (Table 2). A table of the pollen allele frequencies is available from the corresponding author upon request.

Of the 27 loci scored in the megagametophytes, 23 were polymorphic in at least one population (Table 2) and four were monomorphic (*Fdh, Idh-1, Pgi-1,* and *Pgm*) in all eight populations. In addition, *Gdh* was monomorphic in the pollen. In both the trees and the pollen pool, 14 alleles were private, i.e., found in only one population.

Unbiased estimates of expected heterozygosity, H_e, were similar for the trees and the pollen pool (Table 3); the mean for the trees was 0.174 and ranged from 0.128 to 0.201. For both the seed trees and the pollen pool, the percentage polymorphic loci, P_p, averaged between 50 and 60%, and the number of alleles per locus, A, was ~1.8–1.9. Observed heterozygosity was less than expected heterozygosity in every population, suggesting inbreeding (Table 3). The mean of the inbreeding coefficients, 0.116, was similar to Wright's F_{IS}, 0.140 (Table 4). An F between 0.116 and 0.140 suggests that the parents of the present generation were related on average as half-sibs because the inbreeding coefficient is half the relationship coefficient of the parents, and the relationship coefficient of half-sibs is 0.250.

For 30 weeping piñon at Bajada al Arroyo de Orduña, mean dbh and height were 9.1 cm and 3.2 m, respectively. Mean age, by ring count at 1.5 m above the ground, averaged ~38 yr. The tallest tree was only 4.5 m in height and 12.5 cm dbh, with 38 rings. The oldest tree was 69 yr old and only 3.0 m in height. For the seven trees sampled at Cañon la Yesera, mean height and dbh were even less than that at Bajada al Arroyo de Orduña, 2.6 m and 6.5 cm, respectively, and the trees were younger, ~30 yr old on average. At Bajada al Arroyo de Orduña, where data were sufficient for regression analysis, mean annual basal area increment was not related to individual heterozygosity, but mean annual height increment was ($r = 0.51$, $P < 0.05$; Fig. 2).

Wright's F_{ST} was 0.152, which indicates substantial variation among populations, i.e., 15.2% of the genic diversity is among populations. However, much of this variation was a result of the two southern populations, Cañon la Yesera and Bajada al Arroyo de Orduña. Within the six populations in the northern cluster, F_{ST} was only 0.056.

Nei's genetic distance (D) also expresses population differentiation. Nei's D was minimal among populations in the northern cluster of Coahuila and Zacatecas, but much greater between the northern populations and those representing the

TABLE 2. Allele frequencies for 23 polymorphic loci in eight populations[a] of weeping piñon. Sample sizes[b] are given in parentheses following population abbreviations.

Locus	Allele	Cap (34)	DdA (30)	Zap (50)	Fern (22)	Mina (32)	Laja (36)	Yes (14)	Ord (60)
Aco-1	1	0.767	0.533	0.660	0.591	0.563	0.722	1.000	0.981
	2	0.067	0.000	0.040	0.000	0.094	0.167	0.000	0.000
	3	0.167	0.467	0.300	0.409	0.344	0.111	0.000	0.019
Adh-1	1	0.588	0.667	0.540	0.727	0.469	0.389	0.714	0.800
	2	0.412	0.333	0.400	0.227	0.500	0.528	0.000	0.200
	3	0.000	0.000	0.060	0.045	0.031	0.083	0.286	0.000
Ald-1	1	0.550	0.556	0.625	0.700	0.550	0.438	1.000	0.796
	2	0.150	0.333	0.325	0.100	0.250	0.563	0.000	0.204
	3	0.300	0.111	0.050	0.200	0.200	0.000	0.000	0.000
Ald-2	1	0.964	0.962	0.955	1.000	0.857	0.929	0.929	0.891
	2	0.036	0.038	0.045	0.000	0.107	0.071	0.071	0.109
	3	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000
Cadh	1	1.000	1.000	1.000	1.000	1.000	1.000	0.714	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.286	0.000
Cat	1	1.000	1.000	0.940	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000
Fest	1	0.294	0.533	0.340	0.273	0.313	0.529	0.143	0.017
	2	0.706	0.467	0.660	0.636	0.688	0.441	0.857	0.983
	3	0.000	0.000	0.000	0.091	0.000	0.029	0.000	0.000
Gdh	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.983
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
Got-1	1	1.000	1.000	1.000	0.909	1.000	0.941	1.000	1.000
	2	0.000	0.000	0.000	0.091	0.000	0.059	0.000	0.000
Got-2	1	0.844	0.967	0.880	0.800	0.867	0.867	1.000	1.000
	2	0.125	0.000	0.060	0.050	0.133	0.133	0.000	0.000
	3	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000
	5	0.031	0.033	0.000	0.050	0.000	0.000	0.000	0.000
Got-3	1	0.882	0.967	0.920	0.909	0.969	0.941	1.000	1.000
	2	0.088	0.000	0.080	0.091	0.031	0.029	0.000	0.000
	3	0.029	0.000	0.000	0.000	0.000	0.029	0.000	0.000
	4	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
Idh-2	1	1.000	1.000	1.000	1.000	1.000	1.000	0.857	0.931
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.069
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
Lap-1	1	1.000	1.000	0.958	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000
Lap-2	1	0.219	0.429	0.354	0.611	0.500	0.472	0.000	0.040
	2	0.563	0.571	0.500	0.167	0.500	0.417	0.000	0.300
	3	0.156	0.000	0.104	0.056	0.000	0.056	1.000	0.620
	4	0.063	0.000	0.042	0.167	0.000	0.056	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040
Mdh-2	1	0.588	0.767	0.740	0.864	0.594	0.917	0.214	0.833
	2	0.412	0.233	0.260	0.136	0.406	0.083	0.786	0.167
Mdh-3	1	1.000	0.967	0.900	0.955	0.906	1.000	0.571	0.983
	2	0.000	0.000	0.060	0.045	0.063	0.000	0.000	0.000
	3	0.000	0.000	0.040	0.000	0.031	0.000	0.429	0.017
	4	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
Mnr-1	1	0.941	0.933	0.920	0.955	0.844	0.889	0.857	0.967
	2	0.059	0.067	0.080	0.045	0.156	0.111	0.143	0.033
Mnr-2	1	1.000	1.000	1.000	1.000	0.969	1.000	1.000	0.983
	2	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.017
6pg-1	1	0.700	0.833	0.881	0.850	0.893	0.962	0.357	0.587
	2	0.133	0.167	0.048	0.050	0.036	0.000	0.643	0.239
	3	0.033	0.000	0.000	0.100	0.000	0.038	0.000	0.000
	4	0.133	0.000	0.071	0.000	0.071	0.000	0.000	0.174
Pgi-2	1	0.647	0.633	0.760	0.909	0.844	0.861	0.643	0.867
	2	0.294	0.367	0.240	0.091	0.156	0.139	0.000	0.000
	3	0.029	0.000	0.000	0.000	0.000	0.000	0.357	0.133
	4	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skd-1	1	0.971	0.967	0.880	0.909	0.938	1.000	0.786	0.650
	2	0.000	0.033	0.080	0.091	0.031	0.000	0.000	0.017
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.214	0.333
	4	0.000	0.000	0.040	0.000	0.031	0.000	0.000	0.000
	5	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skd-2	1	0.941	1.000	0.960	0.864	1.000	0.917	0.714	0.933
	2	0.059	0.000	0.000	0.000	0.000	0.000	0.286	0.033
	3	0.000	0.000	0.000	0.045	0.000	0.083	0.000	0.033
	4	0.000	0.000	0.040	0.091	0.000	0.000	0.000	0.000

TABLE 2. Continued.

Locus	Allele	Cap (34)	DdA (30)	Zap (50)	Fern (22)	Mina (32)	Laja (36)	Yes (14)	Ord (60)
Tpi	1	0.607	0.846	0.853	0.667	0.808	1.000	1.000	1.000
	2	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.357	0.154	0.147	0.333	0.192	0.000	0.000	0.000

[a] Cap = Ejido de Capulin, DdA = Dos de Abril, Zap = Sierra Zapalinamé, Fern = Jagüey de Ferniza, Mina = Sierra la Mina, Laja = Cañon de la Laja, Yes = Cañon la Yesera, Ord = Bajada al Arroyo de Orduña.
[b] $2N$, the number of genomes, or twice the number of trees.

central and southern portions of the range (Table 5). A distance Wagner tree based on Rogers' (1972) genetic distance graphically reflects the relationships among populations (Fig. 3). The association between geographic distance and Nei's genetic distance was weak and nonlinear; using Mantel's generalized regression procedure, $r = 0.54$ (Table 5, Fig. 4). The correlation was weak because Cañon la Yesera is genetically unique and is at intermediate distance from the other populations. Genetic distance between weeping piñon and maxipiñon was about twice as great as the maximum distance between populations of weeping piñon and nearly two orders of magnitude greáter than distances between populations in Coahuila and Zacatecas.

The number of migrants per generation, Nm, estimated from Wright's F_{ST} was 1.39. Calculated from private alleles, Nm was similar, 1.89 or 2.09. However, within the northern cluster of populations, Nm was higher, 4.21. Between populations in the northern cluster and Bajada al Arroyo de Orduña, Nm averaged 2.13, and between the northern populations and Cañon la Yesera, it averaged 0.99. Between Bajada al Arroyo de Orduña and Cañon la Yesera, Nm was only 1.66. Therefore, much of the differentiation observed was a result of the inclusion of Cañon la Yesera and Bajada al Arroyo de Orduña. Not only do the central and southern fragments appear genetically isolated from the northern part of the range, they are largely isolated from each other.

The multilocus outcrossing rate ranged from 0.836 ($0.732 < t_m < 0.940$; $P = 0.95$) at Bajada al Arroyo de Orduña, the southernmost population sampled, to 0.897 ($0.823 < t_m < 0.971$; $P = 0.95$) at Sierra Zapalinamé, in the north (Table 6). The lower estimate, at least, suggests significant selfing, in agreement with the inbreeding coefficient, F_{IS}, of 0.140. In every case, the mean single-locus outcrossing rate was lower than the multilocus rate (Table 6), and the standard errors indicated that the difference approached statistical significance at Bajada al Arroyo de Orduña and Sierra Zapalinamé. The difference between t_m and t_s suggests that some inbreeding occurred by crosses among relatives, in addition to inbreeding by selfing. The difference might also be due to the Wahlund effect if our sampling scheme included different subpopulations.

The correlation of outcrossed paternity, rp, among progeny within a family varied from 0.042 at Sierra Zapalinamé to 0.346 at Cañon la Yesera. An rp of 0.346 is high and means that the probability that two randomly chosen outcrossed progeny within a maternal family were full-sibs (i.e., had the same pollen parent) was 34.6%. Therefore, at Cañon la Yesera there was a predominance of biparental crosses, crosses among near neighbors or with similar flowering phenology. However, large standard errors were associated with the estimates of rp.

DISCUSSION

Estimates of genetic diversity in weeping piñon were substantially higher than average for long-lived, woody endemics but close to average for outcrossing endemic plant species in

TABLE 3. Genetic diversity and fixation indices in weeping piñon: H_e = expected heterozygosity (unbiased estimate), H_o = observed heterozygosity, P_p = percent polymorphic loci, A = number of alleles per locus, F = fixation index (standard errors in parentheses).

	Pollen				Trees					
Population	n[a]	H_e	P_p	A	n[b]	H_e	H_o	P_p	A	F[c]
Sierra la Mina	60.7	0.184 (0.044)	58.3	1.9 (0.2)	15.2	0.193 (0.041)	0.167 (0.038)	59.3	1.9 (0.2)	0.082
Jagüey de Ferniza	41.0	0.169 (0.039)	58.3	2.0 (0.2)	10.4	0.188 (0.040)	0.135 (0.029)	59.3	1.9 (0.2)	0.195
Sierra Zapalinamé	97.1	0.191 (0.041)	66.7	2.2 (0.2)	24.1	0.192 (0.038)	0.172 (0.037)	66.7	2.0 (0.2)	0.097
Dos de Abril	57.4	0.169 (0.042)	58.3	1.8 (0.2)	14.3	0.166 (0.041)	0.137 (0.034)	55.6	1.6 (0.1)	0.083
Ejido el Capulin	64.6	0.179 (0.044)	54.2	2.1 (0.3)	16.1	0.201 (0.045)	0.156 (0.035)	55.6	2.0 (0.2)	0.118
Cañon de la Laja	66.9	0.174 (0.042)	58.3	1.9 (0.2)	16.7	0.154 (0.040)	0.137 (0.041)	51.9	1.7 (0.2)	0.181
Cañon la Yesera	60.4	0.133 (0.037)	45.8	1.5 (0.1)	7.0	0.167 (0.039)	0.143 (0.040)	44.4	1.5 (0.1)	0.076
Bajada al Arroyo de Orduña	114.5	0.160 (0.040)	58.3	1.8 (0.2)	28.5	0.128 (0.035)	0.103 (0.027)	59.3	1.8 (0.2)	0.099
Mean		0.170	57.3	1.9		0.174	0.144	56.5	1.8	0.116

[a] Mean number of genomes sampled per locus at 24 loci.
[b] Mean number of trees sampled per locus at 27 loci.
[c] Calculated as the mean of the fixation indices for all polymorphic loci.

TABLE 4. Estimates of Wright's (1965) *F* statistics for 23 polymorphic loci in weeping piñon.

Locus	F_{IS}	F_{IT}	F_{ST}
Aco-1	0.001	0.143	0.142
Adh-1	−0.049	0.057	0.101
Ald-1	0.325	0.412	0.129
Ald-2	0.228	0.248	0.026
Cadh	−0.400	−0.037	0.259
Cat	−0.064	−0.008	0.053
Fest	0.237	0.334	0.127
Gdh	−0.017	−0.002	0.015
Got-1	1.000	1.000	0.061
Got-2	0.307	0.346	0.056
Got-3	0.025	0.058	0.034
Idh-2	−0.105	−0.021	0.076
Lap-1	−0.043	−0.005	0.037
Lap-2	0.150	0.397	0.290
Mdh-2	0.175	0.347	0.208
Mdh-3	0.241	0.412	0.226
Mnr-1	−0.020	0.003	0.023
Mnr-2	−0.027	−0.006	0.020
6 pgl	0.148	0.318	0.199
Pgi-2	0.144	0.237	0.109
Skd-1	0.023	0.157	0.137
Skd-2	0.063	0.169	0.114
Tpi	0.268	0.377	0.149
Mean	0.140	0.271	0.152

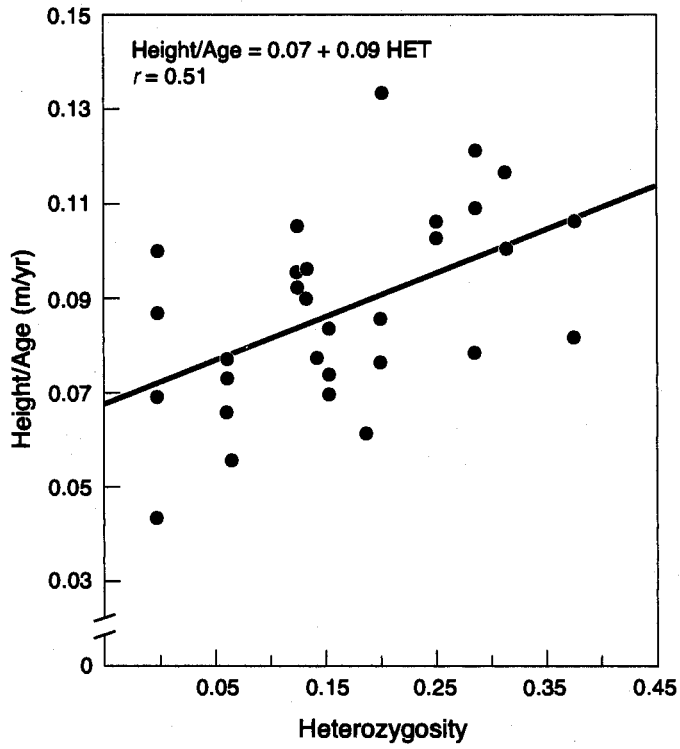

Fig. 2. The relation of mean annual height increment to heterozygosity (heterozygous loci as a proportion of 13–16 polymorphic loci scored per tree) in 30 weeping piñon at Bajada al Arroyo de Orduña, Querétaro.

general; mean H_e was only 0.056 for 20 long-lived woody endemics (Hamrick, Godt, and Sherman-Broyles, 1992) and 0.142 for 57 outcrossing endemic plants (Hamrick and Godt, 1996). For the weeping piñon mother-tree data (27 loci), unbiased H_e ranged from 0.128 to 0.201, and averaged 0.174. Percentage polymorphic loci (Pp) in weeping piñon was 44.4–66.7%, about the same as the mean for outcrossing endemics (54.4%) but twice as high as that for woody endemics (26.3%) reviewed by Hamrick and Godt (1996) and Hamrick, Godt, and Sherman-Broyles (1992). Of course, the category "endemics" spans a range of geographic scales.

Genetic structure of weeping piñon was as expected for endemics with fragmented ranges; populations were strongly differentiated. About 15.2% of the observed diversity in weeping piñon was among populations compared to 17.9% for all outcrossing plant endemics (Hamrick and Godt, 1996) and 14.1% for woody endemics (Hamrick, Godt, and Sherman-Broyles, 1992).

The combination of high levels of diversity and high levels of population differentiation appears to be common in Mexican conifers. Molina-Freaner et al. (2001) found high genetic diversity and high differentiation in weeping piñon, Laguna pine (*Pinus lagunae* Passini), and Mexican populations of bishop pine (*Pinus muricata* D. Don). In weeping piñon H_e was 0.373 and F_{ST} was 0.247, based on 13 isozyme loci and five populations (Molina-Freaner et al., 2001). In nine populations of the Mexican endemic Rzedowski piñon, H_e averaged 0.219, and F_{ST} was 0.175 (Delgado et al., 1999), even though the geographic distance between the most widely separated populations was only ~25 km. Other Mexican pines with similar genetic structure are Gregg's pine (*Pinus greggii* Engelmann ex Parlatore; Ramírez-Herrera and Vargas-Hernández, 1996) and Apache pine (*Pinus engelmannii* Carrière; Bermejo-

TABLE 5. Half-matrices of Nei's (1978) unbiased genetic distances (*D*, above diagonal) calculated from frequencies of 27 loci scored in megagametophytes and geographic distances (in kilometers, below diagonal) between pairs of weeping piñon populations, and a comparison with maxipiñon.

Population[a]	Cap	DdA	Zap	Fern	Mina	Laja	Yes	Ord	Pmax[b]
Cap		0.007	0.003	0.011	0.003	0.021	0.077	0.031	0.202
DdA	49		0.000	0.008	0.003	0.009	0.104	0.045	0.214
Zap	17	63		0.005	0.000	0.006	0.086	0.029	0.204
Fern	20	64	2		0.006	0.018	0.096	0.036	0.257
Mina	42	32	47	48		0.009	0.095	0.040	0.198
Laja	91	85	87	86	57		0.120	0.047	0.191
Yes	307	319	293	291	288	235		0.042	0.243
Ord	516	530	501	499	499	446	211		0.238
Pmax	488	466	485	484	449	398	323	377	

[a] Cap = Ejido de Capulin, DdA = Dos de Abril, Zap = Sierra Zapalinamé, Fern = Jagüey de Ferniza, Mina = Sierra la Mina, Laja = Cañon de la Laja, Yes = Cañon la Yesera, Ord = Bajada al Arroyo de Orduña, Pmax = *Pinus maximartinezii*.

[b] Genetic distances with maxipiñon based on 24 common loci scored in both species.

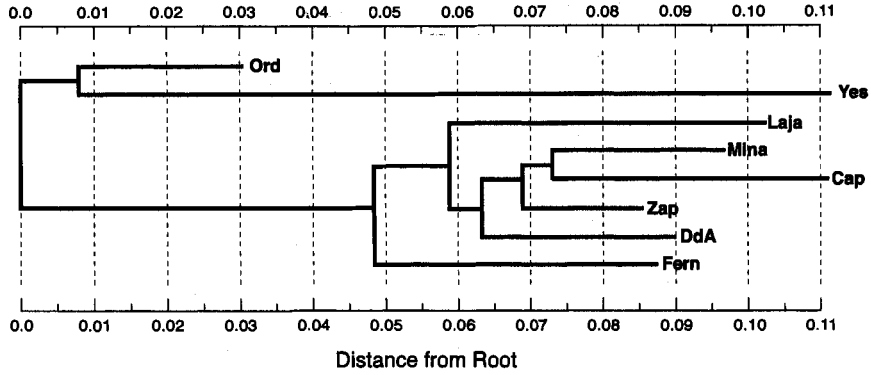

Fig. 3. Distance Wagner tree for eight populations of weeping piñon, calculated with Rogers' (1972) genetic distance. Abbreviations are: Cap = Ejido de Capulin, DdA = Dos de Abril, Zap = Sierra Zapalinamé, Fern = Jagüey de Ferniza, Mina = Sierra la Mina, Laja = Cañon de la Laja, Yes = Cañon la Yesera, Ord = Bajada al Arroyo de Orduña.

Velázquez, 1993). Populations of some Mexican firs (*Abies* spp. Linnaeus) were also well differentiated (Aguirre-Planter, Furnier, and Eguiarte, 2000).

Low rates of gene flow may lead to differentiation among populations because of random genetic drift, and estimates of Nm over the range of weeping piñon were relatively low for pines, between 1.39 and 2.09. Nm in pines generally ranges between 4.6 and 17.2 in rangewide studies (reviewed in Ledig, 1998). The value of 4.6 was recorded in Table Mountain pine (*Pinus pungens* Lambert), a species with scattered populations, like weeping piñon. Lower values of Nm have been found in another Mexican endemic, Chihuahua spruce (*Picea chihuahuana* Martínez), and in Coulter pine (*Pinus coulteri* D. Don), both species with highly fragmented ranges in which the evidence for genetic drift was unmistakable (Ledig et al., 1997; Ledig, 2000).

Much of the differentiation among populations was associated with gaps between the northern cluster of populations and the central and southern fragments. For example, mean values of Nm between populations in the northern cluster and the central and southern populations were 0.99 and 2.13, respectively, while within the northern cluster, Nm was 4.21. The latter value approaches the typical range for pines. Cañon la Yesera may differ from the other populations because of its isolation, coupled with the small area occupied by weeping piñon within the central fragment, or it may be an artifact of our sample size. Because Nm takes many hundreds or thousands of generations to reach equilibria, our estimates probably reflect past gene exchange (Slatkin and Barton, 1989). Thus, the estimates are mainly valuable by comparison to other species.

The mixed mating model for the estimation of outcrossing rate assumes loci are in linkage equilibrium. Although the number of progeny in our families was too small to estimate disequilibrium or even the recombination rate between loci, some of the loci that we used are almost certainly linked. Linkage arrangements are highly conserved in pine, and Conkle (1981) reported tight linkage between *Ald-1* and *Got-3* and

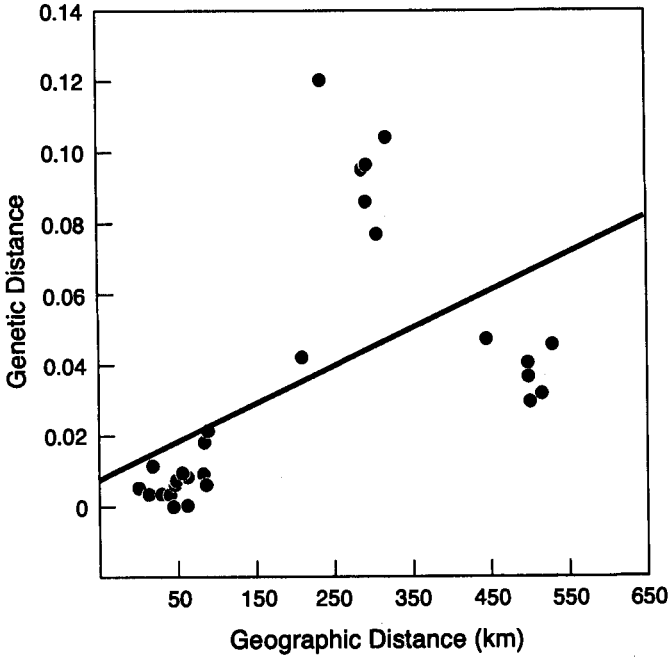

Fig. 4. Nei's unbiased genetic distance in relation to geographic distance among stands of weeping piñon.

TABLE 6. Single-locus and multilocus outcrossing estimates[a] in three populations of weeping piñon (standard errors in parentheses).

Locus[a]	Bajada al Arroyo de Orduña	Cañon la Yesera	Sierra Zapalinamé
Aco-1	0.261 (0.384)	—	0.931 (0.090)
Adh-1	1.057 (0.112)	0.834 (0.304[b])	1.045 (0.169[b])
Ald-2	—	0.773 (0.169)	—
Cat-1	—	—	0.901 (0.168)
Fest	—	0.857 (0.163)	0.798 (0.142)
Got-2	—	—	0.712 (0.147)
Got-3	—	—	0.537 (0.215)
Idh-2	0.063 (0.190)	0.289 (0.015[b])	—
Lap-2	0.673 (0.107)	—	0.976 (0.099)
Mdh-2	0.903 (0.157)	0.845 (0.128)	0.960 (0.127)
Mdh-3	—	0.814 (0.247[b])	0.796 (0.151)
Mnr-1	—	—	0.544 (0.254)
Pgi-2	0.699 (0.194[b])	1.066 (0.202[b])	0.553 (0.155)
Skd-1	1.053 (0.144[b])	1.223 (0.200[b])	0.785 (0.142)
Skd-2	—	0.710 (0.177)	—
t_s[b]	0.762 (0.055)	0.829 (0.071)	0.832 (0.073)
t_m	0.836 (0.052)	0.876 (0.047)	0.897 (0.037)
rp[b]	0.172 (0.094)	0.346 (0.193)	0.042 (0.132)

[a] Calculated with Ritland's (1990b) MLTF unless otherwise noted.
[b] Calculated with Ritland's (1994) MLTR.

between an *Idh* locus and an *Aco* locus. He also reported tight to moderate linkage between *Got-2* and *Lap-2* in several pine species, and found moderate to weak linkage between *Adh-1* and *Pgi-2* and weak linkage between *Adh-1* and *Lap-2*. At least some of Conkle's (1981) loci must be homologous to the loci that we scored in weeping piñon, and because linkage is likely, our estimates of t_m deserve closer scrutiny. Having said that, we feel the estimates are valid because the means of the single-locus estimates, t_s, of outcrossing were similar to t_m. The single-locus model does not, of course, depend on the assumption of linkage equilibrium between loci. Therefore, linkage probably had little effect on the estimate of t_m reported here.

Despite the low density of the woodlands where weeping piñon is dispersed among Mexican piñon, juniper, sclerophyllous vegetation, or succulents, outcrossing is predominant. Although the data suggest a tendency toward higher rates of selfing in the southern fragments of the species, larger samples are needed to determine whether the trend is real. Based on the differences between single-locus and multilocus estimates of outcrossing, some crossing among relatives may be occurring. In the southern and central populations, the correlation of outcrossed paternity, rp, suggests that a high proportion of matings may occur between neighbors or pairs of synchronously flowering trees, reducing the opportunities for recombination.

Selfing and/or crossing between related trees was reflected in excess homozygosity. Inbreeding coefficients were positive in all of the eight populations sampled, and overall, F_{IS} was 0.140. At Bajada al Arroyo de Orduña, we were able to test the relationship between homozygosity and growth, a fitness surrogate, to detect inbreeding depression. Results were equivocal. Mean annual height increment was correlated positively with individual heterozygosity (Fig. 2), but mean annual basal area increment was not. Inbreeding depression is most pronounced under competition and is cumulative over time (Ledig, Guries, and Bonefeld, 1983). Neither factor was likely to accentuate the relationship in weeping piñon. The trees at Bajada al Arroyo de Orduña were widely spaced and were, on average, only 38 yr old. Further studies are needed.

The equilibrium inbreeding coefficient, F_e, is related to the outcrossing rate, t, as (Allard, Jain, and Workman, 1968):

$$F_e = (1 - t)/(1 + t).$$

Substituting our estimates of t_m for t yields an estimated F_e of 0.088 for Cañon la Yesera, 0.066 for Bajada al Arroyo de Orduña, and 0.058 for Sierra Zapalinamé, compared to observed fixation indices of 0.076, 0.099, and 0.097 (Table 3), respectively. Thus, observed F approximates F_e well, suggesting that these populations may be at or near equilibrium with respect to inbreeding and selection against selfs.

The inbreeding depression of selfed progeny can be estimated from the inbreeding coefficient and the rate of selfing (Ritland, 1990c):

$$w = 2[(1 - s)F/(1 - F)s],$$

where w is the fitness of selfs relative to outcrosses, F is the inbreeding coefficient estimated for the parental generation (Table 3), and s is the rate of selfing ($t = 1 - s$). This assumes that the population is in equilibrium, i.e., adult inbreeding coefficients and rates of selfing are constant from generation to generation, an assumption partially reinforced by the agreement between observed F and F_e. If the assumption is violated, the model will tend to overestimate w and thus provide an upper bound to the fitness of selfs. For Bajada al Arroyo de Orduña, Cañon la Yesera, and Sierra Zapalinamé, w = 1.12, 1.16, and 1.87, respectively, suggesting that inbreds were as fit as outcrosses. However, these estimates are imprecise and should be considered upper limits.

The distance Wagner tree suggests two clades, one of the six northern populations and the other of Cañon la Yesera and Bajada al Arroyo de Orduña. Bajada al Arroyo de Orduña, the southernmost population in our sample, is closest to the root of the tree, the hypothetical common ancestor. The central population and the northern populations all have diverged from the root to a similar degree. However, these relationships are tentative because of the limited sampling of populations from the southern and central fragments of the range. Increased sampling might demonstrate three clades.

The genetic distance beween weeping piñon and maxipiñon (Table 5) is of a different order of magnitude than the distance between populations within weeping piñon, as it should be for two morphologically distinct and allopatric species. The important question is whether weeping piñon could be a progenitor of maxipiñon. Maxipiñon seems to have gone through an extreme bottleneck, and some of us (Ledig et al., 1999) predicted that its progenitor must be polymorphic for at least the loci variable in maxipiñon. Maxipiñon is polymorphic for *Mpi*, but we could not score *Mpi* in weeping piñon. However, weeping piñon met the criterion for all other loci at which maxipiñon was polymorphic; i.e., *Ald-2, Got-3, Lap-2, Mnr-1, 6Pg–1, Pgi-2, Skd-1,* and *Tpi-1* were polymorphic in maxipiñon and weeping piñon, although not every population of weeping piñon was polymorphic for all eight loci.

The genetic distance is least between maxipiñon and Cañon de la Laja in Zacatecas. Cañon la Yesera is the geographically closest population to maxipiñon, but the genetic distance between Cañon la Yesera and maxipiñon is among the highest. Maxipiñon is known from a single population near the southern end of the Sierra Madre Occidental. Cañon la Yesera and maxipiñon are in different cordillera, separated by the arid Meseta Central of México. A more direct migration route connects Cañon de la Laja to the southern Sierra Madre Occidental, because the morphotectonic provinces of the Sierra Madre Occidental and the Sierra Madre Oriental are in contact at the northern end of the Sierra Madre Oriental (Ferrusquía-Villafranca, 1993).

The genetic structure of weeping piñon provides a hopeful note with regard to its conservation, which stands in contrast to the concerns expressed by IUCN (Farjon and Page, 1999) and Perry (1991). Although it may be a paleorelict, weeping piñon does not seem to be in a genetic extinction vortex. Genetic diversity, based on 27 loci, is among the highest recorded in our laboratory. Nor is weeping piñon suffering from high rates of inbreeding. Although some selfing is indicated, it is not nearly as high as we expected in populations where trees were widely scattered. Furthermore, F seems to be close to equilibrium conditions, perhaps suggesting that the present population structure and mating system have existed for many generations.

Nevertheless, grazing by domestic animals is a relatively new phenomenon, dating no earlier than the Spanish conquest in 1519–1521. Weeping piñon now is threatened by population pressure, as are many species; the greater the human population, the more grazing animals. Grazing animals destroy seed-

lings, and the reproductive potential of mature trees is reduced because limbs are cut for firewood.

We recommend that areas be set aside for protection of weeping piñon, where grazing, cutting for firewood, and seed collection for consumption can be excluded. A minimum of three areas should be established, at least one each in the areas represented by (1) Coahuila and Zacatecas, (2) San Luis Potosí, and (3) Querétaro and Hidalgo, because of the strong genetic differentiation among these major fragments of the species' range. A program of ex situ conservation is also desirable. Weeping piñons could be planted for the value of their edible seeds in the market, and they would also be valuable in horticulture because of their attractive weeping form and their drought resistance. Ecological studies are needed to determine the timing of seed crops, seed longevity, the effect of seed predators, natural agents of seed dispersal, and the type of seed bed and climatic conditions conducive to the germination and survival of weeping piñon seedlings, to better manage the species for long-term survival. The role of humans in the ecology of weeping piñon should not be neglected, for it may be that pre-Hispanic people were an important component in sustaining the relict piñon ecosystem (Bye, 1993).

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